Validation of Quantification of Lesional KSHV DNA Content for Diagnosis of Kaposi Sarcoma in Africa

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Aggrey Semeere[1], Jeffrey Martin[2], Racheal Ayanga[1], Andrea Gardner[3], Juan Boza[4], Maite Ibáñez de Garayo[3], Xinying Chu[4], Miranda McGaskey[3], Jason Manning[4], Miriam Laker-Oketta[1], Megan Wenger[2], Charles Kasozi[5], Michael Kanyesigye[6], Esther Freeman[7], Robert Lukande[1], Tim McCalmont[2], Phil LeBoit[2], Toby Maurer[8], David Erickson[4], and Ethel Cesarman[3].

[1]Infectious Diseases Institute, Uganda; [2]University of California, San Francisco; [3] Weill Cornell Medicine; [4]Cornell University;[5]Masaka Regional Referral Hospital, Uganda;[6]Mbarara Immune Suppression Syndrome Clinic, Uganda; [7]Harvard Medical School; and [8]Indiana University

Background

In East and Southern Africa:

- KS is common, advanced disease is often present at diagnosis, and survival is poor
- Inaccessibility and inaccuracies of histopathologic diagnosis undoubtedly contribute to the delays in KS diagnosis
  - May also contribute to erroneous treatment with potentially toxic chemotherapy
- We have hypothesized that quantification of KSHV DNA from suspicious skin lesions can distinguish KS from non-KS lesions and can ultimately serve as the basis for …
  - A diagnostic test without need for histopathologic interpretation
- Our earlier work on quantification of lesional KSHV DNA by qPCR — using sub-optimally transportable Ct values — had high sensitivity and specificity for the diagnosis of histopathologically-confirmed KS.

Objective

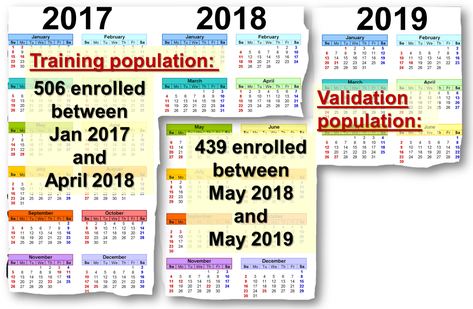
- Assess accuracy of qPCR of lesion-derived KSHV DNA for the diagnosis of KS in an external validation population using a more transportable copy number readout.

Study design

- Cross-sectional design to obtain biopsies from mucocutaneous lesions from consecutive patients in Uganda who presented to skin biopsy services because of clinical suspicion of KS.
- Histopathologic interpretation of biopsies served as gold standard and KSHV DNA copy number quantified in each biopsy.
- An optimal threshold of KSHV DNA copy number was identified in the **training population** and subsequently assessed in an **external validation population**.

Participants

- Consecutive patients who presented to one of three clinical biopsy services in Kampala, Masaka, and Mbarara, Uganda because of clinical suspicion of KS
- Biopsy performed if at least some clinical suspicion of KS by biopsy practitioner
- Training population enrolled prior to validation population. Both used identical procedures.



Statistical Analysis

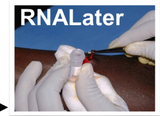
- U.S.-based histopathologic interpretation used as gold standard.
- Receiver operator characteristics (ROC) curve identified optimal threshold of the mean KSHV copy number for KS diagnosis in training population
- Optimal threshold in training population was assessed in external validation population

- Established a **KSHV DNA copy number threshold to** distinguish skin KS from Non-KS
- KSHV DNA lesional qPCR had **high sensitivity and specificity for KS diagnosis** in both **training and external validation** populations.

Biopsy, Histopathology and qPCR

5 mm cylindrical punch
Gelfoam® for hemostasis





Bisect

Histopathology

Uganda

- **Anti-LANA stain available upon discretion of pathologist**

U.S. (Cornell & UCSF)

- **Examination of original slide**
- **New section and/or anti-LANA stain available upon discretion of pathologist**
- **Final interpretation: consensus ≥ 2 readers**

Quantification of KSHV DNA under optimal conditions

U.S. (Cornell)

- **DNA extraction by Qiagen kit**
- **Quantitative PCR**
  - **Purified DNA standardized to 2 ng/µl**
  - **5 µl input**
  - **Target: KSHV ORF 26**
  - **40 cycles**
  - **KSHV copy number read from standard curve**
  - **Mean of 2 replicates used as final value**

U.S.-based Histopathologic Interpretation

	Training Population N=506	Validation Population N=439
KS	341 (67%)	338 (76%)
Not KS	150 (30%)	89 (20%)
Indeterminate	15 (3%)	12 (2.7%)

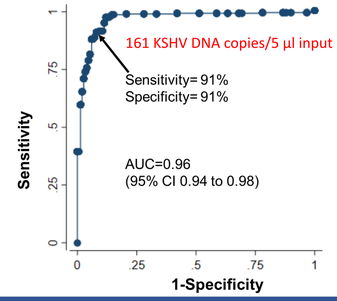
Characteristics of Participants

	Training N=506	Validation N=439
Sociodemographic/Economic		
Age, years *	33 (28 to 41)	33 (29 to 40)
Female	38%	36%
Travel cost, round trip in USD *	$2.73 ($1.64 to $5.45)	$5.39 ($2.70 to $16.19)
Travel time (min), one-way *	60 (60 to 120)	120 (60 to 300)
Clinic performing biopsy		
IDI, Mulago – Kampala	79%	75%
ISS Clinic – Mbarara	5.5%	9.1%
Uganda Cares – Masaka	15%	16%
Clinical history		
Living with HIV infection	94%	94%
Using ART (among HIV-infected)	85%	90%
Lesion Morphology		
Macule	22%	22%
Plaque	64%	60%
Nodule	14%	18%
Tumor	0.6%	0.2%

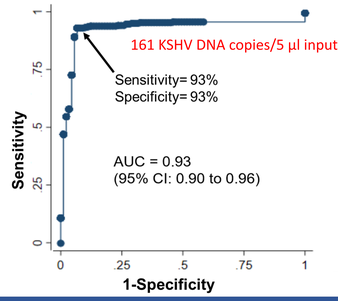
*Median (Interquartile range)

ROC Curves Depicting Performance of KSHV DNA Copy Number For Diagnosis of Histopathologically-Confirmed KS

Training Population



External Validation Population



Conclusions

In an external validation population of consecutive patients with KS-suspicious skin lesions in Uganda:

- qPCR of lesional KSHV DNA had high sensitivity and specificity for diagnosis of KS, performing similarly to our training population.
- Because of ambient endemic KSHV infection in Africa, mere qualitative detection of KSHV DNA in skin lesions is non-specific for KS diagnosis.
- Instead, precise quantification of KSHV DNA is needed.
- A KSHV DNA copy number threshold that balances sensitivity and specificity has now been established.

Implications

- This validation of qPCR performance motivates a point-of-care nucleic acid-based diagnostic test for KS, which we are currently developing.
- The accuracy of KSHV DNA copy number for KS diagnosis, while better than visual macroscopic diagnosis (and often better than local histopathologic interpretation), is still inferior to gold standard histopathologic interpretation.
  - Room for further innovation to improve accuracy of nucleic acid-based KS diagnosis

Correspondence:
Aggrey S. Semeere, Infectious Diseases Institute Makerere University College of Health Sciences
Kampala, Uganda asemeere@idi.co.ug

U01 CA269199
UH3 CA202723
U54 CA254571
U01 AI069911
U54 CA190153