SPATIAL PROFILING OF CORONARY PLAQUE SUGGESTS UNIQUE IMMUNE PATHWAYS IN HIV

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BACKGROUND

Persons with HIV (PWH) have **twice the risk** of developing cardiovascular disease (CVD) compared to HIV-negative individuals. PWH also have aa greater burden of non-calcified coronary plaque, which may be more likely to rupture.

Although higher levels of systemic and local inflammation may contribute to altered plaque formation, there are few studies on coronary plaque components in PWH versus HIV-negative controls.

We *hypothesized* that HIV present in coronary arteries alters the inflammatory response within the coronary plaques, contributing to the increased risk of CVD.

METHODS

- Coronary arteries with varying stages of atheroma from deceased PWH (n=13) and matched HIV-negative donors (n=15) were obtained from CVPath Institute.
- We used droplet digital polymerase chain reaction (ddPCR) to quantify HIV, cytomegalovirus (CMV), and human endogenous retrovirus (HERV K) within plaque.
- We performed an unbiased analysis using NanoString's GeoMX spatial whole transcriptomic approach (n=7 per group) and defined specific immune markers by targeted spatial transcriptomics, proteomics, and immunohistochemistrv.

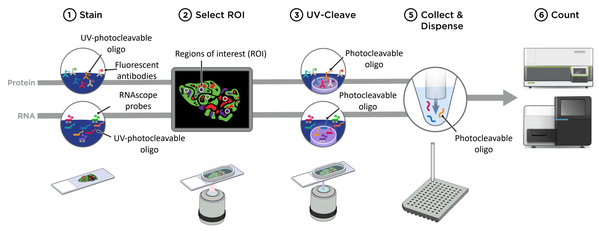


Figure 1. **GeoMX experimental workflow.** Sections on glass slide are stained with anti-CD68, and RNAscope reagents against HIV and cytomegalovirus (CMV). We scanned the fluorescence, and selected ROI through the software. Once ROIs are selected, UV irradiation photocleaved RNA probes from selected ROIs using a microcapillary system. The probes were dispensed into a 96 well microtiter plate. Those barcodes were read out on an Illumina sequencer.

- Comparisons of immunohistochemistry results used Mann-Whitney U tests, and comparisons of multiple regions of interest used linear models for microarray data (LIMMA) with Benjamini Hochberg correction.

The presence of HIV in coronary arteries is associated with increased local inflammatory gene expression, including IFN-γ & NMD decay pathways, which may accelerate atherosclerosis.

RESULTS

- Among coronary arteries matched by plaque type (pathological intimal thickening, early and late atheroma), plaque area, and stenosis (**Figure 2A**), we detected viral RNA sequences for HIV in 77% (10/13) of coronary arteries from PWH, while CMV was detected in 46% (6/13) of PWH and 53% (8/15) of controls. Coronary arteries from all donors had detectable HERV-K, which did not differ by HIV status.

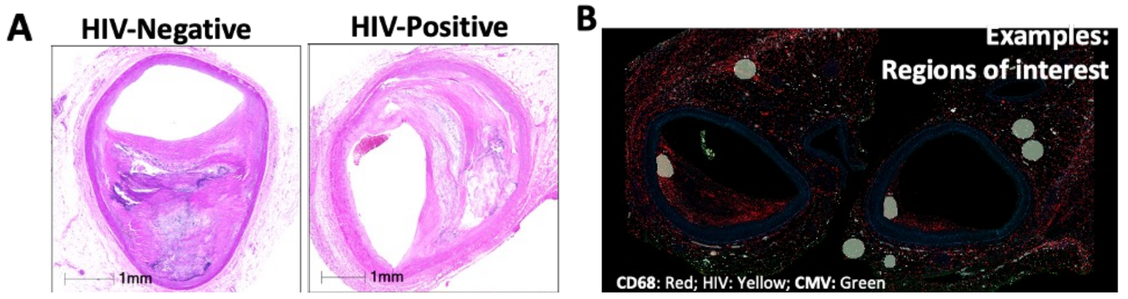


Figure 2. **Characterization of coronary plaques.** (A) Representative coronary plaques from HIV-positive and HIV-negative individuals were stained with H&E stain. (B) Representative image showing regions of interest selected for spatial transcriptomics.

- The RNA transcriptomes of the coronary arteries differed by HIV status with enrichment of genes in several inflammatory pathways including the interferon signaling and nonsense-mediated decay pathway in PWH (**Figure 2B and Figure 3A**).

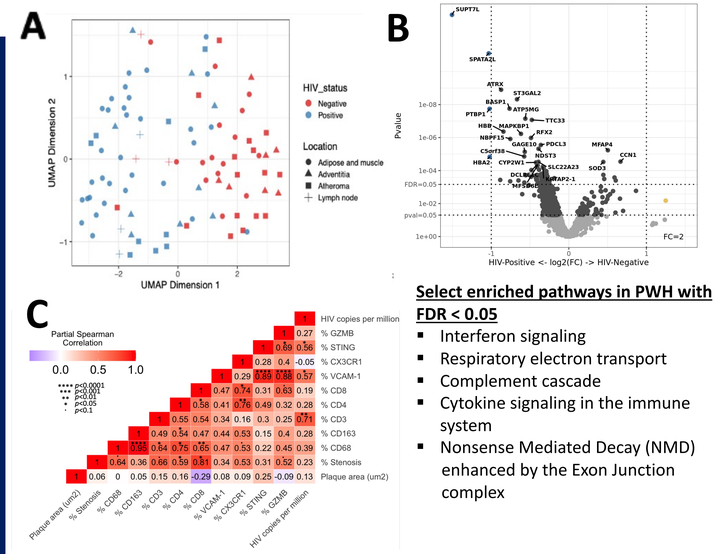


Figure 3. **Coronary plaques form PWH are enriched for inflammatory genes compared to HIV-negative.** UMAP showing regions of interest clustered by HIV status (A), with differential gene expression and select pathways highlighted (B). Partial spearman correlation plots of % immune markers expressed/um2 in coronary plaques from PWH (n=13) adjusted for plaque type (C).

Select enriched pathways in PWH with FDR < 0.05

- Interferon signaling
- Respiratory electron transport
- Complement cascade
- Cytokine signaling in the immune system
- Nonsense Mediated Decay (NMD) enhanced by the Exon Junction complex

- Partial Spearman rank correlation adjusted for coronary plaque type in the PWH showed a significant relationship between HIV copies with stimulator of interferon gamma (STING), CD3, and VCAM1 protein expression (**Figure 3B**).

CONCLUSIONS

- The presence of HIV within the coronary arteries alters the immune environment with stimulation of several pathways such as interferon-gamma and the non-sense mediated decay pathway.
- Notably, the presence of other chronic viruses such as CMV or HERV K does not appear to differ by HIV status. HIV may be an important accelerator of atherosclerosis and increased CVD risk in PWH.
- Further studies are underway to establish whether intact virus, viral sequences or modified host DNA stimulate inflammation within coronary plaques.

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