# The contribution of KSHV to mortality in hospitalized HIV-infected patients being investigated for tuberculosis in South Africa

Running title: Elevated KSHV viral load and mortality

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**Summary**

Given the association of mortality with elevated KSHV viral load in critically ill HIV-infected patients with suspected but not microbiologically confirmed tuberculosis, KSHV viral load and KICS criteria may guide diagnostic evaluation and determine appropriate treatment strategies.

**Footnote Page**

***Conflicts of interest***

The authors state no conflict of interest.

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**Abstract**

***Background:*** Despite increasing numbers of human immunodeficiency virus (HIV)-infected South Africans receiving antiretroviral therapy (ART), tuberculosis remains the leading cause of mortality. Approximately 25% of patients treated for tuberculosis have microbiologically unconfirmed diagnoses. We assessed whether elevated Kaposi's sarcoma-associated Herpes Virus (KSHV) viral load (VL) contributes to mortality in hospitalized HIV-infected patients investigated for tuberculosis.

***Methods:*** 682 HIV-infected patients admitted to Khayelitsha Hospital, South Africa, were recruited, investigated for tuberculosis, and followed for 12-weeks. KSHV-serostatus, peripheral blood KSHV-VL, and KSHV-associated clinical correlates were evaluated.

***Results:*** Median CD4 count was 62 cells/μL (range: 0-526); KSHV-seropositivity was 30.7% (95%CI: 27-34%); 5.8% had detectable KSHV-VL (median 199.1; range: 13.4-2.2x$10^6$ copies/$10^6$ cells); 22% died. Elevated KSHV-VL was associated with mortality (adjusted OR=6.5 [95%CI: 1.3, 32.4]) in patients without tuberculosis or other microbiologically confirmed co-infections (n=159). Six patients had "possible KSHV-inflammatory cytokine syndrome (KICS)": five died, representing significantly worse survival (p<0.0001), and one was diagnosed with KSHV-associated multicentric Castleman disease at autopsy.

**Conclusion:** Given the association of mortality with elevated KSHV-VL in critically ill HIV-infected patients with suspected but not microbiologically confirmed tuberculosis, KSHV-VL and KICS criteria may guide diagnostic and therapeutic evaluation.

**Keywords:** HIV, tuberculosis, Kaposi's sarcoma, Kaposi's sarcoma-associated Herpes Virus, MCD, KICS, mortality, epidemiology, South Africa

## Background

Acquired immunodeficiency syndrome (AIDS)-related deaths have declined from an estimated 1.9 million in 2005 to 1.0 million in 2016, due to global scale-up of antiretroviral therapy (ART). Of those, 730,000 occurred in Sub-Saharan Africa (SSA) [1]. Although ART scale-up has led to a global shift in the proportion of deaths from communicable diseases towards chronic non-communicable conditions [2, 3], in SSA, tuberculosis (TB) remains the leading cause of mortality among human immunodeficiency virus (HIV)-infected individuals, resulting in a third of all AIDS-related deaths [4, 5].

The high burden of suspected TB in South Africa has led to overdiagnosis and overtreatment, and associated delay in diagnosis of cancers such as lymphoma and lung cancer given their overlapping clinical findings [6, 7]. Symptoms of Kaposi's sarcoma-associated herpesvirus (KSHV, or human herpesvirus-8)-associated diseases may also mimic TB. KSHV is the etiological agent of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman disease (MCD), which primarily occur in HIV-infected patients [8-10]. KS is the commonest AIDS-related malignancy worldwide and of particular significance in SSA where KSHV-seroprevalence is elevated. KS incidence in HIV-infected individuals on ART in SSA is estimated to be 286/100,000 person years [11]. In Africa, there were an estimated 32,446 new cases and 17,659 deaths in 2018 [12]. KS often presents with cutaneous disease, but advanced visceral disease with limited or no cutaneous involvement may occur. In contrast, KSHV-MCD is a rare (although most certainly underreported [13]) B-cell lymphoproliferative disorder associated with KSHV lytic activation and interleukin-6 (IL-6) and IL-10 associated inflammatory syndromes. A recently described KSHV-inflammatory cytokine syndrome (KICS) is also associated with KSHV lytic activation and similar cytokine dysregulation. KICS has only been described in two clinicopathologic series of six [14] and ten [15] HIV/KSHV co-infected patients in the US. Most also had KS, and two had PEL, and it has been proposed that KICS contributes to the inflammatory symptoms seen in some patients with severe KS or PEL [15]. KICS in the absence of a KSHV-associated malignancy has also been reported [14, 15].

KSHV has latent and lytic phases characterized by distinct viral gene expression [16, 17]. In KS and PEL, KSHV expresses a limited number of latent phase genes [18, 19], and KS patients generally do not have elevated KSHV viral load (VL) in the blood [14, 20]. In contrast, lytically active KSHV in MCD and KICS expresses a broader range of genes that contribute to pathogenesis [8, 14]. KSHV-VL is elevated in MCD patients [14], and both MCD and KICS are characterized by overproduction of host IL-6, KSHV-encoded vIL-6, and other cytokines, giving rise to inflammatory symptoms such as fever, wasting, hypoalbuminemia, cytopenia, hyponatremia and elevated C-reactive protein (CRP) [14]. Untreated KSHV-MCD and KICS have a high mortality [15]. KSHV-MCD diagnosis requires histologic confirmation, while KICS is a proposed clinical diagnosis requiring exclusion of KSHV-MCD [15] and other serious intercurrent infections. Rituximab is a highly effective therapy for KSHV-MCD, while management of KICS is directed at treating associated malignancies. A working case definition of KICS has been proposed [15] that may serve as a surveillance tool for individuals with HIV/KSHV co-infection who are at high risk of mortality.

Few cases of MCD and no cases of KICS have been reported from SSA despite the high HIV/KSHV prevalence [13, 15, 21]. KS is an independent risk factor for death in HIV-infected people, and a broader range of KSHV-associated diseases with lytic syndromes may play an unrecognized role in HIV-associated morbidity and mortality in SSA. We hypothesized that KSHV may contribute to clinical features and mortality in hospitalized HIV-associated TB-patients in South Africa, and/or be an underrecognized cause of disease in the setting of culture-negative TB.

## Methods

### *Study design*

We conducted a retrospective analysis of an existing hospitalized HIV-associated TB cohort (n=682) in South Africa. The primary objective was to evaluate whether elevated KSHV-VL, defined as >100 copies/106 cells, predicted 12-weeks mortality in the entire cohort, or in a subset who were culture-negative for TB. Secondarily, we evaluated associations of KSHV-VL and serologic assays with clinical features in the cohort, as well as the use of clinical parameters that define KICS to predict mortality. Due to the retrospective nature of this study, no prospective sample size calculation was performed.

### *Study cohort*

HIV-infected adults presenting with clinical syndromes compatible with pulmonary or extrapulmonary TB were recruited at Khayelitsha Hospital, Cape Town, South Africa from January 2014 to October 2016 in the context of a study entitled “Defining interventions to reduce mortality in severe HIV-associated tuberculosis” (UCT HREC/REF: 057/2013). Emergency room and medical ward patients were screened, eligible patients enrolled and written consent obtained. Eligible patients with a depressed level of consciousness were enrolled and followed up daily until they regained capacity to consent. If a patient died prior to providing consent, we obtained approval from UCT HREC to use the patient’s data.

Clinical details, including physical exam with evaluation of skin and oral mucosa, and samples were collected at enrolment. CD4 count, HIV-VL, CRP, full blood and differential count and renal and liver function tests were performed by the National Health Laboratory Services, as well as serum cryptococcal antigen lateral flow assays (IMMY CrAG LFA). Citrate whole blood and plasma was stored at -80°C for KSHV-VL and immunology assays. The standardised TB diagnostic work-up included sputum induction if required. TB blood culture in Myco/Flytic bottles (Becton Dickinson Biosciences), sputum Xpert MTB/RIF assay, sputum TB culture, urine lipoarabinomannan (LAM) and urine Xpert MTB/RIF on concentrated urine were performed during enrolment. Bacterial blood cultures were

performed in all patients who had not received intravenous antibiotics prior to presentation to hospital. Patients were followed for 12-weeks to ascertain vital status.

***Definition of patient groups***

Patients were grouped into four overlapping categories based on the presence or absence of microbiologically confirmed infections. Group 1 (n=675) consisted of the total patient cohort analysed; Group 2 (n=500) included all microbiologically confirmed TB-patients (*Mycobacterium tuberculosis* on culture or GeneXpert on any clinical sample or urine LAM-positive); Group 3 (n=175) included the remainder of the total patient cohort without microbiologically confirmed TB; Group 4 (n=159) consisted of Group 3 patients without another microbiologically confirmed infection (e.g. bacterial blood stream infection or *Cryptococcus spec.*), although this group included some patients who were treated for TB despite negative microbiology. These groups are not mutually exclusive.

***Definition of "possible KICS"***

We evaluated Group 4 patients for KICS. The working case definition of KICS requires at least two clinical manifestations from at least two of three categories [15]: A. Symptoms (including fever, fatigue, oedema, cachexia, respiratory symptoms, gastrointestinal disturbance, arthralgia and myalgia, altered mental state and neuropathy); B. Laboratory abnormalities (anaemia, thrombocytopenia, hypoalbuminemia and hyponatremia); and C. Radiographic abnormalities (lymphadenopathy, splenomegaly, hepatomegaly and body cavity effusions), together with evidence of systemic inflammation (elevated CRP [>10mg/L]), evidence of KSHV lytic activity (elevated (>100 copies/$10^6$ cells) KSHV-VL in peripheral blood) and exclusion of MCD. As this analysis was done retrospectively, MCD could not be excluded for all patients, hence the designation, "possible KICS" patients.

***KSHV and IL-6 assays***

KSHV assays were performed for all patients. Cryopreserved plasma was tested by ELISAs for antibodies against latency associated nuclear antigen (LANA, ORF73) and a lytic structural glycoprotein (K8.1), following established specifications [22]. Participants were considered KSHV-seropositive if antibodies to either antigen were detected [22]. Plasma IL-6 was measured using the Human IL-6 SimpleStep ELISA kit (Abcam), with a minimum detectable dose of 1.6pg/mL (the reference median for IL-6 in well HIV-positive patients being 1.80pg/mL (IQR 1.20-2.89) [23]).

DNA was extracted from peripheral blood mononuclear cells (PBMCs) with plasma removed using the QIAamp DNA Blood Mini kit (Qiagen). DNA concentration was adjusted to 25ng/µL, with 10µL used per PCR reaction (total volume: 50µL) to detect KSHV DNA using 100pmole K6 gene region forward and reverse primers, 5pmole FAM/TAMRA labelled probe [24] and 2X Universal Master Mix (Applied Biosystems). KSHV DNA was quantified against a K6-plasmid standard curve on a LightCycler®480II System (Roche) as follows: 2min 50°C; 8min 95°C; 45 cycles: 15sec 95°C; 1min 60°C. Cellular equivalents were determined using a quantitative assay for human endogenous retrovirus 3 (ERV-3) [25]. Samples were tested in triplicate, averaged and reported as viral DNA copies per million cells.

***Post-mortem histology***

After obtaining consent from the family, an excisional cervical lymph node biopsy was performed 2 days post-mortem on a patient with possible KICS. Tissue was fixed in 10% formal saline for 48h, processed overnight in a Tissue-Tek Vacuum Infiltration Processor (Sakura Finetek) and embedded in paraffin. Tissue sections were cut at 4µm thickness and stained with haematoxylin-and-eosin and ORF73 immunoperoxidase (Cell Marque) and the Benchmark XT automated staining platform with the Ventana ultraView Universal DAB Detection kit (Roche Diagnostics). Immunostaining for kappa and lambda light chains was also performed. Photomicrographs were obtained with an Olympus SC30 3.3 megapixel USB digital colour camera attached to an Olympus BX41 microscope using analySIS getIT 5.1 digital imaging software (Olympus Soft Imaging Solutions).

***Statistical analysis***

KSHV-VL was treated both as a categorical variable (elevated >100 copies/$10^6$ cells versus ≤100 copies/$10^6$ cells or non-detectable) and a continuous variable and assessed for association with mortality using the Chi-square, Fisher exact, or Wilcoxon rank sum tests, as appropriate. The relationship between KSHV-VL and mortality was assessed by binomial logistic regression, controlling for age, sex, CD4 count and ART status. Linearity of the continuous variables with respect to the logit of the dependent variable was confirmed via the Box-Tidwell procedure [26] and studentized residuals with values less than 2.5 standard deviations were accepted.

To compare "possible KICS" patients to the remainder of the cohort, associations of categorical variables (sex, receiving ART, KSHV-seropositivity, presence of skin KS) and continuous variables (age, weight, HIV-VL, CD4 count, KSHV-VL, K8.1 OD, ORF73 OD, IL-6, CRP, haemoglobin, white cell count, platelet count, albumin, sodium) were assessed by Fisher exact or Wilcoxon rank sum tests, respectively. To assess the independent associations of KSHV-seropositivity or KSHV-antibody levels (OD) with mortality, binomial logistic regression or multiple linear regression was performed, respectively. Continuous variables were transformed, where appropriate, to approximate normal distributions. Survival analysis was performed using Kaplan-Meier method and log-rank sum test. P-values are two-tailed and considered significant if <0.05. Statistical testing was performed using SPSS version 25 (IBM Corp, 2017). Performance characteristics of KICS criteria for predicting death were calculated in R using a confusion matrix.

## Results

### *Assessment of clinical parameters*

Of all the patients recruited (n=682), 7 were excluded (2 withdrew, 1 was HIV-negative, 4 had no blood samples stored). 675 are included in this analysis. 12/675 (1.8%) were lost to follow-up, and 146/675 (22%) were confirmed dead by 12-weeks follow-up. Median CD4 count was 62 cells/µL (range: 0-526 cells/µL) (Table 1). 10 patients had a clinical diagnosis of cutaneous or oral KS at enrolment (4 in Group 2, 6 in Group 3, of which 5 were in Group 4). 207/675 patients (30.7%, 95%CI: 27-34%) were KSHV-seropositive, of whom 39 (5.8%) showed detectable VL in the blood (median 199.1; range: 13.4-$2.2x10^6$ copies/$10^6$ cells) (Table 1). Plasma IL-6 was detected in 559 (82.8%) of all patients with a median concentration of 42.3pg/mL (IQR: 9.8-103.8pg/mL), being highly elevated compared to a reference population of HIV-positive patients [23]. In binomial logistic regression including age, sex, CD4 count, haemoglobin levels and ART status, only CD4 count was significantly associated with KSHV-seropositivity (p=0.001, adjusted OR=1.2 (95%CI: 1.1, 1.3), Supplementary Table 1A) whereas sex (male) was associated with elevated KSHV-VL (p=0.029, adjusted OR=2.4 (95%CI: 1.1, 5.1, Supplementary Table 1B) and defaulted ART status was associated with lower KSHV-VL (p=0.042, adjusted OR=0.3 (95%CI: 0.1, 1.0, Supplementary Table 1B). Anaemia was associated with higher K8.1 antibody levels (p=0.022, unstandardized coefficient=-0.033) when adjusted for age, sex, CD4 count and ART status, but not with anti-ORF73 titers (Supplementary Table 2).

### *Elevated KSHV-VL is associated with mortality in microbiologically unconfirmed TB-patients*

Group 1 (n=675) was assessed for an association between elevated KSHV-VL (i.e. >100 copies/$10^6$ cells) and 12-weeks mortality. We identified 33 patients with elevated KSHV-VL of whom 9 (27.3%) died before 12-weeks, compared to 137 (21.7%) of 630 patients with VL ≤100 copies/$10^6$ cells. This difference was not statistically significant (Table 2A, Figure 1). Proven TB-patients (n=500, Group 2) also showed no significant association of KSHV-VL with mortality (Table 2A, Figure 1). However, in patients without proven TB (n=175, Group 3) and particularly in patients without proven TB or other

196 co-infections (n=159, Group 4), elevated KSHV-VL was detected at a higher frequency (p=0.011,
197 OR=7.1, 95%CI: 1.6, 31.7, Table 2A). Importantly, KSHV-VL was significantly higher in patients who died
198 than those who survived 12-weeks (p=0.0094, Figure 1), and overall 5/8 (62.5%) of Group 4 patients
199 with elevated KSHV-VL died, compared to 28/148 (18.9%) with low or non-detectable KSHV-VL.
200 Binomial logistic regression revealed a statistically significant association of age, CD4 count and
201 elevated KSHV-VL with death among Group 4 patients (Table 2B). The adjusted OR for death given
202 elevated KSHV-VL was 6.5 (95%CI: 1.3, 32.4). No significant relationship between KSHV-seropositivity
203 and mortality was noted (data not shown).
204
205 ***Identification and contribution of possible KICS to mortality***
206 We next evaluated Group 4 for KICS since TB and other microbiologically proven infections as alternate
207 cause of clinical presentation had already been excluded in this group as per KICS definition [15]. We
208 identified 6 "possible KICS" patients with the caveat of not excluding MCD at clinical presentation
209 (Figure 2A). Compared to others in Group 4, "possible KICS" subjects were older (Table 3A), had lower
210 platelet counts (Table 3B, Figure 2B), higher K8.1 and ORF73 antibody levels (Table 3B) and, by
211 definition, elevated KSHV-VL (Table 3B, Figure 2B). HIV-VL and CD4 counts did not differ significantly
212 between "possible KICS" and the remainder of Group 4 patients (Table 3A); neither did IL-6 and CRP
213 although being markedly elevated (Table 3B, Figure 2B), nor did haemoglobin and albumin levels
214 although being abnormally low [15] (Table 3B, Figure 2B).
215 Finally, 12-weeks mortality among "possible KICS" patients was 83% (5/6) and thereby significantly
216 higher compared to 18% for the remainder of the Group 4 patients. Median time to death in "possible
217 KICS" patients was 11 days (95%CI: 0, 51) (Figure 2C), supporting previous reports of markedly
218 elevated risk of death in KICS subjects [15]. KICS criteria identified all patients with elevated KSHV-VL
219 in Group 4 (Table 2A) who died within 12-weeks. Moreover, KICS criteria were found to be specific for
220 predicting death in this cohort, with the following characteristics (95%CI): sensitivity 0.15, specificity
221 0.99, positive predictive value 0.83, negative predictive value 0.81.

***Post-hoc description of the identified "possible KICS" patients***

Of the six "possible KICS" patients, one was diagnosed with KSHV-associated MCD at autopsy. This patient displayed the highest KSHV-VL of the entire cohort (2,165,642 copies/106 cells) and was positive for K8.1 but not ORF73, suggesting a highly lytically active KSHV-infection. In the absence of any clinical measure of KICS or KSHV on presentation, this patient was empirically treated for TB for six months with no improvement prior to admission. He presented with further deterioration after completion of TB-treatment and died on the day of enrolment. His CD4 count was 328 cells/μL, and HIV-VL was undetectable. The patient had evidence of systemic inflammation (CRP=304mg/L) and cytokine activation (IL-6=3307pg/mL), as well as severe anaemia (haemoglobin=6.9g/dL), thrombocytopenia (platelet count=124(x$10^3$/μL)), hypoalbuminemia (albumin=16g/L), hyponatremia (sodium=125mEq/L), lymphadenopathy and hepatomegaly. KICS-associated symptoms included respiratory symptoms (cough), weight loss, nausea, body pain and weakness. Histologic examination of lymph nodes was consistent with KSHV-associated MCD (Figure 2D). There was no evidence of PEL. Two other "possible KICS" patients' deaths were retrospectively likely attributable to KICS in the setting of KS [15]. One had biopsy-confirmed KS, was re-started on ART but died before assessment for chemotherapy. One patient, after deterioration on empiric anti-tuberculosis therapy, had skin KS confirmed on biopsy and features of lung KS on computed tomography scan 2 weeks prior to death. The other "possible KICS" patients did not have identified KSHV-associated malignancies despite elevated VL assessed retrospectively. One was started on TB-treatment empirically, deteriorated on treatment and was diagnosed with an invasive keratinizing moderately differentiated squamous cell carcinoma during evaluation of an upper gastrointestinal bleed. The other patient suffered from chronic renal failure due to urethral stricture and was admitted with an episode of acute kidney injury. The patient was treated for suspected bacterial sepsis but died within the study period. The sixth patient was treated for suspected bacterial meningitis, improved and survived the follow-up period.

## Discussion

South Africa has one of the highest global rates of both HIV and TB [27]. Improved diagnostics for treatable diseases that mimic TB are needed to limit unnecessary empiric TB-treatment. Utilizing a large well characterized patient cohort presenting to a Cape Town hospital with suspected TB, we retrospectively evaluated KSHV as a contributor to mortality.

KSHV-seroprevalence is high in SSA with variable regional prevalence [28]. We found 30.7% (95%CI: 27-34%) KSHV-seroprevalence in Cape Town, which is in agreement with other South African estimates of 30-40% from Soweto, Johannesburg and Kwa-Zulu Natal. Of KSHV-seropositive patients, 18.8% (5.8% of the entire cohort) showed detectable virus in the blood, suggestive of poor immune control of KSHV [29].

Focusing on the 33 (5%) patients with elevated (>100 copies/$10^6$ cells) KSHV-VL, we found no higher mortality within the context of either the entire patient cohort or the cohort with confirmed TB, suggesting that KSHV does not play a significant role in potentiating TB mortality. However, in patients with neither microbiologically proven TB nor alternative co-infection, elevated PBMC-associated KSHV-VL was associated with 6.5 higher odds of mortality when adjusted for age, sex, CD4 count and ART status. In contrast, KSHV-seropositivity alone was not associated with mortality (data not shown), suggesting that it is the burden of KSHV that contributes to the observed association [8, 14]. Similarly, elevated plasma KSHV-VL, a marker of circulating tumour DNA, has been noted as a risk factor for death in people with established KS [30]. Although a strong association between elevated KSHV-VL and mortality among microbiologically unconfirmed TB-patients was identified, additional factors such as KSHV-associated malignancies, functional immune dysregulation (cytokine syndromes) or other pathological processes (e.g. co-infections or other cancers) likely also contribute to death.

We further investigated whether application of KICS criteria [15] identified patients with a high mortality and found six “possible KICS” patients, of whom five died, with a median survival of 11 days. Three had identified untreated KSHV-associated malignancies. Although “possible KICS” patients had elevated IL-6 and CRP, these were not distinguishing features compared to other patients with TB or

other critical illnesses. This suggests that CRP is a less useful screening tool than KSHV-VL in this population.

To our knowledge, this is the first systematic evaluation of KICS in South Africa and has implications for other countries with high prevalence of HIV/KSHV co-infection. A study from Uganda reports that three in every ten patients with HIV-associated lymphoma had a possible misdiagnosis and were treated for TB before a final diagnosis of lymphoma was made, and our data suggest that KSHV-associated diseases are important to include in the differential diagnosis of suspected TB [6].

A limitation of our study was that only one of the six “possible KICS” patients had a pathological examination of a lymph node biopsy. This was performed following death and demonstrated KSHV-MCD. Another had possible pulmonary KS diagnosed based on chest X-ray and confirmed cutaneous KS. It is possible that the other patients also had undiagnosed KSHV-associated diseases such as KS or MCD. Although we have not definitively established that “possible KICS” patients died of KSHV-associated malignancies in most cases, our study demonstrates that elevated KSHV-VL in the peripheral blood represents a significant parameter associated with mortality. Our data support that patients meeting KICS criteria should be evaluated for KSHV-MCD, visceral KS, or PEL, particularly in settings with oncology capacity to manage these treatable KSHV-associated malignancies.

Another important finding was that the majority of the entire patient cohort was anaemic, which was significantly associated with elevated antibody levels to the KSHV lytic antigen K8.1 in KSHV-seropositive Group 4 patients. This is consistent with results from a recent study from Uganda, which reported a link between elevated KSHV-serumpositivity and anaemia in the setting of malaria. Additional studies are required to evaluate this association. For example, anaemia may lead to reactivation of KSHV through relative tissue hypoxia [31], or KSHV reactivation may lead to anaemia or chronic inflammation mediated through IL-6.

In sum, our data suggest that elevated KSHV-VL should be considered as an important pathology in HIV-infected patients investigated for TB, and that for those meeting other KICS criteria evaluation for KSHV-VL should be considered. Increasing implementation of PCR-based TB diagnostics should

298 facilitate more rapid TB diagnostic work-up, thereby facilitating selection of patients for whom KSHV-
299 testing may be indicated. In selected patients, KSHV-VL has a strong prognostic value. KSHV-VL has
300 been linked to an increased risk of KSHV-associated malignancies [32]; therefore, HIV-positive patients
301 with elevated KSHV-VL should be evaluated for KSHV-related malignancies and treated appropriately.
302 KSHV-associated malignancies are treatable, and earlier diagnosis may improve survival. Given its high
303 mortality, evaluation of therapeutic strategies for KICS and KICS-like syndromes are urgently needed.

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409 **Figure Legends**

410

411 **Figure 1: 12-weeks mortality versus KSHV viral load** in the entire patient cohort (Group 1, n=675);
412 patients with proven TB (Group 2, n=500); patients without proven TB (Group 3, n=175); patients
413 without microbiologically proven co-infections (Group 4, n=159). 12 patients of the total cohort were
414 lost to follow-up. P value is by Wilcoxon rank sum test, assessing the association of the level of KSHV-
415 VL (continuous variable) with mortality of patients with confirmed vital status at the end of the 12-
416 weeks study period. Data are log transformed. The dotted line indicates "elevated KSHV viral load"
417 >100 copies/106 cells, and the solid lines indicate the median.

418

419 **Figure 2: Identification and contribution of possible KICS to mortality in a cohort of critically ill**
420 **patients investigated for TB. A)** Schematic flow chart showing the diagnosis of "possible KICS" by
421 exclusion in the entire cohort. Patients were excluded if they had microbiologically proven TB or other
422 bacterial or fungal infections and those who remained were further evaluated according to the criteria
423 previously described in the KICS working case definition [15]. 2 patients were excluded on the basis of
424 alternative diagnoses: TB meningitis and community-acquired pneumonia, respectively. As this
425 analysis was done retrospectively, MCD could not be excluded at clinical presentation, hence the
426 designation, "possible KICS" patients. **B)** Selected KICS-defining parameters (KSHV viral load, IL-6 level,
427 CRP level, platelet count, albumin, haemoglobin) in "possible KICS" patients (n=6) compared to the
428 remainder of Group 4 patients (n=153). The dotted lines mark abnormal levels (KSHV-VL>100
429 copies/$10^6$ cells; IL-6>1.8pg/mL [23]; CRP>10mg/L, platelet count<186(x$10^3$/µL), albumin<35g/L and
430 haemoglobin<12g/dL), and the solid lines indicate the median. P values are by Wilcoxon rank sum test.
431 Data are log transformed where necessary. C) Overall confirmed survival at end of the 12-weeks study
432 period in "possible KICS" patients (n=6) compared to the remainder of Group 4 patients (n=153,
433 including 3 patients who were lost to follow-up). P value is by log-rank test. **D)** Histopathological
434 assessment of post-mortem lymph node biopsies taken from a "possible KICS" patient with the highest

435 KSHV-VL of the entire patient cohort. Top panel, from left to right: haematoxylin and eosin stain
436 showing a regressed germinal center with sheets of plasma cells in the mantle zone among prominent
437 capillaries, (20x objective magnification); haematoxylin and eosin stain showing an infiltrate of
438 numerous benign plasma cells (40x objective magnification); immunohistochemical stain of KSHV-
439 ORF73 showing aggregates of KSHV-positive cells in the lymph node staining brown (10x objective
440 magnification). Bottom panel, from left to right: immunohistochemical stain showing brown granular
441 nuclear KSHV-ORF73 positivity among the numerous background plasma cells (40x objective
442 magnification); immunohistochemistry for kappa light chains demonstrate few of the plasma cells in
443 an area of ORF73-positive cells are kappa restricted (40x objective magnification);
444 immunohistochemistry of lambda light chains in the area of ORF73-positive cells demonstrate a large
445 number of the plasma cells are lambda restricted cells (40x objective magnification).