Title

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Arterial and Lymph Node Inflammation Associate with Distinct Inflammatory Pathways in HIV

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Abstract

Background—HIV infection is associated with a high risk of cardiovascular diseases (CVD) and increased arterial inflammation. In HIV, inflammation is also increased within lymph nodes (LN), tissues known to harbor the virus even among treated and suppressed individuals. We tested the hypothesis that arterial inflammation is linked to HIV disease activity and to inflammation within HIV-infected tissues (LN).

Methods—Seventy-four individuals were studied: 45 HIV-infected individuals and 29 uninfected controls. Arterial and LN inflammation were measured using ^{18}F-fluorodeoxyglucose positron emission tomography, (FDG-PET). Detailed immunophenotyping was performed, along with measurement of viral activity/persistence, and circulating inflammatory biomarkers.

Results—Median age was 53 years, 100% male. Lymph node inflammation was higher in HIV-infected individuals and correlated with markers of viral disease activity (viral load, CD8$^+$ T cells,

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Author contributions: Dr. Tawakol, Takx, Ishai, Kaiser, and Pampaloni were responsible for obtaining images on study subjects, image analysis, statistical analysis, and data interpretation. Mr. Li and Ms. Hur helped to recruit study subjects and assisted with data preparation and drafting of the manuscript. Mr. Rupert performed the inflammatory assays for the study. Dr. Hsu and Dr. Sereti performed the T-cell activation and monocyte studies. Dr. Fromentin and Dr. Chomont performed the viral persistence assays and helped with data analysis and editing the manuscript. Dr. Deeks, Dr. Tawakol, Dr. Ganz, and Dr. Hsue helped to design the study, oversaw study recruitment, data interpretation, writing and editing the manuscript. Dr. Hsue had full access to all the data in the study and had final responsibility for the decision to submit for publication.

CD4/CD8 ratio) and $CD4^+$ T cell activation. Arterial inflammation was modestly increased in HIV-infected individuals and was positively correlated with circulating inflammatory biomarkers (hsCRP, IL-6) and activated monocytes ($CD14dimCD16^+$; non-classical) but not to markers of HIV. While LN and arterial inflammation were increased in HIV, inflammatory activity in these tissues was not related (r=0.09, p=0.56).

Conclusion—While LN and to a lesser degree, the arterial wall are inflamed in HIV, inflammation in these tissues is not closely linked. Namely, measures of HIV disease activity strongly associate with LN inflammation but not arterial inflammation. These data suggest that LN and arterial inflammation do not share underlying pathways of immune activation and suggests therapeutic interventions that reduce viral disease activity may not predictably reduce arterial inflammation in HIV or its down-stream consequence (CVD).

INTRODUCTION

Current treatment regimens allow human immunodeficiency virus (HIV)-infected individuals to have a life expectancy that approaches that of the general population.[1] However, there has been a concomitant rise in non-HIV-related morbidity and mortality, notably due to atherosclerotic diseases, among HIV-infected individuals.[2] After correcting for traditional cardiovascular risk factors, the incidence of myocardial infarction (MI), sudden cardiac death, and stroke are higher in HIV-infected individuals, even in the setting of treated and suppressed HIV infection.[3–5]

Several mechanisms likely contribute to the increased prevalence of cardiovascular diseases (CVD) in HIV-infection. HIV-infected individuals may have a higher burden of traditional CVD risk factors,[6,7] and anti-retroviral therapies (ART) may lead to atherogenic changes in lipid metabolism.[8,9] However, those factors alone do not explain the excess CVD risk seen in HIV. Untreated, and to a lesser extent, treated HIV disease associate with chronic up-regulation of a number of inflammatory pathways implicated in the development of atherosclerosis.[3] Multiple immunologic changes, such as T-cell activation and senescence and macrophage activation, have been linked to CVD in HIV-infection.[10] Interleukin 6 (IL-6), D-dimer, and to a lesser degree, C-reactive protein (CRP) are elevated in HIV disease and are strongly associated predict the risk of developing CVD independently of traditional CVD risk factors in HIV.[11] Moreover, arterial inflammation, a key pathobiological hallmark of atherosclerosis[12] which independently predicts the development of CVD events in non-infected individuals[13,14], is increased in HIV, after controlling for traditional CVD risk factors[15,16]. Accordingly, there is substantial interest in deciphering which inflammatory pathways may be responsible for the excess arterial inflammation seen in HIV.

HIV-infection is associated with multiple chronic immunologic changes.[10] HIV preferentially infects memory $CD4^+$ T cells, most of which reside in the secondary lymphoid tissues such as lymph nodes (LN). In the absence of ART, HIV replication in these tissues causes local inflammation and $CD4^+$ T cell loss.[17,18] While effective ART prevents the spread of HIV, it does not cure the disease; namely, previously infected cells continue to produce virions, especially within HIV “reservoirs” such as the LN. As a consequence of HIV persistence in these secondary lymphoid tissues, as well as other factors,[19] HIV-

associated inflammation persists within lymphoid tissues, even in the presence of effective ART. Accordingly, there is substantial interest in studying LN inflammation in treated and suppressed HIV disease. Furthermore, it is hypothesized that the inflammatory pathways associated with LN inflammation may be related to those that potentiate arterial inflammation in HIV.

^{18}F-fluorodeoxyglucose-positron emission tomography / computed tomography (FDG-PET/CT) is employed to assess inflamed and/or infected tissues,[20] including atherosclerotic inflammation,[21,22] leveraging the fact that FDG accumulates in immune cells due to their unusually high metabolic rates.[23] FDG-PET/CT is clinically used to localize and measure inflammatory and infectious diseases,[20,24–27] including within lymph nodes[28] and the artery wall[15,16] in HIV-infected cohorts. However, despite the importance of atherosclerotic inflammation, it remains unknown as to whether the abundant arterial inflammation in HIV infection relates to inflammatory mechanisms associated with the virus itself (such as inflammation within viral reservoirs) or to other causes of chronic inflammation.[10] Accordingly, FDG-PET/CT imaging provides a unique tool to study potentially linked activity between these tissues.

In the present study, we tested whether arterial inflammation in HIV is linked to: 1) HIV disease activity (e.g., treatment status, viral load, CD4/CD8$^+$ T cell counts, T cell activation), and 2) tissue inflammation within foci of persistent HIV infection (namely lymphoid tissues). To address these questions, we performed FDG-PET imaging in HIV-infected individuals, assessed markers of inflammation, immune activation, and HIV persistence, and assessed their relationships to inflammation within the arterial wall and lymphoid tissues.

METHODS

Study population

Participants were recruited from the SCOPE cohort, a clinic-based cohort of individuals receiving care at San Francisco General Hospital and the San Francisco Veteran's Affairs Medical Center. Prior to study entry, HIV infection status was confirmed using HIV antibody testing. Participants were not pre-selected based on cardiovascular risk factors and were consecutive volunteers from the SCOPE study who agreed to participate. Our study population included the following groups based on their treatment status and virologic control at the time of enrollment into our substudy: (1) antiretroviral-treated with undetectable viral loads using conventional assays (typically < 40 copies/ml) (ART suppressed), (2) antiretroviral-untreated or treated with detectable viremia ("non-controllers"), and (3) antiretroviral-untreated with undetectable viremia ("elite controllers"). Uninfected controls were recruited from the San Francisco community. All controls were confirmed to be HIV antibody negative. Controls were matched to HIV-infected study participants by age, gender, and Framingham Risk Score (FRS). The UCSF Committee on Human Research approved this study, and all individuals provided written informed consent before enrollment.

Laboratory Assays

Details of the laboratory analyses are provided in the supplement. Briefly, blood was drawn in the fasting state and used to measure lipids and soluble markers of inflammation. Additionally, cryopreserved peripheral blood mononuclear cells (PBMCs) from the time point closest to the PET/CT scan, median 4 days, were thawed in batches. Cells were stained with viability dye, washed then stained with fluorescent conjugated antibodies to cell surface markers to measure CD4+ and CD8+ T cell activation, to identify monocytes (lineage negative, HLA-DR+ cells) and 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.[29]

Integrated HIV DNA—The size of the HIV reservoir was estimated by measuring the frequency of CD4+ T cells harboring integrated HIV DNA, as previously described.[30] Briefly, CD4+ T cells were isolated from cryopreserved PBMCs by negative selection (StemCell) and subjected to an Alu gnested-PCR to quantify the number of integrated HIV genomes.

Imaging

FDG PET/CT Image Acquisition—FDG-PET/CT imaging of the carotid arteries and ascending aorta was performed using a validated approach.[22,31] Briefly, FDG was administered intravenously (10 mCi) after an overnight fast and imaging was performed 120 minutes later using FDG-PET/CT (Siemens Biograph 64, Siemens, Forshiem Germany). PET imaging of the neck and chest was performed (7 minutes per bed position). Attenuation correction scanning was performed using voltage of 140 kVp and current of 35 mAs. The reconstruction of attenuation-corrected images was performed using OSEM algorithm. All subjects had a blood sugar concentration of < 200 mg/dL at the time of imaging.

Image analysis—Image analysis was performed by a radiologist blinded to all clinical data, using a workstation that enables multi-modal standard image fusion (Leonardo–TrueD, Siemens Solutions, Malvern, Pennsylvania).

Arterial Inflammation: FDG-uptake in the arterial wall was evaluated by placing circular regions of interest (ROI) in the axial plane, every 3 mm, starting 1 cm above the aortic valve and continuing to the bottom of the aortic arch. FDG-uptake in the superior vena cava (SVC) was evaluated for background correction. Aortic FDG-uptake was expressed as target-to-background ratio (TBR), by dividing the maximum standardized uptake values (SUV) in the aortic wall by the mean SUVs in the superior vena cava. Additionally, in the subset of individuals, arterial FDG uptake was measured in the carotid arerty as previously described.[32]

Lymphatic Tissue Activity: FDG-uptake in the lymphatic tissue was evaluated by placing circular ROIs in the axial plane over visualized lymph nodes in the axillary lymph nodes (and, in a subset of individuals, in the cervical lymph nodes as well). For each the axillary

and cervical lymph nodes, at least 2 ROIs were drawn on each side. The maximum SUV were recorded for each ROI and the activity was expressed as TBR, defined as the ratio of the average maximum SUV calculated in the lymph node tissue to background blood activity derived from the SVC. Additionally, activity in the spleen was measured as described previously.[33]

Statistical analysis

Continuous parametric variables are presented as mean with standard deviation (SD), continuous non-parametric variables as median with interquartile range (IQR), and nominal variables as frequency (percentage). Group comparisons for normally distributed continuous variables (such as arterial FDG-uptake) were assessed using an independent samples Student *t* test, and non-normally distributed continuous variables (such as lymph node FDG-uptake) were compared using a Mann-Whitney U test. Nominal data (such as gender, diabetes) was assessed using Pearson's chi-square test. Correlations were tested using either the Pearson correlation coefficient (r) for continuous normally distributed variables, or Spearman's rank correlation coefficient (ρ) for continuous non-normally distributed values. For further comparison of arterial inflammation between groups, we matched uninfected controls to HIV-positive individuals that were treated and virally suppressed based on age, gender, race, and FRS, excluding statin-users. All statistical analyses were performed with IBM SPSS Statistics version 23.

RESULTS

Clinical characteristics

The study cohort included 45 HIV-infected individuals and 29 uninfected controls (Table 1). All study participants were male. Of the 45 individuals with HIV, 33 were treated and suppressed, 7 were elite controllers, and 5 were non-controllers. The HIV-negative group had a higher proportion of subjects who were African-American (48.3% compared to 22.2%; p=0.019). HIV-infected individuals and controls were well matched in age, gender, traditional risk factors including cigarette smoking, and FRS with the exception of family history of CVD which was more common in HIV-infected individuals (36% vs. 10%; p=0.015).

Arterial and lymph node inflammation are increased in HIV

Arterial inflammation, when assessed across all study participants, did not differ between the HIV-infected and HIV-uninfected groups [see Supplemental Figure 1]. However, arterial FDG-uptake associates with FRS and differs among races, and can be attenuated by statin therapy.[15,32,34] Accordingly, we matched 15 statin-naïve HIV-positive individuals on ART with an undetectable viral load to 15 statin-naïve HIV-uninfected subjects based on age, race, and FRS. In these matched groups, arterial inflammation was higher in HIV-positive individuals (aortic TBR 3.63±0.61) than in HIV-negative controls (3.26±0.51; p=0.037, Figure 1).

Additionally, arterial inflammation was generally concordant across arterial beds. In the subset of individuals who also provided carotid arterial PET data (N=71), carotid FDG uptake correlated with aortic FDG uptake (R=0.30, p=0.012).

Lymph node activity was substantially higher in HIV-infected individuals than in the uninfected controls (2A and 2B). Uninfected controls had the lowest lymph node activity (max axillary LN SUV: 1.53±0.56), the elite controller and ART-suppressed groups had intermediate levels of lymph node activity (2.12±0.87, and 2.32±1.79, respectively), and the non-controllers had the highest activity (8.82±3.08). Compared to non-infected controls, the level of lymphoid inflammation was higher in each of the HIV-infected groups (Figure 2B).

Further, there was general concordance of activity across lymphoid tissues. In the subset of individuals who provided spleen data (N=64) and cervical LN data (N=71), axillary lymph node activity correlated with activity derived from the spleen (R=0.29, p=0.02), and cervical lymph nodes (R=0.47, p<0.001).

Lymph node inflammation associates with measures of HIV-disease activity

Among elite controllers, the size of the reservoir as estimated by frequency of $CD4^+$ T cells harboring integrated HIV DNA was associated with levels of lymph node inflammation (r=0.85; p=0.015, Supplemental Table 1). There was no consistent association between HIV DNA and lymph node inflammation among those on ART.

Moreover, across all HIV-infected subjects, higher lymph node inflammation was associated with a higher viral load (r=0.302; p=0.047, Table 2). higher $CD8^+$ T cell count (r=0.513; p=0.002, Table 2), and lower CD4:CD8 ratio (r=−0.412; p=0.016). We found no significant associations between lymph node activity and $CD4^+$ T cell count or CD4 nadir.

Arterial inflammation does not associate with measures of HIV-disease activity

In distinct contrast to lymph node activity, arterial inflammation did not relate to any measure of HIV-disease activity (see Table 2). Furthermore, arterial inflammation did not correlate with lymph node activity, either in all HIV-positive individuals or in treated and suppressed HIV-infected-individuals (Supplemental Figure 1).

Inflammatory cell subsets and inflammatory biomarkers differentially associate with arterial and lymph node inflammation

Lymph node activity correlated with higher frequency of circulating classical monocytes ($CD14^+CD16^-$, Table 3) and activated $CD4^+$ T cells ($HLA^-DR^+CD38^+$). In contrast, arterial inflammation correlated with higher frequency non-classical monocytes ($CD14dimCD16^+$); none of the peripheral blood $CD4^+$ or $CD8^+$ T cell markers associated with arterial activity. Arterial inflammation also positively correlated with proportion of $CX3CR1^+$ monocytes and negatively with proportion of $CCR2^+$ monocytes; neither were predictive of lymph node activity. Inflammatory markers were predictive of both arterial and lymph node inflammation; namely, IL-6 and hsCRP were correlated with arterial inflammation while IL-6 correlated with higher lymph node activity. Additionally, the coagulation marker, D-dimer, associated with lymph node activity.

DISCUSSION

We performed a comprehensive assessment of arterial and lymphoid tissue inflammation in HIV-infected and uninfected adults. We found that inflammation in lymphoid tissues was consistently higher in HIV-infected adults. The impact of HIV disease on arterial inflammation was less striking, becoming significant only after controlling for confounders known to affect atherosclerotic inflammation. Moreover, using detailed, state-of-the-art measures of immune cellular subsets, inflammatory biomarkers, and measures of viral persistence, we observed distinctly separate patterns of immune activation for lymphoid tissues vs. arterial inflammation. Specifically, we found that markers of HIV disease activity and persistence were associated with lymphoid tissue (but not arterial) inflammation. Importantly, despite the effect of HIV on lymphoid tissues, we observed no consistent effect of these markers on arterial inflammation. Instead, we observed that markers of innate system inflammation (hsCRP, IL-6, and non-classical monocytes) were associated with arterial (but not LN) inflammation. Many of these markers have been consistently associated with risk of CVD in both the general population and the HIV-infected population.[32–34] Accordingly, these data demonstrate that arterial inflammation is not closely linked to viral disease activity or to chronic inflammation within viral reservoirs such as lymphoid tissues. These findings have important therapeutic implications both in emerging efforts to reduce the burden of cardiovascular disease in HIV, and to cure HIV infection.

The observation of distinct patterns of immune activation for arterial inflammation and infected lymphoid tissue in HIV-infected humans may have important therapeutic implications. Current studies are testing the hypothesis that reducing viremia may result in lowered arterial inflammation in HIV (NCT01766726). However, our findings suggest that arterial inflammation does not closely follow viral activity. Indeed, in a small study of previously untreated HIV-infected humans, initiation of ART therapy resulted in a marked reduction in lymph node activity (assessed using FDG PET), while the arterial inflammatory signal increased modestly.[28] Hence, the findings from our study provide some insights into the observation of Zanni and colleagues, and provide further data to suggest that control of viremia alone may not result in a reduction in arterial inflammation, and thus may be insufficient for reducing CVD events. Interventions that target other pathways—including monocyte/macrophages—may prove to be more effective in reducing burden of cardiovascular disease in HIV infection. These findings are broadly consistent with various cohort studies that have consistently demonstrated an association between monocyte activation, IL-6, D-dimer, sCD14, and sCD163 and either CVD progression or all-cause mortality.[11,30,36–43]

The association between arterial inflammation and non-classical monocyte subsets may have physiologic relevance. Non-classical monocytes are elevated in the setting of HIV[44], and have been linked to atherosclerosis progression in HIV.[45] The activated monocyte is the target of several therapeutic studies aiming to reduce atherosclerotic inflammation in HIV-infected individuals including low dose methotrexate and IL-1β inhibition (NCT01949116, NCT02312219, and NCT02272946).

In distinction to immune cell subsets and measures of HIV disease activity, soluble inflammatory biomarkers associated with both arterial inflammation as well as lymph node activity and did not separate into distinct pathways. One likely explanation is that the soluble markers are downstream products of chronic HIV infection and are less directly reflective of HIV disease activity as compared to CD4/CD8 ratio and T cell activity. Indeed, in HIV, inflammatory and coagulation markers (e.g. IL-6, sTNFR-I and II, KT ratio, and D-dimer) are known to more strongly associate with non-AIDS events compared to measures of viral disease activity.[39]

Up-regulation of inflammation in HIV disease was much more striking in the lymphoid system as compared to arterial system. Indeed, significant differences for arterial inflammation were only seen when the groups were carefully matched and individuals taking statins (which is known to reduce arterial inflammation in uninfected individuals[32,34]) were excluded. This observation may help to explain the inconsistent findings of heightened arterial inflammation in HIV[15,46,47] since in prior studies, individuals on statins were not consistently excluded and groups were not matched.

We observed a surprisingly high degree of lymphoid inflammation in aviremic states (“elite” control and during ART). Given the association between viral load and inflammation across the entire cohort, and the association between HIV DNA and inflammation in the “elite” controllers, it is likely that HIV production and replication directly stimulate an inflammatory process. The persistent immune dysfunction likely also contributes to inflammation, given the association between CD4/CD8 ratio and lymph node activity. These findings are generally in agreement with prior studies from our group and others including evidence for persistent inflammation and immune dysfunction during ART in gut (a major lymphoid organ).[48–52] We are now actively investigating the degree to which these pathways remain elevated in the lymph nodes of long-term treated adults and the degree to which this phenomenon can be quantified by imaging.

Our findings of a persistent inflammatory environment in the lymphoid tissues of individuals on long-term apparently effective ART has direct implications for emerging efforts to eradicate or control HIV in absence of ART. Theoretically, inflammation within these tissues can cause counter-regulatory immunosuppressive responses, reducing the capacity of the adaptive immune response to eliminate the reservoir. Alternatively, higher levels of T cell activation (a correlate of lymphoid inflammation in this study) could lead to excess production and spread of the virus, even during ART. Finally, excess inflammation may contribute to antigen-specific and cytokine-driven CD4+ T cell proliferation, a major factor contributing to HIV persistence during ART.[53,54] Prospective interventional studies in which these pathways are specifically inhibited in a controlled manner will be necessary to unravel the many complex interactions that likely contribute to HIV persistence and inflammation during ART.[55] Such studies are now ongoing.

Our study has the limitation that it was a cross-sectional study and the findings are associative in nature. Additionally, we did not correct for multiple testing in this exploratory analysis, hence the findings require replication. As with any cross-sectional study, unmeasured confounders may be present. In addition, only male subjects were recruited.

In conclusion, using multi-modal imaging, and HIV viral disease and immune system measures, we observe divergent patterns of immune activation in association with arterial inflammation and lymphoid tissue inflammation. These findings may have important therapeutic implications in both cardiovascular risk modification and curative strategies in HIV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Samji H, Cescon A, Hogg RS, et al. Closing the Gap: Increases in Life Expectancy among Treated HIV-Positive Individuals in the United States and Canada. PLoS. ONE. 2013; 8(12):e81355. [PubMed: 24367482]
2. Hemkens LG, Bucher HC. HIV infection and cardiovascular disease. European Heart Journal. 2014; 35(21):1373–1381. [PubMed: 24408888]
3. Freiberg MS, Chang CC, Kuller LH, et al. HIV infection and the risk of acute myocardial infarction. JAMA internal medicine. 2013; 173(8):614–622. [PubMed: 23459863]
4. Tseng ZH, Secemsky EA, Dowdy D, et al. Sudden Cardiac Death in Patients With Human Immunodeficiency Virus Infection. Journal of the American College of Cardiology. 2012; 59(21): 1891–1896. [PubMed: 22595409]
5. Chow FC, Regan S, Feske S, Meigs JB, Grinspoon SK, Triant VA. Comparison of ischemic stroke incidence in HIV-infected and non-HIV-infected patients in a US health care system. Journal of acquired immune deficiency syndromes (1999). 2012; 60(4):351–358. [PubMed: 22580566]
6. Kaplan RC, Kingsley LA, Sharrett AR, et al. Ten-year predicted coronary heart disease risk in HIV-infected men and women. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2007; 45(8):1074–1081. [PubMed: 17879928]
7. Saves M, Chene G, Ducimetiere P, et al. Risk factors for coronary heart disease in patients treated for human immunodeficiency virus infection compared with the general population. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2003; 37(2):292–298. [PubMed: 12856222]
8. Friis-Moller N, Weber R, Reiss P, et al. Cardiovascular disease risk factors in HIV patients--association with antiretroviral therapy. Results from the DAD study. AIDS (London, England). 2003; 17(8):1179–1193.

9. Riddler SA, Smit E, Cole SR, et al. Impact of HIV infection and HAART on serum lipids in men. Jama. 2003; 289(22):2978–2982. [PubMed: 12799406]
10. Hsue PY, Deeks SG, Hunt PW. Immunologic basis of cardiovascular disease in HIV-infected adults. The Journal of infectious diseases. 2012; 205(Suppl 3):S375–S382. [PubMed: 22577211]
11. Duprez DA, Neuhaus J, Kuller LH, et al. Inflammation, Coagulation and Cardiovascular Disease in HIV-Infected Individuals. PLoS. ONE. 2012; 7(9):e44454. [PubMed: 22970224]
12. Libby P. Inflammation in atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2012; 32(9):2045–2051.
13. Rominger A, Saam T, Wolpers S, et al. 18F-FDG PET/CT identifies patients at risk for future vascular events in an otherwise asymptomatic cohort with neoplastic disease. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2009; 50(10):1611–1620.
14. Figueroa AL, Abdelbaky A, Truong QA, et al. Measurement of Arterial Activity on Routine FDG PET/CT Images Improves Prediction of Risk of Future CV Events. JACC Cardiovasc Imaging. 2013; 6(12):1250–1259. [PubMed: 24269261]
15. Subramanian S, Tawakol A, Burdo TH, et al. Arterial inflammation in patients with HIV. Jama. 2012; 308(4):379–386. [PubMed: 22820791]
16. Tawakol A, Lo J, Zanni MV, et al. Increased arterial inflammation relates to high-risk coronary plaque morphology in HIV-infected patients. Journal of acquired immune deficiency syndromes (1999). 2014; 66(2):164–171. [PubMed: 24828267]
17. Zeng M, Southern PJ, Reilly CS, et al. Lymphoid tissue damage in HIV-1 infection depletes naive T cells and limits T cell reconstitution after antiretroviral therapy. PLoS Pathog. 2012; 8(1):e1002437. [PubMed: 22241988]
18. Doitsh G, Galloway NL, Geng X, et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. Nature. 2014; 505(7484):509–514. [PubMed: 24356306]
19. Deeks SG. HIV infection, inflammation, immunosenescence, and aging. Annu. Rev Med. 2011; 62:141–155. [PubMed: 21090961]
20. Sarrazin JF, Philippon F, Tessier M, et al. Usefulness of fluorine-18 positron emission tomography/computed tomography for identification of cardiovascular implantable electronic device infections. J Am Coll Cardiol. 2012; 59(18):1616–1625. [PubMed: 22538331]
21. Rudd J, Warburton E, Fryer T, et al. Imaging atherosclerotic plaque inflammation with [18F]-Fluorodeoxyglucose positron emission tomography. Circulation. 2002; 105:2708–2711. [PubMed: 12057982]
22. Tawakol A, Migrino RQ, Bashian GG, et al. In vivo 18F-fluorodeoxyglucose positron emission tomography imaging provides a noninvasive measure of carotid plaque inflammation in patients. J Am Coll Cardiol. 2006; 48(9):1818–1824. [PubMed: 17084256]
23. Tawakol A, Singh P, Mojena M, et al. HIF-1alpha and PFKFB3 Mediate a Tight Relationship Between Proinflammatory Activation and Anerobic Metabolism in Atherosclerotic Macrophages. Arterioscler Thromb Vasc Biol. 2015
24. Blankstein R, Osborne M, Naya M, et al. Cardiac positron emission tomography enhances prognostic assessments of patients with suspected cardiac sarcoidosis. Journal of the American College of Cardiology. 2014; 63(4):329–336. [PubMed: 24140661]
25. Osborne MT, Hulten EA, Singh A, et al. Reduction in (1)(8)F-fluorodeoxyglucose uptake on serial cardiac positron emission tomography is associated with improved left ventricular ejection fraction in patients with cardiac sarcoidosis. Journal of nuclear cardiology : official publication of the American Society of Nuclear Cardiology. 2014; 21(1):166–174. [PubMed: 24307261]
26. Ricciardi A, Sordillo P, Ceccarelli L, et al. 18-Fluoro-2-deoxyglucose positron emission tomography-computed tomography: an additional tool in the diagnosis of prosthetic valve endocarditis. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2014; 28:219–224. [PubMed: 25093540]
27. Saby L, Laas O, Habib G, et al. Positron emission tomography/computed tomography for diagnosis of prosthetic valve endocarditis: increased valvular 18F-fluorodeoxyglucose uptake as a novel major criterion. Journal of the American College of Cardiology. 2013; 61(23):2374–2382. [PubMed: 23583251]

28. Zanni MV, Toribio M, Robbins G, et al. Discordant Effects of Antiretroviral Therapy on Systemic Immune Parameters and Arterial Inflammation among Treatment-Naive Individuals with HIV. JAMA Cardiology. 2016 in press.
29. 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. [PubMed: 25122785]
30. Baker JV, Hullsiek KH, Singh A, et al. Immunologic predictors of coronary artery calcium progression in a contemporary HIV cohort. AIDS. 2014; 28(6):831–840. [PubMed: 24370480]
31. Rudd J, Myers K, Bansilal S, et al. 18Fluorodeoxyglucose positron emission tomography imaging of atherosclerotic plaque inflammation is highly reproducible: implications for atherosclerosis therapy trials. J Am Coll Cardiol. 2007; 50:892–896. [PubMed: 17719477]
32. Tawakol A, Fayad ZA, Mogg R, et al. Intensification of Statin Therapy Results in a Rapid Reduction in Atherosclerotic InflammationResults of a Multicenter Fluorodeoxyglucose-Positron Emission Tomography/Computed Tomography Feasibility Study. Journal of the American College of Cardiology. 2013; 62(10):909–917. [PubMed: 23727083]
33. Emami H, Singh P, MacNabb M, et al. Splenic metabolic activity predicts risk of future cardiovascular events: demonstration of a cardiosplenic axis in humans. JACC Cardiovasc Imaging. 2015 Feb 8.(2):121–130. [PubMed: 25577441]
34. Tahara N, Kai H, Ishibashi M, et al. Simvastatin Attenuates Plaque InflammationEvaluation by Fluorodeoxyglucose Positron Emission Tomography. Journal of the American College of Cardiology. 2006; 48(9):1825–1831. [PubMed: 17084257]
35. Borges AH, O'Connor JL, Phillips AN, et al. IL-6 is a stronger predictor of clinical events than hsCRP or D-dimer during HIV infection. The Journal of infectious diseases. 2016
36. Nordell AD, McKenna M, Borges AH, et al. Severity of cardiovascular disease outcomes among patients with HIV is related to markers of inflammation and coagulation. J Am Heart Assoc. 2014; 3(3):e000844. [PubMed: 24870935]
37. Sandler NG, Wand H, Roque A, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. The Journal of infectious diseases. 2011; 203(6):780–790. [PubMed: 21252259]
38. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS. Med. 2008; 5(10):e203. [PubMed: 18942885]
39. Tenorio AR, Zheng Y, Bosch RJ, et al. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect. Dis. 2014; 210(8):1248–1259. [PubMed: 24795473]
40. Boulware DR, Hullsiek KH, Puronen CE, et al. Higher levels of CRP, D-dimer, IL-6, and hyaluronic acid before initiation of antiretroviral therapy (ART) are associated with increased risk of AIDS or death. J Infect. Dis. 2011; 203(11):1637–1646. [PubMed: 21592994]
41. Burdo TH, Lentz MR, Autissier P, et al. Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after anti-retroviral therapy. The Journal of infectious diseases. 2011; 204(1):154–163. [PubMed: 21628670]
42. So-Armah KA, Tate JP, Chang CH, et al. Do Biomarkers Of Inflammation, Monocyte Activation And Altered Coagulation Explain Excess Mortality Between HIV Infected and Uninfected People? J Acquir Immune Defic Syndr. 2016
43. Hsu DC, Ma YF, Hur S, et al. Plasma IL-6 levels are independently associated with atherosclerosis and mortality in HIV-infected individuals on suppressive ART. Aids. 2016
44. Tippett E, Cheng W-J, Westhorpe C, et al. Differential Expression of CD163 on Monocyte Subsets in Healthy and HIV-1 Infected Individuals. PLoS. ONE. 2011; 6(5):e19968. [PubMed: 21625498]
45. Zungsontiporn N, Tello RR, Zhang G, et al. Non-Classical Monocytes and Monocyte Chemoattractant Protein-1 (MCP-1) Correlate with Coronary Artery Calcium Progression in Chronically HIV-1 Infected Adults on Stable Antiretroviral Therapy. PLoS. ONE. 2016; 11(2):e0149143. [PubMed: 26867220]
46. Knudsen A, Hag AM, Loft A, et al. HIV infection and arterial inflammation assessed by (18)F-fluorodeoxyglucose (FDG) positron emission tomography (PET): a prospective cross-sectional

study. Journal of nuclear cardiology : official publication of the American Society of Nuclear Cardiology. 2015; 22(2):372–380. [PubMed: 25467249]

47. Yarasheski KE, Laciny E, Overton ET, et al. (18)FDG PET-CT imaging detects arterial inflammation and early atherosclerosis in HIV-infected adults with cardiovascular disease risk factors. Journal of Inflammation (London, England). 2012; 9:26–26.
48. Sanchez JL, Hunt PW, Reilly CS, et al. Lymphoid fibrosis occurs in long-term nonprogressors and persists with antiretroviral therapy but may be reversible with curative interventions. J Infect. Dis. 2015; 211(7):1068–1075. [PubMed: 25344521]
49. Somsouk M, Estes JD, Deleage C, et al. Gut epithelial barrier and systemic inflammation during chronic HIV infection. Aids. 2015; 29(1):43–51. [PubMed: 25387317]
50. Favre D, Mold J, Hunt PW, et al. Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of TH17 to regulatory T cells in HIV disease. Sci Transl. Med. 2010; 2(32):32ra36.
51. Macal M, Sankaran S, Chun TW, et al. Effective CD4+ T-cell restoration in gut-associated lymphoid tissue of HIV-infected patients is associated with enhanced Th17 cells and polyfunctional HIV-specific T-cell responses. Mucosal Immunol. 2008; 1(6):475–488. [PubMed: 19079215]
52. Mutlu EA, Keshavarzian A, Losurdo J, et al. A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. PLoS Pathog. 2014; 10(2):e1003829. [PubMed: 24586144]
53. Maldarelli F, Wu X, Su L, et al. HIV latency. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. Science. 2014; 345(6193):179–183. [PubMed: 24968937]
54. Wagner TA, McLaughlin S, Garg K, et al. HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. Science. 2014; 345(6196):570–573. [PubMed: 25011556]
55. Barouch DH, Deeks SG. Immunologic strategies for HIV-1 remission and eradication. Science. 2014; 345(6193):169–174. [PubMed: 25013067]

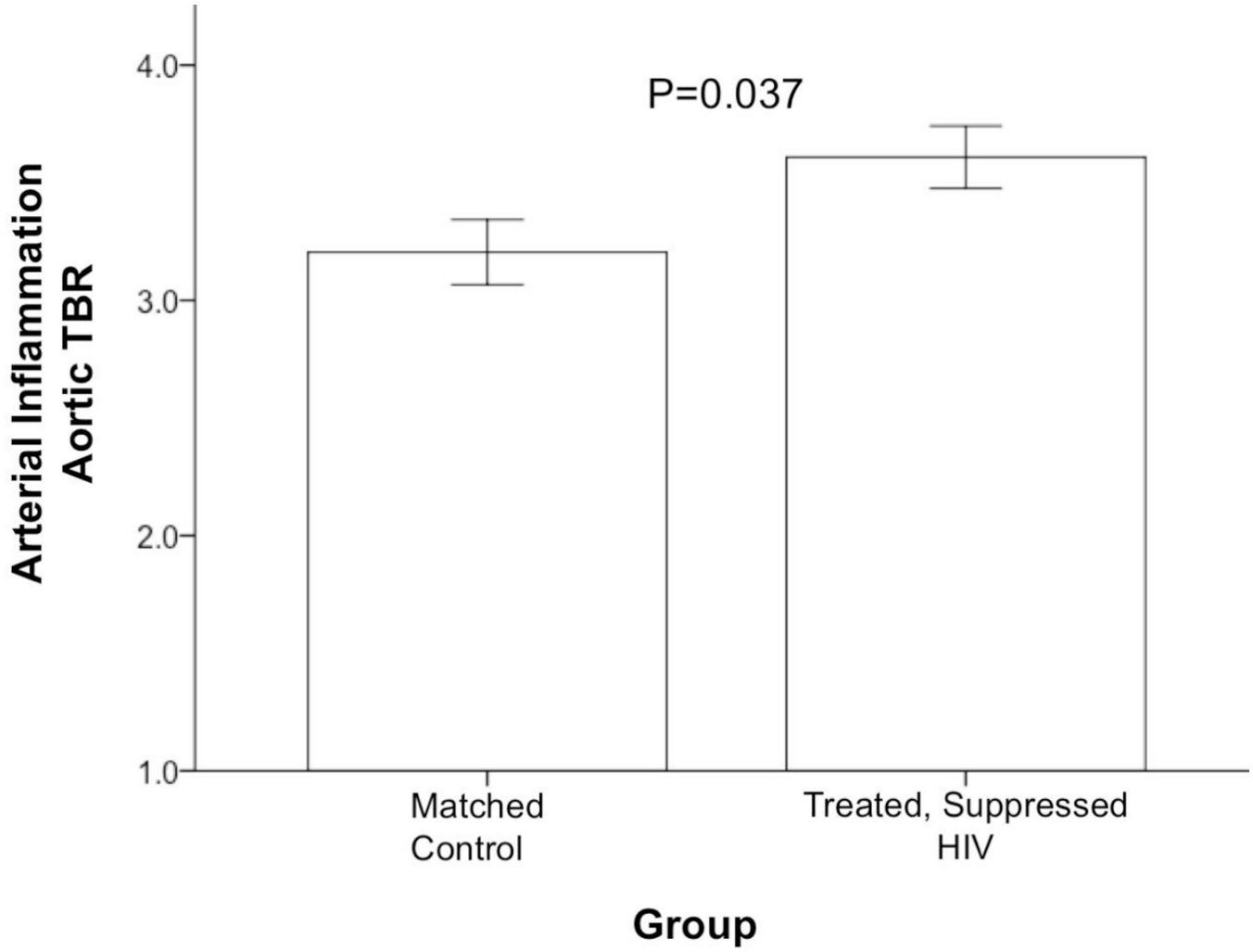
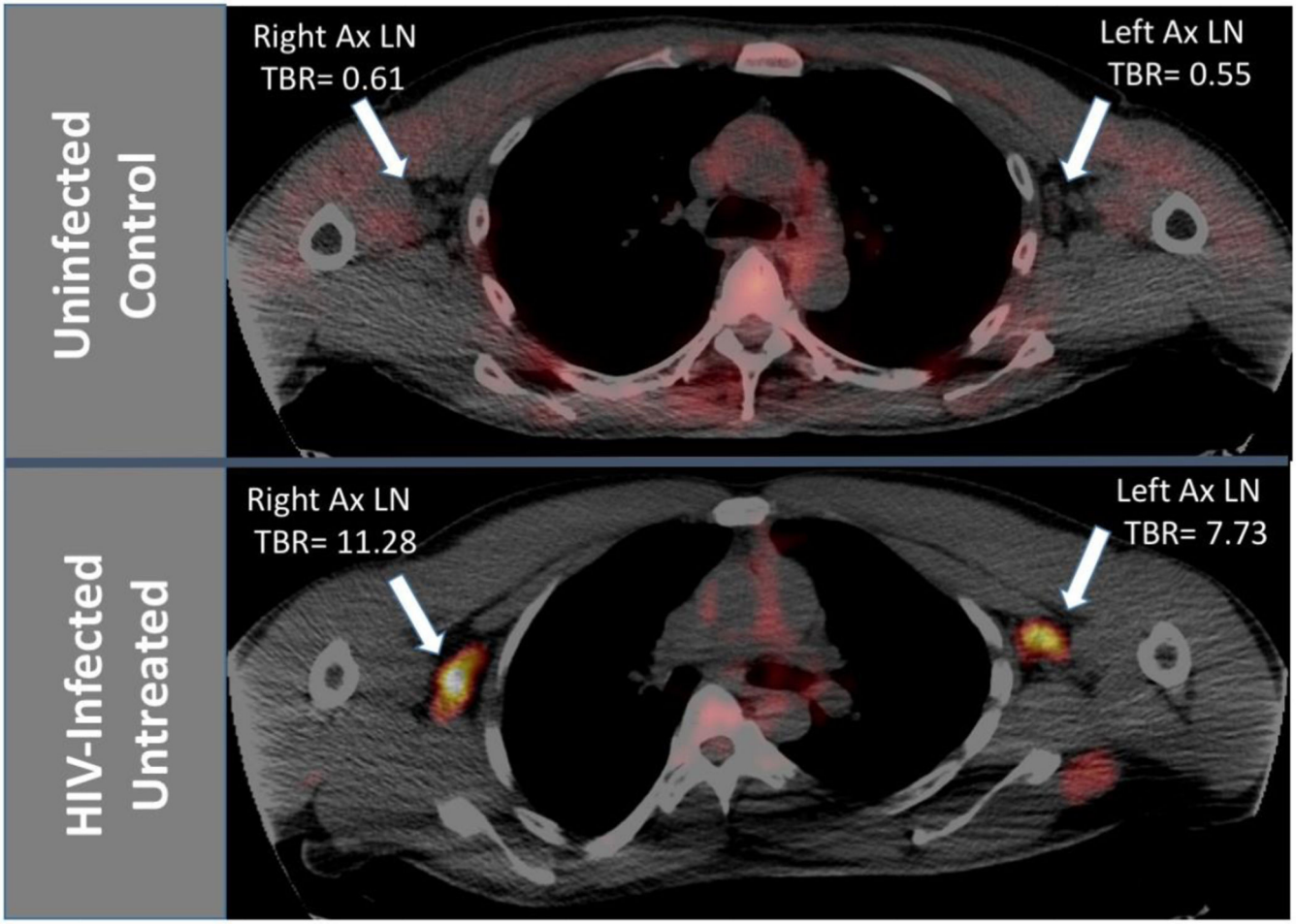


Figure 1.



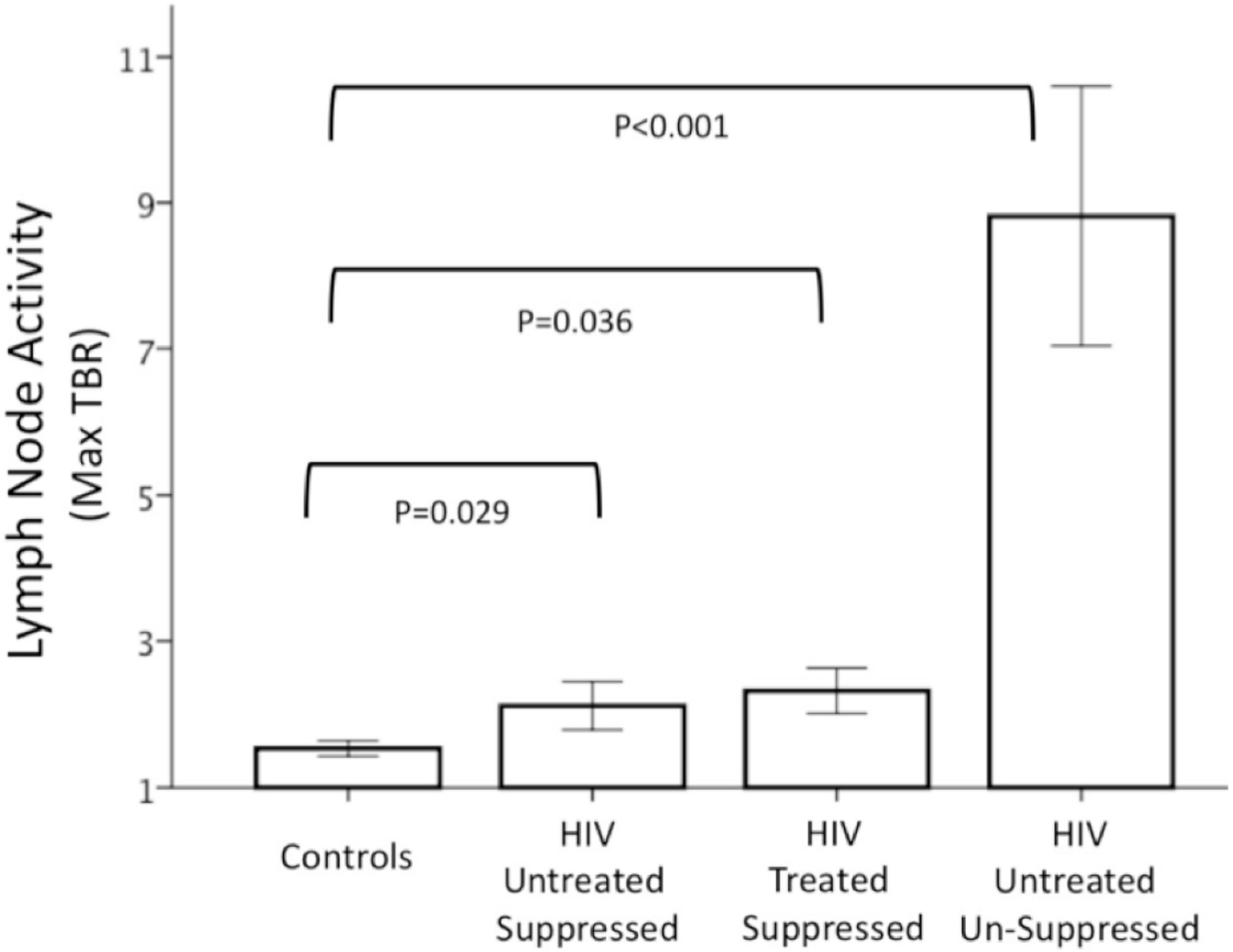


Figure 2.

Table 1

Clinical characteristics of study subjects

Parameter	HIV− (n=29)	HIV+ (n=45)	p-value
Demographic Factors			
Age (years)	53 (49, 59)	52 (46, 56)	0.143
Race (%)			0.118
Caucasian	12 (41)	30 (67)	
African-American	14 (48)	10 (22)	
Latino	2 (7)	4 (9)	
Other	1 (4)	1 (2)	
Male	29 (100)	45 (100)	
BMI (kg/m^2)	27.0, (23.1, 29.2)	26.2 (24.0, 28.7)	0.550
Comorbidities			
Family History	3 (10)	16 (36)	**0.015**
Hypertension	6 (21)	11 (24)	0.708
Diabetes	0	2 (4)	0.250
Statins	1 (5)	5 (11)	0.238
Smoke ever	18 (62)	29 (64)	0.836
Aspirin use	3 (10)	11 (24)	0.131
History of MI/Stroke	1 (4)	1 (2)	0.751
Hep C	2 (7)	10 (22)	0.081
Labs			
Total Cholesterol (mg/dL)	173 (154, 199)	178 (152, 206)	0.987
LDL (mg/dL)	108 (84, 120)	110 (90, 129)	0.469
HDL (mg/dL)	49 (44, 63)	46 (37, 53)	0.068
Triglycerides (mg/dL)	85 (63, 117)	91 (70, 132)	0.499
Creatinine (mg/dL)	0.91 (0.81, 1.04)	0.96 (0.86, 1.07)	0.189
Inflammatory Markers			
D-Dimer (ug/mL)	0.39 (0.28, 0.53)	0.34 (0.25, 0.49)	0.410
CRP (mg/L)	1.19 (0.41, 5.08)	1.29 (0.53, 2.69)	0.834
sCD14 (ug/mL)	1.52 (1.34, 1.75)	1.75 (1.40, 1.95)	0.082
sCD163 (ng/mL)	307.46 (224.23, 431.61)	455.73 (344.37, 650.55)	**0.002**
IL-6 (pg/mL)	0.87 (0.61, 1.38)	0.86 (0.56, 1.76)	0.849
MCP-1 (pg/mL)	213.76 (158.53, 259.17)	210.09 (159.55, 360.06)	0.442
TF (pg/mL)	71.66 (52.95, 81.96)	68·83 (55.30, 83.34)	0.995
FRS (%)	5 (3, 9)	6 (3, 7)	0.986

Values represent number (percentage), or median values (IQR)

Abbreviations: LDL – low-density lipoprotein cholesterol; HDL – high-density lipoprotein cholesterol; CRP – high sensitivity C-reactive protein; sCD14 – soluble CD14; sCD163 – soluble CD163; IL-6; interleukin 6; MCP1 – monocyte chemoattractant protein-1; TF – tissue factor; FRS – Framingham Risk Score

Table 2

Correlations between viral load, CD4 and CD4 Nadir with tissue FDG-uptake in all HIV-positive individuals (n=45)

Disease Activity Measure	Lymph node activity		Arterial inflammation	
	Correlation coefficient	p-value	Correlation coefficient	p-value
Viral load	**0.302**	**0.047**	0.012	0.938
CD4	−0.109	0.481	0.112	0.470
CD4 Nadir	−0.12	0.449	−0.128	0.419
CD8	**0.513**	**0.002**	0.090	0.614
CD4:CD8	**−0.412**	**0.016**	−0.138	0.438

Table 3

Correlations with biomarkers in all treated suppressed individuals (n=33)

Group	Marker	Arterial inflammation		Lymph node activation	
		Correlation	P value	Correlation	P value
Markers of inflammation	D-dimer	0.324	0.080	**0.371**	**0.043**
	CRP	**0.543**	**0.002**	0.299	0.108
	IL6	**0.383**	**0.037**	**0.386**	**0.035**
	sCD163	−0.104	0.579	0.136	0.474
	sCD14	−0.047	0.802	0.259	0.167
CD4$^+$ T cell markers	% CD4$^+$ cells	0.063	0.739	−0.344	0.063
	% HLA$^-$DR$^+$CD38$^+$	0.137	0.471	**0.474**	**0.008**
	% CD28$^-$CD57$^-$	0.148	0.435	0.189	0.316
	% CD28$^-$CD57$^+$	0.281	0.133	0.030	0.877
	% CX3CR1$^+$	0.314	0.091	0.036	0.851
CD8$^+$ T cell markers	% HLA$^-$DR$^+$CD38$^+$	0.196	0.300	0.319	0.086
	% CD28$^-$CD57$^-$	−0.027	0.886	−0.008	0.968
	% CD28$^-$CD57$^+$	0.167	0.148	−0.199	0.291
	% CX3CR1$^+$	0.070	0.712	−0.137	0.471
Monocyte Markers	% CD14$^+$CD16$^+$	−0.316	0.089	−0.091	0.633
	% CD14$^+$CD16$^-$	0.293	0.116	**0.384**	**0.036**
	% CD14^{dim}CD16$^+$	**0.381**	**0.038**	0.033	0.862
	% CX3CR1$^+$	**0.372**	**0.043**	0.170	0.369
	% CCR2$^+$	**−0.412**	**0.024**	−0.191	0.312
	% TF	0.180	0.332	0.328	0.077

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