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Maskew, Mhairi

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The Epidemiology and Effects of Kaposi's Sarcoma Herpesvirus in the Setting of the Southern African HIV Epidemic

By

Mhairi Maskew

Thesis presented for the degree of
Doctor of Philosophy
in the School of Public Health, Faculty of Health Sciences
University of the Witwatersrand

October 2013

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Mhairi Maskew
MBBCh, MSc (Med)

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October 2013

Supervisors: Dr Matthew Fox (Boston University) and Professor Patrick MacPhail (University of the Witwatersrand)

This thesis is presented in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in the School of Public Health, Faculty of Health Sciences, University of the Witwatersrand. The work on which this thesis is based is original research and has not, in whole or in part, been submitted for another degree elsewhere. The contents of this thesis are entirely the work of the named student. The contribution of the student to multi-authored papers is outlined in the preface to the thesis.



Mhairi Maskew
24 October 2013

ABSTRACT

Background

Immunosuppression and co-infection with oncogenic viruses substantially increase the risk of cancers in HIV-infected patients. Since there has been greater access to antiretroviral therapy (ART), increased longevity among those infected with HIV has made morbidity and mortality from cancers associated with HIV increasingly more common. Viral associated cancers including cervical cancer, non-Hodgkin's lymphoma and Kaposi sarcoma (KS) are prominent among HIV-infected individuals. Kaposi sarcoma is the most common tumour in HIV-infected individuals in Africa and is preceded by infection with Kaposi sarcoma herpes virus (KSHV). The prevalence of KSHV in sub-Saharan Africa is, in fact, among the highest in the world and the region also bears the greatest burden of disease due to HIV. Kaposi sarcoma was relatively common in South Africa (up to 5 per 1000 population at risk) prior to the AIDS epidemic but the incidence increased dramatically as the epidemic escalated. The incidence of KS has decreased in the US and Europe with the introduction of ART but the impact of ART in Africa where the underlying prevalence of KSHV is higher has yet to be determined.

The World Health Organization estimates that just over 5 million HIV-1-infected people were receiving ART in sub-Saharan Africa by the end of 2010. Combination ART has been used to successfully treat early stage KS for some time, achieving regression of KS lesions and successfully reducing KS-related mortality. Despite this, the influence of KS on response to ART is not well defined in resource-limited settings. Additionally, it is unclear if co-infection with oncogenic viruses such as KSHV places untreated HIV-infected patients at increased risk even without clinically apparent illness. KSHV typically establishes a persistent latent infection in its host during which time only latent genes (Orf73) are expressed and only viral particles sufficient to maintain infection are produced. In the presence of HIV-1 co-infection however, immune suppression and cytokine release promotes reactivation of KSHV lytic genes which include K8.1 and active replication and increase in

KSHV viral progeny occurs. Previous in vitro studies have suggested interactions between these two viruses including an increase in HIV-1 viral load in the presence of KSHV and induced reactivation of HIV-1 replication in chronically infected cells. Despite this, there are few analyses describing the effect of co-infection with KSHV on HIV treatment outcomes after initiation of ART.

Several clinical and laboratory markers have been associated with advanced disease stage among untreated HIV-infected individuals, including the T-lymphocyte subpopulations, CD4+ and CD8+ which play an important role in the response to viral infections. While KSHV-specific CD8+ T cell epitope responses have been shown to increase after initiation of HAART, it has yet to be determined if T-lymphocyte subpopulations are also a marker of advanced disease stage in ART naive patients infected with KSHV (as seen among the HIV-infected population) and if this has implications for treatment initiation guidelines.

Methods

First, cohort data from two large urban HIV care and treatment programs in Johannesburg and Cape Town, South Africa, was analysed to assess the effect of KS on survival, loss to follow-up and immunologic and virologic responses to ART. Differences in mortality between those with and without KS at ART initiation were estimated with Cox proportional hazard models. Log-binomial models were used to assess differences in CD4 count response and HIV virologic suppression within a year of initiating treatment.

Secondly, a prospective cohort of HIV-infected adults initiating ART in Johannesburg, South Africa was enrolled. Data from this cohort was used to examine the effect of KSHV seropositivity on immunologic and virologic outcomes in the first year of ART among a cohort of HIV-infected adults attending a large, urban HIV care and treatment program in Johannesburg, South Africa. Subjects were defined as seropositive to KSHV if reactive to either KSHV lytic K8.1 or latent Orf73 antigen or

both. Subjects were followed from ART initiation until 18-months on treatment. HIV viral load and CD4 counts were tested 6 monthly. Linear generalized estimating equations and log-binomial regression models were used to estimate the effect of KSHV infection on immunologic recovery and response as well as HIV viral load suppression within 18-months after ART initiation.

Results

The first study used data from two large HIV clinics to identify 13,847 HIV-infected adults who initiated ART at the sites during the study period. Of those, 2% (n=247) presented with KS at ART initiation. The group was similar to those without KS (n=13,600; 98%) with respect to age, presenting CD4 count and proportion on TB treatment. Subjects with KS were, however, over three times more likely to have died at any time after ART initiation (hazard ratio [HR] =3.62; 95% CI 2.71-4.84) than those without KS. Those with KS also gained, on average, 29 fewer CD4 cells (95% CI 7-52 cells/mm^3) and were less likely to increase their CD4 count by 50 cells from baseline (relative risk [RR]=1.43; 95%CI 0.99-2.06) within the first 6-months of treatment.

The second study, a prospective cohort study, enrolled and screened 404 study participants at ART initiation for antibodies to KSHV lytic K8.1 and latent Orf73 antigens. Seropositivity to KSHV was defined as positive to either lytic KSHV K8.1 or latent KSHV Orf73 antibodies. Of the 404 participants, 193 (48%) tested positive for KSHV at ART initiation; with 76 (39%) reactive to lytic K8.1, 35 (18%) to latent Orf73 and 82 (42%) to both. One individual presented with clinical KS at ART initiation. KSHV seropositivity was not associated with body mass index, tuberculosis status, WHO stage, HIV RNA levels, full blood count or liver function tests at initiation. Those with detectable KSHV viraemia (n=19), however, appeared to present with signs of more advanced HIV disease including anaemia and WHO stage 3 or 4 defining conditions compared to those in whom the virus was undetectable. Over the 18 months of follow up, the KSHV positive group gained a similar number of CD4 cells at 6-

(difference of 10 cells/mm^3, 95%CI:-11-31), 12- (3cells/mm^3, 95%CI:-19-25) and 18-months (24cells/mm^3, 95%CI:-13-61) on ART compared to the KSHV negative group. Adjusted relative risk of failure to suppress viral load to <400 copies/mL (1.03; 95%CI: 0.90-1.17) was also similar for the KSHV positive and KSHV negative groups after ART initiation.

Conclusions

The prospective cohort study demonstrated a high prevalence of KSHV among HIV-infected adults initiating ART in a large urban public-sector HIV clinic in South Africa. KSHV viraemia but not KSHV seropositivity may be associated with markers of advanced HIV disease. HIV-positive adults co-infected with KSHV achieved similar immunologic and virologic responses to ART early after treatment initiation compared to those KSHV negative. However, the prognosis for those who have developed clinical disease due to KS is substantially worse than those without KS. HIV-infected adults presenting with KS demonstrated increased risk of mortality even after initiation of ART with the greatest risk in the first year. Even among those who survive the first year on therapy, subjects with KS demonstrated a poorer immunologic response to ART than those without KS.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks and acknowledge the following colleagues and friends who contributed to this thesis:

I am indebted to my supervisors, Professor Patrick MacPhail and Dr Matthew Fox for their patience and constant support throughout the years developing this thesis. I consider myself blessed to have two incredible mentors to turn to.

Dr Denise Whitby provided and developed the KSHV serological assays locally in South Africa and I am grateful to her and Carole Wallis for all their technical support and assistance.

I thank the IeDEA-SA collaboration for supporting the KSHV prospective cohort study and Professor Matthias Egger for providing invaluable critical review of the manuscripts derived from that study.

I am grateful to Kate Shearer for the assistance with the analysis of the KSHV and mortality manuscript.

Khensani Chauke and Constance Mongwenyana assisted with data collection for the KSHV prospective cohort study and their hard work is much appreciated.

I thank the directors and clinical staff of the Themba Lethu Clinic for hosting the KSHV prospective cohort study and for their dedication to the treatment of HIV in South Africa.

Finally, I am most grateful to my family and particularly my husband, Darryl, for seeing this process through with me to its completion.

LIST OF PAPERS

1. Mhairi Maskew, Patrick Macphail, Denise Whitby, Matthias Egger, Carole L Wallis and Matthew P Fox. Prevalence and predictors of Kaposi Sarcoma Herpes Virus seropositivity: A cross-sectional analysis of HIV-infected adults initiating ART in Johannesburg, South Africa. *Infectious Agents and Cancer* 2011, **6**:22

2. Mhairi Maskew, A Patrick MacPhail, Denise Whitby, Matthias Egger, Matthew P. Fox. Kaposi sarcoma-associated herpesvirus and response to antiretroviral therapy: A prospective study of HIV-infected adults. *JAIDS* 2013; 63:442–448

3. Mhairi Maskew, Kate Shearer, Patrick MacPhail, Matthew P. Fox. Kaposi sarcoma herpes virus and mortality following initiation with antiretroviral therapy: a prospective study among HIV infected adults in South Africa. *Prepared for submission to AIDS*

4. Mhairi Maskew, Matthew P. Fox, Gilles van Cutsem, Kathryn Chu, Patrick MacPhail, Andrew Boulle and Matthias Egger for IeDEA Southern Africa. Treatment Response and Mortality among Patients Starting Antiretroviral Therapy with and without Kaposi Sarcoma: A Cohort Study. *PLOS ONE* 2013; 8(6): e64392. doi:10.1371/journal.pone.0064392)

ROLE OF STUDENT ON EACH OF THE PAPERS

The following four papers are formally included as part of the thesis:

Paper 1: Mhairi Maskew, Patrick Macphail, Denise Whitby, Matthias Egger, Carole L Wallis and Matthew P Fox. Prevalence and predictors of Kaposi Sarcoma Herpes Virus seropositivity: A cross-sectional analysis of HIV-infected adults initiating ART in Johannesburg, South Africa. *Infectious Agents and Cancer* 2011, **6**:22

Paper 2: Mhairi Maskew, A Patrick MacPhail, Denise Whitby, Matthias Egger, Matthew P. Fox. Kaposi sarcoma-associated herpesvirus and response to antiretroviral therapy: A prospective study of HIV-infected adults. *JAIDS* 2013; 63:442–448

Paper 3: Mhairi Maskew, Kate Shearer, Patrick MacPhail, Matthew P. Fox. Kaposi sarcoma herpes virus and mortality following initiation with antiretroviral therapy: a prospective study among HIV infected adults in South Africa.

Paper 4: Mhairi Maskew, Matthew P. Fox, Gilles van Cutsem, Kathryn Chu, Patrick MacPhail, Andrew Boulle and Matthias Egger for IeDEA Southern Africa. Treatment Response and Mortality among Patients Starting Antiretroviral Therapy with and without Kaposi Sarcoma: A Cohort Study. *PLOS ONE* 2013; 8(6): e64392. doi:10.1371/journal.pone.0064392)

In summary, the candidate was the lead and corresponding author on all of the included papers, and drafted all versions of the manuscripts. All co-authors critically reviewed and approved the submitted manuscripts, and any comments were addressed and where appropriate integrated by the candidate. The candidate personally conducted all of the analyses in the included papers (as outlined in the methods sections of the papers). In addition, three of the papers report on specific

analyses from a prospective cohort study nested in an on-going HIV clinical cohort, the Themba Lethu Clinical Cohort. The student designed the study and supervised enrolment of study participants and primary data collection. The student also played a key role over a six year period in the establishment and on-going maintenance of the datasets that enable other observational analyses such as the analysis of Kaposi sarcoma presented here, including being involved in primary data collection through the provision of clinical care at the site's Oncology clinic for some of this time. The student worked closely with collaborators and prepared the datasets to conduct the first linkages between the Themba Lethu Clinical Cohort and the National Vital Registration Infrastructure Initiative.

ABBREVIATIONS

3TC	lamivudine
AHR	adjusted hazard ratio
ART	antiretroviral therapy
AZT	zidovudine
BMI	body mass index
cART	combination ART
CCMT	Comprehensive care management and treatment
d4T	stavudine
EFV	efavirenz
HIV	Human Immunodeficiency Virus
IeDEA	International Epidemiologic Databases to Evaluate Aids
KS	Kaposi sarcoma
KSHV	Kaposi sarcoma herpesvirus
MSF	Médecins Sans Frontières
MSM	men who have sex with men
NGO	non-governmental organisation
NNRTI	non-nucleoside reverse transcriptase inhibitors
NRTI	nucleoside reverse transcriptase inhibitors
NVP	nevirapine
PEPFAR	President's Emergency Plan for AIDS Relief
PI	protease inhibitor
TDF	tenofovir
WHO	World Health Organization

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CHAPTER 1: INTRODUCTION AND BACKGROUND

Introduction

This thesis seeks to describe the epidemiology and effects of Kaposi sarcoma and its aetiologic virus, the Kaposi sarcoma herpesvirus in the setting of the South African HIV epidemic. In this introductory chapter, the concept of a "disease transition" occurring in the maturing HIV epidemic is presented and the rationale for exploring Kaposi sarcoma and Kaposi sarcoma herpesvirus as part of this transition is outlined. The existing body of literature around these topics are reviewed and the chapter concludes with a summary of gaps in the evidence to date and how this thesis aims to address some of these.

Background

"The greatest mistake in the treatment of diseases is that there are physicians for the body and physicians for the soul, although the two cannot be separated."

~ Plato

Throughout the history of Western medicine, health care providers have sought to classify and categorise disease into clearly delineated compartments. Even in the broadest of conceptual frameworks, we have created a well-defined distinction between communicable and non-communicable disease. Communicable or infectious diseases are illnesses with an infective causative agent that is frequently transmissible. The pathogenic agents include viruses, bacteria, fungi and parasites. The focus of the treating physician is usually on identifying the pathogen, rapidly initiating care and preventing further transmission of the infection. Non-communicable diseases are conditions that are non-infectious and cannot be transmitted between people. They are often referred to as "chronic" diseases as they frequently take a longer period of time to develop and

manifest than their infectious counterparts. Important non-communicable diseases include cancers, cardio-vascular and metabolic diseases and attention is focused on long-term care and management of the condition as well as changing and improving behaviour and lifestyle choices which led to or could exacerbate the condition. The classification of disease into communicable and non-communicable is not limited to textbooks. Medical school training and hospital departments are also organised that way and as a result, so is patient care. The criticism of this divide is as old as epidemiologic theory itself [Barrett-Connor, 1979; Lower, 1982] and yet the classification remains. For those practicing modern day medicine and epidemiology, however, the distinction between communicable and non-communicable disease can be blurred and overlap between these is not uncommon.

While communicable diseases have historically been more significant in terms of associated mortality and disability-adjusted life years (DALYs) [World Health Organisation, 2004], advances in medical technology as well as drug and vaccine development in the 21st century have shaped a new dynamic, particularly for the developed world. This theory of "epidemiologic transition" developed over 40 years ago [Omran 1971] describes how societies progress through several stages in which the major causes of morbidity and mortality shifts from epidemic infectious diseases and malnutrition to non-communicable disease and injuries with a resultant lower overall mortality rate which peaks in older age groups. Others have since pointed out the limitations of this theory [Frenk et al, 1989]; particularly in developing nations where despite some advances, the transition is incomplete and communicable and non-communicable disease both remain important health concerns. This "double-disease" burden poses major challenges in settings where health care resources and infrastructure are often the most limited [Mayosi et al, 2009]. Examples of this interplay between communicable and non-communicable disease include:

- Increased risk of tuberculosis among diabetics [Jeon et al 2008]
- Association between incidence and mortality of tuberculosis and exposure to cigarette smoke [WHO, 2007]
- Prominence of infection-related cancers associated with HIV and AIDS [Sitas et al, 1997; Ullrich et al, 2011]

This thesis will illustrate and expand on this concept using *cancer with a viral aetiology in the setting of a major infectious disease epidemic* as an example. Viruses are small pathogens only capable of replication within another organism's cells. Late in the 19th century the tobacco mosaic virus became the first virus discovered and since then over 5000 different types have been identified and detailed. Viruses are responsible for a vastly diverse range of diseases. The spectrum of disease includes such variety as the relatively innocuous common cold and childhood illnesses such as measles and chicken pox and extends to acutely life threatening disease such as that due to Ebola. Viruses are also the source of many major global pandemics such as smallpox, H1N1 avian flu outbreaks and the major modern pandemic of interest in this discussion, the human immunodeficiency virus (HIV).

The rise of the Human Immunodeficiency virus (HIV)

The physicians who described a new syndrome of rare opportunistic infections and cancers resistant to treatment in the early 1980s in California and New York [Friedman-Kien, 1981, Gottlieb et al 1981; Siegal et al, 1981] could not have foreseen the global impact their discovery would soon make. By the time the viral aetiology of the syndrome was discovered a few years later [Barre-Sinoussi et al, 1983; Gallo et al, 1984; Popovic et al 1984], AIDS was already a disease of clinical and social significance. The causative agent, the human immunodeficiency virus, is now arguably the most well described virus known to man. HIV is a member of the retrovirus family; a lentivirus which attacks the immune system of its host resulting in destruction of CD4 T lymphocytes, macrophages and

dendritic cells. The resultant loss in cell-mediated immunity leaves the infected individual susceptible to infections with various opportunistic pathogens.

Within 30 years of its discovery in 1983, HIV had infected over 60 million people globally and resulted in the deaths of nearly 30 million people [UNAIDS, 2012]. At the end of 2011, 34 million people globally were living with HIV with low and middle income countries bearing the major burden of disease due to HIV and AIDS. Nearly 70% of those infected live in sub-Saharan Africa [UNAIDS, 2012]. Over five and a half million people were estimated to be living with HIV in South Africa by the end of 2011, for an overall national prevalence of 11% [UNAIDS, 2012]. There is wide geographic variability in estimated prevalence with the highest figures (25.8%) in Kwa-Zulu natal and the lowest (5.3%) in the Western Cape province [HSRC, 2009].

The spread of the HIV pandemic has in many ways stalled or even reversed the progress made in the epidemiologic transition for many developing nations, and has reverted the shift back to a focus on communicable illness – the so-called "counter transition" [Frenk, 1989] as millions of people worldwide became infected with the virus and became ill due to other communicable and opportunistic infections associated with HIV. The high rates of tuberculosis (TB) co-infection among those with HIV have been described as the "dual epidemic". TB infects a third of all HIV positive individuals, has become one of the leading causes of morbidity among those with HIV/AIDS and results in a quarter of all HIV related deaths [World Health Organisation, 2012]. HIV is also recognised as the most important risk factor for tuberculosis [World Health Organisation, 2012].

Blurring the borders of disease classification

The advent of antiretroviral therapy has dramatically changed the landscape of disease and prognosis for those with HIV. As the HIV epidemic progressed, treatment options expanded from monotherapy with huge doses of zidovudine (AZT) to single drug combinations of three or four drugs

referred to as combination ART (c-ART) or highly active antiretroviral therapy (HAART). Four classes of antiretroviral drugs are in current use in South Africa: nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase Inhibitors (NNRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs) and protease inhibitors (PIs). Each class targets a different step in the HIV life cycle as it infects new lymphocytes. The profile of each of these is summarised in Table 1.

Current South African National Department of Health Antiretroviral therapy guidelines advocate use of two NRTIs (or one NRTI and one NtRTI) with either an NNRTI or a PI for first and second-line treatment regimens.

The World Health Organization (WHO) estimates that just over 5 million HIV-1-infected people were receiving antiretroviral therapy in sub-Saharan Africa by the end of 2010, for an estimated coverage of patients eligible for ART of 56% [World Health Organisation, 2011]. An estimated two thirds of those requiring ART in South Africa were on treatment by the end of 2011. Country level indicators suggest the epidemic is stabilising in South Africa as the number of new infections annually across all age groups decreases with expanding access to treatment [UNAIDS, 2012].

The prognosis for HIV-infected individuals has considerably improved since the large-scale introduction of ART into the management of HIV. Disease progression and mortality rates have dropped significantly worldwide [Hogg et al, 1998; Jensen-Fangel, 2004] and individuals infected with HIV can expect a quality and prolongation of life that would not have been possible in the pre-ART era. Recent mortality data from the United Kingdom suggests that the average life expectancy of an HIV infected person on stable suppressive ART with a CD4 count >350 cells is close to that of an HIV-uninfected person [May et al, 2012; Nakagawa et al, 2012]. In fact there is even evidence to suggest that HIV infected subjects might even exceed life expectancy of the general population past the age of 60 years due to increased medical monitoring and access to treatment [Sabin, 2012].

Table 1: Profile of Antiretroviral drugs on the South African National ART programme stratified by drug class

Drug Class	Drug names	Mechanism of Action	Prominent Class Effects	Laboratory monitoring
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)	Abacavir (ABC) Didanosine (ddI) Emtricitabine (FTC) Lamivudine (3TC) Stavudine (d4T) Zidovudine (AZT)	Competitive inhibition of HIV reverse transcriptase and termination of the DNA chain	Hyperlactataemia Steatohepatitis Lipodystrophy Mitochondrial toxicity Pancreatitis	Haemoglobin (AZT) Lactate levels (where clinically indicated)
Nucleotide Reverse Transcriptase Inhibitors (NtRTIs)	Tenofovir (TDF)	Competitive inhibition of HIV reverse transcriptase and termination of the DNA chain	Nephrotoxicity	Creatinine clearance
Non- Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)	Efavirenz (EFV) Nevirapine (NVP)	Noncompetitive binding and conformational change of HIV reverse transcriptase limiting its activity	Hypersensitivity rash Hepatotoxicity	Alanine Transaminase levels (NVP)
Protease Inhibitors (PIs)	Atazanavir (ATV) Darunavir (DRV) Lopinavir/Ritonavir (LPV/r)	Competitive inhibition of HIV protease preventing subsequent cleavage of proteins during viral capsid formation	Hepatitis Dyslipidaemia Hyperglycaemia	Lipid profile Glucose

As a result, HIV has had its own "epidemiologic transition" within the course of the pandemic. In Southern Africa, this change is two-fold. First, in the setting of incomplete ART coverage, the incidence of AIDS-defining cancers, particularly Kaposi sarcoma, remains high [Di Lorenzo et al, 2007; DeVita, 2008; Cook-Mozaffari et al, 2008]. Second, for those receiving ART, this new longevity has changed the scope of illness that the HIV infected patient may experience. Metabolic illnesses, cardiovascular complications and non-AIDS related cancers are increasing in significance among those maintained on long term stable antiretroviral therapy [Bonnet et al, 2008]. A combination of increased overall survival and a decrease in mortality due to opportunistic infections has increased the time a treated HIV infected person is exposed to risk of developing cancer. In addition, high prevalence of hepatitis B and C virus among the HIV infected as well as the effect of immune suppression due to HIV have been associated with an increased risk of cancer in HIV-infected populations [Lewden et al, 2005]. This transition has meant that the treatment focus of HIV has evolved beyond that associated with communicable illnesses and now must adapt to long term care and maintenance; not unlike living with a chronic disease.

The malignant pandemic

Though the incidence of AIDS-related cancers has substantially decreased in the presence of ART in the developed world [Palella et al, 1998; CASCADE Collaboration, 2000], they remain important causes of mortality in settings where ART coverage is incomplete [Parkin et al, 2008]. These cancers typically have an infectious aetiology; a further interplay between communicable and non-communicable disease. Viral-associated cancers are the most prominent cancers among the HIV-infected and include cervical cancer (caused by human papilloma virus), non-Hodgkin's lymphoma (associated with Epstein-Barr virus) and liver malignancies (associated with hepatitis B and C infection) [Parkin et al, 2008; Stein et al, 2008]. The most common cancer among HIV-infected patients in Africa is Kaposi sarcoma (KS).

History and discovery of KS

KS is a vascular endothelial tumour which has a variable clinical presentation. Typically this includes single or multiple cutaneous or muco-cutaneous lesions varying in colour from pink to dark purple or brown. The lesions initially are flat patches which then develop into plaques and eventually raised nodules that can coalesce. More advanced disease can present with significant and disabling lymphoedema of affected areas, usually the lower limbs or face. Visceral involvement can include lesions in the gastrointestinal tract or lungs. The latter is frequently difficult to distinguish from other opportunistic infections, particularly TB. Histopathologically, the tumour is characterized by angiogenesis, oedema and growth of spindle-shaped cells which involve either the reticular dermis during the earlier patch stage or the full thickness of the dermis in the more advanced nodular and plaques stages [Ensoli et al, 2001].

KS was initially described in 1872 by Dr Moritz Kaposi, a Hungarian dermatologist working at the University of Vienna [Kaposi, 1872]. Interestingly, Dr Kaposi was initially of Jewish birth and his original family name was Cohen. After purportedly converting to Catholicism in 1871, he changed his name to Kaposi after Kaposvár, his birth town in Hungary. He originally described a cancer of the skin in five elderly male patients which he termed "idiopathic multiple pigmented sarcoma". The disease was originally thought to be a chronic skin disease with an indolent course restricted to elderly men, typically of Mediterranean origin. Since then four distinct epidemiologic forms have been recognised:

1. *Classic KS*

   This is the form originally described by Dr Kaposi; a chronic indolent skin condition that rarely spreads to other organs and may even regress spontaneously. Cases are typically elderly men of Meditteranean or Jewish origin.

2. *Iatrogenic or immunosuppressive KS*

KS has also been observed among those exposed to long term immunosuppressive therapy such as that administered after an organ transplant [Penn 1979]. Its course is typically chronic with spontaneous remission after immunosuppressive therapy is ceased.

3. *Endemic African KS*

African KS was recognised in the 1950s among subjects of varying ages in sub-Saharan Africa. The course of the tumour also varies widely from benign disease with few lesions to widely disseminated forms with organ involvement and almost 100% fatality rates [Taylor et al, 1971].

4. *AIDS-related KS*

In the early 1980's a cluster of cases of disseminated, aggressive KS among men who have sex with men (MSM) occurred in the US [Friedman-Kien et al, 1981]. This new epidemic form of KS was subsequently associated with the then recently identified AIDS syndrome. In Africa, the age-specific incidence of KS closely resembles the prevalence pattern of HIV [Parkin et al, 2008]:

- a small increase in incidence for children <5 years old
- decreasing incidence through to 15 years of age
- increasing to its peak at 35-39 in men and 25-29 in women

This body of work refers specifically to AIDS-related KS and is hereafter referred to as KS.

KS in the setting of the HIV epidemic

Since the recognition of AIDS-related epidemiologic form, KS has been included as a stage IV AIDS-defining event by the World Health Organisation's Disease Staging system for HIV infection and Disease in Adults and Adolescents [World Health Organisation, 2005]. This system was originally developed in 1990 and then revised in 2005 and offers a clinical approach to the categorization of disease severity in HIV infected person, intended for use in resource-limited settings with limited laboratory facilities for CD4 count testing. It is a collection of clinical conditions that characterise each advancing stage of HIV disease progression with Stage 1 being the earliest and least severe and Stage 4 the most advanced disease stage. Patients are assigned to a particular stage when they demonstrate at least one clinical condition in that stage's criteria. The stages are summarized in Table 2.

The clinical benefit of ART for adults with advanced HIV/AIDS as determined clinically or immunologically is undisputed and the WHO recommends initiation of antiretroviral therapy in adults and adolescents in clinical stage 4 irrespective of CD4 cell count [WHO, 2005]. The WHO Clinical Staging system has been widely implemented in resource-limited setting including Africa and is a practical and cost-effective way to manage HIV-infected patients. Several African studies have demonstrated agreement between the clinical conditions included in the WHO staging system and traditional laboratory markers used for HIV disease monitoring including CD4 cell count [Malamba et al, 1999; Kassa et al 1999; Kagaayi et al, 2007].

Table 2: World Health Organisation Clinical Staging of HIV/AIDS (2005)

WHO Clinical Stage	Stage-defining conditions
Stage 1	Asymptomatic Persistent generalized lymphadenopathy
Stage 2	Moderate unexplained weight loss (<10% of presumed or measured body weight) Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media and pharyngitis) Herpes zoster Angular chelitis Recurrent oral ulceration Papular pruritic eruptions Seborrhoeic dermatitis Fungal nail infections
Stage 3	Unexplained severe weight loss (>10% of presumed or measured body weight) Unexplained chronic diarrhoea for longer than one month Unexplained persistent fever (above 37.6°C intermittent or constant, >1 month) Persistent oral candidiasis Oral hairy leukoplakia Pulmonary tuberculosis (current) Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis or bacteraemia) Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 × 109 per litre) or chronic thrombocytopaenia (<50 × 109 per litre)
Stage 4	HIV wasting syndrome Pneumocystis pneumonia Recurrent severe bacterial pneumonia Chronic herpes simplex infection (orolabial, genital or anorectal >1 month's duration or visceral at any site) Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs) Extra pulmonary tuberculosis Kaposi's sarcoma Cytomegalovirus infection (retinitis or infection of other organs) Central nervous system toxoplasmosis HIV encephalopathy Extra pulmonary cryptococcosis including meningitis Disseminated non-tuberculous mycobacterial infection Progressive multifocal leukoencephalopathy Chronic cryptosporidiosis (with diarrhoea) Chronic isosporiasis Disseminated mycosis (coccidiomycosis or histoplasmosis) Recurrent non-typhoidal Salmonella bacteraemia Lymphoma (cerebral or B-cell non-Hodgkin) or other solid HIV-associated tumours Invasive cervical carcinoma Atypical disseminated leishmaniasis Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy

Even in the era of wider access to ART, KS remains a significant contributor to morbidity and mortality in sub-Saharan Africa [Bassett et al, 1995; Cook-mozaffari et al, 1998; Parkin et al, 1999; Chokunonga et al, 2000; Stein et al, 2008]. Today, KS is one of the most common cancers in Africa and is the most common tumour in HIV infected individuals [Boshoff et al, 2002] (representing up to 9% of all tumours). Kaposi sarcoma was relatively common in South Africa (up to 5 per 1000 population at risk) prior to the AIDS epidemic [Cook-Mozaffari et al, 1998] but the incidence increased dramatically as the epidemic escalated [Parkin et al, 2008; Stein et al, 2008]. Estimated incidence rates as high as 20 per 1000 were reported in a case-control in South Africa between 1995 and 1999 [Sitas et al 2000], considerably higher than rates (0.005 per 1000) reported in developed nations [Grulich et al, 1992]. In fact, KS has been reported to comprise as much as 40% of all cancers in rural parts of Kwa-Zulu Natal [Dedicoat et al, 2003] and has been found to be associated with advanced HIV disease and high mortality among patients attending primary care clinics [Chu et al, 2010].

Staging of disease

In 1988, the AIDS Clinical Trials Group (ACTG) developed a staging system for KS based on tumour extent (T), severity of immunosuppression (I), and other systemic HIV-associated illness [Krown et al, 1989]. The ACTG staging system was later validated and a summary of the system and the prognostic value of each of its components are presented in Table 3. This validation was done before the widespread use of highly active antiretroviral therapy and others have since failed to replicate the predictive value of CD4 count in determining survival in patients with AIDS-related KS receiving ART [Nasti, 2003] though both studies agreed that tumour extension (T) was significantly associated with survival. More recent work among a British cohort of patients presenting with T1 stage disease did not show significantly reduced survival compared with those patients who presented with cutaneous disease only [Stebbing et al, 2006].

Table 3: ACTG TIS staging system for AIDS-related KS and prognosis

Characteristics	Less advanced stage of KS disease (0)	More advanced stage of KS disease (1)	Median survival from diagnosis (0 versus 1 in all cases)
Tumour (T)	T0: tumour confined to skin/ lymph nodes and minimal oral disease	T1: Tumour-associated oedema or ulceration, extensive oral KS or spread to other non-nodal organs	27 vs. 15 months
Immune system (I)	I0: CD4 cell count ≥200	I1: CD4 cell count <200	40 vs. 13 months
Systemic illness (S)	S0: No history of opportunistic infections and absence of "B" symptoms	S1: History of opportunistic infections and/or presence of "B" symptoms	22 vs. 16 months
Overall survival	Longer survival	Shorter survival	

Adapted from Krown et al (1997)

"B symptoms": unexplained fever, drenching night sweats, >10% involuntary weight loss, or diarrhoea persisting more than 2 weeks

Treatment options for KS

In the pre-ART era, localised KS disease has been treated with various modalities. These include radiation, cryotherapy, laser therapy, surgical removal or topical chemotherapy. For disseminated disease, systemic chemotherapy with either one drug or multiple drugs in combination has traditionally been used. These chemotherapeutic agents include doxorubicin (A), vincristine (V), vinblastine, bleomycin (B) and etoposide (ET). ET was one of the earliest chemotherapeutic agents used in the treatment of KS among HIV infected persons and tumour responses have been demonstrated in 36% of subjects administered ET in a randomized trial in Zimbabwe [Olweny et al, 2005]. Combinations of bleomycin (B) and vincristine (V), with or without doxorubicin (A), the so-called ABV or BV regimens, have also been widely used with complete or partial response reported in up to 80-90% of patients in trials conducted both in the US [Ireland-Gill et al, 1992] and at multiple sites by the AIDS Clinical Trials Group (ACTG) [Gill et al, 1992 and 1994]. Other multicenter trials have

yielded more disappointing outcomes with response rates as low as 25% for those receiving ABV [Northfelt et al, 1998]. Pegylated liposomal doxorubicin and taxols have also been shown to be superior to other combination treatments with significantly better toxicity profiles [Stewart et al, 1998; Gill et al, 1996] but the high cost of these drugs precludes their widespread use in resource-limited settings. No chemotherapy option has been shown to significantly improve the long term survival for KS patients in the absence of ART but co-administration of chemotherapy and ART has a considerably better prognosis.

The introduction of ART dramatically altered the course of disease for those with KS. Widespread access to ART in the US and Europe has brought about a decrease in the incidence of KS [Jacobson et al, 1999; Ledergerber et al, 1999; Rabkin, 2001; Mocroft et al, 2004]. In particular, combination ART has been used for some time to successfully treat early stage KS [Bower et al, 1999; Krown 2004; Tirelli et al, 2001], achieving regression of KS lesions [Lynen et al, 2005; Bower et al, 2009] and successfully reducing KS-related mortality [Lynen et al, 2005; Palella et al, 1998]. Notably, suppression of replication of the HIV virus has been associated with remission of KS [Martinez et al, 2006]. In the United Kingdom, survival at 5 years among patients diagnosed with KS in the era of ART was estimated to be 98.4% in patients who had CD4 cell counts above 150 cells/ mm^3 and with skin or lymph node involvement only and no other symptomatic disease [Stebbing et al, 2006].

Currently, advanced or rapidly progressive KS disease is treated with both ART and chemotherapy. As the bulk of the efficacy trials for chemotherapy for KS were conducted prior to widespread access to ART, the choice of chemotherapy regimens that will yield the best response in combination with ART use is not yet clear. Despite advances in treatment options, and indications that chemotherapy and ART can reduce KS-related mortality [Borok et al, 2007], this and other AIDS-defining malignancies remain among the leading causes of HIV-related deaths [Bonnett, 2008]. Up to 1 in 5 deaths in Africa are attributed to cancer [Parkin et al 2008] and up to 30% mortality reported in several African settings despite access to ART [Mosam et al, 2005; Asiimwe et al, 2007].

Kaposi sarcoma herpesvirus

Background

The discovery of the aetiology of KS occurred over 100 years after the tumour was first described. In 1994, the Kaposi sarcoma herpes virus (KSHV) was first isolated from KS tumour cells [Chang et al, 1994] and within a few years, KSHV had been confirmed as the aetiologic virus associated with KS [Whitby et al, 1995; Kedes et al, 1996; Sarad et al, 1999; Sitas et al 1999]. Infection with this KSHV has also been shown to lead to the development of multicentric Castleman's disease [Soulier et al, 1995] and primary effusion lymphomas [Cesarman et al, 1995].

The herpesvirus family are a group of enveloped, linear double stranded DNA viruses with complex genomes and are grouped into three subfamilies - alpha (α), beta (β) and gamma (γ). KSHV is a member of the gamma herpesvirus family and, as the most recently described of the family, is also known as human herpes virus 8 (HHV-8). Gamma herpesviruses (unlike alpha or beta) are notable in their ability to cause malignancies. The KSHV viral particle, as with all herpesviruses, comprises the envelope with protruding glycoprotein spikes, a tegument and a core of double-stranded DNA surrounded by a capsid (Figure 1).

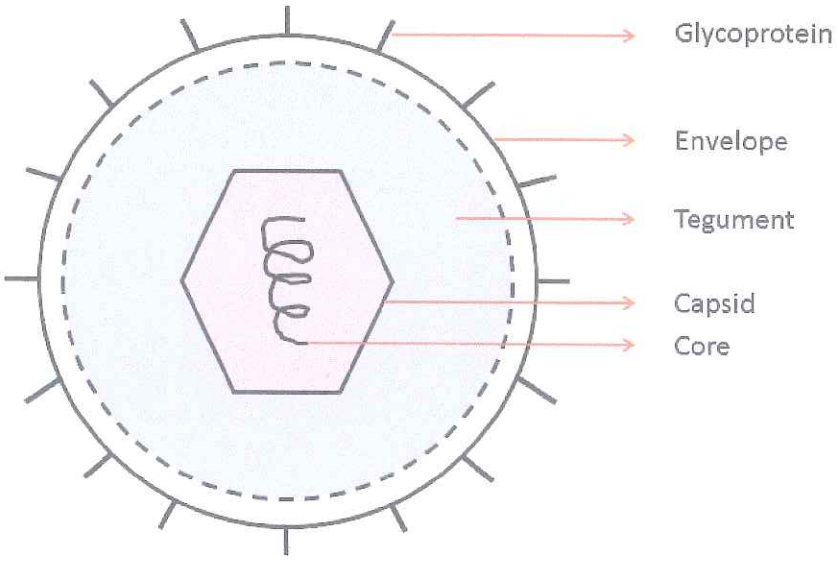


Figure 1: Basic structure of the KSHV viral particle

KSHV Life cycle and antigen expression

Like other herpesviruses, KSHV has a biphasic life cycle. Wilkinson et al [2002] hypothesize that, like all herpesviruses, KSHV establishes a persistent latent infection and the number of KSHV-infected cells are controlled by the intact immune system. During this phase of latent infection only a limited number of viral genes (latent genes) are expressed and new viral particles are not produced. A lytic phase triggered by an unknown regulatory signal is characterized by active replication and production of viral particles. The virus transitions between these phases and expresses different antigens at each phase [Adang et al, 2006]. During periods of latency, LANA (latency associated nuclear antigen) which is encoded by the open reading frame (ORF) 73 is expressed. The lytic phase of the cycle involves assembly of viral particles and several structural proteins including K8.1 are expressed. K8.1 is a glycoprotein found on the envelope of the virus (Figure 1). Transition from latent to lytic phases of the cycle has been associated with progression of KSHV-associated disease [Kedes et al, 1996; Taylor et al, 2004].

Though KSHV expresses considerably more antigens than ORF 73 and K8.1 in the latent and lytic phase respectively, these two are mentioned specifically as they are frequently involved in the laboratory detection of KSHV and were used to ascertain KSHV serostatus among the study participants described in this thesis (see Chapter 2 Methods: Laboratory techniques). Antibodies to KSHV lytic and latent cycle proteins can be detected in blood and other tissues using immunofluorescence assay (IFA), immunoblots or enzyme-linked immunosorbent assays (ELISA).IFA is a technique whereby chemically conjugated antibodies are bound to a fluorescent dye allowing for colourful visualization of targeted proteins or antigens in cells or tissue biopsies under the microscope. Immunoblot assays (sometimes referred to as Western blot) use gel electrophoresis to separate out proteins by structure or length of polypeptide. ELISA use application of an enzyme-linked antibody to a specific antigen that produces a colour change in the presence of the enzyme's substrate. Immunoblot assays using KSHV ORF 73 and ORF57 proteins have demonstrated better

sensitivity in the detection of KSHV than IFA (Yang et al, 2009; Wang et al, 2002; Zhu et al, 1999). IFA appears well-suited to populations with a low prevalence of KSHV but has been used successfully in South African studies [Sitas et al, 1999]. ELISA also performs well in terms of sensitivity and has the advantage of processing large batched samples. This has made this technique particularly useful in sero-epidemiological studies with high volumes of samples requiring testing and ELISAs have been used in more than 30 studies internationally to detect KSHV. As detection of antibodies to a single antigen has been shown to potentially underestimate the prevalence of KSHV, generally two ELISAs are recommended (one lytic and one latent) to provide a more accurate assessment of KSHV antibody status [Mbisa et al, 2010].

Prevalence and transmission of KSHV

Unsurprisingly, the pattern of KSHV prevalence worldwide appears to resemble that of KS [International Agency for Research on Cancer, 1997]. Estimates of the prevalence of KSHV infection vary widely with geographic and population differences. Broadly speaking, there is a high prevalence in sub-Saharan Africa, intermediate prevalence in Mediterranean areas and very low prevalence in Northern Europe and North America. While prevalence as low as 5% have been reported in some North American and European populations [Ablashi et al, 1999], KSHV infection is very common in Africa with nearly 40% of the general adult population infected [Ablashi et al, 1999; Klaskala et al, 2005; Adjei et al 2008] though figures as high as 70% and 87% have been reported in South Africa [Malope et al, 2008] and Botswana [Engels et al 2000] respectively. More recent estimates from South Africa indicate that the prevalence of KSHV infection can vary from 35% to 49% across different municipalities within one province [Malope-Kgokong et al, 2010].

Transmission modalities of KSHV are yet to be clearly defined. Several routes of infection appear possible. These include both sexual and non-sexual modes of transmission. Non-sexual person to person transmission appears to require close contact and can occur through saliva and possibly

blood products including transplanted organs [Engels et al, 1999; Cannon et al, 2001; Stein et al 2004]. Among populations with a high prevalence of KSHV seropositivity, the mode of infection is likely to be saliva and is acquired during childhood and early adult life [Mbulaiteye et al, 2004; Malope et al 2007 and 2008]. A seroprevalence study in the KwaZulu Natal province of South Africa showed that KSHV prevalence ranged from 37.5% among infants <18 months of age to 62.8% among the 35-69 years age group [Wilkinson et al, 1999]. In a prospective cohort study in South Africa, children were 2.6 times more likely to become infected with KSHV if their mother had high KSHV antibody titres [Dedicoat M et al, 2004] suggesting risk of transmission in these groups is related, in part at least, to KSHV viral load.

Sexual and non-sexual transmission of KSHV seems to occur in populations presumably at high risk for sexual acquisition of the virus such as men who have sex with men and sex-workers [Smith et al, 1999; Martin et al, 1998; Pauk et al, 2000; Malope et al, 2008]. KSHV prevalence also appears to be high among pregnant women [Malope-Kgokong et al, 2010] further adding to theories of a sexual route of transmission for KSHV [Martin et al, 1998]. Analysis of bodily fluids demonstrate that KSHV levels are consistently higher in saliva than in semen or prostate secretions so it is likely that saliva is involved in both sexual and non-sexual transmission modes [Pauk et al, 2000; Koelle et al, 1997; Pellet et al, 1999]. Additionally, KSHV viral particles have been demonstrated in oral epithelial cells and shedding occurs regardless of a subject's immune status [Widmer et al, 2006].

KSHV and HIV co-infection

The clinical course of infection with KSHV in the presence of an intact immune system is typically indolent and asymptomatic. In the presence of HIV-1 co-infection however, this course is altered. It has been postulated that the immune suppression caused by HIV-1 as well as direct action of the HIV-1 Tat proteins and cytokine release promotes reactivation of KSHV lytic genes [Ensoli et al, 2004;

Mercader et al, 2000] and enhances KSHV entry into endothelial cells [Aoki et al, 2004], though the role of these proteins in assisting the virulence of other oncogenic viruses such as KSHV is unclear at this stage. Several pro-inflammatory cytokines including tumour necrosis factor and interleukin 1 and interleukin 6 are produced in greater quantities among those infected with HIV and have been shown to stimulate proliferation of KS cells *in vitro* [Ensoli et al, 1990 and 1994; Miles 1996]. The resultant increase in KSHV viral progeny eventually results in destruction of the host cell and progression to the development of Kaposi's sarcoma, and the often aggressive course seen in HIV-1 positive individuals [Aoki et al, 2004; Lukac et al 1999]. Longitudinal studies of KSHV and HIV co-infected subjects found that 30% progressed to KS after HIV seroconversion [Rezza et al, 1999] and the risk of development of KS is roughly two-fold if KSHV seroconversion occurs after HIV infection [Jacobson et al, 2000]. Progression to clinical KS is also associated with low CD4 cell counts, high KSHV titres and high KSHV viral loads [Whitby et al, 1995; Gao et al 1996; Jacobson et al, 2000; Engels et al, 2003].

The debate about the effect of KSHV on HIV-1 among those co-infected is ongoing. *In vitro* and *in vivo* studies demonstrated interaction between these two viruses and an increase in HIV-1 viral load in the presence of KSHV [Aoki et al, 2004; Mercader et al, 2001]. Other *in vitro* work also showed that KSHV increased HIV-1 replication in acutely infected cells but also induced reactivation of HIV-1 replication in chronically infected cells [Lukac et al, 1999; Caselli et al, 2005]. This would suggest that KSHV might be an important contributor to progression of HIV disease. Clinical studies, however, failed to demonstrate any influence of KSHV on the progression of HIV-1 infection in terms of CD4 cell count decline, HIV-1 viral load increase or CD4 cell viraemia [Ait-Arkoub et al, 2003] among a cohort of long term non-progressor of HIV MSMs. The authors concluded that KSHV acted as an opportunistic agent rather than an HIV-cofactor among co-infected individuals.

Obstacles to studying the epidemiology of cancer

Describing trends in cancer in Africa is fraught with multiple challenges. The ideal data for comparing cancer risk across population is from population-based cancer registries, where the number of cancer cases is recorded among a well-defined and enumerated population. Developing registries to calculate incidence of cancers in settings like Southern Africa is challenging. Limited access to laboratories to confirm histo-pathological cancer diagnosis creates difficulty in obtaining an accurate numerator and the migrant nature of many populations groups in the region make defining a denominator difficult. In the absence of such registries, estimating the frequency of cancers has historically been done through case series of cancer patients described by clinicians and pathologists [Parkin et al, 2008].

Population based cancer registries do exist in certain African settings and estimates suggest coverage is up to 11% in these populations [Parkin, 2006; Mqoqi et al, 2004]. In South Africa, the National Cancer Registry was established in 1986 and, as a pathology-based registry, collects data on cancer cases diagnosed in pathology laboratories around the country. Though the coverage is incomplete and incidence rates of cancers are likely underestimated in this way, the data collected by the National Cancer Registry provides some age and gender stratified cancer rates among the South African population [Available at http://www.nioh.ac.za/?page=cancer_statistics&id=163]. Studies published by the pioneers of the Registry describe trends and estimates of burden of disease due to cancer among South Africans enabling comparisons with other settings [Sitas and Isaäcson, 1992; Sitas 1994] and guide policy makers in terms of prevention and control strategies. Additionally, recent amendments to the Regulations Relating to Cancer Registration Act have made notification of cancer cases compulsory [National Health Act, 2011].

Determining mortality related to cancers like KS presents further difficulty. Comprehensive vital registration systems are also not common in Africa [Parkin et al, 2008] and those that are available are often incomplete. In South Africa, mortality data is available from several sources including:

1. ***National Vital Registration system***

The national death registry is the oldest source of mortality data in South Africa having commenced in the early 1900s for white populations. By 1989, the registry was collecting data for all South Africans and attempts to record all deaths (sensitivity among adults is estimated at 90%) and codes cause of death to the ICD coding system [Dorrington et al, 2001, Statistics SA 2005]. Data collection is through collection of death certificates completed by health care workers at the time of death. Cause of death is mainly done by health practitioners (especially in urban areas), but in other places, other forms of certification are allowed, for example verbal autopsy may be a useful alternative in certain rural settings [Kahn et al, 2000].

2. ***National census***

   The National Census was developed in South Africa in 1996 following the country's political transition to democracy. Since then 2 censuses have been completed (2001 and 2011). The census divides the country into enumeration areas and surveys all households within each area and collects data on deaths occurring within the previous 12 months. No data on specific cause of death is available and estimates likely underreport mortality rates [Dorrington et al, 2004].

3. ***South African Demographic and Health Survey***

   The SADHS was first conducted in 1998 and surveys have occurred every five years since then. A sample of 10,000 households drawn from the National census enumeration areas are targeted for inclusion and are interviewed with a more in-depth questionnaire than the census.

Even where mortality data is recorded, linking that data back to a cancer diagnosis or an individual in an HIV treatment cohort is difficult. The National vital registration system records South Africa national identification numbers and this is one possibility for linking these sources of data. Several HIV cohorts have done this in several provinces across South Africa [Fox et al 2010, Boulle et al 2012, Fairall et al 2008] but challenges remain. Low proportions of valid national identification numbers

are often available in treatment cohorts, particularly among those lost to follow up. Cohort attrition due to loss to follow up is a major obstacle to assessing true mortality across HIV treatment programmes [Rosen et al, 2007; Brinkhof et al 2009; Fox et al 2010] as some proportion of patients who are lost from care do not continue care elsewhere and are likely to die. A recent systematic review estimated that only 70% of those initiated onto ART across 39 treatment cohorts in sub-Saharan Africa were alive and in care within 24 months of commencing therapy [Fox et al. 2010]. This figure decreased to 65% by 36 months on ART. Among those who drop out of care, mortality has been estimated as high as 40% [Brinkhof et al, 2009]. Strategies to track and locate patients who are lost or are transferring from one facility to another are needed [Geng et al, 2008]. In the absence of these, other methods which improve ascertainment of mortality such as linkage to vital registration systems should be implemented where possible.

Study rationale

AIDS-related cancers such as Kaposi sarcoma remain important contributors to morbidity and mortality among those infected with HIV in South Africa. In resource-limited settings, lack of adequate population based cancer registries and vital registration systems means that under ascertainment of the burden of disease due to KS in HIV treatment cohorts is problematic and accurate estimates of mortality among those with KS are not readily available. Despite much rigorous basic science work in the KSHV field, clinical studies (particularly prospective ones) are rare and several issues related to the long term prognosis of those co-infected with KSHV and HIV remain unclear. The bulk of the work on KSHV to date has been conducted in the absence of any antiretroviral therapy and the clinical impact of these drugs on the interaction between HIV and KSHV is not well described. While it is evident that ART alone can successfully treat early stage Kaposi's sarcoma [Aversa et al, 2005], the exact mechanism through which this occurs is unknown.

ART has been shown to reduce KSHV viral loads to undetectable levels [Gill et al, 2002] yet the impact of ART in Africa where the underlying prevalence of KSHV is higher is yet to be determined.

Clinical disease due to Kaposi sarcoma is known to be a marker of advanced HIV disease and is one of the WHO stage 4 and AIDS-defining illnesses, but the question remains as to whether co-infection with oncogenic viruses such as KSHV places untreated HIV-infected patients at similar risk of HIV disease progression even without clinically apparent illness. Several clinical and laboratory markers have been associated with advanced disease stage among untreated HIV-infected individuals, including the T-lymphocyte subpopulations, CD4+ and CD8+ [Bonnet et al, 2005; Macias et al 2001; Langford et al, 2007; Cozzi et al 2004; Phillips et al, 2004 2006 and 2007] which play an important role in the response to viral infections. CD8+ lymphocytes are involved in the cellular immune response to several viruses including HIV-1 and herpesvirus. These virus-specific cytotoxic T lymphocytes respond to both lytic and latent antigens of KSHV. Evidence of HAART-induced immune reconstitution to KSHV (indicated by an undetectable KSHV viral load) was associated with an increase in CD8+ lymphocytes, suggesting restoration of these cells may be important in the control of KSHV infection [Wilkinson et al, 2002] and ultimately control or even prevention of KS. While KSHV-specific CD8+ T cell epitope responses have been shown to increase after initiation of ART [Bourboulia et al, 2004], it has yet to be determined if T-lymphocyte subpopulations are also a marker of advanced disease stage in ART naive patients infected with KSHV (as seen among the general HIV-infected population) and if this has implications for treatment initiation guidelines.

Conclusions

We began by considering how disease is currently conceptualised and how a disease transition has altered the landscape of coexisting diseases that the HIV-infected adult experiences. When considering the case of HIV, it quickly becomes evident that in order to treat this condition effectively, one needs to look beyond the boundaries of communicable and non-communicable

illness and consider how HIV is managed among those on treatment, how the virus interacts with other viruses to produce disease, the impact that increased longevity will have on risk of developing conditions previously thought of as "chronic" or "non-communicable".

This thesis considers the effect of KS and its causative agent KSHV has on HIV-infected adults using prospective cohorts of HIV-infected adults initiating ART in South Africa. We estimate the impact of clinical KS throughout the course of antiretroviral therapy and contribute to the understanding not only of co-infection with KSHV but also what impact actively replicating virus with detectable viral burden has on clinical treatment outcomes in the HIV infected person. In doing so, this work offers some insight into the complexity of the interplay between communicable and non-communicable disease and the need for critical thinking that encompasses this shift.

AIMS AND OBJECTIVES

The overarching aim of this work is twofold. Firstly, we aim to determine the effect of ***clinical disease due to Kaposi sarcoma*** among HIV-1 infected adults receiving antiretroviral therapy. Secondly, we aim to estimate the impact ***co-infection with KSHV*** and HIV on HIV-1 infected adults receiving antiretroviral therapy.

Specifically, our objectives include:

1. To estimate the effect of Kaposi sarcoma on risk of mortality and poor immunologic and virologic response to ART (Paper 1).
2. To determine the prevalence of KSHV infection and clinical characteristics among an HIV-1-infected adult cohort initiating ART (Paper 2).
3. To measure the effect of KSHV/HIV-1 co-infection on clinical and immunological outcomes after initiation of ART both in the short (6 months) and medium term (12 months) after treatment initiation (Paper 3 and 4).

THESIS THEMES

The findings of this body of work are presented in their relation to three central themes:

1. A "disease transition" of increasing significance of viral-related cancers in the HIV epidemic
2. Treatment response to ART: impact of viral-related cancers
3. Limitations of data systems documenting the impact of cancers

Table 4 presents a thematic matrix outlining key messages from each of the papers and the integrating narrative.

Table 4: Epidemiology of KS and KSHV: matrix of themes by paper and integrating narrative

THEMES	PAPERS				
	PAPER 1: Prevalence and Predictors of KSHV	**PAPER 2: KSHV and response to ART**	**PAPER 3: KSHV and mortality**	**PAPER 4: KS mortality and response to ART**	**INTEGRATING NARRATIVE**
Health transition to viral-associated cancers	The prevalence of KSHV is high among HIV-infected adults initiating ART		Risk of attrition from care due to mortality and loss to follow up is less among KSHV co-infected	Risk of mortality associated with KS is very high, particularly in the first year on ART	The shift from communicable to non-communicable disease patterns is complex and incomplete in HIV
Viral associated-cancer and treatment response to ART	Seropositivity to KSHV alone is not a predictor of advanced HIV disease stage	Co-infection with KSHV is associated with better immune recovery after ART initiation		Sluggish immune response to ART noted among those with KS	Co-infection with a cancer-causing virus is different from clinical disease due to a virus
Limitations of data systems	Survivor bias in prospective cohort studies examining KSHV among HIV-infected populations may limit the understanding of co-infection		Under ascertainment of mortality, particularly among those LTFU can bias estimates of impact of KSHV	Diagnosis of cancer and ascertainment of mortality likely under-estimated in this population	Data linkage activities and epidemiologic methods including quantitative bias analysis can help address under ascertainment of the impact of cancers

CHAPTER 2: METHODS

Introduction

The methods implemented during the design and conduct of the two studies contained within this thesis is outlined in this chapter. The study design, study sites and study population are described. Next the laboratory techniques used are described as well as the study variables and statistical analysis plan. The chapter concludes with an Ethics statement.

Study design

This work comprises two overarching aims and these were addressed by two studies with different study designs:

1. **Study 1** aimed to estimate the effect of **clinical disease with KS** on response to ART and in order to achieve this, data from a large collaboration that pools HIV treatment cohort data (IeDEA Southern Africa) was analysed.
2. **Study 2** set out to determine the impact of **co-infection with KSHV** on response to ART. For this, a prospective cohort study was designed and conducted among HIV-infected adults initiating ART at a large outpatient treatment facility in Johannesburg, South Africa.

Study sites

1. International epidemiological Databases to Evaluate AIDS in Southern Africa (IeDEA-SA)

The International epidemiological Databases to Evaluate AIDS (IeDEA) is a large collaboration of ART treatment programmes established in 2005 by the National Institute of Allergy and Infectious Diseases (NIAID) to provide a rich resource for globally diverse HIV/AIDS data [www.iedea.org]. IeDEA collects HIV/AIDS data from seven international regional data centers, including four in Africa, and one each in the Asia-Pacific region, the Central/South America/Caribbean region, and North America. Each cohort in the network treats people with HIV, and collects clinical data prospectively

and electronically. This type of data and resource pooling allows researchers to address unique and evolving research questions that individual cohorts are unable to answer. IeDEA develops and implements methodology to effectively pool the collected data, thus providing a cost effective means of generating large data sets to address high priority research questions.

Data for the KS analyses came from two of the cohorts within the Southern African network of the collaboration (IeDEA-SA): the Themba Lethu Clinic (TLC) and the three clinics of the Khayelitsha ART programme in Cape Town. Data were collected prospectively at each site as part of routine HIV treatment monitoring. Data from the different sites were transferred to the IeDEA-SA database managed by the Universities of Cape Town, South Africa and Bern, Switzerland.

2. The Khayelitsha clinics

Médecins Sans Frontières or "Doctors without Borders" is an international medical humanitarian organisation active in over 60 countries worldwide [www.msf.org.za] and has provided medical staffing and assistance in South Africa since 2000. The Khayelitsha HIV treatment programme was set up as a partnership between Médecins Sans Frontières (MSF) and the Western Cape Provincial Department of Health and is now run by the City of Cape Town. The three public sector primary health care clinics that comprise the programme are located in Khayelitsha, a peri-urban township in Cape Town, home to more than half a million residents. The clinics were the site for the first community-based antiretroviral therapy programme in South Africa [Coetzee et al, 2004]. By August 2010, more than 16,000 patients had been initiated on ART at the three clinics [Boulle et al, 2010].

Initially, treatment regimens consisted of zidovudine (AZT) with lamivudine (3TC) and either nevirapine (NVP) or efavirenz (EFV). When the National rollout of ART commenced in 2004, the site replaced AZT with stauvidine (d4T) as the NNRTI backbone as required by the then newly published National Antiretroviral therapy guidelines [South African Ministry of Health, 2004]. Since then the site has initiated and monitored ART according to the most current version of the national guidelines

[2004, 2010]. Care at all clinics was provided according to the South African National Department of Health guidelines in place during the study period [South African National Department of Health, 2004]. This included 6 monthly CD4 cell counts and HIV viral load tests as well as haemoglobin monitoring for those on AZT based regimens, liver function monitoring for those on NVP based regimens and creatinine clearance for patients using tenofovir (TDF). The guidelines, which were revised in 2010, indicate that HIV treatment monitoring tests are to be done twice in the first year on treatment then yearly thereafter [South African National Department of Health, 2010]. Data from patient medical charts is captured onto an electronic patient information system (eKapa) developed in collaboration with MSF and the University of Cape Town. Civil identification numbers are linked with the National death registry in order to enhance ascertainment of mortality at the sites [Boulle et al, 2012]. The data from the three Khayelitsha clinics were aggregated for this analysis.

3. The Themba Lethu Clinic (TLC)

Data from TLC, as part of the IeDEA-SA collaboration, was used in the KS analysis in **Study 1** as described previously. The prospective cohort in **Study 2** investigating the effect of KSHV on treatment outcomes after ART initiation was also conducted at this site. TLC is an urban HIV comprehensive care management and treatment facility based at Helen Joseph Hospital, Johannesburg, Gauteng. The TLC opened in 2004 as a public-sector clinic with non-government organisation support provided through the President's Emergency Plan for AIDS relief (PEPFAR). Currently, TLC is one of the largest treatment facilities in South Africa, with over 30,000 HIV infected adults ever enrolled in its comprehensive HIV care, management and treatment program [Fox et al, 2012]. Since the National rollout of ART in 2004, the site has provided HIV care and management to over 30,000 HIV-infected persons and over 23,000 individuals have been initiated on ART at the clinic according to the guidelines from the South African National Department of Health [2004, 2010]. Patient data at TLC is captured and stored on an electronic patient record database, TherapyEdge-HIV™. Patient laboratory blood tests are taken at ART initiation and monitoring

laboratory tests (viral load, CD4 count, full blood count and liver and kidney function tests) are conducted at 6 months, then yearly thereafter. Up to three attempts are made by clinic counsellors to contact patients who do not return for scheduled clinic appointments. Information on deaths is recorded through passive surveillance and through linkage with the National Vital Registration System [Boulle et al, 2012; Fairall et al, 2008; Fox et al, 2010] which was last conducted in September 2011.

During the period of analysis, standard public-sector ART regimens included stavudine with lamivudine and either nevirapine or efavirenz. The latter was recommended when TB co-infection is present [Boulle et al, 2008] and nevirapine was recommended for women of childbearing age without reliable contraception due to the potential teratogenic effects of efavirenz in the first trimester of pregnancy, despite limited evidence [Ford et al, 2010]. TLC is an outpatient HIV treatment centre and patients requiring other medical services are seen at the secondary level hospital on site (Helen Joseph Hospital) or in the case of Oncology services, are referred to nearby tertiary centres.

Study population

Study 1: The effect of clinical disease with KS on response to ART

For **Study 1**, eligible subjects included in the analysis of **KS** were:

- HIV-positive treatment naïve patients
- Adults $\geq$ 18 years of age
- Subjects who initiated ART at a study clinic between 01 January 2001 and 31 December 2007

The analysis was limited to patients starting standard South African public sector first-line ART regimens (stavudine [d4T] or zidovudine [AZT] with lamivudine [3TC] and either efavirenz [EFV] or nevirapine [NVP]). During the study period, the National guidelines' eligibility criteria for initiation of

ART were either a CD4 cell count <200 cells/mm^3 or a WHO stage 4 illness (such as KS) regardless of CD4 count [South African Ministry of Health, 2004].

Study2: The effect of co-infection with KSHV on response to ART

Enrolment for the **Study 2 prospective KSHV cohort study** occurred between November 2008 and March 2009 at TLC. Through convenience sampling of the adult ART treatment readiness group counselling sessions, potential study participants were identified. Eligible participants were those who met the following criteria:

- HIV-positive treatment naïve patients
- Adults ≥ 18 years of age
- Were eligible for initiation of ART according to National guidelines (CD4 count <200 cells/mm^3 or WHO stage 4 defining illness)
- Attended group ART treatment readiness counselling sessions at Themba Lethu Clinic

All subjects who were assessed by the clinic's ART wellness nurse as ready and eligible for initiation of ART at Themba Lethu Clinic were provided with information about the study (Patient Information and Informed Consent Sheet; Appendix 4) by the study nurse and then invited to participate in the study. All enrolled subjects provided informed consent prior to commencing study procedures.

Laboratory techniques

Routine ART laboratory tests

Both the TLC and Khayelitsha sites performed routine laboratory testing prior to ART initiation as well as on-going treatment monitoring according to the National treatment guidelines (2004). This included CD4 cell counts, haemoglobin levels and alanine transaminase (ALT) levels prior to ART

initiation and six monthly thereafter unless more frequent testing was clinically indicated. More frequent testing of ALT was performed for those initiating NVP-based regimens to monitor ART-related hepatotoxicity within the first month of treatment. Haemoglobin levels were tested more frequently in the first six months among those initiating an AZT based regimen or in whom anaemia was present at the time of ART initiation. HIV viral load testing was not routinely indicated for those initiating ART but was performed within the first six months of treatment and then six-monthly thereafter. Laboratory testing at both sites is conducted by the National Health laboratory Services (NHLS). CD4+ T-cell lymphocytes counts are performed using pan-leucogated CD4+ flow cytometry (FlowCount Fluorospheres, Beckman Coulter-Immunotech, France) while HIV-1 RNA viral load tests are conducted using NucliSENS EasyQ® HIV-1 assay (bioMérieux Clinical Diagnostics, France).

KSHV laboratory testing

To ascertain the study participants KSHV serostatus in **study 2 (The effect of co-infection with KSHV on response to ART)**, KSHV serology tests were required. Laboratory testing for KSHV serology and viral load was not routinely available at the time the KSHV study was designed. Through collaboration with the Viral Oncology Section, AIDS and Cancer Virus Program at the National Cancer Institute (NCI) in Maryland, enzyme-linked immunosorbent assays (ELISA) for detection of antibodies to lytic K8.1 and latent open reading frame (ORF) 73 KSHV antigens (see Chapter 1: Introduction - KSHV Life cycle and antigen expression) were set up at Contract Laboratory Services (a joint venture between the National Health Laboratory Service and the Wits Health Consortium) in Johannesburg, South Africa. All KSHV serology testing was performed by Contract Laboratory Services and National Health Laboratory Service while KSHV viral load testing was performed by the Haematology and Molecular Medicine Department of the University of the Witwatersrand. Detection of antibodies to a single antigen has been shown to potentially underestimate the prevalence of KSHV, therefore, an ELISA to detect antibodies to latency associated nuclear antigen was performed in addition to the lytic K8.1 ELISA to provide a more accurate assessment of KSHV antibody status [Mbisa et al, 2010].

All samples that tested serologically positive to KSHV were then tested for KSHV viral load using quantitative TaqMan PCR [de Sanjosé et al, 2002] performed on the ABI Prism 7900 sequence detection system (Applied Biosystems, Forster City, CA). Subject and control samples were run in triplicate. The KSHV viral load assay has a linear dynamic range of 8 logs and is calibrated to detect a single copy of viral DNA in 150ng genomic DNA.

Study variables

Each study had a primary exposure and several primary outcomes. The two **primary exposure variables** for these studies were:

1. ***Study 1: Clinical disease due to Kaposi sarcoma***

   Study subjects were considered to have KS at initiation of ART if their electronic patient record documented a diagnosis of KS between 6 months prior to up to 6 months after ART initiation. At both sites, KS is typically diagnosed clinically based on the appearance of one or more cutaneous or muco-cutaneous lesions. Histological or pathological confirmation of a KS diagnosis through tissue biopsy is infrequent unless the subject was initially seen in a specialist Dermatology clinic prior to an HIV diagnosis being made. Though radiology services are available to both study sites, radiographic diagnosis of pulmonary KS is also infrequent and is not documented on the HIV clinic databases.

2. ***Study 2: Co-infection with KSHV***

   Venous blood samples were drawn from all the prospective cohort study participants prior to initiation of ART to determine KSHV serostatus. Seropositivity to KSHV was defined as a positive reaction to either lytic KSHV K8.1 or latent KSHV Orf73 antibodies as described above (see KSHV laboratory testing). The KSHV positive group was then further stratified by the presence or absence of detectable KSHV DNA. To determine if treatment outcomes were influenced by the phase of KSHV infection at ART initiation, a positive KSHV result was

further stratified into three categories: 1) positive to lytic k8.1 alone (which may indicate active replication), 2) positive to latent Orf73 alone (may indicate latent infection) or 3) positive to both (may indicate transition between active and latent infection).

The **primary outcome variables** were the same for both **Study 1** and **Study 2**:

1. ***Mortality***

   Mortality is ascertained through passive surveillance and through linkage with the National Vital Registration System. Data for those not returning to both TLC and the Khayelitsha study sites was verified at the end of 2010 with the South African National Vital Registration system for patients in whom a civil identification number was available (42% of those lost to care in Themba Lethu [Fox et al, 2010] and 47% in Khayelitsha [Boulle et al, 2012]). South African National identification numbers from each cohort were linked with the death registry through the Department of Home affairs by a Department official. We assumed a six month delay in capturing of record of deaths into the registry.

2. ***Loss to follow up (LTFU)***

   LTFU was defined as having not attended the clinic in the previous 4 months. Up to three attempts are made by clinic counsellors to contact patients who do not return for scheduled clinic appointments. At TLC, this is done telephonically initially and then followed up with a home-based visit if initial telephone contact is unsuccessful.

3. ***Virologic response to ART***

   Subjects were considered to have demonstrated a virologic response to ART if they achieved suppression of HIV viral load to ≤400 copies/ml. This outcome was measured after six months and then one year on treatment.

4. ***Immunologic response to ART***

As there is no universally accepted definition of what constitutes an acceptable immunologic response to ART, we measured recovery of CD4 cell count in two ways:

i. a linear increase in mean CD4 cell count after ART initiation; and

ii. a dichotomised increase in CD4 cell count (CD4 count increase of > 50 cells/mm3 at 6 months and > 100 cells/mm3 at 12 months after ART initiation).

Additional demographic and clinical data was extracted from the electronic patient records. These included gender, age at ART initiation, initiating ART regimen, WHO clinical stage, body mass index (BMI), tuberculosis status as well as laboratory results for full blood counts and liver function tests.

Statistical analysis

*1. **Description of study participants***

Demographic and clinical features of the study participants at ART initiation were stratified by exposure status and summarized as simple proportions or medians with interquartile ranges.

*2. **Estimates of mortality and loss to follow up***

Cause-specific Cox proportional hazard models were used to estimate the effect of each of the exposures on mortality and loss to follow up on ART. As the hazard of mortality was not consistent over time, for both the mortality and LTFU outcomes, we considered the effect of exposure on each of these events at any time point after initiation of treatment. We then further stratified the analysis into the first year after ART initiation and after the first year on ART. Person-time was calculated from the date of ART initiation to the earliest of: 1) death or loss to follow up; 2) transfer to another facility; or 3) end of study period (31 December 2008 for the KS analysis and 18 months after treatment initiation for the KSHV prospective cohort).

*3. **CD4 cell recovery***

For the continuous CD4 count outcome, we used mixed linear models with a random intercept and an unstructured correlation matrix to estimate CD4 trajectories over time, accounting for repeated observations on an individual. Time was estimated as a quadratic function using a random intercept. We fit a separate model for each exposure level in order to allow for different curves of CD4 count over time by exposure group and not force the curves to be parallel. The association of exposure with change in CD4 count from baseline to 6 and 12 months was estimated using a multivariable linear generalized estimating equation model.

For the dichotomized CD4 cell count outcome, we used log-binomial regression to estimate the impact of each exposure on CD4 response (>50 cells/mm3 and >100 cells/mm3)

*4. **HIV viral load suppression***

Log-binomial regression was again used to estimate the effect of each exposure on failure to achieve viral load suppression at the time points considered.

*5. **Investigation of confounding and bias***

For both **Study 1 (The effect of KS on response to ART)** and **Study 2 (The effect of KSHV on response to ART)**, potential confounding factors such as age, gender, baseline CD4 count, CD3 count, CD8 count, haemoglobin level, tuberculosis treatment status, body mass index (BMI) and initiating treatment regimen were investigated. Age, gender and baseline CD4 count were included in all models as well as other covariates that altered the relative risk of the exposure on each outcome by 10% or more.

Our study variables in **Study 1 (The effect of KS on response to ART)** are also prone to misclassification. First, the method of ascertaining KS exposure status in the cohorts described creates the potential for exposure misclassification. This is due to the fact that patients with systemic KS requiring chemotherapy are treated at an outside facility and may not have their diagnosis known or recorded at the ART clinic. Early disease may present with a single small lesion in

a location that is not regularly examined or is not immediately obvious to the treating clinician. As histological confirmation is not always sought before a diagnosis of KS is made, skin lesions resembling KS may also be mistakenly diagnosed as KS. Second, our measure of death relied on the sites patient records and thus likely suffered from some misclassification. In these datasets, mortality was validated against the national vital registration records, but still some deaths are likely missed, particularly as records of civil identification number are not complete. For both the exposure and the outcome variables that may be prone to misclassification in the KS analysis, as they were assessed independent of each other they are therefore likely non-differentially misclassified.

Probabilistic sensitivity analysis is a method that can be used to estimate the magnitude and direction of the impact of misclassification [Lash et al, 2009]. Using assumptions about the bias in observed associations, quantitative bias analysis can be used to simulate the data that would have been observed had the bias been absent. In doing this, uncertainty intervals (UI) can be created which account for the total study error (systematic and random) rather than just random error alone. We used a customizable and freely available spreadsheet program [https://sites.google.com/site/bias analysis/] to conduct probabilistic sensitivity analysis to explore the impact of:

i. Exposure misclassification (KS)

ii. Outcome misclassification (Death)

iii. The combined effects of both

First, a traditional logistic regression model of the effect of a diagnosis of Kaposi's sarcoma on mortality was estimated. Next, the extent of the misclassification was was speculated on, the sensitivity (Se) and specificity (Sp) of the misclassification was summarized and trapezoidal distributions were specified for each classification parameter. It was assumed for both sources of error that sensitivity of classification was poor (70-85%), but specificity was near perfect (99-100%). Using Monte Carlo (MC) simulation to sample 5,000 times from each of these distributions, the

sampled values of Se and Sp were used to correct for the misclassification using standard formulas. The distributions of corrected estimates were summarized using the median as the point estimate and created a 95% simulation interval (SI) from the 2.5th to 97.5th percentile of the distribution. Random error was also included in the final estimates by subtracting from each corrected estimate a random normal deviate times the standard error of the conventional estimate.

Ethics

Ethical approval for the body of work presented here, including use of data from the Themba Lethu Clinic, was granted by the Human Research Ethics Committee of the University of the Witwatersrand (Protocol number M060813; Appendix 2). The analysis of data for the KS study was nested within on-going cohort studies of routine ART outcomes at the sites in Cape Town and Johannesburg. Use of the data from the Khayelitsha sites was approved by the Ethics Committee of the University of Cape Town (REC REF 440/2007; Appendix 3). The pooling of data in IeDEA-SA was approved by Ethics Committees at the Universities of Bern and Cape Town. Individual patient consent was not needed, consistent with the South African Medical Research Council's Guidelines on Ethics for Medical Research and the Declaration of Helsinki. As this was a retrospective analysis of routine clinical service records, no additional data collection or procedures were undertaken from or on patients, all patient information was entered into the database using coded identification numbers, and no information that could reveal patient identity was available in the analytic datasets.

CHAPTER 3: RESULTS

Introduction

This chapter summarises the main results and findings from the studies. The results are organized by the exposure used in each study. First results from **Study 1 (effect of clinical disease due to KS on response to ART)** are presented, followed by results from the quantitative bias analysis applied to that analysis. Next, the results from Study 2 (effect of co-infection with KSHV on response to ART) are summarised. Finally, the differences in results between the two studies are compared and contrasted.

The impact of clinical disease due to KS

Description of the study population

Prospectively collected data from a cohort of HIV-infected adults initiating ART at two HIV clinics in South Africa (Khayelitsha and Themba Lethu Clinic) were used to assess the effect of KS on survival and immunologic and virologic treatment responses at 6- and 12-months after initiation of ART. By the time of closure of the dataset for extraction (31 December 2008), a total of 13,847 patients met the eligibility criteria for inclusion in the analysis (Table 5). This included 247 individuals (1.8%) diagnosed with KS within six months of ART initiation. The Khayelitsha sites contributed a total of 6,736 patients to the analysis including 153 (2.2%) with KS at initiation. TLC contributed a further 7,111 subjects of which 94 (1.5%) were identified with KS. At initiation of ART, the KS group were similar to those without KS with respect to median age (35 vs. 35 years) and first-line ART regimen (68% vs. 69% initiated on d4T-3TC-EFV). Those with KS were more likely to be male (49% vs. 36%) and though they presented with similar median CD4 counts at initiation compared to those without KS (74 vs. 85 cells/mm^3), the KS group were almost twice as likely to have a CD4 count in the 200-350

cells/mm^3 category (12.3% vs. 7.2%). The proportion of patients on TB treatment was also higher among those with KS (37% vs. 30%).

Table 5: Baseline characteristics of 13,847 adults initiating ART in Cape Town and Johannesburg, South Africa, stratified by presence of Kaposi sarcoma

Characteristic at ART Initiation		No Kaposi Sarcoma (n=13,600)	Kaposi Sarcoma (n =247)
Sex	Male	4893 (36.0%)	121 (49.0%)
Age (years)	Median (IQR)	35 (30-41)	35 (30-41)
Year of ART Initiation	Before 2004	581 (4.3%)	20 (8.1%)
	2004	1947 (14.3%)	42 (17.0%)
	2005	3185 (23.4%)	74 (30.0%)
	2006	4149 (30.5%)	64 (25.9%)
	2007	3738 (27.5%)	47 (19.0%)
CD4 count (cells/mm^3)	Median (IQR)	85 (33-150)	74 (29 -152)
	0-50	4256 (34.3%)	86 (37.9%)
	51-100	2747 (22.1%)	46 (20.3%)
	101-200	4518 (36.4%)	67 (29.5%)
	200-350	899 (7.2%)	28 (12.3%)
First-line ART Regimen	D4T/3TC/EFV	9200 (68.1%)	169 (69.3%)
	D4T/3TC/EFV	3000 (22.2%)	52 (21.3%)
	Other	1562 (11.7%)	23 (9.4%)
TB Diagnosis recorded		3247 (29.5%)	71 (36.6%)

Retention in care

Overall, attrition from care was high at the study sites. By the end of the study period, only 76% of those who initiated ART were known to be alive and receiving care at the study sites or had transferred to another treatment facility. In total, 10% (1,312) had died and 14% (1,837) were LTFU at some point after ART initiation. Median follow-up time for those who died or were lost to follow up was 4.5 (IQR 1.5-12.5) and 9.6 (IQR 4.4-19.3) months, respectively. Mortality was highest within the first 12 months after starting ART with 74% of deaths occurring in the first 12 months of treatment. Though the groups were reasonably similar at ART initiation, those with KS experienced

greater attrition than their counterparts without KS. The KS group only received a median of 12.3 months (IQR: 2.3-29.8) of ART compared to 19.1 months of ART (IQR: 7.8-32.0) among those without KS.

Mortality was substantially higher in the KS group (Figure 2). Over the total follow up, more than a quarter (27%) of those with KS had died compared to less than 10% among those without KS. The risk of mortality was not proportional over time and so we stratified the results into two distinct time periods: first year on ART and after the first year on ART. Individuals with KS had a higher rate of mortality at all durations after ART initiation compared to those without KS though the greatest differences in mortality occurred within the first year on treatment: 28.3/100 person-years (100py) vs. 7.4/100py within the first year and 4.1/100py vs. 1.8/100py after the first year.

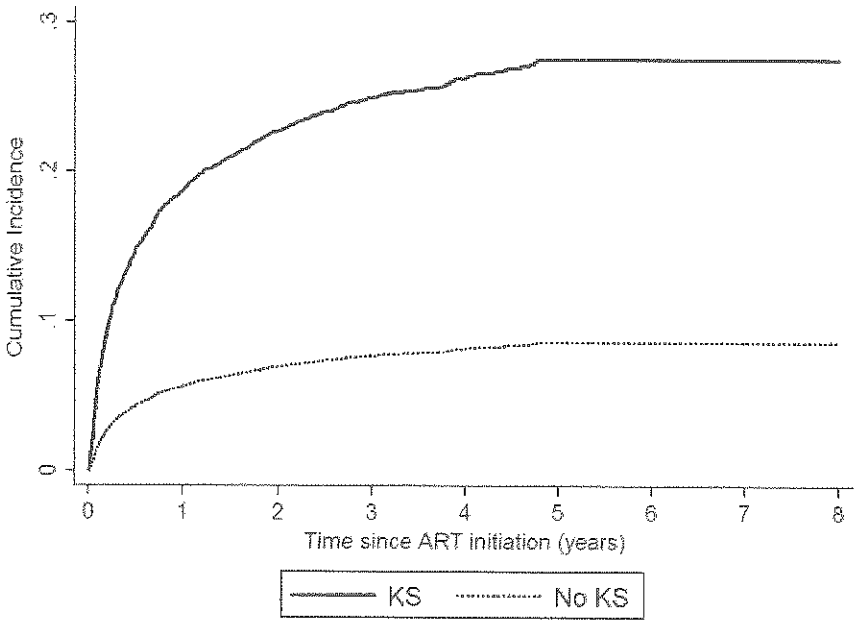


Figure 2: Cumulative incidence of mortality after ART initiation by KS status.

The risk of death for those with KS was over three times that of those without KS at any time point after ART initiation (adjusted HR: 3.62; 95%CI: 2.71-4.84) and four times greater within the first year after ART initiation (adjusted HR: 4.05; 95%CI: 2.95-5.55) (Table 6). Among those who survived to a

year on treatment, the risk of death was still greater in the KS group though the magnitude of this effect was smaller (adjusted HR: 2.30; 95%CI: 1.08-4.89).

Table 6: The effect of Kaposi Sarcoma on mortality after initiation of ART among 13,065 adult HIV-infected patients initiating ART.

	Deaths	Person time (years)	Rate/100 pys*	Crude HR (95% CI) †	Adjusted HR (95% CI)‡
Over total follow up					
No KS	1248 (9.7%)	32345	3.9	1.0	1.0
KS	64 (27.2%)	491	13.0	3.22 (2.51-4.15)	3.62 (2.71-4.84)
Within the first year of ART					
No KS	913 (7.1%)	12317	7.4	1.0	1.0
KS	54 (23.0%)	191	28.3	3.63 (2.76-4.77)	4.05 (2.95-5.55)
After the first year of ART					
No KS	335 (3.1%)	18745	1.8	1.0	1.0
KS	10 (6.5%)	244	4.1	2.03 (1.08-3.80)	2.30 (1.08-4.89)

† HR = hazard ratio, CI = confidence interval, KS = Kaposi sarcoma, ART = antiretroviral therapy, pys = person years, hazard ratios from a Cox proportional hazards regression model
‡ Models adjusted for sex, baseline CD4 count, age, treatment site, tuberculosis at ART initiation, year of ART initiation
*pys= person years

Similarly, a greater proportion of individuals with KS were LTFU after ART initiation compared to those without KS (18% vs. 14%). The rate of LTFU after ART initiation was 8.8/100py among those with KS compared to 5.5/100py among those without KS. Among those with KS, the rate of LTFU was also greatest in the first year after initiation of ART (13.6/100py in the first 12 months vs. 7.0/100py after 12 months) and though adjusted proportional hazards models lacked precision with respect to LTFU comparing those with KS to those without, point estimates in both in first year (adjusted HR: 1.55; 95%CI: 0.85-2.82) and after a year on treatment (adjusted HR: 1.21; 95%CI: 0.54-2.70) suggested an increased risk for the KS group.

The trends between mortality and LTFU are similar in this data. It does follow that some proportion of those LTFU is unrecognized mortality. This is due to the fact that a certain proportion of those lost from care have died but their death has not been ascertained. Verification of vital status in the TLC cohort was performed by linkage to the National death Registry for the first time in 2009 [Fox et al, 2010]. It revealed a startling number of unrecorded deaths (Table 7), among those LTFU. Mortality among those LTFU was estimated as high as 26.6% (95% CI 23.7-29.7) and the proportion recorded deceased in the clinic dataset increased from 4.4% prior to the linkage to 8.6% after linkage and updating was completed.

Table 7: Vital status outcomes among ART patients LTFU after linkage to the National Vital Registration System

	Vital Registration System		
Themba Lethu Clinic records	Registered as dead	Not registered as dead	Total
Died	**227**	**26**	**253**
LTF	**223**	**615**	**838**
Alive and in care	**49**	**4638**	**4687**
Total	**499**	**5279**	**5778**

Adapted from Fox et al, 2010

Immunologic and virologic failure

Despite starting on very similar CD4 cell counts at ART initiation, those with KS appeared to show a slower immunologic response to ART within the first year on treatment. Both linear increases in CD4 count as well as dichotomised outcomes of failing to achieve a CD4 increase of $\geq$50 cells/mm^3 by 6

months on treatment and 100 cells/mm^3 by 12 months were estimated. The median increase in CD4 count by 6 months on ART was 98 cells/mm^3 (IQR 58-164 cells/mm^3) among the KS group and 121 cells/mm^3 (IQR 66-190 cells/mm^3) for those without KS. Patients with KS gained, on average, 29 fewer CD4 cells (95% CI: 7-52 cells/mm^3) than those without KS over the same time period. Nearly a quarter of patients (23.7%; 95% CI: 17.3-32.7%) with KS had failed to achieve a CD4 increase of ≥50 cells/mm^3 by six months on treatment compared to 18.1% (95% CI: 17.5-19.1) of those without KS and the risk of failing to achieve a 50 cell increase was nearly one and a half times greater (RR 1.43; 95%CI: 0.99-2.06) among the KS group. The median increase in CD4 count by 12 months on ART was 150 cells/mm^3 (IQR 90-225 cells/mm^3) among the KS group and 175 cells/mm^3 (IQR: 105-260cells/mm^3) for those without KS. Those with KS gained fewer CD4 cells over the first year of treatment compared to those without KS and the latter group retained consistently higher CD4 cell counts after treatment initiation (Figure 3).

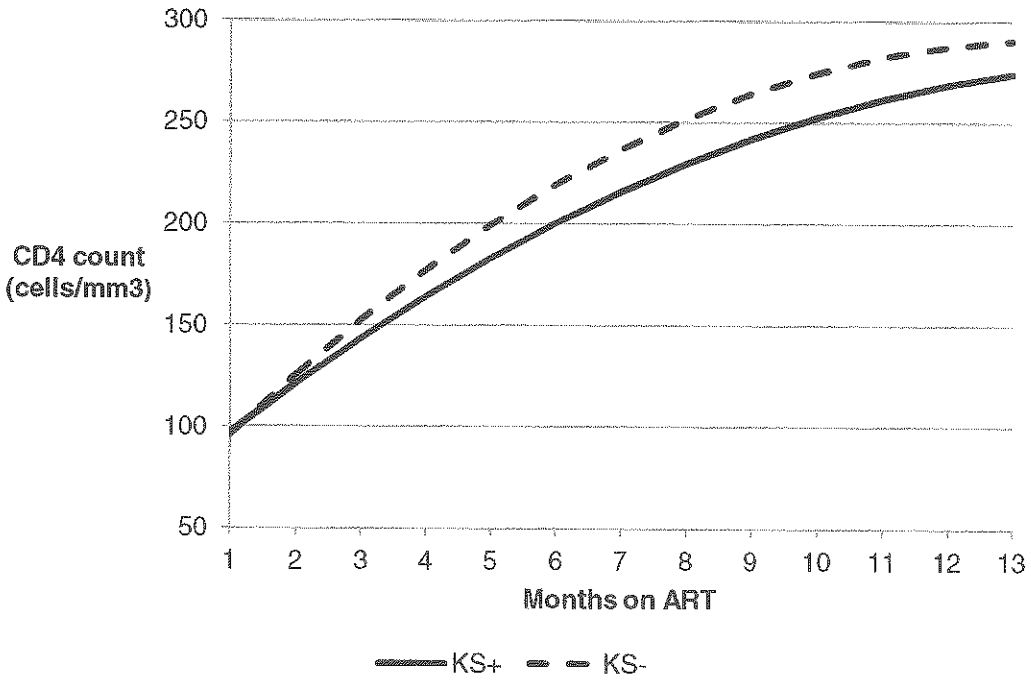


Figure 3: Mean predicted* CD4 cell count increase from ART initiation stratified by KS status.

* Trajectories were estimated using two separate mixed linear models, one for the KS+ and one for the KS- to allow the curves to depart from being parallel. Curves were fitted using time as a quadratic function and a random intercept with an unstructured correlation matrix for repeated measures

Virologic response to ART was favourable among both groups. By 6 months on treatment, only 11% of those with KS had failed to suppress HIV viral load to <400 copies/mL while just under 8% of those without KS had failed to achieve suppression. Among those who survived to a year on treatment, similar proportions failing to achieve virologic responses were noted (7% vs. 10%). The risk ratio for failure to achieve virologic suppression suggests that KS patients may have fared slightly better at 6 (RR: 0.82; 95%CI: 0.38-1.79) and 12 months (RR: 0.25; 95%CI: 0.06-1.00) after initiation of ART compared to those without KS though these estimates lacked precision.

Bias analysis

Addressing the effects of exposures such as co-infection with viruses like KSHV or clinical disease due to cancers requires some epidemiologic methodology approaches. Because clinical disease or infection cannot be randomized, inferences must be made using observational data. The use of observational data is subject to several sources of bias, one of these being bias due to misclassification. Exposure misclassification has been shown to be common in epidemiologic research and the impact on study results can be dramatic. In this analysis of the relationship between KS and mortality in a cohort of HIV-infected patients, our measure of KS, which relied on patient records, likely suffered from some misclassification as did our measure of mortality. We hypothesize that KS (exposure) misclassification arises because patients requiring chemotherapy can be treated at an outside facility and have their diagnosis missed and early KS disease can also be missed. In addition, some cases of KS were diagnosed on clinical grounds only and were not confirmed by histopathological diagnosis. Also, despite validating mortality against national vital registration records some deaths (outcome) are likely missed. For both the exposure and outcome, variables were assessed independent of each other and are therefore likely non-differentially misclassified.

The extent of the misclassification was speculated on and trapezoidal distributions were specified (Figure 4) for the sensitivity and specificity of each classification scheme to represent the bias, assuming that sensitivity was poor (70-85%), but specificity was near perfect (99-100%) as shown in Table 8 below.

Table 8. Distributions specified for non-differential sensitivity and specificity of exposure and outcome classification in a Monte Carlo simulations adjusting for exposure and outcome misclassification in a study of the effect of Kaposi sarcoma on mortality among patients on ART

	Minimum	Lower Mode	Upper Mode	Maximum	Justification
Nondifferential exposure (KS) classification					
Sensitivity	0.70	0.75	0.80	0.85	Some KS will be missed due to patients seeking care at other institutions and early disease can be missed
Specificity	0.99	0.99	1.00	1.00	Few patients are likely classified as having KS who do not
Nondifferential outcome (mortality) classification					
Sensitivity	0.70	0.75	0.80	0.85	Despite validating patients against the national death registry, not all deaths will be recorded in the registry
Specificity	0.99	0.99	1.00	1.00	Few patients will be classified as not alive who are in reality alive

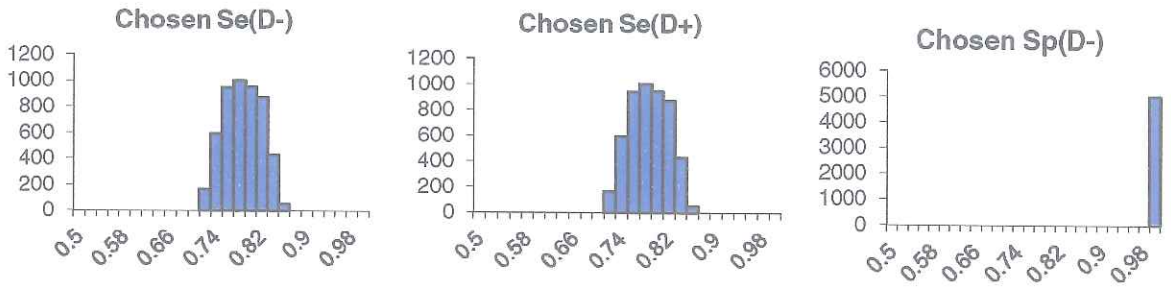


Figure 4. Output distribution of sensitivity and specificity from 10,000 Monte Carlo simulations to explore the impact of exposure and outcome misclassification

Using Monte Carlo (MC) simulation we sampled from each distribution 10,000 times, and used the sampled estimates to correct for the misclassification. The simulations were then summarized using the median and a 95% simulation interval (SI) was created from the 2.5th - 97.5th percentile of the distribution. The results are summarized in Table 9. After correcting for exposure (KS) misclassification, the median of the simulations shifted away from the null to 4.79, but the interval accounting for the misclassification and random error widened (95% SI 3.54-8.42) compared to the conventional. Despite assuming very poor sensitivity, after correcting for outcome (mortality) misclassification, the median of the simulations barely moved away from the null to 3.86, while the interval accounting for the misclassification and random error widened minimally (95% SI 2.90-5.28) compared to the conventional.

Table 9. Results of Monte Carlo simulation adjusting for exposure and outcome misclassification in a study of the effect of Kaposi sarcoma on mortality among patients on ART

Analysis	**OR Median (2.5^{th} - 97.5^{th} percentile)**
Conventional (Random error)	3.46 (2.59 - 4.63)
Adjusted for exposure misclassification (Systematic error)	4.79 (3.54 - 8.42)
Adjusted for exposure misclassification and random error (Total error)	4.86 (3.04 - 9.10)
Adjusted for outcome misclassification (Systematic error)	3.89 (3.70 – 4.09)
Adjusted for outcome misclassification and random error (Total error)	3.86 (2.9 – 5.28)

In this way, an attempt is made to account for the bias rather than speculating on it and despite poor non-differential sensitivity of exposure and outcome misclassification, the estimates of the effect of KS on mortality were likely biased only minimally towards the null.

The effect of KSHV co-infection on ART treatment outcomes

Description of the study population

Between November 2008 and March 2009, a total of 404 HIV-positive treatment naïve patients > 18 years of age who met the National guidelines criteria for initiation of ART (CD4 count <200 cells/mm^3 or WHO stage 4 defining illness) and who were attending group counselling sessions at TLC were invited to participate in the study. Response rates were high: over 95% of those invited to participate agreed. In order to determine if the recruited sample was representative of the untreated population accessing HIV care at that time, this sample was compared to the general population presenting for treatment initiation at TLC during the recruitment period that were either not invited or refused to participate in the study. The presenting features of the study group were very similar to the TLC general population at ART initiation in terms of age, CD4 cell count, HIV viral load, proportion with WHO stage 3 or 4 defining illness, haemoglobin level and BMI. The TLC general population had a slightly lower proportion of females (61% vs. 65%) and slightly lower proportion presenting with tuberculosis (11% vs. 14%) compared to the study group. The median age of the group was 38 years (IQR 32-45years) and none had evidence of clinical Kaposi sarcoma. They did however present with features of advanced HIV infection; the median CD4 count at ART initiation was 87 (40-149 cells/mm^3) and over a third (37%) presented with a WHO stage III/IV defining condition.

Prevalence of KSHV

Screening for KSHV at ART initiation revealed a high prevalence of KSHV among this group of HIV-infected adults. 48% (193/404) tested positive for KSHV with an overall prevalence of KSHV estimated at 48% (95%CI: 43-53%). Of these, 73 (39%; 95%CI: 33-46%) were reactive to lytic K8.1 alone, 34 (18%; 95%CI: 13-24%) to latent Orf73 and 77 (42%; 95%CI:36-50%) to both. The groups were similar in terms of age, gender distribution and HIV disease stage (Table 10).

Table 10: Presenting features of 404 ART naïve adults in care at Themba Lethu in Johannesburg, South Africa stratified by KSHV[&] status

Characteristics[#]		KSHV+[&] (n =193)	KSHV-[&] (n=211)
Female		127 (66%)	135 (64%)
Age (yrs.)	Median (IQR)	37 (32-47)	38 (32-45)
WHO Stage	I/II	116 (64%)	114 (61%)
(n, %)	III/IV	64 (36%)	74 (39%)
BMI	<18.5	38 (21%)	57 (29%)
(n, %)	18.5-24.9	98 (56%)	94 (48%)
	25-30	23 (13%)	31 (16%)
	>30	15 (8%)	14 (7%)
Hemoglobin (n, %)	≤ 8.0 g/dL	14 (7%)	13 (6%)
CD4 count (cells/mm^3)	Median (IQR)	101 (46-168)	89 (34-164)
CD4 cell count category	0-50	47 (28%)	63 (34%)
(n, %)	51-100	39 (23%)	39 (21%)
	101-200	65 (38%)	56 (30%)
	200-350	20 (12%)	30 (16%)
Tuberculosis (n, %)	Yes	26 (14%)	30 (15%)
HIV RNA	Median (IQR)	15500 (6400-33500)	19500 (7300-39000)

[#] Characteristics at eligibility for initiation of antiretroviral therapy

[&] KSHV = Kaposi sarcoma herpes virus

Of the 404 subjects enrolled in the study, 385 (95%) initiated treatment at the site and continued follow up on the study. These participants contributed a total of 466.6 person years of follow up and

the mean follow up time between the groups was similar: 13.9 months (95%CI:12.9-14.8) for the KSHV negative group compared to 15.2 months (95%CI:14.4-16.1) for the KSHV positive group.

KSHV viral load

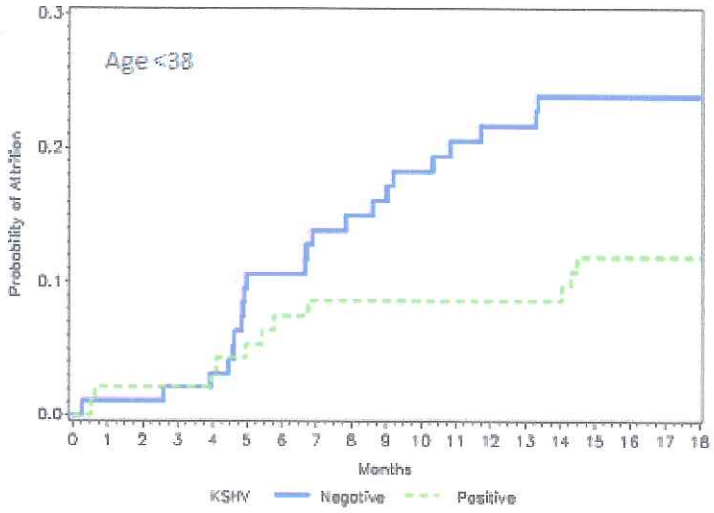
Among those participants with KSHV positive serology results, 167 (87%) samples were also tested for KSHV viral load. 19 (11%) of these had a detectable viral load at initiation of ART. The group reactive to both KSHV antigens (K8.1 and Orf73) had a greater proportion with a detectable KSHV viral load (n=15; 20%) when compared to those reactive to lytic K8.1 (n=2; 3%) or latent Orf73 (n=2; 8%) alone. The estimates suggested that those with a detectable KSHV viral load were more likely to be male (PR=1.43; 95%CI 0.61-3.35), have a WHO stage III/IV illnesses (PR=1.51; 95%CI 0.65-3.50) and haemoglobin $\leq$ 8g/dL (PR=1.42; 95%CI 0.37-5.43) compared to those without a detectable KSHV viral load, although these results lacked precision (as indicated by the wide confidence intervals).

Attrition from care

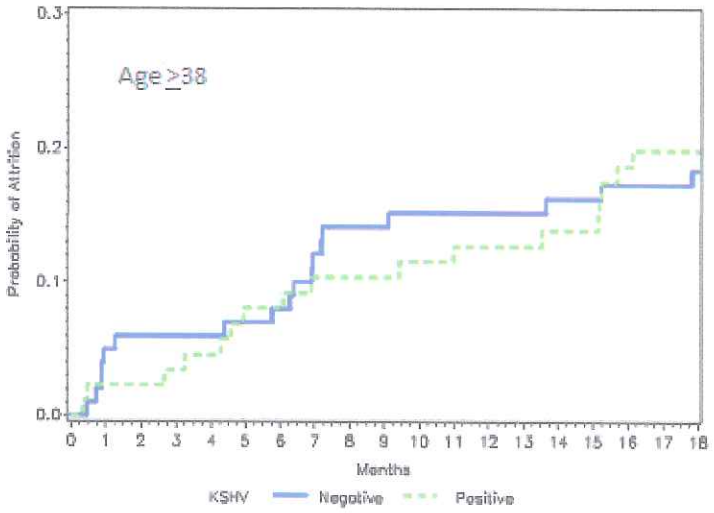
After 18 months of follow up, 310/385 (77%) participants who initiated ART were still alive and in care. This included 154/184 (80%) of the KSHV positive group and 155/201 (73%) in the KSHV negative group. A further 29 participants (6%; n=12 from the KSHV positive group and 8%; n= 17 from the KSHV negative group) had transferred to another treatment facility. In this analysis attrition from care was a combined outcome comprising both known deaths (n=31, 8%) and those lost to follow up whose vital status was unknown (n=35; 9%). Though the study numbers were small and the estimates subsequently lacked precision, the direction of the point estimates suggested the KSHV positive group were less likely to experience attrition than their KSHV negative counterparts at both 12- (aHR=0.57; 95% CI 0.29-1.11) and 18-months (aHR=0.77; 95% CI 0.44-1.35) after ART initiation compared to the KSHV negative group.

Effect measure modification of the effect of KSHV on attrition from care by both age (Figure 5) and CD4 cell count (Figure 6) was noted on the relative scale. The protective effect of co-infection with

KSHV [aHR=0.50; 95% CI 0.22-1.14] is demonstrated among those less than 38 years old (the median age of the cohort) but not for the participants aged ≥38.0 [aHR=0.92; 955 CI 0.41-2.05]. Similarly, among those participants with low baseline CD4 counts, KSHV co-infection was associated with a decreased risk of attrition compared to the KSHV negative group [aHR= 0.62; 95% CI 0.32-1.18]. The effect is not seen among those with CD4 counts >100 cells at ART initiation [aHR= 1.02; 95% CI 0.27-3.89].

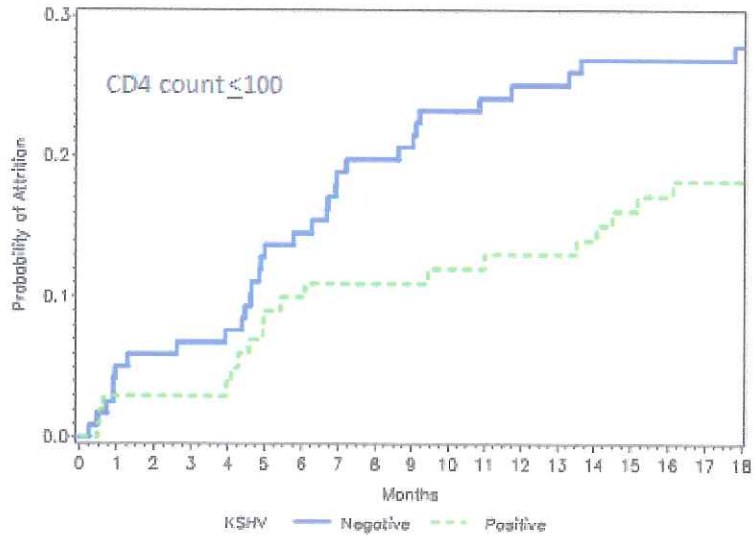


aHR= 0.50 (95% CI 0.22-1.14)

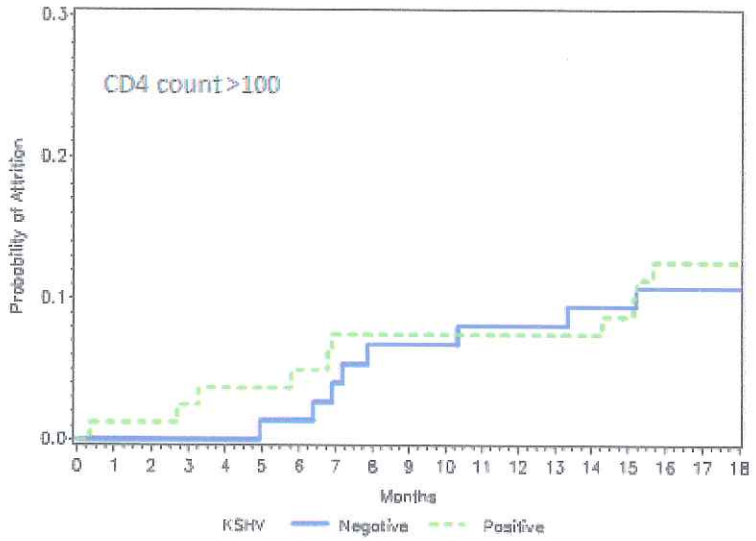


aHR= 0.92 (95% CI 0.41-2.05)

Figure 5: The relation between KSHV and attrition from care stratified by age



aHR= 0.62 (95% CI 0.32-1.18)



aHR= 1.02 (95% CI 0.27-3.89)

Figure 6: Attrition from care by KSHV status stratified by CD4 count

When the KSHV positive group was stratified by reactivity to KSHV antigen, the substrata did not all experience the same risk (Table 11). The risk of attrition for those reactive to lytic k8.1 alone was increased by 6-, 12- and 18-months of follow up on ART compared to the KSHV negative group. On the contrary, the KSHV positive substrata reactive to Orf73 alone or both K8.1 and Orf73 antigens were less likely to experience attrition from care over all time points considered when compared to the KSHV negative group.

Table 11: The relation between KSHV antibody response and mortality

	Death within 18 months			Death within 12 months			Death within 6 months		
Characteristic	Death/N (%)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Death/N (%)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Death/N (%)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
KSHV Status									
Both	4/77 (5.2%)	0.51 (0.17, 1.49)	0.63 (0.19, 2.09)	3/77 (3.9%)	0.46 (0.14, 1.59)	0.54 (0.13, 2.21)	3/77 (3.9%)	0.69 (0.19, 2.46)	0.72 (0.17, 3.08)
K8.1 only	8/73 (11.0%)	1.16 (0.51, 2.65)	1.73 (0.68, 4.42)	6/73 (8.2%)	1.05 (0.41, 2.67)	1.35 (0.44, 4.18)	5/73 (6.9%)	1.27 (0.44, 3.64)	1.62 (0.46, 5.73)
Orf73 only	2/34 (5.9%)	0.57 (0.13, 2.46)	0.90 (0.19, 4.26)	1/34 (2.9%)	0.35 (0.05, 2.63)	0.58 (0.07, 4.92)	1/34 (2.9%)	0.51 (0.07, 3.98)	0.75 (0.09, 6.51)
Neither	19/199 (9.6%)	Reference	Reference	16/199 (8.0%)	Reference	Reference	11/199 (5.5%)	Reference	Reference
Sex									
Male	15/134 (11.2%)	1.60 (0.80, 3.17)	1.48 (0.66, 3.35)	12/134 (9.0%)	1.63 (0.75, 3.52)	1.33 (0.51, 3.50)	9/134 (6.7%)	1.54 (0.64, 3.72)	0.87 (0.28, 2.68)
Female	18/249 (7.2%)	Reference	Reference	14/249 (5.6%)	Reference	Reference	11/249 (4.4%)	Reference	Reference
Age at initiation									
<30	6/69 (8.7%)	Reference	Reference	5/69 (7.3%)	Reference	Reference	4/69 (5.8%)	Reference	Reference
30-39.9	6/157 (3.8%)	0.42 (0.13, 1.29)	0.34 (0.08, 1.36)	5/157 (3.2%)	0.43 (0.12, 1.48)	0.37 (0.08, 1.82)	5/157 (3.2%)	0.55 (0.15, 2.04)	0.52 (0.10, 2.85)
40-44.9	8/66 (12.1%)	1.38 (0.48, 3.98)	1.76 (0.49, 6.24)	6/66 (9.1%)	1.26 (0.39, 4.14)	1.61 (0.37, 7.07)	5/66 (7.6%)	1.33 (0.36, 4.95)	2.08 (0.43, 10.16)
≥45	13/91 (14.3%)	1.65 (0.63, 4.35)	2.41 (0.79, 7.33)	10/91 (11.0%)	1.55 (0.53, 4.53)	2.23 (0.63, 7.95)	6/91 (6.6%)	1.18 (0.33, 4.16)	1.96 (0.45, 8.60)
Baseline CD4+ Count									
<50	18/120 (15.0%)	3.83 (1.60, 9.16)	2.69 (0.97, 7.47)	14/120 (11.7%)	3.39 (1.30, 8.82)	1.76 (0.54, 5.74)	11/120 (9.2%)	3.91 (1.25, 12.29)	1.82 (0.48, 6.83)
50-100	8/100 (8.0%)	1.92 (0.70, 5.30)	2.08 (0.66, 6.50)	6/100 (6.0%)	1.65 (0.53, 5.12)	1.55 (0.42, 5.71)	5/100 (5.0%)	2.06 (0.55, 7.66)	1.72 (0.42, 7.09)
>100	7/163 (4.3%)	Reference	Reference	6/163 (3.7%)	Reference	Reference	4/163 (2.5%)	Reference	Reference
WHO Stage									
I/II	13/226 (5.8%)	Reference	Reference	8/226 (3.5%)	Reference	Reference	7/226 (3.1%)	Reference	Reference
III/IV	16/133 (12.0%)	2.27 (1.09, 4.72)	3.03 (1.26, 7.31)	14/133 (10.5%)	3.14 (1.32, 7.49)	4.06 (1.42, 11.64)	11/133 (8.3%)	2.77 (1.07, 7.14)	3.22 (1.01, 10.27)
Haemoglobin level									
<8.5	6/33 (18.2%)	2.87 (1.18, 7.01)	2.88 (1.01, 8.20)	6/33 (18.2%)	3.89 (1.55, 9.81)	3.90 (1.26, 12.02)	5/33 (15.2%)	4.38 (1.56, 12.28)	4.05 (1.19, 13.85)
≥8.5	25/339 (7.4%)	Reference	Reference	18/339 (5.3%)	Reference	Reference	13/339 (3.8%)	Reference	Reference

The presence of a detectable KSHV viral load also appeared to increase risk of attrition. The KSHV positive group with a detectable KSHV viral load experienced a two-fold increased risk of attrition compared to the KSHV negative participants by 18 months of follow up (aHR 2.06 95% CI 0.52-8.20) while the KSHV positives with undetectable KSHV viral loads experienced a similar risk to the KSHV negatives (aHR 0.94; 95% CI 0.40-2.22) though the confidence intervals around these estimates were wide and lacked precision.

CD4 count response

Both groups demonstrated good immune responses to treatment. The mean increase in CD4 count among the KSHV+ group by 6 months was 117 cells/mm^3 (95% CI: 102-132) compared to 107 cells/mm^3 (95% CI: 92-121) among the KSHV-. We again considered both linear increases in CD4 count as well as a dichotomised outcome. The KSHV positive group gained a similar number of cells over the duration of follow up compared to the KSHV negative group (Figure 7).

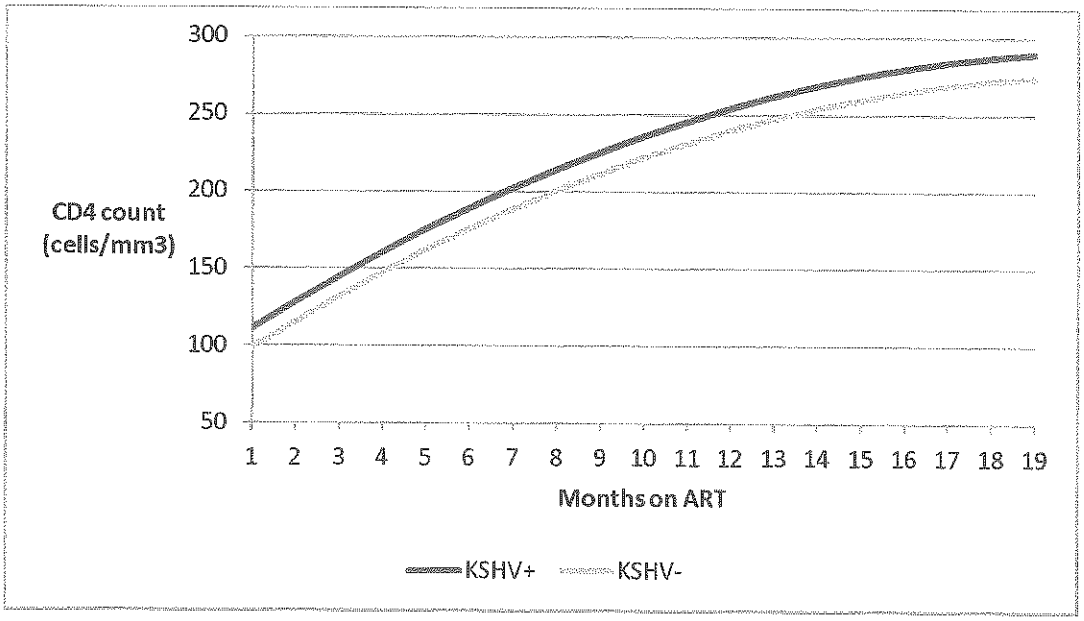


Figure 7: Predicted CD4 cell count increase from ART initiation stratified by KSHV status

The trajectories were estimated using two separate mixed linear models, one for the KSHV+ and one for the KSHV- to allow the curves to depart from being parallel. Despite this, the predicted CD4 trajectories from start of ART were almost perfectly parallel for the groups suggesting that the only differences over time were related to persistence of those differences seen at initiation of ART. The greatest increase in CD4 count was noted shortly after treatment initiation for both groups, though the KSHV positive group retained consistently higher CD4 cell counts at all of the time points observed. There was little difference between the groups in terms of proportions achieving a 50 cell increase in CD4 count at either six months (31% vs.30%) or a year on treatment was demonstrated (32% vs.27%). In fact the point estimates from adjusted models suggested a small protective effect against failure to achieve a CD4 response for patients with KSHV after a year on treatment (RR= 0.75 95% CI: 0.50-1.12) compared to their KSHV- counterparts.

The effect of a detectable KSHV viral load at ART initiation on subsequent CD4 response was also considered. Though the number of events was small and the estimates lacked precision, there was increased risk of poor CD4 response at both 6- (RR=1.88; 95%CI: 1.00-3.53) and 12-months (RR=1.69; 95%CI: 0.78-3.64) on treatment for those with a detectable KSHV viral load. The group with a positive KSHV result were further stratified into reactive to lytic K8.1 only, reactive to latent Orf73 only or reactive to both antigens. Those reactive to both lytic K8.1 and latent Orf73 had the smallest median increase in CD4 count (68; 21-145 cells/mm^3) of all groups. By 6 months on ART, more of those reactive to both antigens (37%, n=25/68) had not increased their CD4 count by 50 cells/mm^3 compared to those reactive to lytic K8.1 (26%, n=15) and latent Orf73 (21%, n=6) groups. Adjusted estimates suggested little difference in CD4 response compared to the KSHV negative group for those reactive either K8.1, Orf73 or both (Table 12). Results were similar at 12 months on treatment.

Table 12: Immune response to ART stratified by reactivity to KSHV antigens

	6-months			12-months		
Exposure	Failure to achieve CD4 response	Crude RR (95% CI)‡	Adjusted† RR (95% CI)‡	Failure to achieve CD4 response*	Crude RR (95% CI)‡	Adjusted† RR (95% CI)‡
KSHV-	48 (31%)	Reference	Reference	28 (27%)	Reference	Reference
Orf73 only	6 (6%)	0.68 (0.32-1.44)	0.82 (0.41-1.67)	9 (41%)	1.53 (0.32-1.44)	1.53 (0.86-2.70)
K8.1 only	15 (26%)	0.84 (0.51-1.37)	1.01 (0.63-1.63)	10 (29%)	1.07 (0.51-1.37)	1.11 (0.66-1.87)
Both	25 (37%)	1.17 (0.79-1.73)	1.14 (0.81-1.61)	16 (30%)	1.11 (0.79-1.73)	1.11 (0.73-1.68)

HIV Viral Load suppression

Achieving HIV virologic suppression on ART was common among both groups. By 6 months on treatment, only 4% of those with KSHV (5/143) had failed to suppress HIV viral load to <400 copies/mL while 10% (14/139) of those without KSHV had failed to achieve suppression. Among those who survived to a year on treatment, similar proportions achieving virologic suppression were noted (6% (6/109) vs. 8% (8/104). Estimates demonstrated similar virologic responses between the KSHV+ and KSHV-- groups at both 6- (RR = 1.03; 95% CI:0.90 - 1.17) and 12-months (RR = 1.01; 95% CI:0.74-1.37) on treatment after adjustment for sex, age, CD4 count, co-infection with tuberculosis, haemoglobin and body mass index.

Contrasting the clinical effect of KS with co-infection with KSHV

The results of these studies suggest quite different effects on ART treatment outcomes for clinical disease with KS compared to co-infection with KSHV. Table 13 summarises and contrasts these findings. Overall, clinical disease with KS at initiation of ART was associated with a poorer response to treatment and a significantly increased risk of mortality. Co-infection with KSHV however, was not associated with risk of poor outcomes after ART initiation and in fact appeared to be protective

against attrition (though the imprecise nature of the estimates limits our ability to make inferences from this).

Table 13: Summary of the effect of KS and KSHV on ART treatment outcomes

	Kaposi sarcoma clinical disease	KSHV co-infection
Presentation at ART initiation	Advanced immune suppression (median CD4 count 74 cells/mm^3)	KSHV not associated with markers of advanced HIV disease
Gender distribution	Over-representation of males (49%)	Representative of general HIV clinic population
Risk of mortality and LTFU	Significantly increased risk of mortality among those with KS (four-fold in the first year on ART). LTFU Also increased among those with KS (1.5 times more likely)	KSHV associated with protective effect against attrition from care. Group with detectable KSHV viral load possible exception
Immunologic response to ART	KS group demonstrated more sluggish recovery of CD4 cells compared to those without KS	Similar immune response to ART for KSHV positive and negative groups
Virologic response to ART	Limited evidence of better virologic suppression among those with KS	No association between KSHV and virologic suppression noted

CHAPTER 4: DISCUSSION

Introduction

The body of work covered in this thesis describes the epidemiology of Kaposi sarcoma and its aetiologic virus and the effect of each of these on treatment outcomes after initiating ART. In doing so, the transition occurring within the maturing HIV epidemic in Southern Africa is explored and the complexity of the spectrum of disease due to viral-associated cancers is uncovered. The limitations in studying the complex nature of the impact of co-infection with cancer-causing viruses and of cancer itself posed by lack of efficient and representative data systems are outlined.

This thesis was developed with specific objectives in mind as outlined in Chapter 1. In setting about meeting these objectives, it is evident that unpacking the epidemiology of KS and KSHV is not straightforward and the answers to these questions are complex. The reasons for this are three-fold:

1. First, the effect of clinical disease due to Kaposi sarcoma and the effect of co-infection with KSHV appear to manifest quite differently among those receiving ART
2. Second, understanding co-infection with KSHV is further complicated by the differences noted between the substrata of the KSHV infected group
3. Third, the backdrop to this work is one where the capacity of health services and data systems to study the impact of cancers and viruses are limited

This chapter highlights the key findings of each of the papers and discusses their relevance to the existing body of evidence as well as the implications for the management of HIV. It concludes by considering how current conceptualisation of disease may limit the appreciation of the complexity of the clinical outcome for the individual and where future research efforts might be focused. The discussion draws on the three themes outlined in the matrix in Table 4 (see Chapter 1: Thesis Themes):

1. Disease transition within the HIV epidemic and the increasing significance of viral-associated cancers and their aetiologic agents
2. Viruses and viral associated cancers and the impact on treatment response to ART
3. Limitations of data systems documenting the impact of cancers on ART

Disease transition within the HIV epidemic

The maturation of the HIV epidemic has brought attention to non-communicable disease within the spectrum of HIV infection. Evidence of this transition is apparent by the increasing significance of conditions such metabolic disease, hypertension, lipid disorders and diabetes but the most significant of these is cancer. The transition proposed to be occurring in the HIV epidemic differs from that described by Omran (1971) in that the underlying backdrop to the transition remains a powerful infectious force and the origins of cancers which are on the rise are typically communicable too. Viral-associated cancers are the most important and here we focus on Kaposi sarcoma and its viral aetiology, the Kaposi sarcoma herpes virus.

Study 2 (The effect of co-infection with KSHV on response to ART) documented a high prevalence (48%) of KSHV in a population of HIV-infected adults initiating ART in an urban HIV outpatient treatment facility (see Chapter 3: Results - Prevalence of KSHV). The association between the HIV and KSHV infection has been described previously and the high prevalence of KSHV demonstrated in this setting is comparable with findings from similar settings among HIV-infected populations [Malope-Kgokong et al, 2010; Sitas et al, 1999]. In fact, KSHV positive pregnant women were four times as likely to have HIV compared to their KSHV negative counterparts in South African antenatal clinics [Malope-Kgokong et al, 2010] where the overall prevalence of KSHV was estimated at 45% and that of HIV at 23%. In the developed world, prevalence of KSHV appears much lower (<10%) though certain populations are at increased risk including men who have sex with men and those with a history of another sexually transmitted infection [Smith et al, 1999]. Such conflicting results

are probably caused by varying routes of transmission of this virus and much geographic variability in prevalence rates [Schulz et al, 2000]. The South African antenatal-clinic based study showed rates of KSHV infection to vary from 35% to 49% across different municipalities within one province [Malope-Kgokong et al, 2010]. Among populations with a high prevalence of KSHV seropositivity, the route of infection is likely to be saliva and is acquired during childhood and early adult life [Mbulaiteye et al, 2004; Malope et al, 2010]. This appears to be independent of HIV transmission which is acquired sexually during adolescence.

Despite this clear association with HIV infection, those co-infected with KSHV at ART initiation did not present with signs of more advanced HIV disease than their KSHV negative counterparts. In fact the KSHV positive group had higher BMI and CD3, 4 and 8 cell counts than their KSHV negative counterparts (see **Paper 1:** Prevalence and predictors of Kaposi Sarcoma Herpes Virus seropositivity: A cross-sectional analysis of HIV-infected adults initiating ART in Johannesburg, South Africa). Among the KSHV infected individuals, however, those with a detectable KSHV viral load presented with signs of more advanced HIV disease including anaemia and WHO stage 3 or 4 defining conditions compared to those in whom the virus was undetectable.

The impact of clinical infection with KS at initiation of ART however was important. The findings of **Study 1 (The effect of clinical disease due to KS on response to ART)** demonstrated a substantially increased risk of mortality associated with KS. The risk of death was four times greater among the KS group in the first year on ART and though the risk decreased thereafter, those with KS were still twice as likely to die after the first year of treatment as those without KS after adjustment for measured confounders. This is consistent with high mortality due to KS in African populations despite increased access to ART. The cumulative one-year survival of KS subjects attending care in Cape Town was 39% among untreated subjects and even among those receiving ART was as low as 60% [Chu et al, 2010]. The one-year survival in a treated Nigerian cohort of KS patients was slightly higher at 77% [Agaba et al, 2009]. These findings highlight the importance of early diagnosis and

initiation of appropriate treatment for HIV-infected subjects with KS at every stage of HIV infection and treatment. The decline of cancer-associated mortality seen in the developed world since widespread use of ART has not followed through to developing nations even though access to ART is increasing. This is partly due to incomplete coverage with ART in these settings but also limited access to several treatment options for AIDS-associated cancers [Sissolak et al, 2005], particularly chemotherapy.

In addition to an increased risk of mortality, the results from Study 1 suggested a recorded KS diagnosis may be associated with poor immunologic response to ART. First, those with KS were roughly twice as likely to have a nadir CD4 count between 200 and 350 cells/mm^3 compared to those without KS. This is likely explained by the fact that KS (as a WHO stage 4-defining condition) was an indication for initiation of ART with CD4 count ≥200 cells/mm^3 at a time when the ART eligibility criteria were otherwise <200. Second, after initiation of ART, those with KS were less likely to increase their CD4 cell counts by 50 and 100 cells at 6 and 12 months on treatment respectively. The KS group also had a smaller mean increase in CD4 cell count at both time periods than those without KS though the actual difference in CD4 gain was small between the groups. This may be due to differences in disease stage at treatment initiation [Nash et al, 2008] or possibly related to the additional suppressive effect of chemotherapy on the KS patients' immune system. CD4 cell counts have been documented to decline by up to 50% during chemotherapy even in the presence of virally suppressive ART [Powles, 2002], an additional immune insult that would not be experienced by those not receiving chemotherapy.

Co-infection with KSHV: on the causal pathway to KS or a detour?

While the negative impact of clinical disease with KS was evident in this population of HIV-infected adults, the effect of co-infection with KSHV was less clear. KSHV has been well-documented as the

virus causing clinical disease with KS and there is plausible biological evidence that co-infection with HIV can act as a trigger for increased replication of KSHV and development of clinical disease with KS. Previous in vitro studies have suggested interactions between these two viruses including an increase in HIV-1 viral load in the presence of KSHV [Mercader et al, 2001] and induced reactivation of HIV-1 replication in chronically infected cells [Caselli et al, 2005]. Additionally, there is evidence that the immune suppression caused by HIV-1 as well as cytokine release promotes reactivation of KSHV lytic genes [Mercader et al, 2000] including K8.1. This increase in viral progeny eventually results in destruction of the host cell and progression to the development of Kaposi sarcoma, and the often aggressive course seen in HIV-1 positive individuals [Aoki et al, 2004; Lukac et al, 1999]. There is also evidence that, in HIV positive individuals, high KSHV viral loads multiply the risk of developing KS many-fold (Sitas et al 1999).

If the causal pathway from infection with KSHV to disease with KS via HIV co-infection is as depicted in Figure 8, it would stand to reason that those infected with KSHV could have more advanced HIV disease at initiation of ART and possibly even poorer outcomes once initiated on ART.

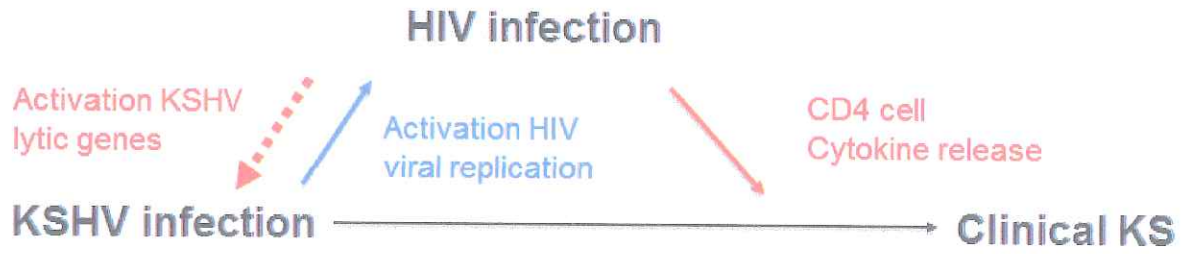


Figure 8: Conceptual causal pathway from KSHV infection to clinical disease due to KS

The findings of **Study 2 (The effect of co-infection with KSHV on response to ART)**, however, differed somewhat from this conceptual hypothesis. The results suggest that those with KSHV co-

infection achieved comparable virologic and immunologic responses to ART when compared to those without KSHV. Though numbers were small and the results were somewhat imprecise, the KSHV groups gained a similar number of CD4 cells over the first 18 months of ART and were at similar risk of failing to achieve a 50 cell increase and 100 cell increase at 6- and 12-months respectively. *In vitro* studies have also suggested that KSHV not only increases HIV-1 replication in acutely infected cells but also induces reactivation of HIV-1 replication in chronically infected cells [Caselli et al, 2005]. In this study population however, little difference was observed in HIV viral load prior to ART initiation between the groups as well as a similar risk of failure to suppress viral load at 6- and 12-months after ART initiation when comparing KSHV+ to KSHV-. The *in vitro* findings were demonstrated in the absence of antiretroviral therapy while the study population described I this thesis was treated with triple drug combination ART and the direct impact of ART on KSHV is not well described. Though there is limited evidence describing the clinical outcomes of those with KSHV, this study's results concur with previous work among a cohort of long term non-progressor MSMs, which also concluded there was no impact of KSHV on the progression of HIV-1 infection in terms of CD4 cell count decline, HIV-1 viral load increase or CD4 cell viraemia [Ait-Arkoub et al, 2003]. Ait-Arkoub and colleagues postulate that KSHV acts as an opportunistic agent rather than an HIV-cofactor among co-infected individuals.

Those with KSHV also appeared more likely to be alive and in care at the end of follow up compared to their KSHV negative counterparts (see Paper 3: Treatment Response and Mortality among Patients Starting Antiretroviral Therapy with and without Kaposi Sarcoma: A Cohort Study). Of interest is that the protective effect of KSHV was restricted to those with low CD4 counts and in younger age categories. It is unclear at this stage exactly why the protective effect of KSHV on attrition was noted only among those with low CD4 counts though reasons are postulated below.

Firstly, it is possible that the lack of association demonstrated between KSHV seropositivity and suppression of the immune system is due, at least in part, to survivor bias. If KSHV infection is, in

fact, associated with more advanced immune suppression and subsequent faster HIV disease progression, one could expect those with KSHV to be at greater risk of mortality before being able to access HIV treatment and care. Thus, the KSHV population presenting for treatment may represent a particularly healthy group of KSHV-infected individuals who have survived the excess risk associated with KSHV co-infection to the point of ART initiation. The finding of higher BMI and T-lymphocyte subpopulations noted in the KSHV infected population in Paper 1: "Prevalence and predictors of Kaposi Sarcoma Herpes Virus seropositivity: A cross-sectional analysis of HIV-infected adults initiating ART in Johannesburg, South Africa" are consistent with this. Additionally, despite this high prevalence of KSHV in an immunologically suppressed population, only one individual (0.2% of the study population) presented with clinical Kaposi sarcoma, further supporting the notion that this KSHV infected group may indeed represent survivors.

Alternately, this group of KSHV infected subjects may represent a relatively young population (see Table 10: Presenting features of 404 ART naïve adults in care at Themba Lethu in Johannesburg, South Africa stratified by KSHV status) who have not had KSHV for long and among whom ART was initiated early on in the course of infection before any negative outcomes could manifest. ART has been shown to be effective in treating "early" KSHV by reducing KSHV viral load [Aversa et al, 2005]; the fact that the protective effect of KSHV was restricted to those in younger age categories may be related to this. A previous study among South African HIV uninfected cancer patients has demonstrated that older age is associated with high anti-KSHV antibody titers [Wojcicki et al, 2003] which in turn are associated with risk of development and progression of KS [Sitas et al, 1999].

Second, there is evidence that KSHV infection can also be associated with *inhibition* of HIV infection of CD4 cells [Boshoff et al, 1997; Lusso 2000]. This inhibitory action is believed to be mediated through beta chemokines. Chemokines are small proteins secreted by cells that primarily act to attract and guide migration of cells. This ability is particularly useful during inflammation when the cells of the immune system are needed at the site of infection or during tissue repair. KSHV-encoded

chemokines have been shown to prevent infection of CD4 cells expressing CCR3, a known entry co-receptor for HIV. Inability of HIV to infect CD4 cells would allow for preservation of the immune system despite the presence of actively replicating HIV. If this protective inhibition is not clinically apparent early on in HIV infection when CD4 cells are in abundance, but manifests only later as cell counts drop to critical levels, it would offer some explanation (at least in part) as to why the protective effect of KSHV was noted only among those with CD4 counts <100 cells.

Finally, it may be that the effect of KSHV is modified by strata of KSHV seropositivity. The impact of these different states of KSHV infection, when combined, could result in estimates that tend towards the null. This is explored in more detail in the following section.

KSHV and HIV: peeling back the layers

As we further unpack the relation between KSHV and HIV, it becomes evident that infection with KSHV is complex and the pathogenesis of the virus is such that different states of infection are quite possible. We stratified our analyses by 1) presence of detectable KSHV viral load and then by 2) reactivity to the KSHV antigens. We found that the effect of KSHV on treatment outcomes sometimes differed by these strata.

First, the KSHV positive group with a detectable KSHV viral load appeared more likely to die or become lost from care within the first 18 months of ART compared to their KSHV negative participants (aHR 2.06 95% CI 0.52-8.20) while the KSHV positives with undetectable KSHV viral loads experienced a similar risk to the KSHV negatives (aHR 0.94; 95% CI 0.40-2.22). The small numbers studied and resultant imprecise nature of these estimates makes it difficult to draw solid conclusions from this data alone but KSHV viremia has previously been associated with the likelihood of development of clinical Kaposi sarcoma and other signs of advanced HIV disease including thrombocytopenia and higher HIV viral loads [Minami et al, 2009; Campbell et al, 2000] and KSHV

DNA is present in all tissue biopsies of clinical KS lesions [Chang et al Science 1994; Boshoff et al Lancet 1995; Kedes et al Nature Med 1996]. The association between high KSHV viral loads and KS disease progression has resulted in suggestions to use KSHV viral load testing in patients with KS to assess further disease progression and to guide initiation of antiherpes treatments such as ganciclovir [Laney et al, 2007]. KSHV DNA in plasma has also been shown to predict death among those with clinical Kaposi sarcoma [El Amari, 2004]. It is hypothesized that detectable KSHV viremia may indicate poor immune control of KSHV infection which in turn may indicate more advanced HIV disease.

Contradicting this theory somewhat though, is the lack of consistent evidence for a negative impact on CD4 cell count and HIV viral load suppression in the presence of detectable KSHV viral load. When the analysis was restricted to the KSHV infected group only, results were similar for those with a detectable KSHV viral load when compared to those where the virus was undetectable at initiation of ART.

Second, the effect of KSHV was stratified by reactivity to two KSHV antigens: 1) reactive to lytic K8.1 alone; 2) reactive to latent Orf73 alone and 3) reactive to both (see Phases of the KSHV cycle in Chapter 1: Introduction). Though the pathogenesis from KSHV infection to clinical disease is complex and includes expansion of latently infected cells, the transition between latency and lytic replication may play a role in the development of progression to clinical disease and possibly even transmission of the virus [Adang et al, 2006; Taylor et al, 2004]. Among those reactive to both lytic K8.1 and Orf73, a state which may indicate transition between phases of the KSHV cycle, a poorer immunological response in terms of number of CD4 cells gained within the first year on ART was noted. Poor immunologic response to treatment and low CD4 cell counts have previously been associated with increased risk of KS morbidity and mortality [Chene et al, 2003; Carrieri et al 2003; Lawn et al; 2008]. The findings of Paper 1 (Prevalence and predictors of Kaposi Sarcoma Herpes Virus seropositivity: A cross-sectional analysis of HIV-infected adults initiating ART in Johannesburg, South

Africa) provided some evidence in support of the clinical impact of the different stages of the KSHV cycle. The optical density for lytic K8.1 KSHV antigen was higher than that for latent Orf73 and more individuals were reactive to the lytic antigen.

In addition to this, those positive to latent Orf73 antigen presented with markers of less immune suppression and less advanced disease at ART initiation. Those with higher BMI, higher CD3, 8 and 4 cell counts were more likely to be reactive to latent Orf73 than those who did not react to latent Orf73 (including the overall KSHV negative group). CD4 and 8 cells play important roles in cell-mediated immunity and control of viremia in the HIV-infected individual [Ogg et al, 1998; Chinen et al 2002] and low absolute numbers of CD4 and 8 cells have been associated with an increased risk of disease progression and mortality among HIV-infected persons [Macias et al, 2001; Langford et al, 2007; Cozzi Lepri et al, 2004; Phillips et al, 2006]. The results suggest this might be true across all strata of KSHV seropositivity and by proxy, phase of KSHV cycle. These differences though, seemed to be restricted to the time prior to ART initiation. Over 18 months of follow up on ART, when stratified by reactivity to K8.1 alone, Orf73 alone or both antigens, all three groups gained a similar number of CD4 cells and were at similar risk of failing to achieve a 50 cell increase and 100 cell increase at 6- and 12-months respectively. The linear models for those KSHV+ and KSHV- were fit separately, allowing for different curves by exposure group. Despite this, the curves remained almost perfectly parallel over the time on study providing further evidence that the only difference between the groups were the differences in baseline CD4 count at treatment initiation.

Though the evidence for clinical differences in these substrata of the KSHV positive group is not as compelling as it could be, it is an intriguing possibility and to some degree, supported by the findings in **Study 2 (The effect of co-infection with KSHV on response to ART)**. The pathogenesis of KSHV is complex though and its effect in the presence of ART is likely more involved than the simple conceptual framework outlined here. Early stage KS has been successfully treated with ART [Aversa et al, 2005], yet the exact mechanism through which ART reduces the tumour is unknown. ART has

been shown to reduce KSHV viral loads to undetectable levels [Gill et al; 2002]. CD8+ lymphocytes are involved in the cellular immune response to several viruses including HIV-1 and herpesvirus; these virus-specific cytotoxic T lymphocytes respond to both lytic and latent antigens of KSHV. Evidence of ART-induced immune reconstitution to KSHV (indicated by an undetectable KSHV viral load) has been associated with an increase in CD8+ lymphocytes, suggesting restoration of these cells may be important in the control of KSHV infection [Wilkinson et al, 2002] and ultimately control or even prevention of KS.

Limitations of studying cancer in the setting of the HIV epidemic

This study offers some insight into, as yet, unanswered questions around the clinical effect of KSHV co-infection. In interpreting and considering the results presented here, one must consider the backdrop against which the clinical care of the study subjects for HIV and cancer is provided and the limitations of the setting in which these health care services operate and this research was conducted.

As noted previously, when studying effects of exposures such as cancer and infection with viruses, randomisation is not possible and reliance was placed on observational cohort data to try to add to the evidence on these topics. Observational cohorts can be prone to bias, particularly that due to loss to follow up. While clinical outcomes from HIV treatment programmes in Southern Africa have been encouraging [Egger et al, 2002; Lawn et al 2005; Ivers et al 2005] and comparable to those achieved in European programmes [Keiser et al, 2008], high rates of loss to follow up reported in African programmes (up to 40%) are of concern [Rosen et al, 2007] and may negatively impact on the ability to accurately study mortality and other overall programme treatment goals. High rates of lost to follow up were observed in the cohort studied and that may have introduced selection bias to the results, though rates of loss to follow up between the groups were similar. Nevertheless, loss to follow up may have led to underestimation of the mortality rates due to different rates between

patients lost with and without KS. As previously noted, anywhere from 20-50% of those lost from HIV care are actually deceased [Fox et al, 2010, Maskew et al 2007, Brinkhof et al, 2009; Geng et al, 2008; Rosen et al, 2010].

In the absence of reliable vital registration systems, HIV treatment programmes have to rely on verbal report from family members or tracing a sample of those lost from care [Geng et al 2008] and adjusting estimates statistically. South Africa has one of few functioning vital registration systems in Africa (Setel et al, 2007) and, in order to improve ascertainment of mortality among those lost; data linkages were conducted with this system through the National Vital Registration Infrastructure Initiative. While the linkages dramatically improved the ascertainment of mortality in the study populations presented here, it is limited to those with a valid national identification number (42% of those lost in TLC and 47% in the Khayelitsha sites).

Compounding this issue of identification is the lack of unique identifiers with which to perform such linkages in the absence of a valid national identification number. To date, the health care services in South Africa do not have national data system which uses a unique patient identifier that is transferrable across health care facilities. Each health facility allocates its own patient identifier and there is frequently complete disconnect between sites. The result is that patients transferring between care facilities for geographic or medical reasons do not have their data linked or transferred across sites. This is typical of the setting in which this study was conducted. While HIV care and treatment was provided at the local decentralised sites, oncology services are centralised at tertiary facilities. The impact on clinical care as well as accuracy of medical records is evident. The prevalence of KS among this HIV-infected treatment naïve population was estimated at 1.8%, very similar to the prevalence of 1.6% seen in Nigeria [Agaba et al, 2009], but much lower than seen among European cohorts (3.8-6.4%) in the late 1990s [El Amari et al, 2008; Dal Maso et al, 2001; Mocroft et al, 2004]. While there may be country-level differences in prevalence of KS, the figures derived from **Study 1 (The effect of clinical disease due to KS on response to ART)** could

underestimate the true prevalence in this setting for several reasons. Firstly, diagnosis of KS in HIV outpatient clinics may be impaired by the limited access to oncology and histopathology services, as well as the remote location of these from the HIV clinics. Additionally, early recognition KS by primary care staff is limited. As a recent study in South Africa noted, 46% of study subjects were diagnosed with KS and HIV at the same time [Khammissa et al, 2008]. The information disconnect results not only in under ascertainment of recorded diagnosis of cancer in HIV cohorts, but also a gap in the patients' medical record at the HIV clinic providing on-going treatment. Data on staging of KS disease and use of chemotherapy is rarely available in HIV cohort datasets and this limits the ability to adjust models for these or estimate the effect of chemotherapy and ART on treatment outcomes. Encouragingly, the National department of Health recently outlined out plans to introduce a single standardised data system across HIV CCMT sites nationally.

While this may improve communication between HIV treatment sites, the challenge of linkage across other health care facilities, including those providing oncology services, remains. Population-based record linkage between HIV registry data and cancer registry data has been used previously in Europe and North America to investigate cancers in HIV infected people [Simard et al, 2011; Grulich et al, 2002; Clark et al 2004; Cooksley et al, 1999]. Data from HIV-infected cohorts linked to cancer registry data not only improves ascertainment of cancer diagnosis within HIV cohorts but provides opportunities to study a wider range of risk factors than those possible with HIV cohort data alone[Polesel et al, 2008; Clifford et al, 2005]. Until recently, the Uganda AIDS-cancer Registry Match Study was the only HIV cancer linkage study to be conducted in sub-Saharan Africa [Mbuliteye et al, 2006]. Towards the end of this study, data linkages between the South African national Cancer Registry and several HIV cohorts in the IeDEA-SA network were commenced. Analysis of this linkage is still underway but it is hoped that activities such as these will provide vital data needed to accurately estimate the impact of cancer in the setting of the southern African HIV epidemic.

For the reasons noted above and outlined in the Methods section (see Quantitative bias analysis), it is likely some cases of KS were misclassified in the populations studied. This can introduce misclassification bias. In the absence of reliable sources against which to verify cancer data, epidemiologic methods must be applied to address such bias. Traditionally, discussions around bias in studies is somewhat speculative; authors acknowledge bias likely exists in their results and sometimes consider how such bias may have impacted on the direction of their estimates but rarely are any efforts made to quantify the effect of such bias. Here well-described epidemiologic methodology [Lash et al, 2009] with easy to use and freely available software was used to quantify the effect of the possible bias that misclassification of both exposure (KS) and outcome (mortality) could have introduced to the results. The analysis demonstrated that despite poor non-differential sensitivity of exposure and outcome misclassification, the estimates of the effect of KS on mortality were likely biased only minimally towards the null. In situations where studies can only be conducted using observational data, quantitative bias analysis is an important tool that can be easily applied to explore and quantify the extent of bias present in our studies.

Conclusion and research horizons

This thesis commenced with an introduction to the interplay between communicable and non-communicable disease in the setting of the HIV epidemic. Using the example of HIV and the AIDS-related cancer Kaposi sarcoma, it is evident that current conceptualizations of a single underlying cause may not account adequately for the contribution to morbidity and mortality of coexisting diseases that the HIV-infected adult experiences. A disease transition has surfaced as the HIV epidemic matures and AIDS-related malignancies remain important, particularly in resource-limited settings. Despite effective ART, cancers such as KS remain a poor prognostic factor for ART treatment outcomes. High rates of mortality and loss to follow up confirm that KS remains a significant problem in these settings. Within this transition lies a paradox in that these settings bear

the greatest burden of disease due to cancers yet are most limited in terms of data systems required to effectively evaluate these conditions.

There is still much to learn about the effects of a transition within a disease such as HIV. While cancers such as KS remain prominent, future research efforts in this field may focus on investigating alternate chemotherapy drugs, particularly those available on an out-patient basis, which could be safely administered at primary care level in order to increase access and reduce delays in treating cancers and resulting mortality. On-going ACTG randomised control trials (A5263 and A5264) aim to compare three chemotherapeutic options (oral etoposide, bleomycin and vincristine, or doxorubicin HCL liposome injection) in combination with ART for treatment of KS in resource-limited settings.

Understanding how infection with KSHV progresses to clinical disease with KS in the HIV-infected patient remains to be determined. As KSHV transmission has been shown to occur even early in life presumably by saliva, temporal relationships between KSHV infection and the factors considered are difficult to establish. Prospective studies following subjects from KSHV infection through to infection with HIV and then clinical disease due to KS are ideal but very difficult to achieve. Use of birth cohorts such as the Birth to Twenty (BT20) which followed a cohort of South African infants from birth through to 20 years of age could prove to be a valuable resource in addressing questions such as these. As national guidelines change and ART is initiated at higher CD4 counts, changes in prevalence and presenting features of those co-infected with KSHV might occur. Studies focused on HIV-infected individuals who are not yet eligible for ART and ideally among a population who have recently HIV seroconverted may shed further light on this point.

As access to ART continues to scale-up and models of HIV care shift away from specialist services to decentralised clinics, investigating factors that impact on the effective response to ART is important. Accurate evaluation of the impact of cancers on ART treatment outcomes is essential at the national level to allow researchers to effectively identify interventions most likely to reduce disease burden thereby enabling policy makers to effectively plan for health care resource allocation. In order for

this to happen strengthening of national health care data systems as well as effective use national registries is critical. Understanding and unpacking the complexity within the course of diseases such as HIV and its associated conditions will only be possible once that is achieved.

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Appendix 1: Approval of study protocol and title from the Faculty of Health Sciences, University of the Witwatersrand

University of the Witwatersrand

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
FACULTY OF HEALTH SCIENCES
ASSESSORS MEETING

CANDIDATE: MIHAIL MASKEW

Date of Assessor Group Meeting: 16/9/2009 School / Department / Division: PUBLIC HEALTH

Yes ☐ No ☐ Is the research question clearly identified and described?

Comments:

Yes ☐ No ☐ Not entirely ☐

Is the design of the study and methods the methods used appropriate for the research question being asked?

Comments: Please number pages of protocol.

Is the study feasible within:

i. the applicant's resources? Yes ☐ No ☐

ii. the departments resources? Yes ☐ No ☐

iii. the time frame? Yes ☐ No ☐

M MASKEW

Do you recommend:

i. shortening / lengthening of the protocol? Please specify and explain.

No

ii. the appointment of a co-supervisor? Yes / ~~No~~

Nominee/s :

Overall recommendation regarding the protocol :

i. revision of the protocol to the Supervisor / Head of Department: (**Candidates:** one copy, list of corrections, supervisor approval letter – submit to PG Office) — Yes / No

ii. revision of the protocol to the satisfaction of the Assessor Group: (**Candidates:** six copies, list of corrections, supervisor approval letter – submit to PG Office) — Yes / No

iii. revision of the protocol and resubmission of the revised protocol to the next Assessor Group Meeting: (**Candidates:** six copies, list of corrections, supervisor approval letter – submit one copy to PG Office / 5 to school assessor group administrator) — Yes / No

iv. candidate goes ahead: — Yes / No

Assessor Names and Signatures :

S NAICKER

U KALA

F VENTER

E LIBHABER

S VAN VUUREN

R DUARTE

S NAICKER

Assessor Group Chair

16/9/09

Date

Appendix 2: Human Research Ethics Committee of the University of the Witwatersrand ethical clearance certificate for study protocol

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Maskew

CLEARANCE CERTIFICATE	PROTOCOL NUMBER M080628
PROJECT	Kaposi's Sarcoma and Kaposi's Sarcoma Herpes Virus infection in the setting of the sub-Saharan HIV Epidemic: A Multicentre Study
INVESTIGATORS	Dr M Maskew
DEPARTMENT	School of Public Health
DATE CONSIDERED	08.06.27
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 08.08.07 CHAIRPERSON .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof AP MacPhail

DECLARATION OF INVESTIGATOR(S)

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

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 3: University of Cape Town Research Ethics Committee approval

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
e-mail: lamees.emedi@uct.ac.za

08 October 2007

REC REF: 440/2007

Dr G Van Cutsem
Infectious Disease Epidemiology Unit
Public Health & Family Medicine

Dear Dr Van Cutsem

PROJECT TITLE: KAPOSI'S SARCOMA IN PATIENTS ON ANTIRETROVIRAL THERAPY IN KHAYELITSHA

Thank you for submitting your study to the Research Ethics Committee for review.

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

This serves to confirm that the University of Cape Town 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 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.

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

Please quote the REC. REF in all your correspondence.

lemjedi

Yours sincerely

A/PROF. M. BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Appendix 4: Patient Information and Consent Sheet for prospective KSHV cohort study

The Effect of Kaposi's Sarcoma Herpes Virus on the Outcome of HIV and the Response to Highly Active Antiretroviral Therapy

Patient Information Sheet and Consent Form

Good Day.

I am Dr Mhairi Maskew, an epidemiologist in the department of Medicine at the University of Witwatersrand. I am doing a research project as a part of a doctoral thesis and would be grateful for your assistance. Before agreeing to participate, it is important that you read the following explanations of the purpose of the study, the study procedures, benefits, risks, discomforts, and precautions as well as your right to withdraw from the study at any time. This information leaflet is to help you to decide if you would like to participate. You should fully understand what is involved before you agree to participate.

Many people in Southern Africa are infected with a virus called Kaposi's sarcoma Herpes Virus (KSHV). Often people can live with this virus for many years without becoming ill but in a few cases infection with the virus leads to a cancer called Kaposi's sarcoma. This cancer affects the skin and may be treated if detected early.

At the same time, many people in the country are being infected with HIV and it is possible that infection with KSHV may affect the way that the HIV virus works. It is also possible that infection with KSHV might affect the way people respond to the antiretroviral drugs which are used to treat HIV. The effect of the KSHV virus in South African patients has not been studied previously.

You are about to start antiretroviral treatment and you are invited to take part in this study by giving us 3 extra tubes of blood at each of your visits to the clinic. This blood will be taken at the same time as your other routine blood tests. One of the tests will be to see if you have KSHV in your blood and if so, another test will be used to determine if the virus changes over time. The blood samples will be safely stored by the University. Part of your blood will be stored so that genetic testing can be done in the future. These genetic tests will only be done to answer questions related to HIV and KSHV infection and will not be used in a way that may discriminate against you.

You will not have to make any extra visits to the clinic and there will be no cost to you. All your results will be kept confidential and your name and personal details will not be revealed to anyone.

It is very important that you should only agree to take part in this study if you are completely happy with the procedures involved. If you don't want to take part, your decision will not affect your treatment in any way. If you need more information before making a decision about participating in the study, please feel free to contact me on (011) 276 8896 and I will gladly answer any questions that you may have. Alternatively, you can contact the Chairman of the Human Research Ethics Committee of the University of the Witwatersrand, Professor Cleaton-Jones on (011) 717 2301.

I hereby confirm that this document has been explained to me and that I understand the procedure involved. I am aware that blood will be taken for analysis. I also understand that my personal details will not be disclosed to anyone.

Participant's Name (print)	Signature	Date
Study staff Conducting Consent Discussion (print)	Signature	Date
Witness Name (As appropriate)	Signature	Date

SCIENTIFIC ARTICLES

PAPER 1:

Prevalence and predictors of Kaposi Sarcoma Herpes Virus seropositivity: A cross-sectional analysis of HIV-infected adults initiating ART in Johannesburg, South Africa.

Maskew et al. Infectious Agents and Cancer 2011, 6:22
http://www.infectagentscancer.com/content/6/1/22

INFECTIOUS AGENTS AND CANCER

RESEARCH ARTICLE Open Access

Prevalence and predictors of kaposi sarcoma herpes virus seropositivity: a cross-sectional analysis of HIV-infected adults initiating ART in Johannesburg, South Africa

Mhairi Maskew[1,2*], A Patrick MacPhail[1,3], Denise Whitby[4], Matthias Egger[5], Carole L Wallis[6] and Matthew P Fox[2,7,8]

Abstract

Background: Kaposi sarcoma (KS) is the most common AIDS-defining tumour in HIV-infected individuals in Africa. Kaposi sarcoma herpes virus (KSHV) infection precedes development of KS. KSHV co-infection may be associated with worse outcomes in HIV disease and elevated KSHV viral load may be an early marker for advanced HIV disease among untreated patients. We examined the prevalence of KSHV among adults initiating antiretroviral therapy (ART) and compared immunological, demographic and clinical factors between patients seropositive and seronegative for KSHV.

Results: We analyzed cross-sectional data collected from 404 HIV-infected treatment-naïve adults initiating ART at the Themba Lethu Clinic, Johannesburg, South Africa between November 2008 and March 2009. Subjects were screened at ART initiation for antibodies to KSHV lytic K8.1 and latent Orf73 antigens. Seropositivity to KSHV was defined as positive to either lytic KSHV K8.1 or latent KSHV Orf73 antibodies. KSHV viremia was determined by quantitative PCR and CD3, 4 and 8 lymphocyte counts were determined with flow cytometry. Of the 404 participants, 193 (48%) tested positive for KSHV at ART initiation; with 76 (39%) reactive to lytic K8.1, 35 (18%) to latent Orf73 and 82 (42%) to both. One individual presented with clinical KS at ART initiation. The KSHV infected group was similar to those without KSHV in terms of age, race, gender, ethnicity, smoking and alcohol use. KSHV infected individuals presented with slightly higher median CD3 (817 vs. 726 cells/mm^3) and CD4 (90 vs. 80 cells/mm^3) counts than KSHV negative subjects. We found no associations between KSHV seropositivity and body mass index, tuberculosis status, WHO stage, HIV RNA levels, full blood count or liver function tests at initiation. Those with detectable KSHV viremia (n = 19), however, appeared to present with signs of more advanced HIV disease including anemia and WHO stage 3 or 4 defining conditions compared to those in whom the virus was undetectable.

Conclusions: We demonstrate a high prevalence of KSHV among HIV-infected adults initiating ART in a large urban public-sector HIV clinic. KSHV viremia but not KSHV seropositivity may be associated with markers of advanced HIV disease.

Keywords: Kaposi sarcoma, Kaposi sarcoma herpes virus, resource-poor setting, antiretroviral therapy

Background

Since there has been greater access to antiretroviral therapy (ART) [1-3], increased longevity among those infected with HIV has made morbidity and mortality from cancers associated with HIV increasingly more common [4]. Viral associated cancers including cervical cancer, non-Hodgkin's lymphoma and Kaposi sarcoma (KS) are prominent among HIV-infected individuals [4,5]. Kaposi sarcoma is the most common tumour in HIV-infected individuals in Africa and is preceded by infection with Kaposi sarcoma herpes virus (KSHV) [6]. Kaposi sarcoma was relatively common in South Africa (up to 5 per 1000 population at risk) prior to the AIDS epidemic [7] but the incidence increased dramatically as

* Correspondence: mmaskew@witshealth.co.za
[1]Clinical HIV Research Unit, Department of Medicine, Faculty of Health Sciences, University of the Witwatersrand (York Avenue), Johannesburg (2193), South Africa
Full list of author information is available at the end of the article

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the epidemic escalated [4,5,8]. The incidence of KS has decreased in the US and Europe with the introduction of HAART [9-11] but the impact of HAART in Africa where the underlying prevalence of KSHV is higher is yet to be determined.

While clinical Kaposi sarcoma is known to be a marker of advanced HIV disease and is one of the WHO stage 4 and AIDS-defining illnesses [12], it is unclear if co-infection with oncogenic viruses such as KSHV places untreated HIV-infected patients at similar risk even without clinically apparent illness. Several clinical and laboratory markers have been associated with advanced disease stage among untreated HIV-infected individuals [13-21], including the T-lymphocyte subpopulations, CD4+ and CD8+ [14,18,22-26] which play an important role in the response to viral infections. While KSHV-specific CD8+ T cell epitope responses have been shown to increase after initiation of HAART [27,28], it has yet to be determined if T-lymphocyte subpopulations are also a marker of advanced disease stage in ART naive patients infected with KSHV (as seen among the HIV-infected population) and if this has implications for treatment initiation guidelines.

We enrolled subjects in a cohort study to determine the impact of KSHV on response to HAART as well as the effects of HAART on KSHV control. This cross-sectional analysis forms part of this larger study and aimed to measure the prevalence of KSHV infection among these HIV-infected adults initiating ART in a large treatment programme in Johannesburg, South Africa and to compare T-lymphocyte subpopulations and other demographic, clinical and laboratory factors between patients seropositive and seronegative for KSHV at enrolment.

Methods

Study design

This cross-sectional study utilized data from patients enrolled in care at the Themba Lethu Clinic in Johannesburg, South Africa. Currently, Themba Lethu has over 23,000 HIV infected adults enrolled in its comprehensive HIV care, management and treatment program. Since inception, over 16,000 of these patients have been initiated on ART at this clinic. Care at the clinic is provided according to the guidelines from the South African National Department of Health [29]. Patient data at Themba Lethu is captured and stored in an electronic patient record, TherapyEdge-HIV™. At enrolment into care, data on demographics, physical examination and clinical diagnoses are recorded. At initiation of ART, laboratory test results, including CD4 lymphocyte counts, full blood counts and liver function tests, are also recorded.

Eligibility Criteria

Between November 2008 and March 2009, all HIV-positive treatment naïve patients > 18 years of age who met the National guidelines criteria for initiation of ART (CD4 count < 200 cells/mm^3 or WHO stage 4 defining illness) and who were attending group counselling sessions at Themba Lethu were invited to participate in the study. Patients who did not meet these criteria or had a history of prior ART use were excluded from the study.

Study variables

Venous blood samples were drawn from all study participants prior to initiation of ART to determine KSHV serostatus. Seropositivity to KSHV was defined as a positive reaction to either lytic KSHV K8.1 or latent KSHV Orf73 antibodies detected using enzyme-linked immunosorbent assays (ELISA). Detection of antibodies to a single antigen has been shown to potentially underestimate the prevalence of KSHV, therefore, the ELISA to detect antibodies to latency associated nuclear antigen was performed in addition to the lytic K8.1 ELISA to provide a more accurate assessment of KSHV antibody status [30]. KSHV viremia was determined using 150 ng of DNA extracted from buffy coat using the QIAamp DNA Blood Midi kit, according to the manufacturer's instructions. Quantitative TaqMan PCR as per previously published methodology [31] was performed on the ABI Prism 7900 sequence detection system (Applied Biosystems, Forster City, CA). Subject and control samples were run in triplicate. The KSHV viral load assay has a linear dynamic range of 8 logs and is calibrated to detect a single copy of viral DNA in 150 ng genomic DNA. CD3, CD4 and CD8 lymphocyte counts (components of the total lymphocyte count) were performed using flow cytometry and standard methodology.

Additional data was extracted from the electronic patient record. Demographic variables of interest included gender, age at study enrolment, race, as well as ethnicity (using mother and father tongue as a proxy). Clinical data on initiating ART regimen, WHO clinical stage, body mass index (BMI), tuberculosis status and HIV RNA level at enrolment were also extracted as well as laboratory results for full blood counts and liver function tests.

Statistical analysis

The prevalence of KSHV among the study group was estimated and is presented with corresponding 95% confidence bounds. Demographic, clinical and laboratory characteristics of the participants at study enrolment were stratified by KSHV status and summarized as simple proportions or medians with interquartile ranges (IQR). Logarithmic transformations of optical densities for lytic K8.1 and latent Orf73 were performed and are presented as geometric means. Crude prevalence ratios for participant presenting features were estimated using log-binomial regression models stratified by KSHV

serostatus. This analysis was also further stratified by reaction to lytic K8.1 or latent Orf73 antigen. Models were adjusted for age, sex and presenting CD4 count where appropriate.

Approval to conduct this study and use of data from the Themba Lethu site was granted by the Human Research Ethics Committee of the University of the Witwatersrand.

Results

All eligible subjects initiating ART at Themba Lethu Clinic between November 2008 and March 2009 were invited to participate and we enrolled 404 of these. Response rates were high: it is estimated that fewer than 5% of those invited to participate refused. However, in order to determine if the sample was representative of the untreated population accessing HIV care at that time, we compared this sample to the general population presenting for treatment initiation at Themba Lethu during the recruitment period that were either not invited or refused to participate in the study. The presenting features of the study group were very similar to the Themba Lethu Clinic general population at ART initiation (Table 1) in terms of age, CD4 cell count, HIV viral load, proportion with WHO stage 3 or 4 defining illness, hemoglobin level and BMI. The Themba Lethu Clinic general population had a slightly lower proportion of females (61% vs. 65%) and slightly lower proportion presenting with tuberculosis (11% vs. 14%) compared to the study group.

The median age of the study group was 38 years (IQR 32-45 years) and 262 (65%) were women. The median CD4 count at ART initiation was 87 cells/μL (IQR 40-149 cells/μL) and nearly 40% presented with a WHO stage III/IV defining condition. The majority of participants were started on standard public-sector first-line ART regimens: 86% on stavudine, lamivudine and efavirenz and 7% on stavudine, lamivudine and nevirapine. The remaining 7% who presented with a contra-indication to one of the standard first-line regimens were initiated on zidovudine, lopinavir/ritonavir or tenofovir-based regimens.

Prevalence of KSHV

Among the study participants, 193/404 tested positive to lytic KSHV K8.1 and/or latent KSHV Orf73 antibodies at ART initiation; the prevalence of KSHV in this urban population was estimated at 48% (95%CI: 43-53%). Of those positive for KSHV, 76 (39%; 95% CI 33-46%) were reactive to lytic K8.1 alone, 35 (18%; 95% CI13-24%) to latent Orf73 and 82 (42%; 95%CI 36-50%) to both. Only one individual presented with clinical KS at ART initiation. This individual was positive to both lytic and latent KSHV antigen but did not have a detectable KSHV viral load.

KSHV viral load

Of the 193 individuals serologically positive to KSHV, 167 (87%) had samples available for KSHV viral load testing. KSHV DNA was detected in 19 (11%) of the buffy coat samples of those serologically positive to

Table 1 Presenting features of 404 ART naïve adults in care at Themba Lethu in Johannesburg, South Africa stratified by KSHV[&] status

Characteristics[#]		* General Clinic Population (n = 679)	KSHV+[&](n = 193)	KSHV-[&](n = 211)
Female		416 (61%)	127 (66%)	135 (64%)
Age (yrs.)	Median (IQR)	37 (31-43)	37 (32-47)	38 (32-45)
WHO Stage	I/II	408 (64%)	116 (64%)	114 (61%)
(n, %)	III/IV	231 (36%)	64 (36%)	74 (39%)
BMI	< 18.5	118 (23%)	38 (21%)	57 (29%)
(n, %)	18.5-24.9	286 (56%)	98 (56%)	94 (48%)
	25-30	72 (14%)	23 (13%)	31 (16%)
	> 30	37 (7%)	15 (8%)	14 (7%)
Hemoglobin (n, %)	≤ 8.0 g/dL	41 (7%)	14 (7%)	13 (6%)
CD4 count (cells/mm^3)	Median (IQR)	100 (39-173)	101 (46-168)	89 (34-164)
CD4 cell count category	0-50	167 (31%)	47 (28%)	63 (34%)
(n, %)	51-100	106 (19%)	39 (23%)	39 (21%)
	101-200	182 (33%)	65 (38%)	56 (30%)
	200-350	89 (16%)	20 (12%)	30 (16%)
Tuberculosis (n, %)	Yes	75 (11%)	26 (14%)	30 (15%)
HIV RNA	Median (IQR)	20000 (8300-39000)	15500 (6400-33500)	19500 (7300-39000)

[#] Characteristics at eligibility for initiation of antiretroviral therapy

* Themba Lethu clinic general population refers to treatment naïve adults eligible for initiation of antiretroviral therapy during the study period who were not recruited into the study

[&]KSHV = Kaposi sarcoma herpes virus

KSHV with a median of 10.6 (IQR 6.7-37.5) copies/uL and values ranging from 1.91 - 109.8 copies/uL. A higher proportion of those positive to both lytic and latent KSHV antibodies had detectable KSHV viral loads (20%) compared to those positive to only lytic (3%) or only latent (8%). The estimates suggested that those with a detectable KSHV viral load were more likely to be male (PR = 1.43; 95%CI 0.61-3.35), have a WHO stage III/IV illnesses (PR = 1.51; 95%CI 0.65-3.50) and hemoglobin ≤ 8 g/dL (PR = 1.42; 95%CI 0.37-5.43) compared to those without a detectable KSHV viral load, although these results lacked precision (as indicated by the wide confidence intervals).

Associations with overall KSHV seropositivity

The KSHV infected group was similar to those without KSHV group in terms of age, race, gender and ethnicity (mother and father tongue). KSHV positive individuals presented with slightly higher median CD3 (817 vs. 726 cells/mm^3) and CD4 (90 vs. 80 cells/mm^3) than KSHV negative subjects (Figure 1). In log-binomial regression models, adjusted prevalence ratios of KSHV seropositivity was increased for those with CD3 and CD8 counts ≥ 500 cells compared to those < 500 cells and also CD4 counts between 51 and 200 cells compared to CD4 counts ≤ 50, (Table 2) although some of these estimates lacked precision (i.e. our confidence intervals are wide). Those with a BMI > 18.5 kg/m^2 were also more likely to be infected with KSHV while those with Zulu as mother tongue were somewhat less likely to be KSHV positive. We found no association between KSHV seropositivity and several other factors including gender, age, WHO stage and tuberculosis status. Estimates were adjusted for gender, age and baseline CD4 count, where appropriate.

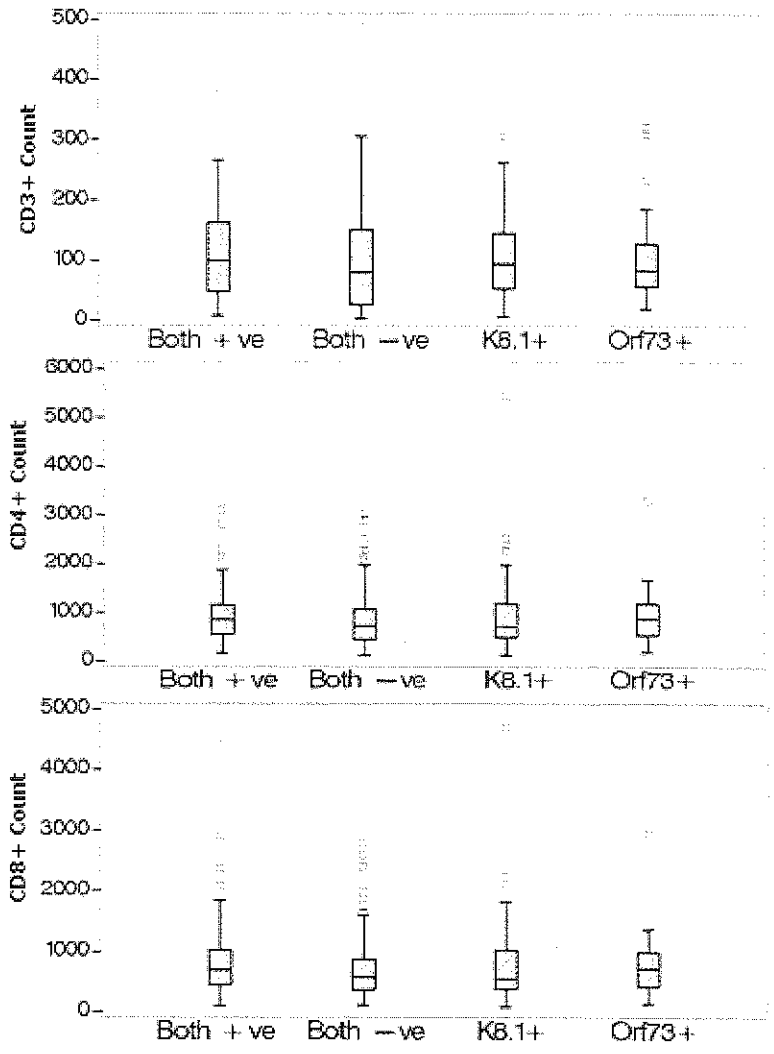


Figure 1 Box Plots of CD3+ (top), CD4+ (middle) and CD8+ (bottom) lymphocyte counts by KSHV serostatus.

Table 2 Associations with lytic/latent KSHV# seropositivity

	Overall KSHV# positive€		Positive to Lytic K8.1 only¥		Positive to Latent Orf 73 only$^{\beta}$	
	Total Positive (n, %)	Adjusted PR# (95%CI)	Total Positive (n, %)	Adjusted PR# (95%CI)	Total Positive (n, %)	Adjusted PR# (95%CI)
Gender						
Female	127 (66%)	1	104 (66%)	1	73 (62%)	1
Male	66 (34%)	0.96 (0.77-1.19)	54 (34%)	0.98 (0.75-1.26)	44 (38%)	1.16 (0.84-1.60)
Age category						
< 40 yrs.	118 (61%)	1	94 (59%)	1	69 (60%)	1
≥ 40 yrs.	75 (39%)	1.06 (0.86-1.31)	64 (41%)	1.00 (0.78-1.29)	48 (41%)	0.99 (0.73-1.35)
Language						
Zulu	43 (32%)	1	35 (33%)	1	29 (35%)	1
Other	90 (68%)	1.21 (0.93-1.58)	71 (67%)	1.17 (0.84-1.62)	54 (65%)	1.09 (0.75-1.59)
Has tuberculosis						
No	160 (86%)	1	131 (87%)	1	97 (87%)	1
Yes	25 (14%)	1.06 (0.78-1.42)	20 (13%)	1.08 (0.74-1.57)	15 (13%)	1.06 (0.67-1.67)
BMI category						
< 18.5	36 (21%)	1	28 (20%)	1	20 (19%)	1
≥ 18.5	137 79%)	1.28 (0.97-1.70)	114 (80%)	1.33 (0.94-1.90)	85 (81%)	1.46 (0.94-2.26)
Hemoglobin						
≥ 8.5 g/dL	166 (89%)	1	136 (89%)	1	96 (85%)	1
< 8.5 g/dL	21 (11%)	1.15 (0.84-1.56)	17 (11%)	1.17 (0.80-1.72)	17 (15%)	1.64 (1.11-2.43)
CD4 count						
0-50 cells	47 (28%)	1	39 (28%)	1	24 (23%)	1
51-100 cells	39 (23%)	1.18 (0.87-1.62)	29 (21%)	1.03 (0.68-1.55)	28 (27%)	1.61 (0.97-2.66)
101-200 cells	65 (38%)	1.28 (0.97-1.68)	53 (38%)	1.16 (0.75-1.81)	37 (36%)	1.29 (0.72-2.30)
201-350 cells	19 (11%)	0.98 (0.65-1.47)	17 (12%)	0.96 (0.52-1.76)	15 (14%)	1.27 (0.59-2.90)
CD3 count						
< 500 cells	41 (21%)	1	34 (22%)	1	22 (19%)	1
≥ 500 cells	152 (79%)	1.35 (1.04-1.76)	124 (78%)	1.31 (0.93-1.84)	95 (81%)	1.62 (1.04-2.54)
CD8 count						
< 500 cells	64 (33%)	1	54 (34%)	1	35 (30%)	1
≥ 500 cells	129 (67%)	1.20 (0.96-1.50)	104 (66%)	1.09 (0.81-1.47)	82 (70%)	1.41 (0.96-2.09)

KSHV = Kaposi sarcoma herpes virus, PR = prevalence ratio estimated from log-binomial regression model. All models adjusted for sex, age and baseline CD4 count

€ KSHV negative individuals used as the comparison group

¥ Those who did not react to lytic K8.1 including the KSHV negative individuals used as the comparison group

$^{\beta}$ Those who did not react to latent Orf73 including the KSHV negative individuals used as the comparison group

Associations with lytic or latent antibody presence

The geometric mean of the optical density was 0.83 (95%CI 0.80-0.87) for lytic K8.1 and 0.34 (95%CI 0.32-0.37) for latent Orf73. Presence of antibodies to latent Orf73 was associated with higher median CD3 (863 vs. 720 cells/mm^3; p = 0.019) and CD8 (706 vs. 569 cells/mm^3; p = 0.026) cell counts. Higher BMI (≥ 18.5 kg/m^2) was associated with reactivity to both lytic K8.1 [PR = 1.33 (0.94-1.90)] and latent Orf73 [PR = 1.46 (0.94-2.26)] in models adjusted for age, gender and CD4 cell count (Table 2), while higher CD3, CD4, CD8 counts as well as a low hemoglobin level were associated more strongly with reactivity to latent Orf73 than lytic K8.1.

Discussion

The prevalence of KSHV is reported to be high in Africa [32-34]. Those co-infected with KSHV and HIV are at high risk for Kaposi sarcoma, a condition associated with poor outcome in HIV-infected patients. This cross-sectional analysis estimated the prevalence of KSHV among adults initiating ART at a large urban clinic in Johannesburg and investigated factors associated with KSHV seropositivity and reactivity to either lytic or latent KSHV antigen. We demonstrated a high prevalence of KSHV in this population and found that few factors associated with advanced HIV disease stage and progression were also associated with KSHV seropositivity. In fact, reactivity to latent KSHV antigen was

associated with better markers of immunity in terms of higher CD3, CD4 and CD8 cell counts.

The high prevalence of KSHV (48%) demonstrated in this setting is comparable with findings from similar settings among HIV-infected populations [34,35]. In contrast, KSHV prevalence appears to be much lower in the developed world among the HIV uninfected but higher among pregnant women [34] and men who have sex with men [36]. These findings have fuelled theories of a sexual route of transmission for KSHV [37]. Such conflicting results are probably the result of varying routes of transmission of this virus and much geographic variability in prevalence rates [38]. A South African study showed rates of KSHV infection to vary from 35% to 49% across different municipalities within one province [34]. Among populations with a high prevalence of KSHV seropositivity, the route of infection is likely to be saliva and is acquired during childhood and early adult life [39,40]. Sexual and non-sexual transmission seems to occur in KSHV naïve adult populations such as men who have sex with men and sex-workers [36,37,41,42].

Advanced age, male gender, nutrition, anemia, concurrent tuberculosis and other opportunistic infections have been demonstrated to be associated with an advanced stage of HIV disease and mortality among untreated individuals [13,14,16,17,19-22,37]. Our finding of a positive association between anemia and KSHV seropositivity among the group reactive to latent Orf73 is in keeping with this. However, we found no evidence of an association with other poor prognostic features; in fact the KSHV positive group had higher BMI and CD3, 4 and 8 cell counts than their KSHV negative counterparts. Among the KSHV infected individuals, however, those with a detectable KSHV viral load presented with signs of more advanced HIV disease including anemia and WHO stage 3 or 4 defining conditions compared to those in whom the virus was undetectable. KSHV viremia has previously been associated with the likelihood of development of clinical Kaposi sarcoma and other signs of advanced HIV disease including thrombocytopenia and higher HIV viral loads [43]. KSHV DNA in plasma has also been shown to predict death among those with clinical Kaposi sarcoma [44]. Detectable KSHV viremia may indicate poor immune control of KSHV infection [43-45] which in turn may indicate more advanced HIV disease. Furthermore, KSHV viremia has been associated with increased risk of clinical Kaposi sarcoma among HIV infected subjects [45] and, therefore, HIV/KSHV co-infected subjects with detectable KSHV viral load may benefit from specific prophylactic strategies and increased monitoring for KSHV related diseases.

The optical density for lytic and latent KSHV was comparable to other work in similar settings with high HIV prevalence [34]. The optical density for lytic K8.1 KSHV antigen was higher than that for latent Orf73 and more individuals were reactive to the lytic antigen. This is in keeping with the theories that lytic antigen represents actively replicating virus, as would be seen in the case of advanced HIV disease, and poor control of the immune system in the untreated individual [28]. In addition to this, in our data, those positive to latent Orf73 antigen presented with markers of less immune suppression and less advanced disease. Those with higher BMI, higher CD3, 8 and 4 cell counts were more likely to be reactive to latent Orf73 than those who did not react to latent Orf73 (including the overall KSHV negative group). CD4 and 8 cells play important roles in cell-mediated immunity and control of viremia in the HIV-infected individual [46,47] and low absolute numbers of CD4 and 8 cells have been associated with an increased risk of disease progression and mortality among HIV-infected persons [18,22-25].

Co-infection with other viruses such as hepatitis, Epstein-Barr and human papilloma has been shown to increase the risk of HIV disease progression and mortality in immune suppressed HIV infected individuals [48-51]. There is plausible biological evidence that suggests KSHV could impact on disease progression through stimulation of HIV *tat* proteins and activation of HIV replication [52]. Despite this, the KSHV seropositive group in this study was associated with less immune suppression and better BMI, particularly among those seropositive to latent Orf73, an antigen expressed less often during active replication of the KSHV virus. One study among a cohort of long term non-progressors also found no effect of KSHV infection on persistence of long term non-progressor status [53].

Our findings must be considered in light of possible limitations of the study. Firstly, due to the cross sectional nature of the analysis, we cannot make inferences about causal relationships between KSHV and the factors under investigation. As KSHV transmission has been shown to occur even early in life [39,40], presumably by saliva, temporal relationships between KSHV infection and the factors considered are difficult to establish. However, information about possible associations between KSHV seropositivity and other known risk factors for HIV disease progression are useful in generating hypotheses about possible interactions between these viruses. Secondly, it is possible that the association we demonstrate between less suppression of the immune system and KSHV seropositivity is due, at least in part, to survival bias. If KSHV infection is, in fact, associated with more advanced immune suppression and subsequent faster HIV disease progression, one could expect those with KSHV to be at greater risk of mortality before being able to access HIV treatment and care. Thus, the KSHV population presenting for treatment may represent a particularly healthy group of KSHV-infected individuals who have survived to that point as suggested by higher BMI and T-lymphocyte subpopulations noted.

Additionally, despite this high prevalence of KSHV in an immunologically suppressed population, only one individual presented with clinical Kaposi sarcoma. As national guidelines change and ART is initiated at higher CD4 counts, we may see changes in prevalence and presenting features of those co-infected with KSHV. Studies focused on HIV-infected individuals who are not yet eligible for ART and ideally among a population who have recently HIV seroconverted may shed light on this point. Thirdly, in light of the geographic and population differences in prevalence and routes of transmission of KSHV, it may not be possible to generalize these results to other populations, such as those in the developed world.

Conclusions

Despite these limitations, this study offers some insight into, as yet, unanswered questions around the clinical effect of KSHV co-infection. We demonstrate a high prevalence of co-infection with KSHV among a group of treatment naïve, HIV-infected adults initiating ART at a large urban clinic in Johannesburg, South Africa. The study population was similar to the general population accessing care at Themba Lethu Clinic with respect to presenting features. The results may, therefore, be reasonably extrapolated to HIV positive individuals accessing care at urban public sector clinics in South Africa. Although there appeared to be some immunological differences in terms of CD3, CD4 and CD8 cell counts between the KSHV positive and negative groups, KSHV seropositivity was not associated with other known risk factors for disease progression such as age, gender and concurrent tuberculosis infection among the HIV-infected untreated population. If we assume that KSHV co-infection has little or no impact among the untreated population, it may follow that KSHV co-infection would have little effect on outcomes among treated individuals, unless KSHV has a large direct impact on the effectiveness of antiretrovirals. Future research efforts in this field should be focused on longitudinal studies to determine if KSHV seropositivity, particularly viremia, poses a risk for HIV treatment outcomes, development of KS immune reconstitution inflammatory syndrome and mortality in the presence of antiretroviral therapy.

Acknowledgements and Funding

We express our gratitude to the directors and staff of the Themba Lethu Clinic, in addition to Right to Care (RTC), the NGO supporting the database and study site through a partnership with USAID. We also thank the Gauteng and National Department of Health for providing for the care of the patients at the Themba Lethu Clinic as part of the Comprehensive Care Management and Treatment plan and Lynne Mcnamara for proofing of the manuscript. This work was supported by the United States Agency for International Development (USAID) under the terms of agreement 674-A-00-08-00007-00 with RTC. MF was also supported by Award Number K01AI083097 from the National Institute of Allergy and Infectious Diseases (NIAID). The content is solely the responsibility of the authors and does not necessarily represent the official views of the USAID, the Themba Lethu Clinic, Right to Care or National Institute of Allergy And Infectious Diseases. USAID and NIAID had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review or approval of the manuscript.

Author details

[1]Clinical HIV Research Unit, Department of Medicine, Faculty of Health Sciences, University of the Witwatersrand (York Avenue), Johannesburg (2193), South Africa. [2]Health Economics and Epidemiology Research Office, Department of Medicine, Faculty of Health Sciences, University of the Witwatersrand (York Avenue), Johannesburg (2193), South Africa. [3]Right to Care (Perth Road), Johannesburg (2192), South Africa. [4]Viral Oncology Section, AIDS and Cancer Virus Program, SAIC-Frederick, NCI-Frederick (Rosemont Avenue), Frederick MD (21702-1201), USA. [5]Division of International and Environmental Health, Institute of Social and Preventive Medicine (ISPM), University of Bern (Finkenhubelweg), Bern (3012), Switzerland. [6]Department of Molecular Medicine and Hematology, Faculty of Health Sciences, University of the Witwatersrand (York Avenue), Johannesburg (2193), South Africa. [7]Center for Global Health and Development, Boston University (Massachusetts Avenue), Boston (02118), USA. [8]Department of Epidemiology, Boston University School of Public Health (Massachusetts Avenue), Boston (02118), USA.

Authors' contributions

All authors contributed to the conception and design of the study. MM, PM, DW and CW contributed to acquisition of data. MM performed the statistical analysis. MM, MF, DW and CW interpreted the results and MM, MF, PM and ME drafted the manuscript. All authors revised the manuscript critically for intellectual content and have approved the submitted version.

Competing interests

The authors declare that they have no competing interests.

Received: 3 August 2011 Accepted: 17 November 2011
Published: 17 November 2011

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doi:10.1186/1750-9378-6-22
Cite this article as: Maskew *et al.*: Prevalence and predictors of kaposi sarcoma herpes virus seropositivity: a cross-sectional analysis of HIV-infected adults initiating ART in Johannesburg, South Africa. *Infectious Agents and Cancer* 2011 6:22.

PAPER 2:

Kaposi sarcoma-associated herpesvirus and response to antiretroviral therapy: A prospective study of HIV-infected adults.

Kaposi Sarcoma-Associated Herpes Virus and Response to Antiretroviral Therapy: A Prospective Study of HIV-Infected Adults

Mhairi Maskew, MBBCh, MSc (Med), A. Patrick MacPhail, PhD, FRCP,† Denise Whitby, PhD,‡ Matthias Egger, MD, MSc, MFPHM, DTM & H,§ and Matthew P. Fox, DSc, MPH*||¶*

Background: The possible impact of coinfection with the Kaposi sarcoma–associated herpes virus (KSHV) on the response to antiretroviral therapy (ART) is unknown. Prospective studies are rare, particularly in Africa.

Methods: We enrolled a prospective cohort of HIV-infected adults initiating ART in Johannesburg, South Africa. The subjects were defined as seropositive to KSHV if they were reactive to either KSHV lytic K8.1 or latent Orf73 antigen or to both. The subjects were followed from ART initiation until 18 months of treatment. HIV viral load and CD4 counts were tested 6 monthly. Linear generalized estimating and log-binomial regression models were used to estimate the effect of KSHV infection on immunologic recovery and response and HIV viral load suppression within 18 months after ART initiation.

Results: Three hundred eighty-five subjects initiating ART from November 2008 to March 2009 were considered to be eligible including 184 (48%) KSHV+. The KSHV+ group was similar to the KSHV− in terms of age, gender, initiating CD4 count, body mass index, tuberculosis, and hemoglobin levels. The KSHV+ group gained a similar number of cells at 6 [difference of 10 cells per cubic millimeter, 95% confidence interval (CI): −11 to 31], 12 (3 cells per cubic millimeter, 95% CI: −19 to 25), and 18 months (24 cells per cubic millimeter, 95% CI: −13 to 61) compared with that gained by the KSHV− group. Adjusted relative risk of failure to suppress viral load to <400 copies per milliliter (1.03; 95% CI: 0.90 to 1.17) were similar for KSHV+ and KSHV− by 6 months on treatment.

Conclusions: In a population with a high KSHV prevalence, HIV-positive adults coinfected with KSHV achieved similar immunologic and virologic responses to ART early after treatment initiation compared with those with KSHV−.

Key Words: Kaposi sarcoma herpes virus, antiretroviral therapy, resource-poor setting, virologic suppression

(*J Acquir Immune Defic Syndr* 2013;63:442–448)

Received for publication January 4, 2013; accepted April 9, 2013.

From the *Health Economics and Epidemiology Research Office, Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; †Clinical HIV Research Unit, Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; ‡Viral Oncology Section, AIDS and Cancer Virus Program, SAIC-Frederick, Frederick National Laboratory for Cancer Research, Frederick MD; §Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern, Switzerland; ||Center for Global Health and Development, Boston, MA; and ¶Department of Epidemiology, Boston University School of Public Health, Boston, MA.

Supported by the National Institute of Allergy and Infectious Diseases (NIAID), Grant 5U01-AI069924-05 to the International epidemiological Databases to Evaluate AIDS in Southern Africa (IeDEA-SA). This study was also made possible by the generous support of the American people through Cooperative Agreement AID 674-A-12-00,029 from the United States Agency for International Development (USAID). M.M., M.F., M.E., and D.W. also receive funding from the NIH.

The authors have no conflicts of interest to disclose.

M.M. designed the study, collected data, performed the statistical analysis, and drafted the article. P.M. assisted with study design, data collection, critical review, and editing of the article. D.W. assisted with data collection, laboratory methodology, critical review, and editing of the article. M.E. assisted with statistical analysis, critical review, and editing of the article. M.F. was involved with study design, statistical analysis, critical review, and editing of the article.

The contents are the responsibility of the authors and do not necessarily reflect the views of the USAID or of the United States Government.

Correspondence to: Dr. Mhairi Maskew, MBBCh, MSc (Med), Health Economics and Epidemiology Research Office, Themba Lethu Clinic, Helen Joseph Hospital, Perth Road, Westdene, Johannesburg 2192, South Africa (e-mail: mmaskew@heroza.org).

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INTRODUCTION

The prevalence of Kaposi sarcoma herpes virus (KSHV) in sub-Saharan Africa is among the highest in the world,[1–3] and the region also bears the greatest burden of disease due to HIV.[4] Infection with KSHV has been shown to lead to the development of Kaposi sarcoma (KS)[5–7] and to multicentric Castleman disease[8] and primary effusion lymphomas.[9] KS, which is associated with significant morbidity and mortality, has now become one of the most common cancers in parts of sub-Saharan Africa and is the most common tumor in HIV-infected individuals.[10] Coinfection with other viruses including cytomegalovirus, hepatitis B, and hepatitis C has been previously associated with HIV disease progression and mortality[11,12] and poor CD4 cell count responses after initiation of antiretroviral therapy (ART).[12,13]

KSHV typically establishes a persistent latent infection in its host during which time only latent genes [of which open reading frame (Orf) 73 is 1 example] are expressed. In the presence of HIV-1 coinfection, however, immune suppression and cytokine release promote the reactivation of KSHV lytic genes, which include K8.1[14] and active replication and increase in KSHV viral progeny occurs. Previous in vitro

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studies have suggested interactions between these 2 viruses including an increase in HIV-1 viral load in the presence of KSHV[15] and induced reactivation of HIV-1 replication in chronically infected cells.[16] Despite this, there are few analyses describing the effect of coinfection with KSHV on HIV treatment outcomes after the initiation of ART.

We examined the effects of KSHV seropositivity on immunologic and virologic outcomes in the first year of ART among a cohort of HIV-infected adults attending a large, urban HIV care and treatment program in Johannesburg, South Africa.

METHODS

Study Design and Site

This prospective cohort study was conducted at the Themba Lethu Clinic (TLC) in Johannesburg, South Africa. Currently, TLC is one of the largest treatment facilities in South Africa, with >30,000 HIV-infected adults ever enrolled in its comprehensive HIV care, management, and treatment program.[17] Since the National rollout of ART in 2004, >23,000 individuals have been initiated on ART at the clinic according to the guidelines from the South African National Department of Health.[18,19] Patient data at TLC are captured and stored on an electronic patient record. Patient laboratory blood tests are taken at ART initiation and monitoring laboratory tests (viral load, CD4 count, full blood count, and liver and kidney function tests) are conducted at 6 months, then yearly thereafter. Up to 3 attempts are made by clinic counselors to contact patients who do not return for scheduled clinic appointments. Information on deaths is recorded through passive surveillance and through linkage with the National Vital Registration System,[20] which was last conducted in September 2011. The program participates in the International epidemiological Databases to Evaluate AIDS in Southern Africa.[21]

Eligibility Criteria

HIV-positive treatment naive patients, >18 years of age, who were assessed as ready and eligible for the initiation of ART at Themba Lethu clinic were invited to participate in the study. Participant enrollment was conducted between November 2008 and March 2009. The population was recruited from the TLC ART initiation groups. All eligible subjects attending a group counseling session before the initiation of ART were approached and invited to participate in the study on consecutive Wednesdays at TLC until the study sample size was enrolled. Patients with a history of ART use or who were unwilling to consent were excluded from the study. All the enrolled subjects provided informed consent before commencing the study procedures.

Laboratory Analysis

Laboratory testing for KSHV serology was conducted by the Contract Laboratory Services and National Health Laboratory Service, whereas KSHV viral load testing was performed by the Hematology and Molecular Medicine Department of the University of the Witwatersrand. Enzyme-linked immunosorbent assays that detect antibodies to lytic K8.1 and latent Orf73 KSHV recombinant protein antigens were developed in the Viral Oncology Section, ACVP, FNLCR, USA, and transferred to the Contract Laboratory Services. The enzyme-linked immunosorbent assays have good sensitivity and specificity and have been used in >30 studies internationally.[22] All samples that tested serologically positive to KSHV were then tested for KSHV viral load using quantitative TaqMan polymerase chain reaction[23] performed on the ABI Prism 7900 sequence detection system (Applied Biosystems, Forster City, CA). Subject and control samples were run in triplicate. The KSHV viral load assay has a linear dynamic range of 8 logs and is calibrated to detect a single copy of viral DNA in 150 ng of genomic DNA.

Study Variables

KSHV status at ART initiation was the exposure variable in this analysis. KSHV status was determined by venous blood samples drawn from all the study participants before the initiation of ART. Additional demographic and clinical data were extracted from the electronic patient record. Seropositivity to KSHV at the time of ART initiation was defined as a positive reaction to either lytic KSHV K8.1 or latent Orf73 antigen. The KSHV+ group was then further stratified by the presence or absence of detectable KSHV DNA. We further stratified a positive KSHV result into 3 categories: (1) positive to lytic k8.1 alone, (2) positive to latent Orf73 alone, or (3) positive to both.

We compared immunologic and virologic outcomes after ART initiation by KSHV status at treatment initiation. Outcome variables were (1) linear increase in the mean CD4 cell count after ART initiation and (2) virologic response (suppression of HIV viral load to ≤400 copies/mL) after 6 and 12 months on ART.

Statistical Analysis

Demographic and clinical features of the study participants at ART initiation were stratified by KSHV status and summarized as simple proportions or medians with interquartile ranges (IQRs). The association of KSHV with a linear increase in CD4 counts from baseline to 6, 12, and 18 months was estimated using mixed linear models. We also estimated estimate CD4 trajectories by modeling the CD4 count over time using a linear regression model with CD4 cell counts after ART initiation as the outcome variable and time estimated in the models as a quadratic function using random slopes and a random intercept with an unstructured correlation matrix for repeated measures. CD4 count trajectory models for those KSHV+ and KSHV− were fit separately to allow for different curves by exposure group. Log-binomial regression was used to estimate the relative risk (RR) of KSHV on viral load suppression (<400 vs. ≥400) by 6 and 12 months on treatment, respectively. Age, gender, and baseline CD4 count were considered a priori confounders and were adjusted for in all models. Other covariates including CD3 count, CD8 count, hemoglobin level, tuberculosis

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treatment status, body mass index (BMI), and initiating treatment regimen were investigated for potential confounding using change-in-estimate criterion. A covariate was considered to be a confounder if the RR varied by ≥10% when the covariate was added to (or removed from) the model. The combined effect of potential confounders was assessed in the same way and was identified by the change in estimate of the RRs after adjustment for all the potential confounding factors.

Approval to conduct this study and to use data from the TLC site was granted by the Human Research Ethics Committee of the University of the Witwatersrand.

RESULTS

The original cohort enrolled 404 consenting adults presenting for the initiation of ART at TLC between November 2008 and March 2009. The baseline characteristics of the original cohort have been described elsewhere.[24] This analysis is restricted to the 385 (95%) participants who initiated ART. The presenting features of this group at ART initiation are summarized in Table 1. The median age of the group was 38 years (IQR 32–45 years), the majority (n = 250, 65%) were women, and none had evidence of KS. The median CD4 count at ART initiation was 87 (40–149 cells per cubic millimeter) and over a third (37%) presented with a World Health Organization (WHO) stage III/IV defining condition. The majority of participants were started on standard first-line ART regimens; 86% on stavudine (d4T), lamivudine (3TC), and efavirenz and 7% on d4T, 3TC, and nevirapine. The remaining 7% were initiated on tenofovir-, zidovudine-, or lopinavir-based regimens. Alternative first-line regimens are used in situations where a preexisting condition such as peripheral neuropathy, hepatic pathology, or a planned pregnancy at ART initiation precludes the use of 1 of the standard regimens.[19]

Participant Retention

The participants contributed a total of 5599.5 person months of follow-up and the mean follow-up time between the groups was similar: 13.9 months [95% confidence interval (CI): 12.9 to 14.8] for the KSHV− group compared with 15.2 months (95% CI: 14.4 to 16.1) for the KSHV+ group. Outcomes at the end of 18 months of follow-up among the KSHV+ group were similar in terms of death (7% vs. 9%), loss to follow-up (7% vs. 10%) and transfer to care at another facility (6% vs. 8%) when compared with that of the KSHV− group (Table 1).

Prevalence of KSHV

Among the study participants, 184/385 tested positive to KSHV with an overall prevalence of KSHV estimated at

TABLE 1. Baseline Characteristics of 385 Adults Initiating ART in Johannesburg, South Africa, Stratified by KSHV Status

Characteristics*		Overall (n = 385)	KSHV+ (n = 184)	KSHV− (n = 201)
Sex	Female	250 (65%)	121 (66%)	129 (64%)
	Male	135 (35%)	63 (34%)	72 (36%)
Age (yrs)	Median (IQR)	38 (32–45)	37 (32–45)	38 (32–44)
WHO stage	I/II	226 (59%)	114 (62%)	112 (56%)
(n, %)	III/IV	133 (35)	62 (34)	71 (35)
	Missing	26 (6)	8 (4)	18 (9)
BMI	<18.5	93 (24%)	36 (20%)	57 (28%)
(n, %)	18.5–24.9	194 (50)	100 (54)	94 (47)
	25–29.9	53 (14)	22 (12)	31 (15)
	>30	29 (8)	15 (8)	14 (7)
	Missing	16 (4)	11 (6)	5 (3)
CD4 count (cells/mm^3)	Median (IQR)	87 (40–149)	90 (52–148)	78 (26–151)
CD4 cell count category	0–50	121 (31%)	47 (28%)	64 (34%)
(n, %)	51–100	100 (26)	39 (23)	39 (21)
	101–200	122 (32)	65 (38)	56 (30)
	200–350	42 (11)	19 (11)	29 (15)
First-line ART regimen	d4T/3TC/efavirenz	332 (87%)	157 (85%)	175 (87%)
(n, %)	d4T/3TC/nevirapine	25 (6)	15 (8)	10 (6)
	Other	28 (7)	13 (7)	15 (7)
Tuberculosis (n, %)	Yes	95 (25)	46 (25)	49 (25)
HIV RNA	Median (IQR)	18,000 (7300–37,000)	15,500 (7600–33,000)	19,500 (7300–39,000)
Outcomes at 18 mos post ART initiation (n, %)	Alive in care	310 (77)	154 (80)	155 (73)
	Died	31 (8)	13 (7)	18 (9)
	LTFU†	35 (9)	14 (7)	21 (10)
	Transferred	29 (7)	12 (6)	17 (8)

*Characteristics at the initiation of ART.
†LTFU = lost to follow-up defined as missing a scheduled clinic visit by >90 days.

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48% (95% CI: 43% to 53%). Of these, 73 (39%; 95% CI: 33% to 46%) were reactive to lytic K8.1 alone, 34 (18%; 95% CI: 13% to 24%) to latent Orf73, and 77 (42%; 95% CI: 36% to 50%) to both. The groups were similar in terms of age, gender distribution, and HIV disease stage. The KSHV+ group presented with a somewhat higher median CD4 cell count (90 vs.78 cells per cubic millimeter) than their KSHV− counterparts. Among those participants with KSHV+ results, a convenience sample of 167 samples was also tested for KSHV viral load. Nineteen (11%) of these had a detectable viral load at the initiation of ART. Those reactive to both K8.1 and Orf73 were more likely to have a detectable KSHV viral load (n = 15, 20%) when compared with those reactive to lytic K8.1 (n = 2, 3%) or latent Orf73 (n = 2, 8%) alone.

CD4 Count Response

Both groups demonstrated good immune responses to treatment. The mean increase in CD4 count among the KSHV+ group by 6 months was 117 cells per cubic millimeter (95% CI: 102 to 132) compared with 107 cells per cubic millimeter (95% CI: 92 to 121) among the KSHV−. The KSHV+ group gained a similar number of cells at 6, 12 and 18 months compared with that in the KSHV− group (Table 2). The predicted CD4 trajectories from the start of ART were also similar for the groups (Fig. 1). The greatest increases occurred shortly after the treatment initiation for both groups, though the KSHV+ group retained consistently higher CD4 cell counts at all time points observed despite overall retention in care being slightly higher among those with KSHV at the end of follow-up (80% vs.73%). We also estimated differences in linear increase in CD4 count with log-transformed data to account for its nonnormal distribution. These results also showed little difference in increase in CD4 count comparing KSHV+ and KSHV− groups at 6, 12, or 18 months after ART initiation.

We then restricted the analysis to the KSHV+ group and considered the effect of a detectable KSHV viral load at ART initiation on subsequent CD4 response. Linear models adjusted for year of ART initiation, baseline WHO stage, hemoglobin, BMI, and tuberculosis status suggested little difference in the number of CD4 cells gained for those with an undetectable KSHV viral load at 6 (24.0 cells per cubic millimeter; 95% CI: −25.2 to 73.5), 12 (2.1 cells per cubic millimeter; 95% CI: −53.2 to 49.1), and 18 months on treatment (14.9 cells per cubic millimeter; 95% CI: −104.6 to 134.5) compared with those whose KSHV viral load was detectable.

HIV Viral Load Suppression

Achieving HIV virologic suppression on ART was common among both groups. By 6 months on treatment, only 4% of those with KSHV (5/143) had failed to suppress HIV viral load to <400 copies/mL while 10% (14/139) of those without KSHV had failed to achieve suppression. Among those who survived to a year on treatment, similar proportions achieving virologic suppression were noted 6% (6/109) vs. 8% (8/104). Estimates demonstrated similar virologic responses between the KSHV+ and KSHV− groups at both 6- (RR = 1.03; 95%CI:0.90-1.17) and 12-months (RR = 1.01; 95%CI:0.74-1.37) on treatment after adjustment for sex, age, CD4 count, co-infection with tuberculosis, hemoglobin and BMI (Table 3).

When we restricted the analysis to the KSHV+ group, those with antibodies to Orf73 only demonstrated the best virologic response of all the groups—100% (25/25) of this group achieved suppression of HIV viral load to <400 copies per milliliter by 6 and 12 months on treatment. All other groups achieved suppression rates >90% though, and there were no differences in the likelihood of achieving virologic suppression when we compared the KSHV− group with those reactive to lytic K8.1 (RR = 1.02; 95% CI: 0.83 to 1.25) or reactive to both antigens (RR = 1.02; 95% CI: 0.87 to 1.21) by 6 months on ART. The results were similar at 12 months of treatment.

DISCUSSION

The clinical effect of KSHV infection on immunologic and virologic outcomes in HIV-positive patients after ART initiation is unclear. In this prospective cohort study, we

TABLE 2. Crude and Adjusted Difference in Mean CD4 Count at 6, 12, and 18 months of Follow-Up From Baseline

		6 mos (n = 327)		12 mos (n = 291)		18 mos (n = 232)	
Variable		Crude (95% CI)	Adjusted* (95% CI)	Crude (95% CI)	Adjusted* (95% CI)	Crude (95% CI)	Adjusted* (95% CI)
KSHV	KSHV+ vs. KSHV−	10.0 (−10.7 to 30.7)	10.2 (−10.5 to 30.9)	2.7 (−19.8 to 25.1)	3.1 (−18.9 to 25.2)	13.8 (−22.1 to 49.8)	23.8 (−13.2 to 60.7)
Gender	Female vs. male	23.3 (1.7 to 44.9)	33.7 (11.7 to 55.7)	15.1 (−8.6 to 38.9)	26.9 (3.2 to 50.6)	33.8 (−3.9 to 71.6)	46.9 (6.9 to 86.9)
Baseline	100–199 vs. 200–350	7.3 (−29.3 to 44.0)	5.7 (−31.1 to 42.6)	0.7 (−38.9 to 40.4)	1.0 (−38.2 to 40.2)	−27.6 (−92.8 to 37.5)	−35.0 (−103.9 to 33.9)
CD4 count	50–99 vs. 200–350	14.0 (−23.9 to 51.9)	8.3 (−30.1 to 46.8)	17.2 (−23.7 to 58.0)	18.1 (−23.0 to 59.3)	−23.7 (−90.9 to 43.4)	−47.2 (−119.9 to 25.6)
(cells/ mm^3)	0–50 vs. 200–350	10.0 (−27.5 to 47.6)	3.2 (−41.3 to 34.9)	21.2 (−19.4 to 61.9)	9.4 (−31.4 to 50.2)	16.6 (−50.7 to 83.9)	−4.8 (−76.4 to 66.9)
Age (yrs)	<40 vs. ≥40	36.4 (15.5 to 57.2)	32.9 (11.5 to 54.2)	43.0 (20.6 to 65.4)	36.8 (13.9 to 59.7)	48.2 (12.1 to 84.3)	39.9 (2.0 to 77.8)

*Mean differences estimated with linear generalized estimating equations adjusted for baseline WHO stage, baseline CD8 count, baseline hemoglobin, baseline BMI, tuberculosis status at ART initiation, year of ART initiation.

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TABLE 3. Virologic Outcomes at 6 and 12 months on ART Stratified by KSHV Status

	6 mos (n = 327)			12 mos (n = 291)		
Exposure	Failure to Suppress Viral load*	Crude RR (95% CI)†	Adjusted RR‡ (95% CI)†	Failure to Suppress Viral load*	Crude RR (95% CI)†	Adjusted‡ RR (95% CI)†
KSHV−	14 (10%)	1	1	8 (8%)	1	1
KSHV+	5 (4%)	1.07 (1.00 to 1.14)	1.03 (0.90 to 1.17)	6 (6%)	1.02 (0.95 to 1.10)	1.01 (0.74 to 1.37)

*Failure to suppress viral load to <400 copies per milliliter.
†Relative risk from a log-binomial regression model.
‡Models adjusted for sex, age, CD4 count, CD8 count, TB treatment status, hemoglobin, and BMI at ART initiation.

followed a group of KSHV+ adults without clinical evidence of KS and a group of KSHV− HIV-infected adults for 18 months after ART initiation. We found that, overall, immunologic and virologic responses to the first year of ART were similar between those coinfected with KSHV compared with their KSHV− counterparts. In general, KSHV infection, did not seem to have a negative impact on CD4 cell count recovery during the first 18 months on ART, though the impact of detectable KSHV viral load on CD4 cell count reconstitution requires further investigation.

The clinical course of infection with KSHV in the presence of an intact immune system is typically indolent and asymptomatic. Like all herpes viruses, KSHV establishes a persistent latent infection in its host and the number of KSHV-infected cells is controlled by the intact immune system.[25] In the presence of HIV-1 coinfection, however, this course is altered. There is evidence that the immune suppression caused by HIV-1 and cytokine release promotes the reactivation of KSHV lytic genes[14] including K8.1. This increase in viral progeny eventually results in the destruction of the host cell and progression to the development of KS, and the often-aggressive course seen in HIV-1 positive individuals.[26,27]

Although the pathogenesis from KSHV infection to clinical disease is complex and includes expansion of latently infected cells, the transition between latency and lytic replication may play a role in the development of progression to clinical disease and possibly even transmission of the virus.[28,29] Although the detection of antibodies to lytic antigens is only a rough approximation of active lytic replication of KSHV (we also did not find a strong correlation between K8.1 antibody detection and detectable KSHV viral load in peripheral blood in this study), we did observe a poorer immunological response in terms of the number of CD4 cells gained within the first year on ART among those reactive to both lytic K8.1 and Orf73. Poor immunologic response to treatment and low CD4 cell counts have previously been associated with an increased risk of KS morbidity and mortality.[30–32]

The debate about the effect of KSHV on HIV-1 among those coinfected is ongoing. In vitro and in vivo studies suggested an interaction between these 2 viruses and an increase in HIV-1 viral load in the presence of KSHV.[15] Conversely, there is evidence that KSHV infection is associated with the inhibition of HIV infection of CD4 cells expressing CCR3 receptors, largely mediated through beta

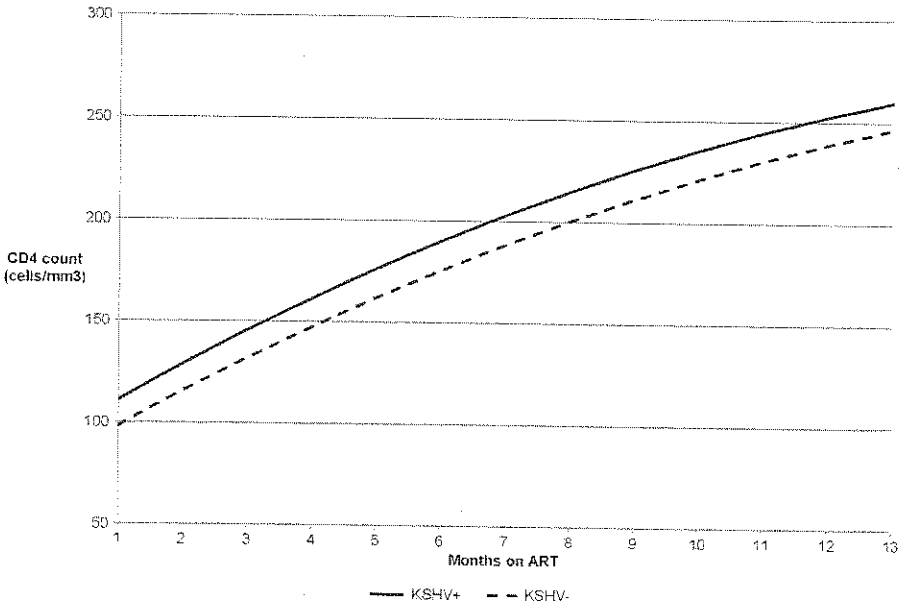


FIGURE 1. Predicted CD4 cell count increase from ART initiation stratified by KSHV status. Trajectories were estimated by using 2 separate mixed linear models, one for the KSHV+ and one for the KSHV. This allowed the curves to depart from being parallel. Curves were fit by using time as a quadratic function with random slopes and a random intercept with an unstructured correlation matrix for repeated measures.

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chemokines.[33,34] In this population, we observed little difference in the HIV viral load before ART initiation between the groups and a similar risk of failure to suppress the viral load at 6 and 12 months after ART initiation when comparing KSHV+ with KSHV−. Other in vitro work also suggested that KSHV increased HIV-1 replication in acutely infected cells and also induced the reactivation of HIV-1 replication in chronically infected cells.[16] Our results suggest that those with KSHV coinfection achieved comparable virologic and immunologic responses with highly active antiretroviral therapy when compared with those without KSHV. Although the numbers were small and the results were somewhat imprecise, we noted that when stratified by reactivity to K8.1 alone, Orf73 alone, or both antigens, all 3 groups gained a similar number of CD4 cells over the first 18 months of ART and were at a similar risk of failing to achieve a 50-cell increase and a 100-cell increase at 6 and 12 months, respectively. The linear models for those KSHV+ and KSHV− were fit separately, allowing for different curves by exposure group. Despite this, the curves remained parallel suggesting that the only difference between the groups were the differences in baseline CD4 count at treatment initiation, and these remained almost perfectly consistent over time. When we restricted the analysis to the KSHV-infected group only, there was a trend to an association between detectable KSHV viral load and poor reconstitution of CD4 cells after ART initiation when compared with those where the virus was undetectable at initiation of ART. We emphasize here that the numbers in this subanalysis were small, limiting our power to detect statistically significant differences. Broadly speaking, our results concur with previous work among a cohort of long term nonprogressor MSMs, which also concluded that there was no impact of KSHV on the progression of HIV-1 infection in terms of CD4 cell count decline, HIV-1 viral load increase or CD4 cell viramia.[35] The authors postulate that KSHV acts as an opportunistic agent rather than an HIV-cofactor among coinfected individuals. It is also possible that the effects are not apparent in the short term but may manifest later.

Our study has several strengths and limitations that should be considered when interpreting these findings. One important strength is that, unlike prior studies that have been conducted in the absence of any ART, our study investigates the clinical impact of the interaction between HIV and KSHV in the presence of ART. Early stage KS has been successfully treated with ART,[36] yet the exact mechanism through which ART reduces the tumor is unknown. ART has been shown to reduce KSHV viral loads to undetectable levels.[37] CD8+ lymphocytes are involved in the cellular immune response to several viruses including HIV-1 and herpes virus; these virus-specific cytotoxic T lymphocytes respond to both lytic and latent antigens of KSHV. Evidence of ART-induced immune reconstitution to KSHV (indicated by an undetectable KSHV viral load) was associated with an increase in CD8+ lymphocytes, suggesting restoration of these cells may be important in the control of KSHV infection[25] and ultimately control or even prevention of KS. All models in this analysis were adjusted for CD8 cell count at the initiation of ART to attempt to account for this. However, as the timing of this immune restoration to KSHV in relation to immune restoration to HIV-1 has not yet been established (estimates vary from as little as 12 weeks[37] to within 1[25] to 2years[38] of initiating ART) it is possible that residual confounding exists in our estimates.

We note that with observational studies, particularly cohort studies, the potential for bias due to the differences in retention and follow-up exists. Although we cannot exclude this possibly completely, we do note that the mean follow-up time was very similar for both KSHV+ and KSHV− groups. Additionally, the proportions lost to follow-up were similar between the groups and <10% in both groups.

Our findings are also strengthened by the relatively large sample size. Although previous work has been conducted predominantly among small cohorts of mostly European males, we investigate this relationship among a relatively large sample of predominantly black heterosexual adults in an urban South African setting with a high prevalence of HIV and KSHV coinfection.

However, although the sample is large compared with that in previous clinical work in this field, we note that the limited precision of some of our estimates might mean that small differences were not detected. In addition, our sample is limited to individuals attending care at 1 urban facility in Johannesburg, South Africa. This may mean our results have limited generalizability to other nonurban settings or populations of men who have sex with men. Results may be different in the developed world especially, considering the differences in ART regimens used in these settings and the geographical and population differences[1–3,6,39–41] in KSHV prevalence previously demonstrated.

CONCLUSIONS

We demonstrate good HIV treatment outcomes after ART initiation among a group of treatment naive, HIV-infected adults initiating ART at a large urban clinic in South Africa. In this population with a high KSHV prevalence, coinfection with KSHV does not seem to negatively impact the immunologic recovery or virologic response to ART in the first 18 months of treatment. The impact of detectable KSHV viral load on CD4 cell count recovery after the initiation of ART requires further investigation.

ACKNOWLEDGMENTS

The authors thank all the study participants, doctors, and nurses at the TLC.

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PAPER 3:

Kaposi sarcoma herpes virus and mortality following initiation with antiretroviral therapy: a prospective study among HIV infected adults in South Africa

Kaposi sarcoma herpes virus and attrition following initiation with antiretroviral therapy: a prospective study among HIV infected adults in South Africa

Authors: Mhairi Maskew[1], Kate Shearer[1], Patrick MacPhail[2], Matthew P. Fox[1,3,4]

Affiliations:

[1] Health Economics and Epidemiology Research Office, Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand

[2] Right to Care, Johannesburg

[3] Center for Global Health and Development, Boston, USA

[4] Department of Epidemiology, Boston University School of Public Health, Boston, USA

Corresponding Author Details:

Health Economics and Epidemiology Research Office

Themba Lethu Clinic, Helen Joseph Hospital

Perth Road, Westdene, Johannesburg, South Africa

Phone: +27-11-276-8850, Fax: +27-86-516-0628

E-mail: mmaskew@heroza.org

Word count: 3381

Abstract body: 510

Key words: Attrition from care; Kaposi sarcoma herpesvirus; HHV-8; ART; resource-poor setting;

Abstract

Background:

Mortality due to Kaposi sarcoma (KS) is high among those infected with HIV. Infection with Kaposi sarcoma herpes virus (KSHV) precedes development of KS but the effect of KSHV on attrition from care among those co-infected with HIV in the presence of antiretroviral therapy is unclear.

Methods:

This prospective cohort study enrolled HIV-infected treatment-naïve adults initiating ART at Themba Lethu Clinic, Johannesburg, South Africa. Subjects were screened for KSHV at initiation of ART. Seropositivity to KSHV was defined as reactive to either lytic KSHV K8.1 or latent KSHV Orf73 antigen or both. Subjects were followed from ART initiation until 18 months on treatment. In this analysis attrition from care was a combined outcome comprising both known deaths and those lost to follow up whose vital status was unknown. We report cumulative incidence of attrition from care and use Co proportional hazard regression models to estimate the effect of KSHV infection and presence of detectable KSHV viral load on attrition within 18-months after ART initiation.

Results:

After 18 months of follow up, 310/385 (77%) participants who initiated ART were still alive and in care. This included 154/184 (80%) of the KSHV positive group and 155/201 (73%) in the KSHV negative group. At 18 months after ART initiation, patients with KSHV were slightly less likely to be dead or lost to follow-up compared to patients without KSHV, both before (HR: 0.72; 95% CI: 0.44, 1.16) and after (aHR: 0.77 (0.44, 1.35) adjustment for other baseline covariates. The risk of attrition was modified by age and immune status. No difference was found between KSHV groups among older patients (aHR= 0.92; 95% CI 0.41-2.05), however KSHV positive patients less than 37.9 years of age were much less likely to die or become lost to follow up 18 months after ART initiation than KSHV negative patients in the same age group (11.7% vs. 22.7%; aHR= 0.50 95% CI 0.22-1.14). No difference in attrition was found by KSHV status for patients with CD4 counts greater than 100 cells/mm^3 (aHR= 1.02; 95% CI 0.27-3.89) but KSHV negative patients with CD4 counts of 100 cells/mm^3 or less were more likely to die or become lost to follow-up than KSHV positive patients with similar baseline CD4 counts (26.9% vs. 17.8%; aHR= 0.62 95% CI 0.32-1.18). The KSHV positive group with a detectable KSHV viral load experienced a two-fold increased risk of attrition compared to the KSHV negative participants by 18 months of follow up (aHR 2.06 95% CI 0.52-8.20) while the KSHV positives with undetectable KSHV viral loads experienced a similar risk to the KSHV negatives (aHR 0.94; 95% CI 0.40-2.22).

Conclusions:

In the setting of high prevalence of KSHV and HIV co-infection, we found those co-infected with KSHV less likely to experience attrition from care after initiation of ART. In particular those with lower CD4 counts and in younger age categories were at reduced risk compared to those with less immune suppression and at an older age respectively. Further prospective studies ideally from time of seroconversion to KSHV are required to determine the reasons behind these findings.

Background

Recent mortality data from the United Kingdom suggests that the average life expectancy of an HIV infected person on stable suppressive antiretroviral therapy (ART) with a CD4 count >350 cells is close to that of an HIV-uninfected person [May et al, 2012; Nakagawa et al, 2012]. A combination of increased overall survival and a decrease in mortality due to opportunistic infections has increased the time a treated HIV infected person is exposed to risk of developing cancer. In the setting of incomplete ART coverage, the incidence of AIDS-defining cancers, particularly Kaposi sarcoma, remains high [Bassett et al, 1995; Cook-Mozaffari et al, 1998; Parkin et al, 1999; Chokunonga et al, 2000; Stein et al, 2008]. Kaposi sarcoma was relatively common in South Africa (up to 5 per 1000 population at risk) prior to the AIDS epidemic [Cook-Mozaffari et al, 1998] but the incidence increased dramatically as the epidemic escalated [Parkin et al, 2008; Stein et al, 2008]. Estimated incidence rates as high as 20 per 1000 were reported in a case-control in South Africa between 1995 and 1999 [Sitas et al 2000], considerably higher than rates (0.005 per 1000) reported in developed nations [Grulich et al, 1992].

Kaposi sarcoma herpesvirus (KSHV) is the aetiologic virus causing KS. The pattern of KSHV prevalence worldwide appears to resemble that of KS. KSHV infection is very common in Africa with up to half of the general adult population infected [Ablashi et al, 1999; Klaskala et al, 2005; Adjei et al 2008, Maskew et al, 2011]. While antiretroviral combination therapy (ART) has been used to successfully treat early stage KS for some time [Bower et al, 1999; Krown 2004; Tirelli et al, 2001], it is as yet unclear what impact co-infection with KSHV might have on mortality among those co-infected with HIV in the presence of antiretroviral therapy, particularly in a setting of endemic KSHV infection. We assessed the effect of KSHV seropositivity on attrition from care in the first year after initiation on ART among a cohort of HIV-infected adults attending a large, urban HIV care and treatment program in Johannesburg, South Africa.

Methods

Study design

We conducted a prospective cohort study at the Themba Lethu Clinic (TLC) in Johannesburg, South Africa. Currently, TLC has enrolled over 30,000 HIV infected adults in its comprehensive HIV care, management and treatment program [Fox et al, 2012] and more than 23,000 of these have been initiated on ART since the National rollout of ART in 2004. HIV care and management at the site is

provided according to the guidelines from the South African National Department of Health [South African National Ministry of Health, 2004 and 2010]. Patient data at TLC is captured and stored on an electronic patient record, TherapyEdge-HIV™. Patient laboratory blood tests (except viral loads) are taken at ART initiation and monitoring labs tests (viral load, CD4 count, etc.) are conducted at 6 months, then yearly thereafter. Up to three attempts are made by clinic counsellors to contact patients who do not return for scheduled clinic appointments. Information on deaths is recorded through passive surveillance and through linkage with the National Vital Registration System [Fox et al, 2010] which was last conducted in September 2011.

Eligibility Criteria

Enrolment for this prospective cohort study occurred between November 2008 and March 2009 at Themba Lethu Clinic. Through convenience sampling of the adult ART treatment readiness group counselling sessions, we identified potential study participants. Eligible participants were HIV-positive treatment naïve adults > 18 years of age who were eligible for initiation of ART according to National guidelines (CD4 count <200 cells/mm3 or WHO stage 4 defining illness) after attending group ART treatment readiness counselling sessions at Themba Lethu Clinic. All enrolled subjects provided informed consent prior to commencing study procedures.

Laboratory Analysis

Samples from all consented study participants were analysed for reactivity to one or both of the KSHV antigens lytic K8.1 and latent open reading frame (ORF) 73. Enzyme linked immunosorbent assays (ELISA) were used to detect antibodies to lytic K8.1 and latent ORF 73. All samples that tested serologically positive to KSHV were then tested for KSHV viral load using quantitative TaqMan PCR [de Sanjosé et al, 2002] performed in triplicate on the ABI Prism 7900 sequence detection system (Applied Biosystems, Forster City, CA). The KSHV viral load assay has a linear dynamic range of 8 logs and is calibrated to detect a single copy of viral DNA in 150ng genomic DNA. Laboratory testing for KSHV serology and viral load was conducted by Contract Laboratory Services (CLS), National Health Laboratory Service (NHLS) and the Haematology and Molecular Medicine Department of the University of the Witwatersrand respectively.

Study variables

The primary exposure variable for this analysis was KSHV serostatus at ART initiation. KSHV serostatus was determined by venous blood samples drawn from all study participants prior to

initiation of ART. Additional demographic and clinical data was extracted from the electronic patient record. Seropositivity to KSHV at the time of ART initiation was defined as a positive reaction to either lytic KSHV K8.1 or latent Orf73 antigen. The KSHV positive group was then further stratified by the presence or absence of detectable KSHV DNA.

Our *primary outcome variable* was attrition from care after ART initiation. Study participants were considered to have experienced attrition if 1) they were confirmed dead during the course of study follow up or 2) they had not attended the clinic in the previous four months (lost to follow up). Mortality is ascertained through active tracing of patients who do not return to the clinic, and data for those lost is also verified with the South African National Vital Registration system for patients in whom a civil identification number was available (42% of those lost to care in Themba Lethu [20]). The effect of KSHV on attrition was estimated at three time points after ART initiation: 1) experienced attrition within 6 months of ART initiation, 2) experienced attrition within the first year after ART initiation and 3) experienced attrition within the first 18 months of follow up. We also considered whether stage of disease at ART initiation modified the effect of KSHV infection attrition after treatment initiation. We repeated the models described above stratified by CD4 cell count at treatment initiation (< 100 cells/mm^3 vs. ≥ 100 cells/mm^3).

Statistical analysis

Demographic and clinical features of the study participants at ART initiation were stratified by KSHV status and summarized as simple proportions or medians with interquartile ranges. We present cumulative incidence of attrition after treatment initiation by KSHV status. Cox proportional hazard regression was used to estimate the hazard of death and LTFU by KSHV serostatus. Potential confounding factors such as age, gender, baseline CD4 count, CD3 count, CD8 count, haemoglobin level, tuberculosis treatment status, body mass index (BMI) and initiating treatment regimen were investigated. Age, gender and baseline CD4 count were included in all models and other covariates that altered the relative risk by 10% or more were included where appropriate.

Approval to conduct this study and use of data from the TLC site was granted by the Human Research Ethics Committee of the University of the Witwatersrand.

Results

The original cohort enrolled 404 consenting adults presenting for initiation of ART at TLC between November 2008 and March 2009 [Maskew et al, 2011]. In total, 193/404 tested positive for KSHV with an overall prevalence of KSHV estimated at 48% (95%CI: 43-53%). Of these, 73 (39%; 95%CI: 33-46%) were reactive to lytic K8.1 alone, 34 (18%; 95%CI: 13-24%) to latent Orf73 and 77 (42%; 95%CI: 36-50%) to both. This analysis is restricted to the 383 (95%) participants who initiated ART. The presenting features of this group at ART initiation are summarized in Table 1. The median (IQR) age of the group was 37.9 years (31.8 – 44.7) and the majority (n=249, 65.0%) were women. On average, study participants presented with signs of advanced HIV disease. The median CD4 count at ART initiation was 87 (40-149 cells/mm^3) and approximately a third (34.7%) presented with a WHO stage III/IV defining condition. The majority of participants were started on standard first line ART regimens; 84.6% on stavudine, lamivudine and efavirenz and 6.8% on stavudine, lamivudine and nevirapine. The remaining 8.6% were initiated on tenofovir, zidovudine or lopinavir-based regimens. Alternative first line regimens are used in situations where a pre-existing condition such as peripheral neuropathy, hepatic pathology or a planned pregnancy at ART initiation precludes use of one of the standard regimens. While most of the baseline characteristics were comparable between patients with and without KSHV, KSHV negative patients were somewhat more likely to be severely immunocompromised at ART initiation with 37.7% of KSHV negative patients initiating ART with a CD4 count <50 cells/mm^3 compared to 24.5% of KSHV positive patients.

The study participants contributed a total of 466.6 person years of follow up and the mean follow up time between the groups was similar: 13.9 months (95%CI:12.9-14.8) for the KSHV negative group compared to 15.2 months (95%CI:14.4-16.1) for the KSHV positive group.

KSHV and attrition

After 18 months of follow up, 310/385 (77%) participants who initiated ART were still alive and in care. This included 154/184 (80%) of the KSHV positive group and 155/201 (73%) in the KSHV negative group. A further 29 participants (6%; n=12 from the KSHV positive group and 8%; n= 17 from the KSHV negative group) had transferred to another treatment facility. In this analysis attrition from care was a combined outcome comprising both known deaths (n=31, 8%) and those lost to follow up whose vital status was unknown (n=35; 9%).

At 18 months after ART initiation, patients with KSHV were slightly less likely to be dead or lost to follow-up compared to patients without KSHV (Figure 1), both before (HR: 0.72; 95% CI: 0.44, 1.16) and after (aHR: 0.77 (0.44, 1.35) adjustment for other baseline covariates. Little difference in

attrition was found between KSHV groups at 6 months after initiation (aHR: 0.91; 0.40, 2.10). However, at 12 months after ART initiation, KSHV positive patients were 43% less likely to die or become lost to follow-up compared to KSHV negative patients, irrespective of adjustment for other baseline covariates (aHR: 0.57; 95% CI: 0.29, 1.11). (Table 2)

Analysis of KSHV substrata

The effect of a detectable KSHV viral load appeared to increase risk of attrition. The KSHV positive group with a detectable KSHV viral load experienced a two-fold increased risk of attrition compared to the KSHV negative participants by 18 months of follow up (aHR 2.06 95% CI 0.52-8.20) while the KSHV positives with undetectable KSHV viral loads experienced a similar risk to the KSHV negatives (aHR 0.94; 95% CI 0.40-2.22) though the confidence intervals around these estimates were also wide.

Attrition and age at ART initiation

We noted effect measure modification on the relative scale of the effect of KSHV on attrition from care by both age (Figure 2). In order to determine if there was a difference in attrition at 18 months between KSHV groups stratified by age, we compared attrition among patients younger than 37.9 and those at least 37.9 years of age. No difference was found between KSHV groups among older patients (aHR= 0.92; 95% CI 0.41-2.05), however KSHV positive patients less than 37.9 years of age were much less likely to die or become lost to follow up 18 months after ART initiation than KSHV negative patients in the same age group (11.7% vs. 22.7%; aHR= 0.50 95% CI 0.22-1.14).

Attrition and baseline CD4 count

We conducted a similar stratified analysis to determine if there were differences in attrition between KSHV groups by CD4 count. We dichotomized baseline CD4 count at 100 cells/mm^3 and compared attrition at 18 months between KSHV groups for patients with CD4 counts less than or equal to 100 cells/mm^3 and for those with CD4 counts greater than 100 cells/mm^3. No difference in attrition was found by KSHV status for patients with CD4 counts greater than 100 cells/mm^3 (aHR= 1.02; 95% CI 0.27-3.89). However, KSHV negative patients with CD4 counts of 100 cells/mm^3 or less were more likely to die or become lost to follow-up than KSHV positive patients with similar baseline CD4 counts (26.9% vs. 17.8%; aHR= 0.62 95% CI 0.32-1.18) (Figure 3).

Discussion

Clinical disease with Kaposi sarcoma has been associated with significant risk of mortality among the HIV-infected population, particularly in settings where ART coverage is incomplete. The effect of co-infection with KSHV on risk of attrition from care after initiation of ART has not been described, despite the fact that the prevalence of KSHV is very high in Southern Africa. KSHV has been well-documented as the virus causing clinical disease with KS and there is plausible biological evidence that co-infection with HIV can act as a trigger for increased replication of KSHV and development of clinical disease with KS. Previous in vitro studies have suggested interactions between these two viruses including an increase in HIV-1 viral load in the presence of KSHV [Mercader et al, 2001] and induced reactivation of HIV-1 replication in chronically infected cells [Caselli et al, 2005]. Additionally, there is evidence that the immune suppression caused by HIV-1 as well as cytokine release promotes reactivation of KSHV lytic genes [Mercader et al, 2000] including K8.1. This increase in viral progeny eventually results in destruction of the host cell and progression to the development of Kaposi sarcoma, and the often aggressive course seen in HIV-1 positive individuals [Aoki et al, 2004; Lukac et al, 1999].

High rates of mortality due to KS are documented in African populations despite increased access to ART. The cumulative one-year survival of KS subjects attending care in Cape Town was 39% among untreated subjects and even among those receiving ART was as low as 60% [Chu et al, 2010]. The one-year survival in a treated Nigerian cohort of KS patients was slightly higher at 77% [Agaba et al, 2009].

In this study however, those with co-infected with KSHV appeared more likely to be alive and in care at the end of follow up compared to their KSHV negative counterparts. We also noted with interest that the protective effect of KSHV was restricted to those with low CD4 counts and in younger age categories. We postulate some explanations for the findings of this study. Firstly, it is possible that the lack of association demonstrated between KSHV seropositivity and suppression of the immune system and is due, at least in part, to survival bias. If KSHV infection is, in fact, associated with more advanced immune suppression and subsequent faster HIV disease progression, one could expect those with KSHV to be at greater risk of mortality before being able to access HIV treatment and care. Thus, the KSHV population presenting for treatment may represent a particularly healthy group of KSHV-infected individuals who have survived the excess risk associated with KSHV co-infection to the point of ART initiation. In support of this is the fact that despite a very high prevalence of KSHV in an immunologically suppressed population, only one individual (0.2%) presented with clinical

Kaposi sarcoma, much lower than estimates of 1-6-2.5% from the region [Agaba et al, 2009; Chu et al, 2010].

Alternately, this group of KSHV infected subjects may represent a relatively young population (Table 1) who have not had KSHV for long and ART was initiated early on in the course of infection before any negative outcomes could manifest. ART has been shown to be effective in treating "early" KSHV by reducing KSHV viral load [Aversa et al, 2005]; the fact that the protective effect of KSHV was restricted to those in younger age categories may be related to this. A previous study among South African HIV uninfected cancer patients has demonstrated that older age is associated with high anti-KSHV antibody titers [Wojcicki et al, 2003] which in turn are associated with risk of development and progression of KS [Sitas et al, 1999].

Second, there is evidence that KSHV infection can also be associated with inhibition of HIV infection of CD4 cells [Boshoff et al, 1997; Lusso 2000]. This inhibitory action is believed to be mediated through beta chemokines. Chemokines are small proteins secreted by cells that primarily act to attract and guide migration of cells. This ability is particularly useful during inflammation when the cells of the immune system are needed at the site of infection or during tissue repair. KSHV-encoded chemokines have been shown to prevent infection of CD4 cells expressing CCR3, a known entry co-receptor for HIV. Inability of HIV to infect CD4 cells would allow for preservation of the immune system despite the presence of actively replicating HIV. If this protective inhibition is not clinically apparent early on in HIV infection when CD4 cells are in abundance, but manifests only later as cell count drop to critical levels, it would offer some explanation (at least in part) as to why the protective effect of KSHV was noted only among those with CD4 counts <100 cells.

When we stratified the KSHV positive group by presence of a detectable KSHV viral load, those with KSHV viremia appeared more likely to die or become lost from care within the first 18 months of ART compared to their KSHV negative participants while the KSHV positives with undetectable KSHV viral loads experienced a similar risk to the KSHV negatives. The small numbers studied and resultant imprecise nature of these estimates makes it difficult to draw solid conclusions from our data alone but KSHV viremia has previously been associated with the likelihood of development of clinical Kaposi sarcoma and other signs of advanced HIV disease including thrombocytopenia and higher HIV viral loads [Minami et al, 2009; Campbell et al, 2000]. The association between high KSHV viral loads and KS disease progression has resulted in suggestions to use KSHV viral load testing in patients with KS to assess further disease progression and to guide initiation of antiherpes treatments such as ganciclovir [Laney et al, 2007]. KSHV DNA in plasma has also been shown to predict death among those with clinical Kaposi sarcoma [El Amari, 2004]. It is hypothesized that

detectable KSHV viremia may indicate poor immune control of KSHV infection which in turn may indicate more advanced HIV disease.

Or study has several strengths and weaknesses. First, in addition to the limitations outlined above, we note that observational cohorts can be prone to bias, particularly that due to loss to follow up. While clinical outcomes from HIV treatment programmes in Southern Africa have been encouraging [Egger et al, 2002; Lawn et al 2005; Ivers et al 2005] and comparable to those achieved in European programmes [Keiser et al, 2008], high rates of loss to follow up reported in African programmes (up to 40%) are of concern [Rosen et al, 2007] and may negatively impact on the ability to accurately study mortality and other overall programme treatment goals. High rates of lost to follow up were observed in the cohort studied and that may have introduced selection bias to the results, though rates of loss to follow up between the groups were similar. Nevertheless, loss to follow up may have led to underestimation of the mortality rates due to different rates between patients lost with and without KSHV. As previously noted, anywhere from 20-50% of those lost from HIV care are actually deceased [Fox et al, 2010, Maskew et al 2007, Brinkhof et al, 2009; Geng et al, 2008; Rosen et al, 2010]. Second, this cohort represents a sample of adults initiating care at one urban HIV treatment facility and thus our findings may have limited generalizability. In particular, results might be quite different in populations in the developed world where the prevalence of KSHV appears much lower and the route of transmission of KSHV is different. Despite these limitations, our study is one of very few prospective studies estimating the clinical impact of co-infection with KSHV and offers valuable contribution to this field.

Conclusions

In the setting of high prevalence of KSHV and HIV co-infection, we found those co-infected with KSHV less likely to experience attrition from care after initiation of ART. In particular those with lower CD4 counts and in younger age categories were at reduced risk compared to those with less immune suppression and at an older age respectively. Further prospective studies ideally from time of seroconversion to KSHV are required to determine the reasons behind these findings.

Table 1: Baseline characteristics of 385 adults initiating ART in Johannesburg, South Africa stratified by KSHV status

Characteristics[#]		Overall (n=385)	KSHV+ (n =184)	KSHV- (n=201)
Sex	Female	249 (65.0%)	121 (65.8%)	128 (64.3%)
	Male	134 (35.0%)	63 (34.2%)	71 (35.7%)
Age (yrs)	Median (IQR)	37.9 (31.8 – 44.7)	37.5 (31.7 – 44.7)	38.1 (32 – 44.8)
WHO Stage	I/II	226 (59.0%)	114 (62.0%)	112 (56.3%)
(n, %)	III/IV	133 (34.7%)	62 (33.7%)	71 (35.7%)
	Missing	24 (6.3%)	8 (4.4%)	16 (8.0%)
BMI	<18.5	92 (24.0%)	37 (20.1%)	55 (27.6%)
(n, %)	18.5 – 24.9	200 (52.2%)	104 (56.5%)	96 (48.2%)
	25-29.9	54 (14.1%)	22 (12.0%)	32 (16.1%)
	≥30	27 (7.1%)	14 (7.6%)	13 (6.5%)
	Missing	10 (2.6%)	7 (3.8%)	3 (1.5%)
CD4 count (cells/mm3)	Median (IQR)	87 (40 – 149)	78 (26 – 151)	90 (51.5 – 147.5)
CD4 cell count category	0-50	120 (31.3%)	45 (24.5%)	75 (37.7%)
(n, %)	51-100	100 (26.1%)	56 (30.4%)	44 (22.1%)
	101-200	122 (31.9%)	66 (35.9%)	56 (28.1%)
	200-350	41 (10.7%)	17 (9.2%)	24 (12.1%)
First-line ART Regimen	d4T/3TC/EFV	324 (84.6%)	152 (82.6%)	172 (86.4%)
(n, %)	d4T/3TC/NVP	26 (6.8%)	16 (8.7%)	10 (5.0%)
	Other	33 (8.6%)	16 (8.7%)	17 (8.5%)
Tuberculosis (n, %)	Yes	95 (24.8%)	46 (25.0%)	49 (24.6%)
HIV RNA	Median (IQR)	38500 (5850 – 175000)	43000 (6000 – 180000)	37000 (5300 – 150000)
Outcomes at 18 months post ART initiation (n, %)	Alive in care	210 (54.8%)	103 (56.0%)	107 (53.8%)
	Died	38 (9.9%)	15 (8.2%)	23 (11.6%)
	LTFU*	77 (20.1%)	40 (21.7%)	37 (18.6%)
	Transferred	58 (51.1%)	26 (14.1%)	32 (16.1%)

[#] Characteristics at initiation of ART

*LTFU = lost to follow up defined as missing a scheduled clinic visit by more than 90 days

Table 2: The effect of KSHV on attrition from care after initiation of ART among adult HIV-infected patients initiating ART

	Attrition within 18 months			Attrition within 12 months			Attrition within 6 months		
Characteristic	**Attrition /N (%)**	**Unadjusted HR (95% CI)**	**Adjusted HR (95% CI)**	**Attrition /N (%)**	**Unadjusted HR (95% CI)**	**Adjusted HR (95% CI)**	**Attrition /N (%)**	**Unadjusted HR (95% CI)**	**Adjusted HR (95% CI)**
KSHV Status									
KSHV -	40/199 (20.1%)	**Reference**	**Reference**	35/199 (17.6%)	**Reference**	**Reference**	18/199 (9.1%)	**Reference**	**Reference**
KSHV +	28/184 (15.2%)	0.72 (0.44, 1.16)	0.77 (0.44, 1.35)	19/184 (10.3%)	0.57 (0.32, 0.99)	0.57 (0.29, 1.11)	14/184 (7.6%)	0.83 (0.41, 1.66)	0.91 (0.40, 2.10)
Sex									
Male	30/134 (22.4%)	1.52 (0.95, 2.46)	1.80 (1.01, 3.21)	26/134 (19.4%)	1.78 (1.05, 3.04)	2.07 (1.08, 3.99)	13/134 (9.7%)	1.30 (0.64, 2.63)	0.90 (0.37, 2.17)
Female	38/249 (15.3%)	**Reference**	**Reference**	28/249 (11.2%)	**Reference**	**Reference**	19/249 (7.6%)	**Reference**	**Reference**
Age at initiation									
<30	15/69 (21.7%)	**Reference**	**Reference**	12/69 (17.4%)	**Reference**	**Reference**	7/69 (10.1%)	**Reference**	**Reference**
30-39.9	21/157 (13.4%)	0.57 (0.29, 1.11)	0.55 (0.23, 1.28)	17/157 (10.8%)	0.60 (0.28, 1.25)	0.53 (0.20, 1.39)	11/157 (7.0%)	0.69 (0.27, 1.77)	1.13 (0.31, 4.05)
40-44.9	14/66 (21.2%)	0.96 (0.46, 1.99)	0.96 (0.38, 2.41)	10/66 (15.2%)	0.87 (0.38, 2.02)	0.87 (0.30, 2.54)	7/66 (10.6%)	1.07 (0.38, 3.05)	1.83 (0.47, 7.15)
≥45	18/91 (19.8%)	0.91 (0.46, 1.81)	1.48 (0.64, 3.38)	15/91 (16.5%)	0.96 (0.45, 2.06)	1.55 (0.61, 3.94)	7/91 (7.7%)	0.79 (0.28, 2.24)	2.09 (0.58, 7.63)
Baseline CD4+ Count									
<50	29/120 (24.2%)	2.45 (1.36, 4.41)	2.25 (1.08, 4.67)	25/120 (20.8%)	3.11 (1.56, 6.19)	2.53 (1.07, 6.00)	18/120 (15.0%)	5.21 (1.93, 14.03)	2.73 (0.92, 8.06)
50-100	21/100 (21.0%)	1.97 (1.05, 3.70)	2.47 (1.17, 5.20)	17/100 (17.0%)	2.35 (1.12, 4.93)	2.86 (1.19, 6.91)	9/100 (9.0%)	2.96 (0.99, 8.82)	1.74 (0.54, 5.62)
>100	18/163 (11.0%)	**Reference**	**Reference**	12/163 (7.4%)	**Reference**	**Reference**	5/163 (3.1%)	**Reference**	**Reference**
WHO Stage									
I/II	27/226 (12.0%)	**Reference**	**Reference**	20/226 (8.9%)	**Reference**	**Reference**	11/226 (4.9%)	**Reference**	**Reference**
III/IV	29/133 (21.8%)	2.02 (1.20, 3.42)	1.73 (0.98, 3.04)	24/133 (18.1%)	2.22 (1.23, 4.02)	1.89 (0.99, 3.61)	16/133 (12.0%)	2.60 (1.20, 5.59)	2.49 (1.05, 5.91)
Hemoglobin									
<8.5	13/33 (39.4%)	3.06 (1.67, 5.62)	2.44 (1.14, 5.23)	9/33 (27.3%)	2.58 (1.26, 5.30)	2.58 (1.08, 6.18)	7/33 (21.2%)	3.71 (1.59, 8.69)	2.40 (0.85, 6.78)
≥8.5	52/339 (15.3%)	**Reference**	**Reference**	42/339 (12.4%)	**Reference**	**Reference**	22/339 (6.5%)	**Reference**	**Reference**

HR = hazard ratio, CI = confidence interval, hazard ratios from a Cox proportional hazards regression model; Attrition from care defined as confirmed dead or not having attended a clinic visit in the last 4 months

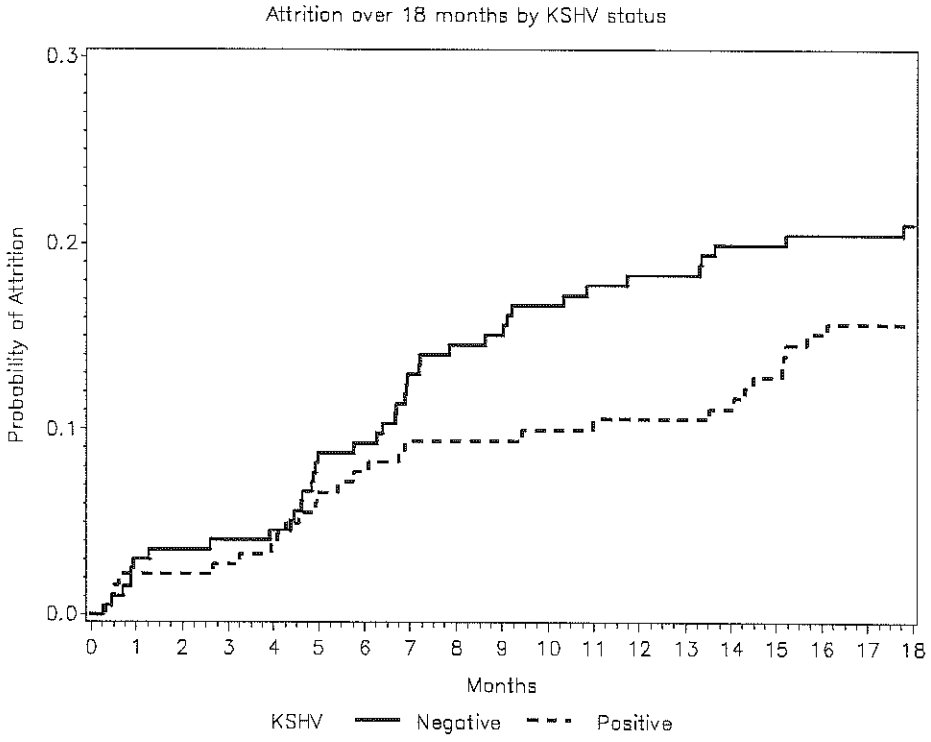


Figure 1: Attrition from care by KSHV status over the first 18 months of ART

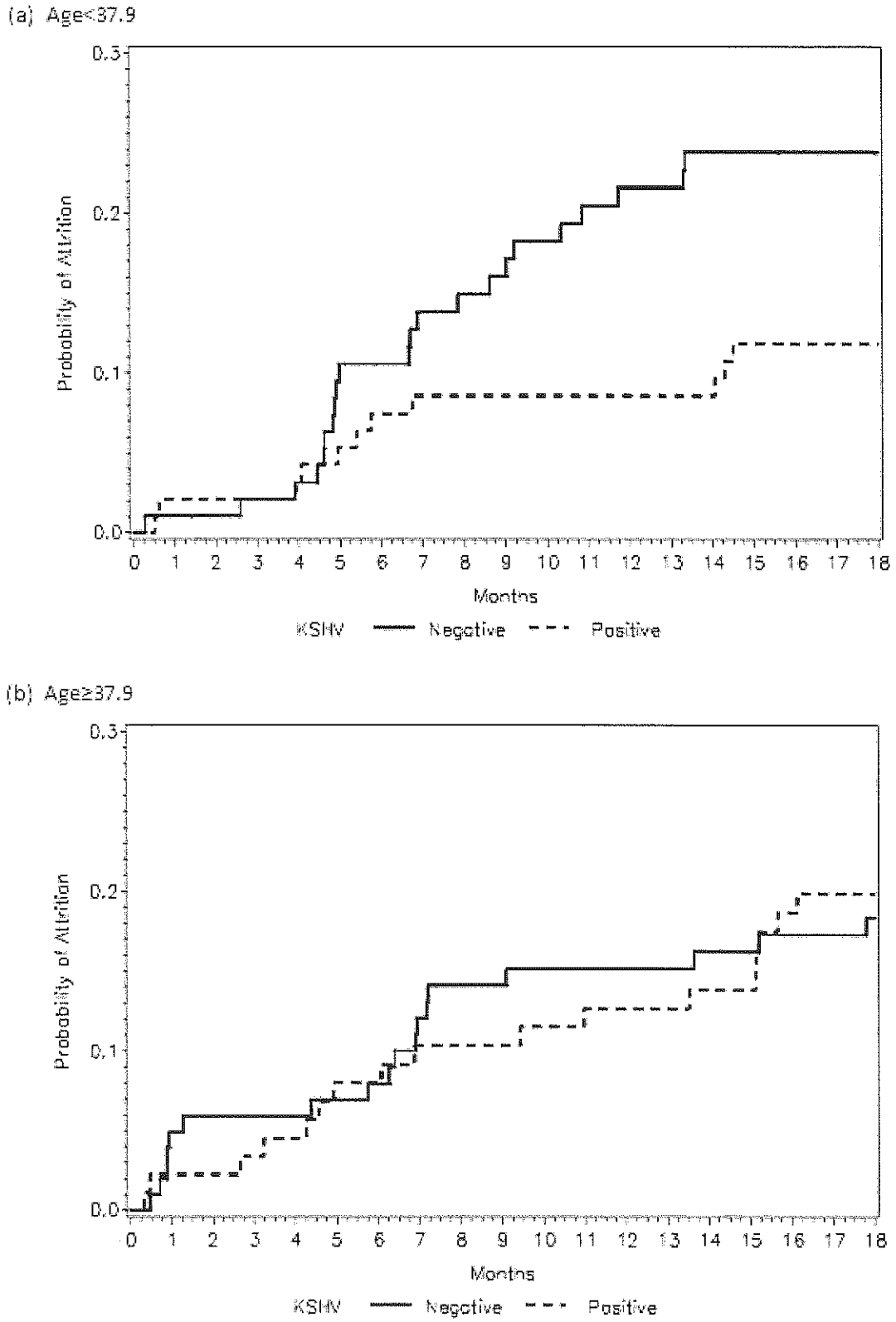
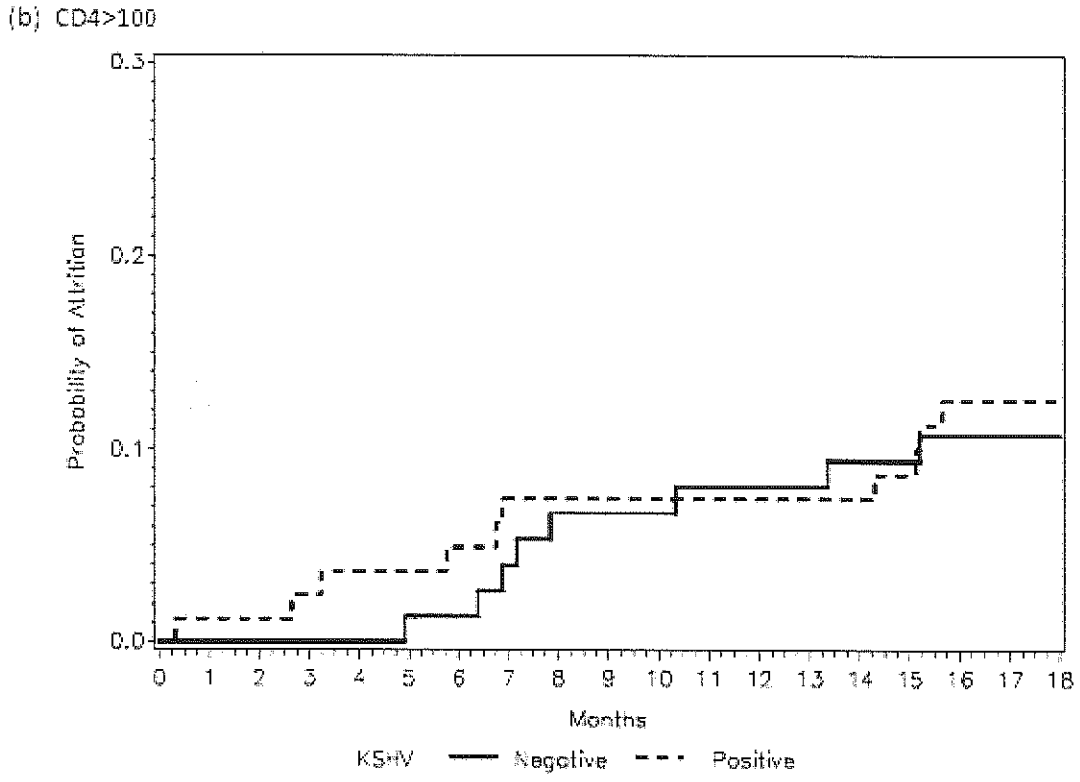


Figure 2: Attrition from care by KSHV status stratified by age



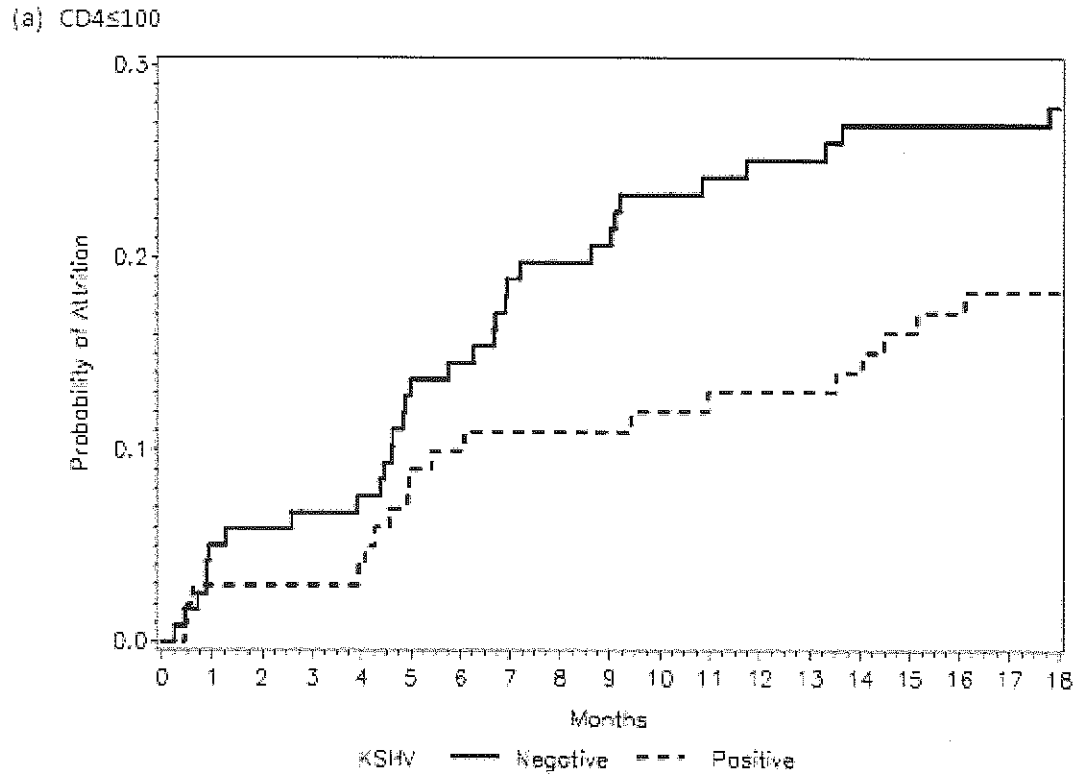


Figure 3: Attrition from care by KSHV status stratified by CD4 cell count

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PAPER 4:

Treatment Response and Mortality among Patients Starting Antiretroviral Therapy with and without Kaposi Sarcoma: A Cohort Study

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Treatment Response and Mortality among Patients Starting Antiretroviral Therapy with and without Kaposi Sarcoma: A Cohort Study

Mhairi Maskew[1]*, Matthew P. Fox[1,2,3], Gilles van Cutsem[4,5], Kathryn Chu[6], Patrick MacPhail[1], Andrew Boulle[5], Matthias Egger[7], for IeDEA Southern Africa

1 Health Economics and Epidemiology Research Office, Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, **2** Center for Global Health and Development, Boston University, Boston, Massachusetts, United States of America, **3** Department of Epidemiology, Boston University School of Public Health, Boston, Massachusetts, United States of America, **4** Medecins Sans Frontieres, Cape Town, South Africa, **5** Centre for Infectious Disease Epidemiology and Research (CIDER), University of Cape Town, Cape Town, South Africa, **6** South Africa Medical Unit (SAMU), Médecins Sans Frontières, Johannesburg, South Africa, **7** Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern, Switzerland

Abstract

Background: Improved survival among HIV-infected individuals on antiretroviral therapy (ART) has focused attention on AIDS-related cancers including Kaposi sarcoma (KS). However, the effect of KS on response to ART is not well-described in Southern Africa. We assessed the effect of KS on survival and immunologic and virologic treatment responses at 6- and 12-months after initiation of ART.

Methods: We analyzed prospectively collected data from a cohort of HIV-infected adults initiating ART in South Africa. Differences in mortality between those with and without KS at ART initiation were estimated with Cox proportional hazard models. Log-binomial models were used to assess differences in CD4 count response and HIV virologic suppression within a year of initiating treatment.

Results: Between January 2001–January 2008, 13,847 HIV-infected adults initiated ART at the study clinics. Those with KS at ART initiation (n = 247, 2%) were similar to those without KS (n = 13600,98%) with respect to age (35 vs. 35yrs), presenting CD4 count (74 vs. 85cells/mm^3) and proportion on TB treatment (37% vs. 30%). In models adjusted for sex, baseline CD4 count, age, treatment site, tuberculosis and year of ART initiation, KS patients were over three times more likely to have died at any time after ART initiation (hazard ratio[HR]: 3.62; 95% CI: 2.71–4.84) than those without KS. The increased risk was highest within the first year on ART (HR: 4.05; 95% CI: 2.95–5.55) and attenuated thereafter (HR: 2.30; 95% CI: 1.08–4.89). Those with KS also gained, on average, 29 fewer CD4 cells (95% CI: 7–52cells/mm^3) and were less likely to increase their CD4 count by 50 cells from baseline (RR: 1.43; 95% CI: 0.99–2.06) within the first 6-months of treatment.

Conclusions: HIV-infected adults presenting with KS have increased risk of mortality even after initiation of ART with the greatest risk in the first year. Among those who survive the first year on therapy, subjects with KS demonstrated a poorer immunologic response to ART than those without KS.

Citation: Maskew M, Fox MP, van Cutsem G, Chu K, MacPhail P, et al. (2013) Treatment Response and Mortality among Patients Starting Antiretroviral Therapy with and without Kaposi Sarcoma: A Cohort Study. PLoS ONE 8(6): e64392. doi:10.1371/journal.pone.0064392

Editor: Thomas F. Schulz, Hannover Medical School, Germany

Received December 31, 2012; **Accepted** April 12, 2013; **Published** June 5, 2013

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Funding: This project was supported by a grant from the National Institutes for Health (Grant number 1 U01 AI069924-01) in collaboration with the IeDEA Southern Africa collaboration. Funding was also provided by the United States Agency for International Development (USAID) under the terms of agreement 674-A-00-08-00007-00 with Right to Care (RTC). The opinions expressed herein are those of the authors and do not necessarily reflect the views of USAID, the Themba Lethu Clinic, or Right to Care. Matthew Fox was also supported by Award Number K01AI083097 from the National Institute of Allergy and Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy And Infectious Diseases or the National Institutes of Health. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors declare the co-author Andrew Boulle is a PLOS ONE Editorial Board member and that this does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: mmaskew@heroza.org

Introduction

Immunosuppression and co-infections with certain oncogenic viruses appears to increase the risk of some cancers in HIV-infected patients [1]. Infection with Kaposi sarcoma herpes virus (KSHV) is a precondition for the development of Kaposi's sarcoma (KS), and the seroprevalence of KSHV infection is high in both HIV-infected and uninfected populations, particularly in Africa [2]. Consequently, there has been a sharp increase in the incidence of KS since the advent of the HIV pandemic, and KS is a significant contributor to morbidity and mortality in sub-Saharan Africa [3–5]. Today KS is one of the most common cancers in Africa and is the most common tumour in HIV-infected individuals [4,6]. In South Africa, the incidence of KS rose dramatically as the HIV epidemic escalated (increasing threefold between 1988 and 1996) [7]. KS has been found to be associated

with advanced disease and high mortality among patients attending primary care clinics [8].

The World Health Organization (WHO) estimates that just over 5 million HIV-1-infected people were receiving antiretroviral therapy (ART) in sub-Saharan Africa by the end of 2010, for an estimated coverage of patients eligible for ART of 56% [9]. Combination ART has been used to successfully treat early stage KS for some time [10–12], achieving regression of KS lesions [13,14] and successfully reducing KS-related mortality [13,15]. In particular, suppression of replication of HIV has been associated with remission of KS [16]. In the United Kingdom, survival at 5 years among patients diagnosed with KS in the era of ART was estimated to be 98.4% in patients who had CD4 cell counts above 150 cells/mm^3 and with skin or lymph node involvement only and no other symptomatic disease [17]. At the other end of the spectrum, 5-year survival was estimated at 8.4% in patients with a history of AIDS, more severe immunosuppression, more advanced KS and other symptomatic disease.

The prognosis of KS is not well defined in resource-limited settings and its influence on response to ART is unclear. In particular, the excess mortality related to KS among those on ART is not well described. We used cohort data from two large urban HIV care and treatment programs in Johannesburg and Cape Town, South Africa, to assess the effect of KS on survival, loss to follow-up and immunologic and virologic responses to ART.

Methods

Ethics Statement

This analysis was nested within ongoing cohort studies of routine ART outcomes at the sites in Cape Town and Johannesburg. Use of data from the Themba Lethu and Khayelitsha sites were approved by the Human Research Ethics Committee of the University of the Witwatersrand and the Ethics Committee of the University of Cape Town, respectively. The pooling of data in IeDEA-SA was approved by Ethics Committees at the Universities of Bern and Cape Town. Individual patient consent was not needed, consistent with the South African Medical Research Council's Guidelines on.

Ethics for Medical Research and the Declaration of Helsinki. As this was a retrospective analysis of routine clinical service records, no additional data collection or procedures were undertaken from or on patients, all patient information was entered into the database using coded identification numbers, and no information that could reveal patient identity was available in the analytic datasets.

Cohort Description

Data for this study came from two HIV treatment cohorts: the Themba Lethu Clinic in Johannesburg [18] and three clinics of the Khayelitsha ART programme in Cape Town [19,20], South Africa. Both Themba Lethu and the Khayelitsha clinics are part of the International epidemiological Databases to Evaluate AIDS in Southern Africa (IeDEA-SA), a large collaboration of ART treatment programmes (www.iedea-sa.org) [21]. Themba Lethu has initiated over 16,000 patients on ART since its inception in 2004. The Khayelitsha clinics were set up by Médecins Sans Frontières (MSF) in 2001 and are now run by the Western Cape Provincial Department of Health. In August 2010, more than 16,000 patients were on ART. The data from the three Khayelitsha clinics were aggregated for this analysis. Care at all clinics was provided according to the South African National Department of Health guidelines in place during the study period [22]. Routine data were collected prospectively at each site to facilitate HIV treatment monitoring. Data from the different sites were transferred to the IeDEA-SA database managed by the Universities of Cape Town, South Africa and Bern, Switzerland.

Eligibility Criteria

We included HIV-positive treatment naïve patients ≥18 years of age who initiated ART at a study clinic between 01 January 2001 and 31 December 2007. We limited the analysis to patients starting standard South African public sector first-line ART regimens (stavudine [d4T] or zidovudine [AZT] with lamivudine [3TC] and either efavirenz [EFV] or nevirapine [NVP]) [22]. During the study period, the National guidelines' eligibility criteria for initiation of ART were either a CD4 cell count <200 cells/mm^3 or a WHO stage 4 illness (such as KS) regardless of CD4 count. We found 13,847 patients were eligible for the current analysis.

Study Variables

We compared ART outcomes by KS status at ART initiation. KS was defined as having a KS diagnosis recorded in the dataset between 6 months prior to and 6 months after ART initiation. KS is diagnosed mostly on a clinical basis at the study sites and while certain individuals may have had histopathological confirmation of disease, this is not routinely done in all cases. Our primary outcomes included: 1) all-cause mortality; 2) loss to follow up (LTFU); 3) failure to achieve virologic response at 6- and 12-months on ART (HIV viral load ≤400 copies/ml); and 4) failure to achieve immunologic response (CD4 count increase of >50 cells/mm^3 at 6 months and >100 cells/mm^3 at 12 months after ART initiation). LTFU was defined as having not attended the clinic in the previous 4 months. Mortality is ascertained through active tracing of patients who do not return to the clinic, and data for those lost was also verified at the end of 2010 with the South African National Vital Registration system for patients in whom a civil identification number was available (42% of those lost to care in Themba Lethu [23] and 47% in Khayelitsha [19]). As the hazard of mortality was not consistent over time, for both the mortality and LTFU outcomes, we considered the effect of KS on each of these events at any time point after initiation of treatment. We then further stratified the analysis into the first year after ART initiation and after the first year on ART.

Statistical Analysis

Baseline characteristics for each group were stratified by KS status and summarized as proportions or medians with inter-quartile ranges. Cause-specific Cox proportional hazard models were used to estimate the effect of KS on mortality and loss to follow up on ART at each time period considered. Person-time was calculated from the date of ART initiation to the earliest of: 1) death or loss to follow up; 2) transfer to another facility; or 3) end of study period (31 December 2008). We used mixed linear models with a random intercept and an unstructured correlation matrix to estimate CD4 trajectories over time, accounting for repeated observations on an individual. Time was specified as a quadratic function. Models for those with KS and those without KS were fit separately to allow for different curves by exposure group. The association of KS with change in CD4 count from baseline to 6 and 12 months was estimated using a multivariate linear generalized estimating equation model. Additionally, log-binomial regression was used to estimate the impact of KS status on CD4 response (>50 cells/mm^3 and >100 cells/mm^3) and VL suppression (<400 vs. ≥400) by 6- and then by 12-months on treatment respectively. All models were adjusted for age, gender,

baseline CD4 count (at ART initiation), tuberculosis treatment status, time period (year of ART initiation) and initiating treatment site.

Results

A total of 13,847 patients met eligibility criteria, including 247 individuals (1.8%) diagnosed with KS at baseline (i.e. 6 months prior to up to 6 months after ART initiation). The prevalence of KS was slightly greater in Khayelitsha than at Themba Lethu (2.2% vs. 1.5%). The patient characteristics at initiation of ART are described in Table 1. Those with KS at ART initiation were similar to those without with respect to median age (35 vs. 35 years) and first-line ART regimen (68% vs. 69% initiated on d4T-3TC-EFV). The median presenting CD4 count was somewhat lower in KS patients (74 vs. 85 cells/mm^3) but those with KS were about twice as likely to have a CD4 count in the 200–350 cells/mm^3 category (12.3% vs. 7.2%). The proportion on TB treatment was also higher among those with KS (37% vs. 30%). As expected, patients with KS were more likely to be male than other patients (49% vs. 36%). Those without KS had received a median of 19.1 months of ART (IQR: 7.8–32.0) compared to 12.3 months (IQR: 2.3–29.8) among those with KS.

Mortality and Loss to Follow Up

Vital status outcomes were ascertained for 13,065 (94%) of the 13,847 subjects (95% for those with KS and 94% for those without KS). Of these, 10% (1,312) died and 14% (1,837) were LTFU at some point after ART initiation (Table 2). Median follow-up time for those who died or were lost to follow up was 4.5 (IQR 1.5–12.5) and 9.6 (IQR 4.4–19.3) months, respectively. Mortality was highest within the first 12 months after starting ART (74% of deaths occurred in the first 12 months).

A greater proportion of individuals with KS died after ART initiation compared to those without KS (27% vs. 10%). The rate of LTFU after ART initiation was 13.0/100 py among those with KS compared to 3.9/100 py among those without KS. Individuals with KS had higher mortality rates at all durations after ART initiation compared to those without KS: 28.3/100 person-years (100 py) vs. 7.4/100 py within the first year and 4.1/100 py vs. 1.8/100 py after the first year.

Cumulative incidence curves showed higher incidence of mortality for those with KS after ART initiation with the greatest differences in mortality occurring within the first year on treatment (Figure 1). The risk of death for those with KS was over three times that of those without KS at any time point after ART initiation (adjusted HR: 3.62; 95%CI: 2.71–4.84) and four times greater within the first year after ART initiation (adjusted HR: 4.05; 95%CI: 2.95–5.55) (Table 2). Among those who survived to a year on treatment, the risk of death was still greater in the KS group though the magnitude of this effect was smaller (adjusted HR: 2.30; 95%CI: 1.08–4.89).

We also analyzed the effect of time of KS diagnosis in relation to ART initiation on mortality. The mortality rate after ART initiation was greater among those diagnosed with KS before ART initiation than those diagnosed with KS after ART initiation (14.2/100 py vs. 9.8/100 py) though both of these were greater than the proportion who died among those without KS (3.9/100 py). The hazard of death among those diagnosed with KS before ART initiation was higher than the hazard among those diagnosed with KS after ART initiation (HR = 4.14 95% CI 2.97–5.77 vs. HR = 2.61 95% CI 1.47–4.62) comparing both groups to those without KS.

Table 1. Baseline characteristics of 13,847 adults initiating ART in Cape Town and Johannesburg, South Africa, stratified by presence of Kaposi sarcoma.

Characteristics		No Kaposi Sarcoma (n = 13,600)	Kaposi Sarcoma (n = 247)
Sex	Male	4893 (36.0%)	121 (49.0%)
Age at ART Initiation (years)	Median (IQR)	35 (30–41)	35 (30–41)
Initiating treatment site	Khayelitsha	6583 (48.4%)	153 (61.9%)
	Themba Lethu	7017 (51.6%)	94 (38.1%)
Year of ART Initiation	Before 2004	581 (4.3%)	20 (8.1%)
	2004	1947 (14.3%)	42 (17.0%)
	2005	3185 (23.4%)	74 (30.0%)
	2006	4149 (30.5%)	64 (25.9%)
	2007	3738 (27.5%)	47 (19.0%)
CD4 at ART Initiation (cells/mm^3)	Median (IQR)	85 (33–150)	74 (29–152)
	0–50	4256 (34.3%)	86 (37.9%)
	51–100	2747 (22.1%)	46 (20.3%)
	101–200	4518 (36.4%)	67 (29.5%)
	200–350	899 (7.2%)	28 (12.3%)
First-line ART Regimen	d4T/3TC/EFV	9200 (68.1%)	169 (69.3%)
	d4T/3TC/NVP	3000 (22.2%)	52 (21.3%)
	Other	1562 (11.7%)	23 (9.4%)
TB at Initiation	Yes	3247 (29.5%)	71 (36.6%)

TB = tuberculosis; IQR = interquartile range, ART = antiretroviral therapy; d4T = stavudine, 3TC = lamivudine, EFV = efavirenz, NVP = nevirapine.
Number of patients (%) are shown unless otherwise stated.
doi:10.1371/journal.pone.0064392.t001

Table 2. The effect of Kaposi Sarcoma on mortality and loss to follow-up after initiation of ART among 13,065 adult HIV-infected patients initiating ART in Cape Town and Johannesburg, South Africa.

	Death					Lost to follow up				
	Deaths	Person time (years)	Rate/100 pys*	Crude HR (95% CI) †	Adjusted HR (95% CI)‡	LTFU**	Person time (years)	Rate/100 pys*	Crude HR (95% CI) †	Adjusted HR (95% CI)‡
Over total follow-up										
No KS	1248 (9.7%)	32345	3.9	1.0	1.0	1794 (14.0%)	32345	5.5	1.0	1.0
KS	64 (27.2%)	491	13.0	3.22 (2.51–4.15)	3.62 (2.71–4.84)	43 (18.3%)	491	8.8	1.58 (1.16–2.14)	1.42 (0.88–2.29)
Within first year of ART										
No KS	913 (7.1%)	12317	7.4	1.0	1.0	985 (7.7%)	12317	8.0	1.0	1.0
KS	54 (23.0%)	191	28.3	3.63 (2.76–4.77)	4.05 (2.95–5.55)	26 (11.1%)	191	13.6	1.69 (1.14–2.49)	1.55 (0.85–2.82)
After first year of ART										
No KS	335 (3.1%)	18745	1.8	1.0	1.0	809 (7.4%)	18745	4.3	1.0	1.0
KS	10 (6.5%)	244	4.1	2.03 (1.08–3.80)	2.30 (1.08–4.89)	17 (11.0%)	244	7.0	1.44 (0.89–2.32)	1.21 (0.54–2.70)

†HR = hazard ratio, CI = confidence interval, KS = Kaposi sarcoma, ART = antiretroviral therapy, pys = person years, hazard ratios from a Cox proportional hazards regression model.
‡Models adjusted for sex, baseline CD4 count, age, treatment site, tuberculosis at ART initiation, year of ART initiation.
*pys = person years.
**LTFU = Lost to follow up defined as having missed a clinic appointment by at least 3-months after the scheduled visit date.
doi:10.1371/journal.pone.0064392.t002

A greater proportion of individuals with KS were LTFU after ART initiation compared to those without KS (18% vs. 14%). The rate of LTFU after ART initiation was 8.8/100 py among those with KS compared to 5.5/100 py among those without KS. Among those with KS, the rate of LTFU was greatest in the first year after initiation of ART (13.6/100 py in the first 12 months vs. 7.0/100 py after 12 months). In adjusted proportional hazards models there was little evidence for a difference in the rate of LTFU in those with KS compared to those without, both in first year (adjusted HR: 1.55; 95%CI: 0.85–2.82) and after a year on treatment (adjusted HR: 1.21; 95%CI: 0.54–2.70). In competing risks analyses with death as the competing event, the rate of LTFU was closely similar but attenuated (HR: 1.02; 95% CI: 0.59–1.78).

Immunologic and Virologic Failure

Among the 12,337 subjects alive and in care at 6 months on treatment, CD4 count values were available for 8,676 (70%) of these (63% of those with KS and 70% of those without KS). By 6 months on treatment, nearly a quarter of patients (23.7%; 95% CI: 17.3–32.7%) with KS had failed to achieve a CD4 increase of $\geq$50 cells/mm^3 compared to 18.1% (95% CI: 17.5–19.1) of those without KS (Table 3). The median increase in CD4 count by 6 months on ART was 98 cells/mm^3 (IQR 58–164 cells/mm^3) among the KS group and 121 cells/mm^3 (IQR 66–190 cells/mm^3) for those without KS. Patients with KS gained, on average, 29 fewer CD4 cells (95% CI: 7–52 cells/mm^3) than those without KS over the same time period. Among the 11,667 patients who survived to a year on treatment, CD4 count values were available for 7,157 (62%) of subjects. 29.9% (95% CI: 21.4–39.6%) of KS patients failed to achieve a 100 cell increase in CD4 count compared to 23.3% (95% CI: 22.3–24.3%) of patients without KS. The median increase in CD4 count by 12 months on ART was 150 cells/mm^3 (IQR 90–225 cells/mm^3) among the KS group and 175 cells/mm^3 (IQR: 105–260cells/mm^3) for those without KS.

Using adjusted generalised estimating equations, those with KS gained an estimated 9 fewer CD4 cells (95% CI -21–40 cells/mm^3) than those without KS over the first year of ART. The predicted CD4 trajectories from start of ART suggested some advantage for those without KS (Figure 2). Despite starting on very similar CD4 cell counts at ART initiation, those with KS gained fewer CD4 cells over the first year of treatment compared to those without KS. By the end of the first year the rate of increase in CD4 count was similar for the groups, though the group without KS retained consistently higher CD4 cell counts after treatment initiation. In log-binomial models, patients with KS were more likely to fail to achieve a 50 cells/mm^3 increase (RR 1.43; 95% CI: 0.99–2.06) and 100 cells/mm^3 increase (RR 1.20; 95% CI: 0.84–1.73) in CD4 count at 6- and 12-months on treatment respectively (Table 3). Sensitivity analyses yielded no qualitative differences in results when attributing either achieving or failing to achieve the outcome to all missing CD4 count responses at 6 or 12 months on treatment (results not shown).

Virologic response to ART was favourable among both groups (Table 3). By 6 months on treatment, only 11% of those with KS had failed to suppress HIV viral load to <400 copies/mL while just under 8% of those without KS had failed to achieve suppression. Among those who survived to a year on treatment, similar proportions failing to achieve virologic responses were noted (7% vs. 10%). The relative risk for failure to achieve virologic suppression suggests that KS patients fared better at 6 (RR: 0.82; 95% CI: 0.38–1.79) and 12 months (RR: 0.25; 95% CI: 0.06–1.00) after initiation of ART compared to those without KS though these estimates lacked precision. Results from sensitivity

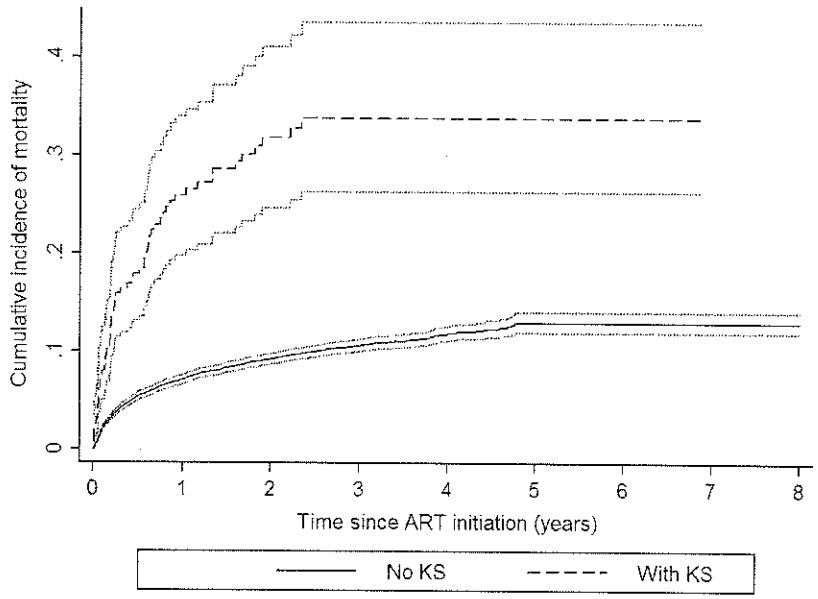


Figure 1. Cumulative incidence of mortality after ART initiation by KS status.
doi:10.1371/journal.pone.0064392.g001

analyses again did not qualitatively change the results at either 6 or 12 months on treatment.

Discussion

AIDS-related malignancies are increasing in significance as the HIV epidemic matures, particularly in resource-limited settings. These settings bear the greatest burden of disease due to cancers yet are most limited in terms of drug options, infrastructure and staffing required to effectively treat these conditions. As access to ART continues to scale-up and models of HIV care shift away from specialist services to decentralised clinics, investigating factors that impact on the effective response to ART is important. ART has shown much promise in the treatment of early stage KS [10–16,24] but the effect of KS on response to ART is not clear.

We found the prevalence of Kaposi sarcoma among this HIV-infected treatment naïve population was 1.8%, very similar to the prevalence of 1.6% seen in Nigeria [25], and somewhat lower than seen among European cohorts (3.8–6.4%) in the late 1990s [26–28]. While there may be country-level differences in prevalence of KS, our figures could underestimate the true prevalence in this setting for several reasons. Firstly, diagnosis of KS in HIV outpatient clinics may be impaired by limited access to oncology and histopathology services, as well as lack of training on early recognition KS by primary care staff. A recent study in South Africa noted that 46% of study subjects were diagnosed with KS and HIV at the same time [29] and earlier studies have shown high pre-ART attrition underestimates KS prevalence; in South Africa the prevalence of KS including pre-ART subjects was estimated at 3.4% [8]. Additionally, limited communication and linkage between oncology and outpatient services hampers the recording of cancer diagnoses in HIV clinic patient records and use of national cancer registry databases to ascertain cancer diagnoses is not routine.

We demonstrated a substantially increased risk of mortality associated with KS. The risk of death was four times greater among the KS group in the first year on ART and though the risk decreased thereafter, those with KS were still twice as likely to die after the first year of treatment as those without KS after adjustment for measured confounders. This is in keeping with previous findings on KS mortality [8,25] and highlights the importance of early diagnosis and initiation of appropriate treatment for HIV-infected subjects with KS at every stage of HIV infection and treatment. We also note that the KS group were more likely to have a diagnosis of tuberculosis at initiation of ART when compared to those without KS. Though TB is not diagnosed primarily by chest x-ray in these settings, the radiographic appearance of the nodular infiltrate associated with pulmonary KS could have been mistaken for TB in some cases. Pulmonary KS is associated with high rates of mortality and though all models were adjusted for diagnosis of TB at ART initiation, this may have contributed to the excess mortality noted in the KS group.

Though the results were imprecise and lacked statistical significance, we note that the majority of estimates suggested those with KS were less likely to fail to suppress HIV viral load. It is possible that this reflects survivor bias in that those with KS who are also poorly adherent to treatment do not survive to have a viral load test done at the intervals described. Though we cannot make inferences from our results, if this effect were real, it might suggest better adherence among those surviving with KS possibly related to more intensive follow up and more frequent attendance at clinic visits for their KS related care. We did also note some immunologic differences. First, those with KS were roughly twice as likely to have a nadir CD4 count between 200 and 350 cells/mm3 compared to those without KS. This is likely explained by the fact that KS (as a WHO stage 4-defining condition) was an indication for initiation of ART with CD4 count ≥200 cells/mm3 at a time when the ART eligibility criteria were otherwise <200. Second, after initiation of ART, those with KS were less likely to increase their CD4 cell counts by 50 and 100 cells at 6 and 12 months on treatment respectively. The KS group also had a smaller mean increase in CD4 cell count at both time periods than those without KS though the actual difference in CD4 gain was

Table 3. Immunologic and Virologic Outcomes at 6 and 12-months on ART stratified by KS status among 8,676 adult HIV-infected patients initiating ART in Cape Town and Johannesburg, South Africa.

6-months				12-months		
Exposure	**Number with failure**	**Crude RR (95% CI)‡**	**Adjusted† RR (95% CI)‡**	**Number with failure**	**Crude RR (95% CI)‡**	**Adjusted† RR (95% CI)‡**
*Immunologic failure**						
No KS	1565 (18.3%)	1.0	1.0	1655 (23.3%)	1.0	1.0
KS	29 (24.4%)	1.33 (0.97–1.83)	1.43 (0.99–2.06)	29 (29.9%)	1.28 (0.94–1.74)	1.20 (0.84–1.73)
*Failure to suppress HIV viral load***						
No KS	642 (7.8%)	1.0	1.0	714 (10.2%)	1.0	1.0
KS	14 (10.7%)	1.37 (0.83–2.26)	0.82 (0.38–1.79)	7 (6.9%)	0.67 (0.33–1.38)	0.25 (0.06–1.00)

†Models adjusted for sex, baseline CD4 count, age, treatment site, tuberculosis at ART initiation, year of ART initiation.
‡VL = viral load, RR = relative risk, CI = confidence interval, relative risk from a log-binomial regression model KS = Kaposi's sarcoma, ART = antiretroviral therapy,
*Failure to achieve a CD4 response defined as an increase of ≥50 cells/mm^3 at 6 months and ≥100 cells/mm^3 at 12 months.
**Failure to suppress VL to <400 copies/ml.
doi:10.1371/journal.pone.0064392.t003

small between the groups. This may be due to differences in disease stage at treatment initiation [30] or possibly related to the additional suppressive effect of chemotherapy on the KS patients' immune system. CD4 cell counts have been documented to decline by up to 50% during chemotherapy even in the presence of virally suppressive ART [31], an additional immune insult that would not be experienced by those not receiving chemotherapy and could partially explain the lack of significant difference in viral load suppression in this study. We emphasize that our findings apply only to those who remained alive and in care with follow-up laboratory results at the time points considered.

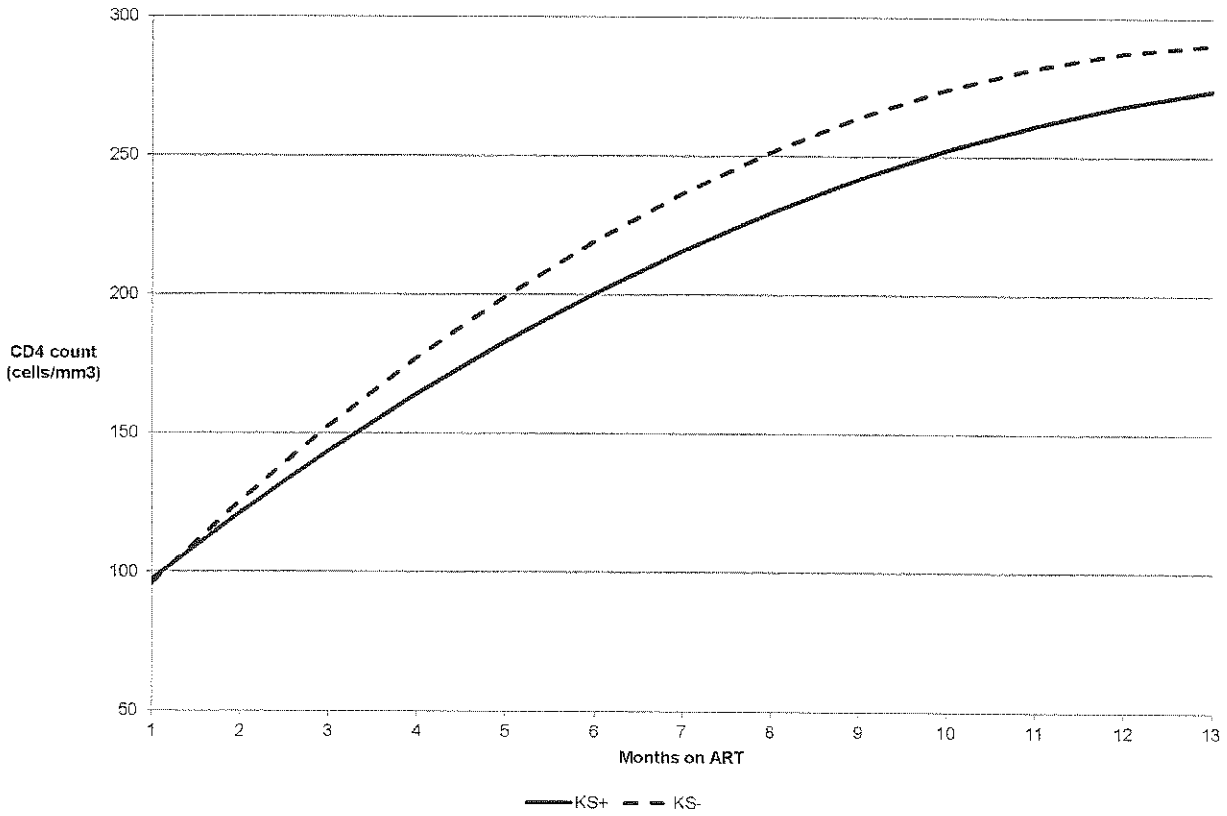


Figure 2. Mean predicted* CD4 cell count increase from ART initiation stratified by KS status. *Trajectories were estimated using two separate mixed linear models, one for the KS+ and one for the KS- to allow the curves to depart from being parallel. Curves were fitted using time as a quadratic function and a random intercept with an unstructured correlation matrix for repeated measures.
doi:10.1371/journal.pone.0064392.g002

Our findings need to be considered in light of the study's limitations. First, the prevalence of KS is likely to be underestimated in these cohorts due to the remote location of oncology services from the HIV outpatient clinics and other reasons explored above. Current linkage projects in collaboration with the National cancer registry are underway which aim to improve ascertainment of cancers among HIV treatment cohorts. Second, high rates of lost to follow up in these cohorts may introduce the possibility of selection bias, though we did not find significant differences in rates of LTFU between the groups. The rate of LTFU may, however, have led to underestimations of the mortality rates due to different mortality rates between patients lost to follow-up with and without KS. As previously noted, anywhere from 20–50% of those lost from HIV care are actually deceased [23,32–34]. Finally, we lacked data on staging of KS disease and use of chemotherapy and were unable to adjust models for these or estimate the effect of chemotherapy and ART on treatment outcomes, particularly among those with visceral advanced KS.

Despite effective ART, clinical KS remains a poor prognostic factor in ART treatment outcomes. High rates of mortality and loss to follow up confirm that KS remains a significant problem in low and middle income countries. Insufficient awareness at primary care level leads to under-diagnosis of KS, especially in its early stages, and result in patients often presenting with advanced disease. Delays in initiation of treatment for AIDS-related malignancies such as KS remain a problem in settings where oncology services and chemotherapy are offered at specialist tertiary centres often remote from primary care services and unable to cope with the high patient burden. Under-diagnosis of disseminated disease and limited chemotherapy options are also likely to be negatively impacting on survival and need to be addressed to improve outcomes for HIV-infected patients presenting for treatment with KS. In particular, chemotherapeutic options, such as paclitaxel, are not readily available in resource-limited settings due to high cost. Future research efforts may focus on investigating alternate chemotherapy drugs, including etoposide [35–37], which could be safely administered at primary care level in order to increase access and reduce delays and resulting mortality.

Acknowledgments

We express our gratitude to the directors and staff of the Themba Lethu Clinic, in addition to Right to Care, the NGO supporting the Themba Lethu database and study site through a partnership with USAID, as well as Médecins Sans Frontières and the Centre for Infectious Disease Epidemiology and Research, who support the Khayelitsha clinics. We also thank the Gauteng, Western Cape and National Departments of Health for providing for the care of the patients at the Themba Lethu and Khayelitsha clinics as part of the Comprehensive Care Management and Treatment plan. We are also grateful to Gcina Mahlangeni of Médecins Sans Frontières for her assistance with data collection.

Author Contributions

Conceived and designed the experiments: MM MPF GVC KC PM ME. Performed the experiments: MM MPF AB. Analyzed the data: MM. Contributed reagents/materials/analysis tools: AB ME. Wrote the paper: MM MPF GVC KC PM AB ME.

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