Saliva Kaposi Sarcoma Herpesvirus Levels as a Diagnostic Marker of Visceral Kaposi Sarcoma

Matthew Witterholt[1], Tishiya Carey[1], Kathryn Lurain[1], Ralph Mangusan[1], Anaida Widell[1], Irene Ekwede[1], Vickie Marshall[2], Nazzarena Labo[2], Kyle Moore[2], Wendell Miley[2], Romin Roshan[2], Elena M Cornejo Castro[2], Denise Whitby[2], Robert Yarchoan[1], Ramya Ramaswami[1]

[1] HIV and AIDS Malignancy Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA [2] Viral Oncology Section, Frederick National Cancer Laboratory, Frederick, MD, USA

BACKGROUND

- Kaposi sarcoma (KS) is an HIV-associated malignancy caused by Kaposi sarcoma herpesvirus (KSHV), also known as human herpesvirus-8 (HHV8).
- Early stage T0 of KS involves the skin, while advanced T1 stage results in visceral KS involvement of the gastrointestinal and/or respiratory tracts.
- Despite the known efficacy of bronchoscopies and endoscopies for the diagnosis of visceral KS, these methods are costly and not readily available in low-resource settings.
- KSHV viral load (VL) levels may act as a cost-effective biomarker for the progression of visceral KS. Among patients with suspected visceral KS involvement, we evaluated whether saliva KSHV VL measurement may be helpful as a diagnostic marker to indicate a diagnosis of visceral KS.

We present findings from a retrospective analysis of patients with KS

METHODS

- Patients on Standard of Care (NCT00923065) and Tissue Procurement (NCT00182858) between 2005-2023 were evaluated. All patients with KS or suspected KAD had a diagnostic bronchoscopy and or endoscopy/ colonoscopy due to symptoms concerning pulmonary and/or GI KS (**Fig 1**).
- Blood and saliva samples were collected within a week of a patient's diagnostic procedures. Peripheral blood mononuclear cells (PBMCs) and saliva measurements of KSHV, CD4+ T cell count, HIV VL, hemoglobin, platelets, albumin, and C-reactive protein levels were evaluated.

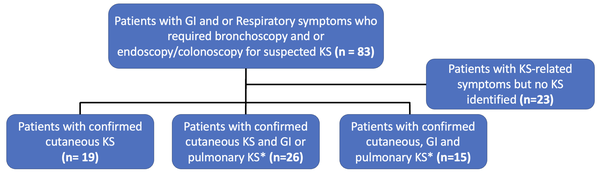


Figure 1 – Consort diagram highlighting the patients by group skin and GI KS confirmed by biopsy; *pulmonary KS was confirmed by visualization and imaging findings only.

Statistical Analysis Baseline characteristics were analyzed using Wilcoxon Rank-Sum tests. Receiver operator curves (ROCs) observed sensitivity and specificity of KSHV saliva VL to diagnosis visceral KS among symptomatic pts. For all participants with visceral disease, Kaplan-Meier (KM) method evaluated overall survival from date of KS diagnoses to death or last follow-up before September 1st, 2023.

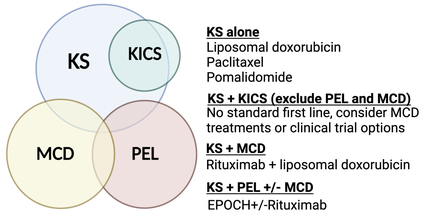


Figure 2 – The overlap of KSHV-associated diseases and treatments administered.

In the 21st century, severe visceral involvement of Kaposi sarcoma persists among people with HIV in the United States that impacts survival outcomes

	All KS (N= 60)	Cutaneous KS only (N= 19)	GI or Pulmonary KS (N= 26)	GI and Pulmonary KS (N= 15)
Cisgender Male, n (%)	59 (98.3)	19 (100)	25 (96.2)	15 (100)
HIV positive, n (%)	57 (95)	18 (94.7)	25 (96.2)	14 (93.3)
Concurrent cutaneous KS, n (%)	55 (91.7)	19 (100)	22 (84.6)	14 (93.3)
MCD, PEL, and or KICS diagnoses, n (%)	38 (63.3)	17 (89.5)	12 (46.2)	9 (60)
Median CD4 count, cells/μL, (IQR)	125 (32.5, 219.5)	212 (128, 380.8)	73 (19, 164) **	38 (33, 164) *
Median HIV VL, copies/mL, (IQR)	87 (0, 1329)	0 (0,61)	189 (0.5, 3281) *	328.5 (104, 16978) ***
Hemoglobin, g/dL, median (IQR)	9.1 (8.1, 11.4)	9.8 (8.5, 11.7)	9 (8.1, 11.3)	8.9 (7.8, 10.5)
Platelets, K/mcL, median (IQR)	222.5 (137.5, 285)	232 (156.5, 286)	236 (159.3, 310.5)	148 (110, 232.5)
Albumin, g/dL, median (IQR)	3 (2.4, 3.7)	3.4 (2.6, 3.9)	3 (2.4, 3.8)	2.4 (2.3, 3.4)
C-Reactive Protein, mg/L, median (IQR)	27.2 (7, 85.2)	23.7 (9.7, 55.8)	38.2 (7.9, 104.8)	45 (4.5, 100.8)
Median saliva KSHV viral load, copies/mL, median (IQR)	1 (0, 6700.3)	1 (0, 3.3)	1 (0, 11661)	1 (1. 6583.5)
Median PBMCs KSHV viral load, copies/mL, median (IQR)	215.5 (.8, 4686.5)	84 (0,2315.5)	52 (0.3, 7128.8)	1692 (403.5, 8902)

Note: Per Wilcoxon analysis with cutaneous and another group: *if p > .05, **if p > .005, ***if p > .0005

Table 1 – Baseline Characteristics

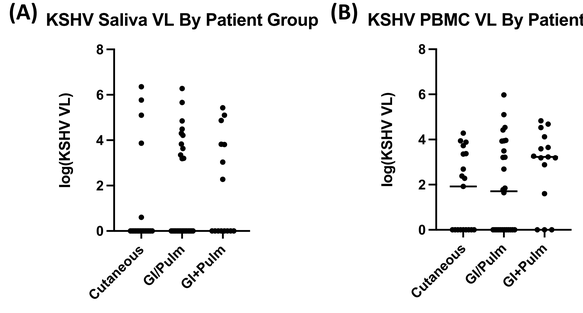
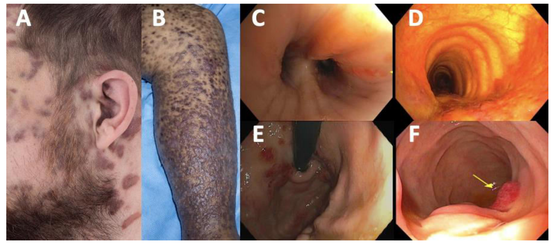


Figure 3 – Scatterplots examining patient KSHV PBMC (**A**) and Saliva (**B**).

RESULTS

- 38 (63.3%) of patients had a KAD, KICS was the most common; It was more common in patients with cutaneous KS compared to patients with cutaneous and visceral KS (**Fig 2**)
- Significant differences were seen in CD4+ count and HIV VL among patient groups (**Table 1**)
- KSHV PBMC and Saliva VL were non-significant between patient groups (**Fig 3**)

Figure 4 – Manifestations of cutaneous (**A,B**) pulmonary (**C,D**) and GI (**E,F**) KS in patients with biopsy confirmations.



RESULTS

- Median OS in pts with skin KS only was not reached, was 9.2 years in pts with GI or pulmonary KS, and 2.6 years in pts with both GI and pulmonary KS (P=0.009, **Fig 5**).
- A saliva KSHV VL level of ≥ 190 copies/10^6 cells had a sensitivity of 46%, specificity of 76%, and positive likelihood ratio of 2 for predicting the presence of visceral disease in symptomatic patients (**Fig 6**)

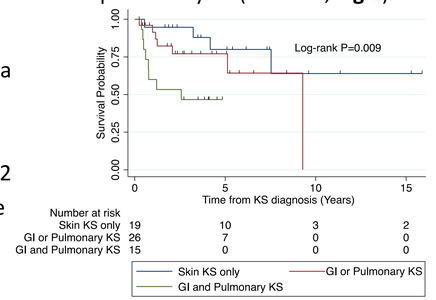


Figure 5 – KM curve highlighting the survival outcomes in patients with skin and/or visceral KS.

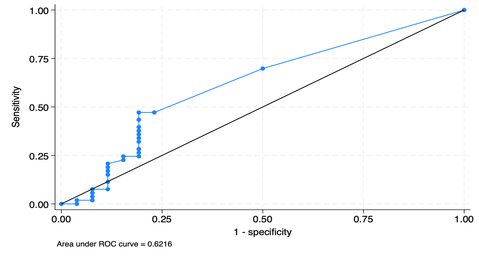


Figure 6 – ROC Curve observing sensitivity and specificity of saliva KSHV VL for GI and/or pulmonary KS.

CONCLUSIONS

Despite well-controlled HIV among patients with KS, the presence of visceral KS impacts overall survival. Saliva KSHV VL, though not a sensitive marker on its own, may work in conjunction with other clinical findings to aid in the diagnosis of visceral KS.

ACKNOWLEDGMENTS

The authors wish to thank the HAMB team, the Whitby Lab and the trial participants and their families.
This study was supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute.

Author Contact Information:
Email: Witterholtmt@nih.gov