# KSHV-associated Mortality in HIV-infected South Africans with Suspected Tuberculosis

Melissa J Blumenthal[1,2], Charlotte Schutz[2,3,4], David Barr[4,5], Michael Locketz[6], Vickie Marshall[7], Denise Whitby[7], Arieh A Katz[1,2], Thomas Uldrick[8], Graeme Meintjes[2,3,4] and Georgia Schäfer[1,2]

[1]Division of Medical Biochemistry and Structural Biology, Department of Integrative Biomedical Sciences, University of Cape Town, South Africa; [2]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa; [3]Department of Medicine, University of Cape Town, South Africa; [4]Wellcome Centre for Infectious Diseases Research in Africa, University of Cape Town, South Africa; [5]Wellcome Trust Liverpool Glasgow Centre for Global Health Research, Institute of Infection and Global Health, University of Liverpool, Liverpool, UK; [6]Division of Anatomical Pathology, National Health Laboratory Service, University of Cape Town, South Africa; [7]Viral Oncology Section, AIDS and Cancer Virus Program, Leidos Biomedical Research, Frederick National Laboratory for Cancer Research, NIH, USA; [8]Fred Hutchison Cancer Research Center, USA.

## Introduction

The oncogenic Kaposi's Sarcoma-associated Herpes Virus (KSHV) is the etiological agent of Kaposi's Sarcoma (KS), Multicentric Castelman's disease (MCD), Primary Effusion Lymphoma and the newly described KSHV inflammatory cytokine syndrome (KICS) [1,2]. KSHV is transmitted via saliva mainly during early childhood and some studies indicate KSHV prevalence to be >50% in South Africa [3,4]. KICS, associated with elevated KSHV lytic activity as well as systemic inflammation and elevated Interleukin-6 (IL-6) levels, presents with a range of severe symptoms (including, among others, gastrointestinal disturbances, respiratory symptoms, effusions and adenopathy) and laboratory abnormalities (namely, anaemia, thrombocytopenia, hypo-albuminemia and hyponatremia) and has a high rate of mortality [1,2]. KICS mimics tuberculosis (TB), which, despite increasing numbers of human immunodeficiency virus (HIV)-infected South Africans receiving antiretroviral therapy (ART), remains the leading cause of mortality [5]. Approximately 25% of patients treated for TB have microbiologically unconfirmed diagnoses. We hypothesize that KSHV-driven KICS has a currently unrecognized contribution to mortality in patients who present with a suspected diagnosis of TB. Although KSHV and HIV coinfection are known to be particularly prevalent in sub-Saharan Africa, the contribution of KSHV and KICS to patient mortality has not been studied in this context yet. This study is an important first step in describing the clinical spectrum and epidemiology of KSHV infection and KICS in the South African context. Based on these data, including tests for KSHV lytic reactivation in the diagnostic work-up of hospitalised HIV-infected patients (once TB has been ruled out) should be considered.

## Aim

To assess the contribution of KSHV and KICS to mortality in patients presenting with a suspected diagnosis of TB in South Africa.

## Methods

- Study cohort: Patients recruited on presentation at Khayelitsha Day Hospital, Cape Town, South Africa with suspected diagnosis of TB and followed for a 12 week period.
- KSHV seroprevalence: ELISA to detect antibodies to KSHV antigens K8.1 or ORF73 in patient plasma.
- KSHV viral load: Quantitative TaqMan PCR to K6, normalized to ERV-3, using DNA extracted from peripheral blood cells.
- IL-6 level: Abcam Human IL-6 SimpleStep ELISA using patient plasma.
- KICS definition: Working case definition was applied (see Figure 1).

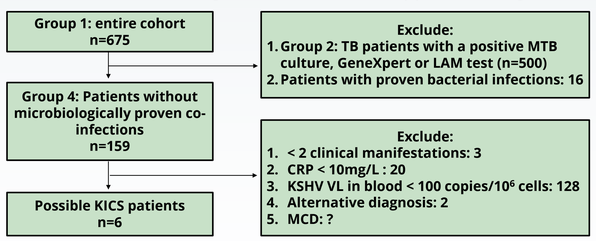



**Figure 1: A schematic showing the definition by exclusion of the study subsets: Non-TB, non-bacterial infection patients and "Possible KICS" patients.** All recruited patients were over 18 years of age, were South African, were HIV+ and presented with a suspected diagnosis of TB. Study subsets were defined by the exclusions shown in the blocks on the right. Abbreviations: LAM, lipoarabinomannan; VL, viral load.

## Results

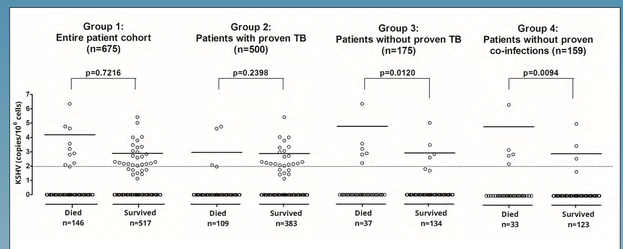



**Figure 2: 12-weeks mortality versus KSHV viral load** in the entire patient cohort (Group 1, n=675); patients with proven TB (Group 2, n=500); patients without proven TB (Group 3, n=175); patients without microbiologically proven co-infections (Group 4, n=159). P value is by Wilcoxon rank sum test. Data are log transformed. The dotted line indicates "elevated KSHV viral load" >100 copies/$10^6$ cells.

**Table 1: Logistic regression of factors assessed for association with mortality based on elevated KSHV VL among Group 4 patients (n=159)**

| | Unadjusted Odds Ratio | 95% CI for Unadjusted OR Lower | Upper | Adjusted Odds Ratio | 95% CI for Adjusted OR Lower | Upper | P |
|---|---|---|---|---|---|---|---|
| Elevated KSHV VL[1] | 7.143 | 1.611 | 31.671 | 6.467 | 1.290 | 32.406 | 0.023 |
| Sex[2] | 0.949 | 0.433 | 2.082 | 0.477 | 0.187 | 1.214 | 0.120 |
| Age[3] | 1.051 | 1.014 | 1.088 | 1.067 | 1.022 | 1.113 | 0.003 |
| CD4 count[4] | 0.892 | 0.742 | 1.072 | 0.777 | 0.619 | 0.975 | 0.029 |
| ART status[5] | | | | | | | 0.980 |
| Defaulted | 0.790 | 0.285 | 2.190 | 1.067 | 0.346 | 3.290 | 0.910 |
| on ART | 0.967 | 0.395 | 2.368 | 1.105 | 0.417 | 2.930 | 0.840 |

[1]KSHV VL is for elevated (>100 copies/$10^6$ cells) compared to non-elevated (<100 copies/$10^6$ cells or non-detectable); [2]Sex is for males compared to females; [3]Age is per 1 year increase; [4]CD4 count is per 50 cell increase; [5]For ART status, naïve is the reference category.

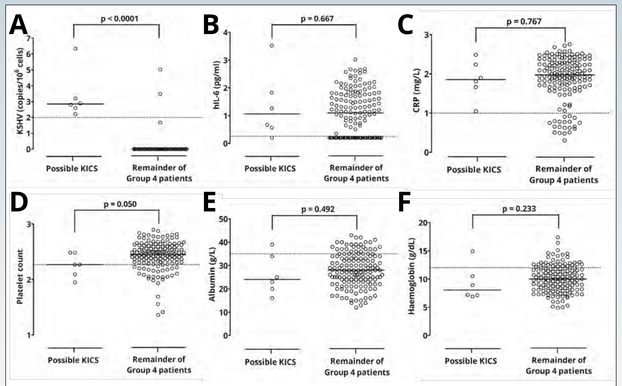



**Figure 3: Selected KICS-defining parameters in "possible KICS" patients**. A) KSHV viral load, B) IL-6 levels, C) CRP levels, D) platelet count, E) albumin and F) haemoglobin in "possible KICS" patients (n=6) compared to the remainder of Group 4 patients (n=153). The dotted lines mark abnormal levels (KSHV VL > 100 copies/$10^6$ cells; IL-6 > 1.8pg/ml [6]; CRP > 10mg/l, platelet count < 186, albumin < 35g/L and haemoglobin < 12g/dL). P values are by Wilcoxon rank sum test. Data are log transformed where necessary.

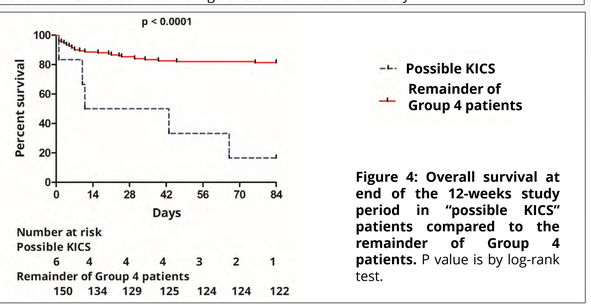



**Figure 4: Overall survival at end of the 12-weeks study period in "possible KICS" patients compared to the remainder of Group 4 patients.** P value is by log-rank test.

**Table 2: Baseline characteristics of KICS subjects (n=6) and all other Group 4 patients (n=153).** Mortality, sex, ART, KSHV seropositivity and KS diagnosis are given as count and percentage; other characteristics are median and range.

| | Possible KICS subjects (n=6) | Remainder of patients without microbiologically proven co-infection (n=153) | P value |
|---|---|---|---|
| Died | 5 (83.3%) | 28 (18.3%) | 0.002 |
| Males | 4 (67%) | 61 (39.9%) | 0.227 |
| Age (years) | 47 (26–74) | 38 (19–81) | 0.049 |
| Weight (kg) | 58 (35–71) | 53.0 (30–99) | 0.912 |
| Receiving ART | 3 (50%) | 63 (41.2%) | 0.290 |
| HIV VL (copies/mL) | 81 298 (29–3 507 840) | 70 363 (29–7 934 692) | 0.978 |
| CD4 (cells/µl) | 106.5 (10–328) | 97 (2–519) | 0.818 |
| KSHV seropositive | 6 (100%) | 55 (35.9%) | 0.003 |
| K8.1 OD | 2.1 (1.3–2.3) | 0.48 (0.10–2.57) | 0.001 |
| ORF73 OD | 3.3 (0.32–7.4) | 0.24 (0.03–8.14) | 0.003 |
| Skin KS | 1 (16.7%) | 4 (2.6%) | 0.177 |
| White cell count | 7.6 (3.5–21.6) | 6.8 (1.0–43.2) | 0.783 |
| Sodium (mEq/L) | 132 (125–138) | 131 (113–150) | 0.692 |

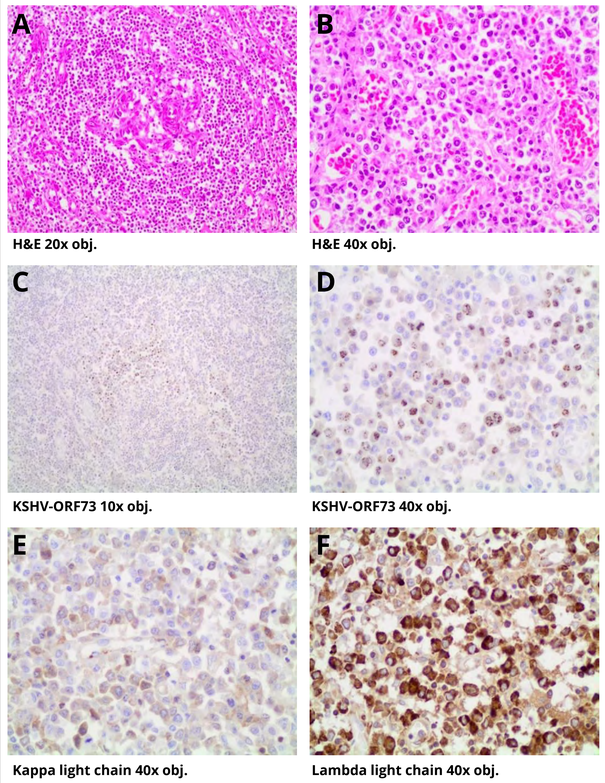



**Figure 5: Histopathological assessment of post-mortem lymph node biopsies taken from a "possible KICS" patient with the highest KSHV VL of the entire patient cohort.** A) Haematoxylin and eosin stain showing a regressed germinal center with sheets of plasma cells in the mantle zone among prominent capillaries. B) Haematoxylin and eosin (H&E) stain showing an infiltrate of numerous benign plasma cells. C) Immunohistochemical stain of KSHV-ORF73 showing aggregates of KSHV-positive cells in the lymph node staining brown. D) Immunohistochemical stain showing brown granular nuclear KSHV-ORF73 positivity among the numerous background plasma cells. E) Immunohistochemistry for kappa light chains demonstrate few of the plasma cells in an area of ORF73-positive cells are kappa restricted; F) immunohistochemistry of lambda light chains demonstrate a large number of the plasma cells are lambda restricted.

## Discussion and Conclusion

With one of the highest rates of both HIV and TB, improved diagnostics for treatable diseases that mimic TB are needed in South Africa to limit unnecessary empiric TB treatment. Utilizing a large well characterized cohort of patients presenting to a Cape Town hospital with suspected TB, we systematically evaluated KSHV as a contributor to mortality. We found a KSHV seroprevalence of 30.7% (95% CI: 27–34%) in Cape Town, which is in agreement with other studies out of South Africa. Focusing on the 33 (5%) patients with elevated (> 100 copies/$10^6$ cells) KSHV VL, we found that they did not have 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 KSHV VL was associated with a 6.5 higher odds of mortality when adjusted for age, sex, CD4 count and ART status. We further investigated whether application of KICS criteria identified patients with a high mortality and identified six "possible KICS" patients, of whom five died, with a median survival of 11 days. While the "possible KICS" patients had elevated IL-6 and CRP, these were not distinguishing features compared to other patients with TB or other critical illnesses, suggesting that CRP is a less useful screening tool than KSHV VL in this population. 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. Nonetheless, our data suggest that lytic reactivation of KSHV 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. KSHV VL has been linked to an increased risk of KSHV-associated malignancies; therefore, HIV-positive patients with elevated KSHV VL should be evaluated for KSHV-related malignancies and treated appropriately. KSHV associated malignancies are treatable and earlier diagnosis may improve survival. Given its high mortality, evaluation of therapeutic strategies for KICS and KICS-like syndromes are urgently needed.

## Acknowledgments

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