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

Mhairi Maskew, MBBCh, MSc Med[1], **A Patrick MacPhail, PhD, FRCP**[2], **Denise Whitby, PhD**[3], **Matthias Egger, MD, MSc, MFPHM, DTM & H**[4], and **Matthew P. Fox, DSc, MPH**[1,5,6]

[1]Health Economics and Epidemiology Research Office, Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg [2]Clinical HIV Research Unit, Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg [3]Viral Oncology Section, AIDS and Cancer Virus Program, SAIC-Frederick, Frederick National Laboratory for Cancer Research, Frederick MD, USA [4]Institute of Social and Preventive Medicine (ISPM), University of Bern, Switzerland [5]Center for Global Health and Development, Boston, USA [6]Department of Epidemiology, Boston University School of Public Health, Boston, USA

Abstract

Background—The possible impact of co-infection with Kaposi's sarcoma associated herpes virus 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. 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 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—385 subjects initiating ART from November 2008-March 2009 were 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 haemoglobin levels. The KSHV+ group gained a similar number of cells at 6- (difference of 10 cells/mm^3, 95% CI: −11–31), 12- (3 cells/mm^3, 95% CI: −19–25) and 18-months (24 cells/mm^3, 95% CI: −13–61) compared to the KSHV− group. Adjusted relative risk of failure to suppress viral load to <400 copies/mL (1.03; 95% CI: 0.90–1.17) were similar for KSHV+ and KSHV− by 6-months on treatment.

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

Corresponding Author and Address for Reprints: Dr Mhairi Maskew, Health Economics and Epidemiology Research Office, Themba Lethu Clinic, Helen Joseph Hospital, Perth Road, Westdene, Johannesburg, South Africa, 2192, Phone: +27-11-276-8850, Fax: +27 86 516 0628, mmaskew@heroza.org.

Keywords

Kaposi sarcoma herpesvirus; antiretroviral therapy; resource-poor setting; virologic suppression

Introduction

The prevalence of Kaposi sarcoma herpesvirus (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] as well as multicentric Castleman's 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 tumour in HIV infected individuals [10]. Co-infection with other viruses including cytomegalovirus, hepatitis B and hepatitis C has been previously associated with HIV disease progression and mortality [11–12] as well as poor CD4 cell count responses after initiation of antiretroviral therapy [12–13].

KSHV typically establishes a persistent latent infection in its host during which time only latent genes (of which Orf73 is one example) are expressed. In the presence of HIV-1 co-infection however, immune suppression and cytokine release promotes reactivation of KSHV lytic genes which include K8.1 [14] 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 [15] and induced reactivation of HIV-1 replication in chronically infected cells [16]. Despite this, there are few analyses describing the effect of co-infection with KSHV on HIV treatment outcomes after initiation of antiretroviral therapy (ART).

We examined 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.

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 over 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, over 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 is 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 three 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 (IeDEA-SA) [21].

Eligibility Criteria

HIV-positive treatment naïve patients, >18 years of age, who were assessed as ready and eligible for initiation of ART at Themba Lethu clinic were invited to participate in the study. Participant enrolment 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 prior to 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 prior ART use or who were unwilling to consent were excluded from the study. All enrolled subjects provided informed consent prior to commencing study procedures.

Laboratory Analysis

Laboratory testing for KSHV serology was conducted by Contract Laboratory Services (CLS) and National Health Laboratory Service (NHLS) while KSHV viral load testing was performed by the Haematology and Molecular Medicine Department of the University of the Witwatersrand. Enzyme linked immunosorbent assays (ELISA) which detect antibodies to lytic K8.1 and latent open reading frame (Orf) 73 KSHV recombinant protein antigens were developed in the Viral Oncology Section, ACVP, FNLCR, USA and transferred to the CLS. The ELISAs have good sensitivity and specificity and have been used in over 30 studies internationally [22]. All samples that tested serologically positive to KSHV were then tested for KSHV viral load using quantitative TaqMan PCR [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 150ng 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 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. We further stratified a positive KSHV result into three 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 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. The association of KSHV with linear increase in CD4 count from baseline to 6, 12, and 18-months was estimated using mixed linear models. We also estimated estimate CD4 trajectories by modeling CD4 count over time using a linear regression model with CD4 cell count 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 of KSHV on VL 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, haemoglobin level, tuberculosis 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 relative risk varied by 10% or more 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 relative risks after adjustment for all the potential confounding factors.

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. 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 Kaposi sarcoma. 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. The majority of participants were started on standard first line ART regimens; 86% on stavudine (d4T), lamivudine (3TC) and efavirenz (EFV) and 7% on stavudine, lamivudine and nevirapine (NVP). The remaining 7% 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 [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% 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. Outcomes at the end of 18 months of follow up among the KSHV positive 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 to the KSHV negative group (Table 1).

Prevalence of KSHV

Among the study participants, 184/385 tested positive to 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. The KSHV+ group presented with a somewhat higher median CD4 cell count (90 vs.78 cells/mm^3) than their KSHV-counterparts. Among those participants with KSHV positive results, a convenience sample of 167 samples were also tested for KSHV viral load. 19 (11%) of these had a detectable viral load at 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 to 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/mm^3 (95% CI: 102–132) compared to 107 cells/mm^3 (95% CI: 92–121) among the KSHV−). The KSHV positive group gained a similar number of cells at 6-, 12- and 18-months compared to the KSHV negative group (Table 2). The predicted CD4 trajectories from start of ART were also similar for the groups (Figure 1). The greatest increases occurred shortly after treatment initiation for both groups, though the KSHV positive 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 non-normal 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 positive 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, haemoglobin, body mass index and tuberculosis status suggested little difference in number of CD4 cells gained for those with an undetectable KSHV viral load at 6- (24.0 cells/mm^3; 95% CI: −25.2–73.5), 12- (2.1 cells/mm^3; 95% CI: −53.2–49.1) and 18-months on treatment (14.9 cells/mm^3; 95% CI: −104.6–134.5) compared to 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, haemoglobin and body mass index.

When we restricted the analysis to the KSHV positive 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/mL by 6- and 12-months on treatment. All other groups achieved suppression rates >90% though and there were no differences in likelihood of achieving virologic suppression when we compared the KSHV negative group to those reactive to lytic K8.1 (RR=1.02; 95% CI: 0.83–1.25) or reactive to both antigens (RR=1.02; 95% CI: 0.87–1.21) by 6-months on ART. Results were similar at 12-months on 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 followed a group of KSHV positive adults without clinical evidence of KS and a group of KSHV negative 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 co-infected with KSHV compared to their KSHV negative counterparts. In general, KSHV infection, did not appear 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 herpesviruses, 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 co-infection however, this course is altered. There is evidence that the immune suppression caused by HIV-1 as well as cytokine release promotes reactivation of KSHV lytic genes [14] 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 [26–27].

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 [28–29]. Though the detection of antibodies to lytic antigens is only a rough approximation of active lytic replication of KSHV (we also did not find 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 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 increased risk of KS morbidity and mortality [30–32].

The debate about the effect of KSHV on HIV-1 among those co-infected is ongoing. In vitro and in vivo studies suggested an interaction between these two 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 inhibition of HIV infection of CD4 cells expressing CCR3 receptors, largely mediated through beta chemokines [33–34]. In this population, we observed little difference in HIV viral load prior to ART initiation between the groups and similar risk of failure to suppress viral load at 6- and 12-months after ART initiation when comparing KSHV+ to KSHV−. Other in vitro work also suggested that KSHV increased HIV-1 replication in acutely infected cells but also induced reactivation of HIV-1 replication in chronically infected cells [16]. Our results suggest that those with KSHV co-infection achieved comparable virologic and immunologic responses to HAART when compared to those without KSHV. Though 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 three 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. 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 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 to those where the virus was undetectable at initiation of ART. We emphasize here that the numbers in this sub-analysis were small, limiting our power to detect statistically significant differences. Broadly speaking, our 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 [35]. The authors postulate that KSHV acts as an opportunistic agent rather than an HIV-cofactor

among co-infected individuals. It is also possible that effects are not apparent in the short term but may manifest later.

Our study has several strengths and limitations which should be considered when interpreting these findings. One important strength is that, unlike prior studies which have been conducted in the absence of any antiretroviral therapy, 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 tumour 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 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) 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 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 a year [25] to two years [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 differences in retention and follow up exists. While we cannot exclude this possibly completely, we do note that the mean follow-up time was very similar for both KSHV positive and negative groups. Additionally, the proportions lost to follow-up were similar between the groups and less than 10% in both groups.

Our findings are also strengthened by the relatively large sample size. While 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 co-infection. However, while the sample is large compared to 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 one urban facility in Johannesburg, South Africa. This may mean our results have limited generalizability to other non-urban 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 [1–3,39–42] in KSHV prevalence previously demonstrated.

Conclusion

We demonstrate good HIV treatment outcomes after ART initiation among a group of treatment naïve, HIV-infected adults initiating ART at a large urban clinic in South Africa. In this population with a high KSHV prevalence, co-infection with KSHV does not appear 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 initiation of ART requires further investigation.

Acknowledgments

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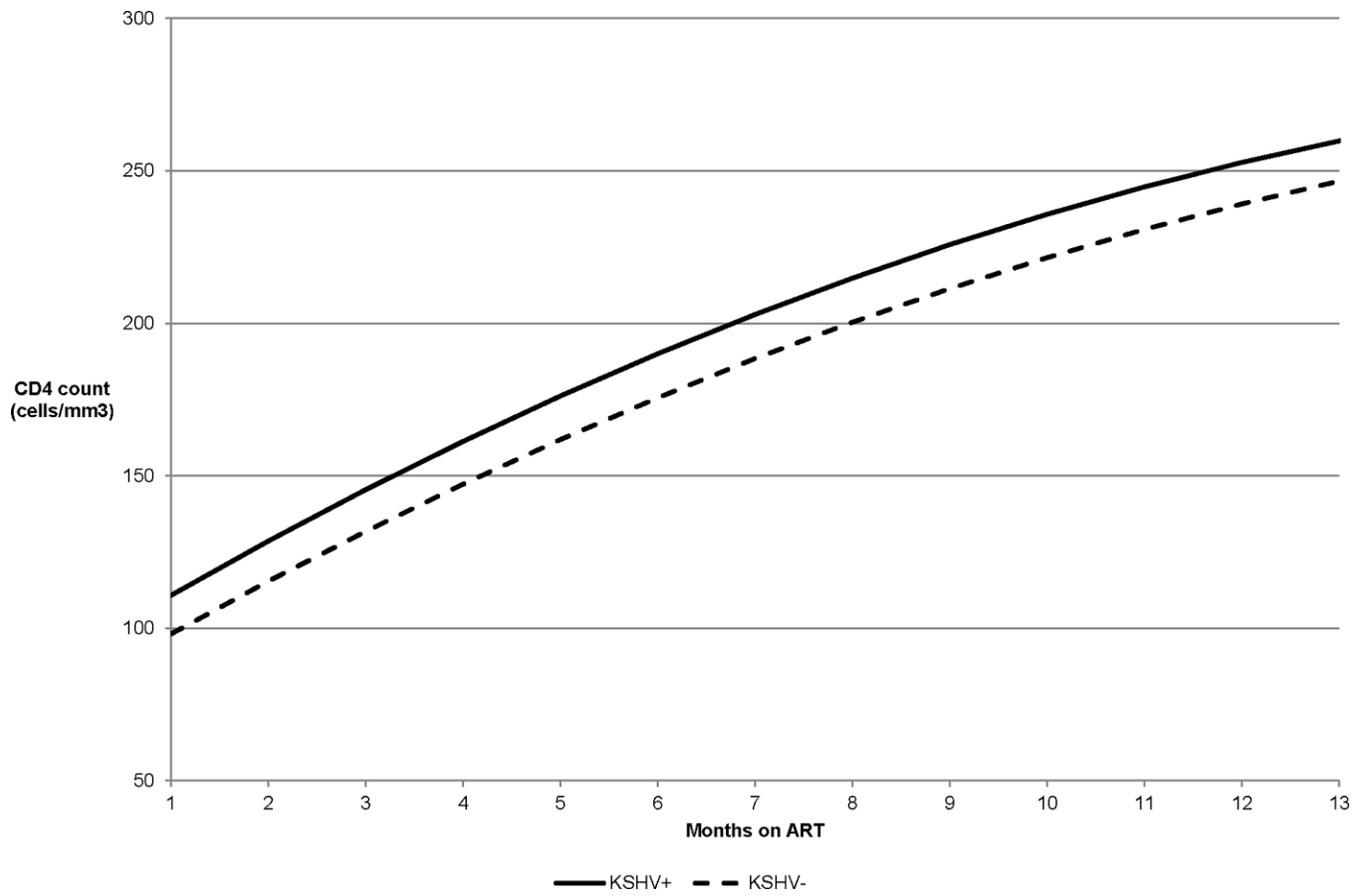
Figure 1.

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/mm3)	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/EFV	332 (87%)	157 (85%)	175 (87%)
(n, %)	d4T/3TC/NVP	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)	18000 (7300–37000)	15500 (7600–33000)	19500 (7300–39000)
Outcomes at 18 months post ART initiation (n, %)	Alive in care	310 (77%)	154 (80%)	
	Died	31 (8%)	13 (7%)	155 (73%)
	LTFU [*]	35 (9%)	14 (7%)	18 (9%)
	Transferred	29 (7%)	12 (6%)	17 (8%)

[#] Characteristics at initiation of ART

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

Table 2

Crude and adjusted difference in mean CD4 count at 6-, 12- and 18-months of follow-up from baseline

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

[£] Mean differences estimated with linear generalized estimating equations adjusted for baseline WHO stage, baseline CD8 count, baseline hemoglobin, baseline body mass index, tuberculosis status at ART initiation, year of ART initiation

Table 3

Virologic Outcomes at 6 and 12-months on ART stratified by KSHV status

Exposure	6-months (n=327)			12-months (n=291)		
	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 (1.00–1.14)	1.03 (0.90–1.17)	6 (6%)	1.02 (0.95–1.10)	1.01 (0.74–1.37)

[†]
Models adjusted for sex, age, CD4 count, CD8 count, TB treatment status, haemoglobin and body mass index at ART initiation

[‡]
VL = viral load, RR = relative risk, CI = confidence interval, relative risk from a log-binomial regression model

[*]
Failure to suppress VL to <400 copies/ml