Presence of Kaposi Sarcoma-Associated Herpesvirus tissue reservoirs in seronegative postmortem cases

627

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BACKGROUND

- Kaposi sarcoma (KS), an HIV/AIDS-associated malignancy, remains one of the most prevalent causes of morbidity and mortality in people living with HIV-1 (PLWH).
- Kaposi Sarcoma-Associated Herpesvirus (KSHV) is the causative agent of Kaposi sarcoma (KS) and multiple other diseases in PLWH and immune suppressed individuals.
- Similar to HIV-1, KSHV can remain latent in an infected individual and could periodically reactivate to cause diseases upon immunosuppression.
- While HIV-1 tissue reservoirs in aviremic individuals were recently identified, little is known about KSHV tissue reservoirs, and whether KSHV seronegative individuals are truly uninfected given the reported observation of sero-reversion in infected individuals.
- Our objective is to identify the potential KSHV tissue reservoirs, and whether KSHV seronegative individuals can harbor KSHV in their tissues.

METHODS

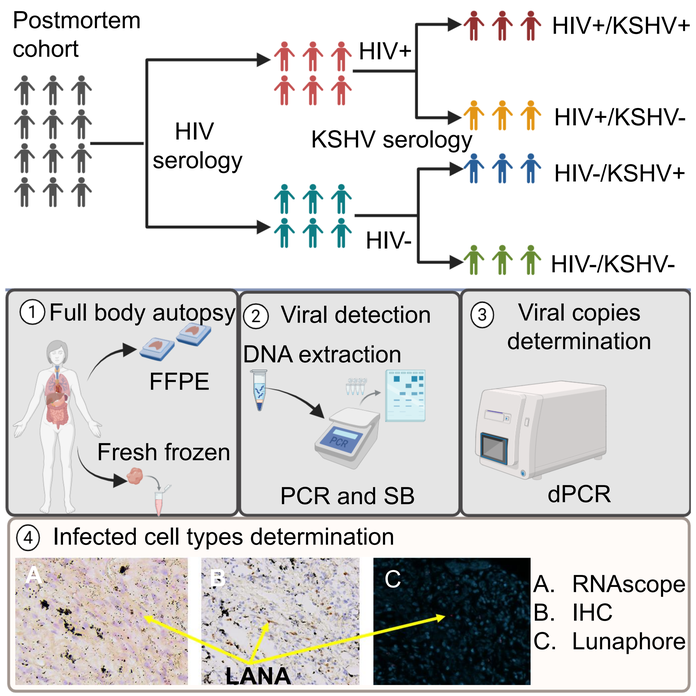


Figure 1. Workflow of postmortem KSHV reservoir study: serostatus stratification, full autopsy tissue collection, viral DNA detection and quantification by dPCR, and infected cell identification by immunohistochemistry, RNAscope and Lunaphore.

KSHV DNA was detected in multiple organs in 30% of KSHV seronegative individuals, demonstrating that serology testing underestimates the true KSHV prevalence and may pose transmission risk in organ transplantation.

RESULTS

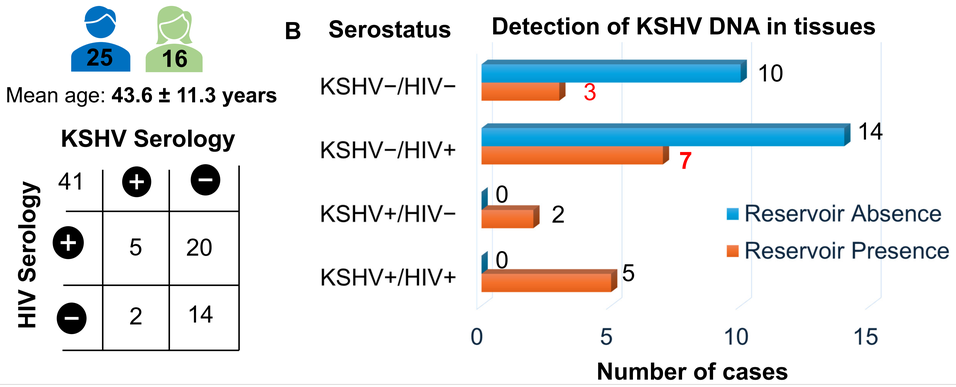


Figure 2. Detection of KSHV tissue reservoir in KSHV seronegative individuals.
(A) Cohort demographics and serologic classification. HIV-1 serostatus was determined by rapid antibody testing, and KSHV serostatus was determined using an in-house immunofluorescence assay (IFA).**(B)** Autopsy cases with and without detectable KSHV DNA in tissue reservoirs stratified by KSHV and HIV-1 serostatus. KSHV tissue reservoirs positivity was defined by southern blot (SB) detection of KSHV viral genes LANA (ORF73) and/or ORF26 DNA.

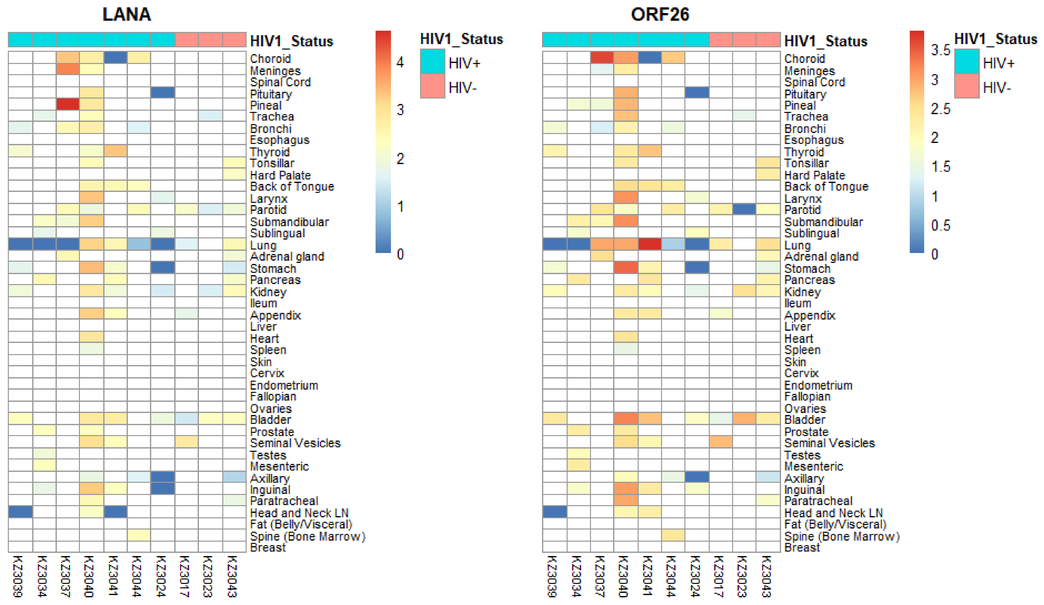


Figure 3. KSHV DNA (LANA and ORF26) copy number in tissues of KSHV seronegative individuals. Heatmaps show KSHV DNA copy number per 10^6 cells across tissues (rows) and cases (columns) measured by LANA (left) and ORF26 (right). Values are log10(x+1) transformed. Warmer colors indicate higher copy number; white indicates tissues that were southern blot negative, hence not analyzed by dPCR. The top bar annotates HIV status of each case. Tissue and sample ordering are fixed (no clustering).

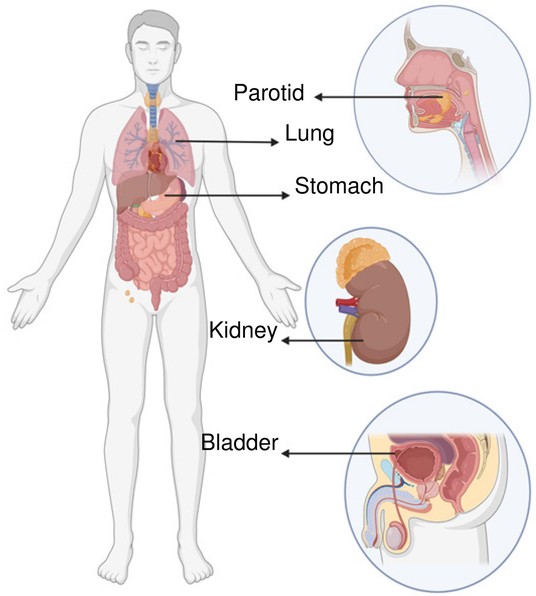


Figure 4. Common KSHV tissue reservoirs in KSHV seronegative individuals. Highlighted organs harbor KSHV DNA (LANA and ORF26) in at least 50% of KSHV seronegative individuals (n = 10).

CONCLUSIONS

- This study provides compelling evidence that KSHV can persist in tissue reservoirs even in individuals who are seronegative by current standard serological assays.
- The detection of viral DNA in multiple organs in 30% of seronegative individuals challenges the prevailing assumption that absence of antibodies equates to absence of infection.
- This finding highlights that KSHV serology testing is inadequate and may underestimate KSHV prevalence, presenting a significant risk in organ transplantation where donors with a negative KSHV serology test could still transmit the virus, leading to KS in the recipient.
- More cases are currently being analyzed to increase our sample size and further define KSHV tissue reservoirs. We will determine the influence of HIV-1 may have on the size and distribution of these KSHV tissues reservoirs. We will also identify which cell types are harboring KSHV in tissues.

ACKNOWLEDGMENTS

This research project is supported NIH grants U54 CA277846 and TW012277. WK is a Fogarty Fellow.