Supplementary Online Content

Tawakol A, Ishai A, Li D, et al. Association of arterial and lymph node inflammation with distinct inflammatory pathways in human immunodeficiency virus infection. *JAMA Cardiol*. Published online December 7, 2016. doi:10.1001/jamacardio.2016.4728

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix

Data Supplement

Supplemental Methods

Assessment of lipids, markers of immune activation, inflammation and coagulopathy

Blood was drawn in the fasting state and used to measure total cholesterol, HDL cholesterol, and triglycerides. LDL cholesterol was calculated by Friedewald's formula except for individuals who had triglycerides ≥ 4.56 mmol/L, where it was measured directly. C-reactive protein was measured using a high sensitivity assay (Dade Behring, Deerfield, Illinois). The nadir $CD4^+$ T cell count was defined as the lowest laboratory-confirmed value prior to the baseline PET scan.

Cryopreserved peripheral blood mononuclear cells (PBMCs) from the time point closest to the PET/CT scan, median 4 days (interquartile range, IQR 0-14), were thawed in batches. Cells were stained with viability dye LIVE/DEAD® Fixable Blue Dead Cell Stain Kit (Life technologies, NY, USA), washed then stained with fluorescent conjugated antibodies to cell surface markers. To measure $CD4^+$ and $CD8^+$ T cell activation, PBMCs were stained with anti-CD3 PerCP (BD, NJ, USA), anti-CD8 pacific blue (BD), anti-CD4 eFluor 605 (eBioscience, CA, USA), anti-CX3CR1 PE (eBioscience), anti-CD28 PE-Cy7 (eBioscience), anti-CD57 FITC (BD), anti-CD38 APC (BD) and anti-HLA-DR APC-Cy7 (BD). PBMCs were stained with anti-CD2, anti-CD3, anti-CD19 and anti-CD20 efluor 450 (eBioscience), anti-CD56 V450 (BD), and anti-HLA-DR efluor 605 (eBioscience), to identify monocytes (lineage negative, HLA-DR^+ cells). PBMCs were also stained with anti-CD14 FITC (eBioscience), anti-CD16 PE-Cy7 (Biolegend, CA, USA), anti-CCR2

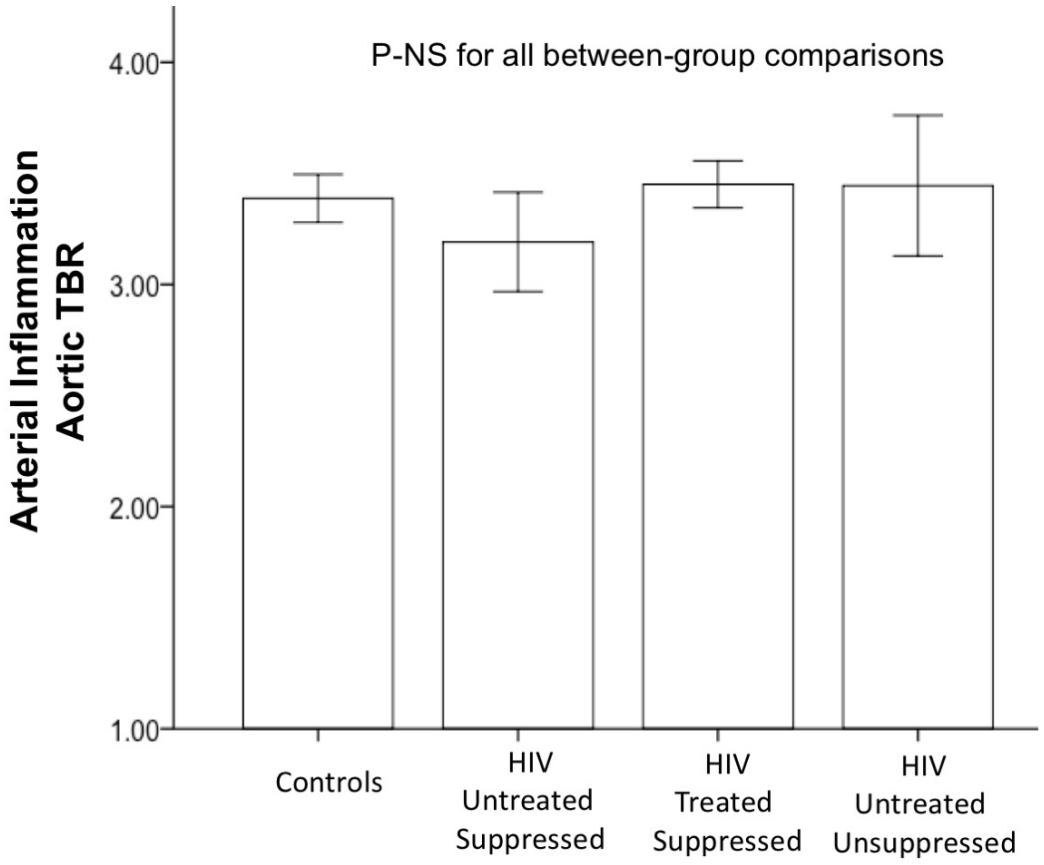
PerCP-Cy5.5 (Biolegend), anti-CX3CR1 APC (Biolegend), anti-TF PE (eBioscience) and anti-CCR5 APC-Cy7 (BD) to evaluate subpopulations of monocytes. Cellular markers were detected by flow cytometry using an LSRII flow cytometer (BD). We evaluated monocytes for expression of CD14 vs CD16 (defining classical monocytes as $CD14^{+}CD16^{-}$, intermediate as $CD14^{+}CD16^{+}$ and patrolling or non-classical as $CD14dimCD16^{+}$), using methods previously described. [1]

Soluble markers of inflammation (IL-6, CRP, and MCP-1) were assessed in cryopreserved plasma samples, using a multiplex electrochemiluminescence assay (Meso Scale Discovery, MD, USA). ELISA was used to analyse TF and sCD14 (R&D Systems, Minneapolis M.N.) as well as levels of sCD163 (Aviscera Biosceience Santa Clara C.A.). D-dimer results were obtained using ELFA (Enzyme Linked Flourescent Assay, BioMerieux, Durham N.C.).

eTable. Correlations between tissue FDG-uptake and biomarkers in HIV-infected elite controllers

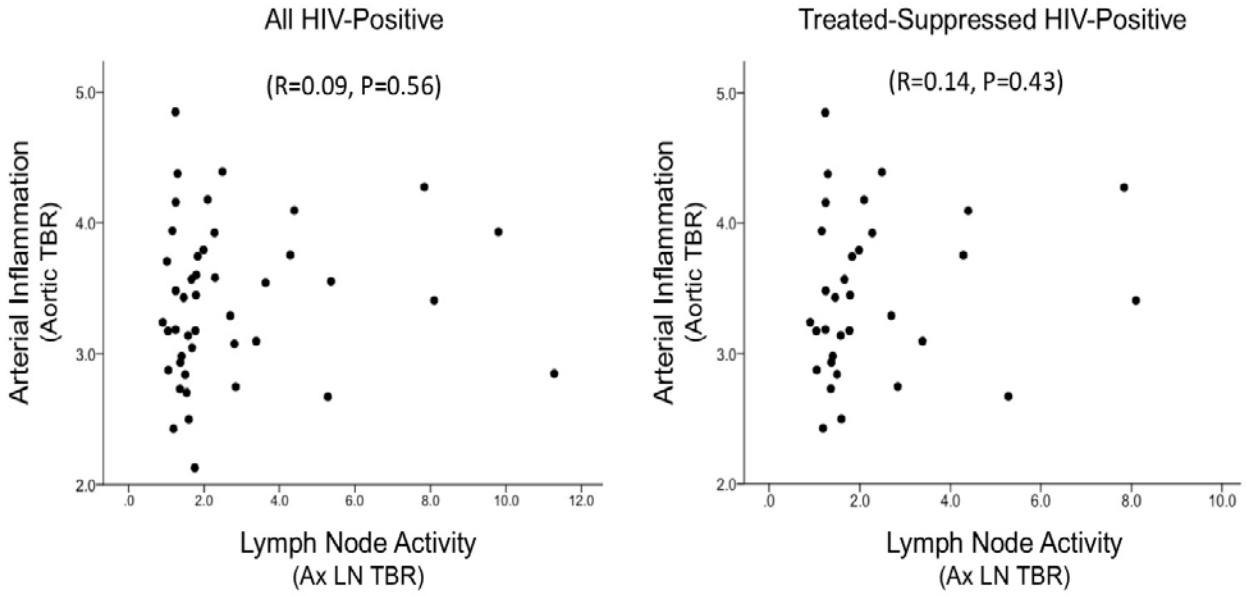
Correlations with lymph node activity in HIV+ controllers (n=7)			
Group	**Marker**	**Correlation coefficient**	**p-value**
Viral markers	Integrated HIV DNA	**0.778**	**0.039**
Markers of inflammation and thrombosis	D-dimer	-0.429	0.397
	CRP	-0.429	0.397
	IL6	-0.429	0.397
$CD4^+$ T cell markers	CD4	-0.179	0.702
	CD4 Nadir	-0.714	0.071
	% $CD4^+$	0.257	0.623
	% $HLA^-DR^+CD38^+$ in $CD4^+$ T cells	-0.029	0.957
	% $CX3CR1^+$ in $CD4^+$ T cells	-0.314	0.544
	% $CD28^-CD57^-$ in CD4+ T cells	**-0.829**	**0.042**
	% $CD28^-CD57^+$ in $CD4^+$ T cells	-0.314	0.544
$CD8^+$ T cell markers	% $HLA^-DR^+CD38^+$	-0.086	0.872
	% $CD28^-CD57^-$ in $CD8^+$ T cells	-0.116	0.827
	% $CD28^-CD57^+$ in $CD8^+$ T cells	0.314	0.544
Monocyte Markers	% $CD14^+CD16^-$ monocytes	0.371	0.468
	% $CD14^+CD16^+$ monocytes	-0.257	0.623
	% $CD14^{dim}CD16^+$ monocytes	-0.257	0.623
	% $CX3CR1^+$ monocytes	-0.371	0.468
	% $CCR2^+$ monocytes	0.371	0.468

eFigure 1: Arterial inflammation in HIV-infected individuals and Controls



Levels of arterial inflammation overall were not significantly different among HIV-infected individuals and controls (p=NS for all comparisons). Also, levels of arterial inflammation among the different HIV categories (i.e. untreated, treated) were not different (p=NS for all comparisons). Error bars depict ±1 SEM.

eFigure 2. Scatterplot of arterial inflammation vs lymph node activity



Lymph node activity did not correlate with arterial inflammation among all HIV-infected individuals (left hand plot, p=0.56) or among the treated suppressed individuals only (right hand plot, p=0.43).

eReference

1. Vandergeeten C, Fromentin R, Merlini E, et al. Cross-clade ultrasensitive PCR-based assays to measure HIV persistence in large-cohort studies. *Journal of virology.* 2014;88(21):12385-12396.