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## Serum amyloid P (SAP) is associated with impaired brachial artery flow-mediated dilation in chronically HIV-1 infected adults on stable antiretroviral therapy

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### Abstract

**Objective**—This study aimed to evaluate the relationship between inflammatory biomarkers and endothelial dysfunction (ED), as measured by brachial artery flow-mediated dilation (FMD).

**Methods**—We conducted a cross-sectional analysis utilizing baseline data of 135 participants with HIV infection on stable antiretroviral therapy (ART) in the Hawaii Aging with HIV-Cardiovascular (HAHC-CVD) study who had available baseline inflammatory biomarkers and brachial artery FMD measurements.

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#### Disclaimer statements

The findings and conclusions of this study do not necessarily represent the views of The Queen's Medical Center.

#### Contributors

All authors were involved in study conception or design, or acquisition of data or analysis, and interpretation of data. All authors participated in revising the manuscript critically for important intellectual content, and approved the final version for publication.

#### Conflict of interest

None of the authors have any relevant conflicts of interest to disclose.

#### Ethics approval

The study was approved by the University of Hawaii Committee on Human Subjects and was performed in accordance with the Declaration of Helsinki, all International Conference on Harmonization Good Clinical Practice guidelines, and applicable local regulatory requirements and laws. Written informed consents were obtained from all participants.

**Results**—We observed significant associations between brachial artery FMD and baseline brachial artery diameter, age, male gender, traditional cardiovascular disease (CVD) risk factors such as BMI, waist to hip ratio, hypertension, systolic blood pressure (BP), diastolic BP, and LDL cholesterol, and 10-year coronary heart disease (CHD) risk estimated by Framingham risk score (FRS). Of all biomarkers tested, higher level of C-reactive protein (CRP) (beta =  $-0.695$ ,  $P=0.030$ ) and serum amyloid P (SAP) (beta =  $-1.318$ ,  $P=0.021$ ) were significantly associated with lower brachial artery FMD in univariable regression analysis. After adjusting for baseline brachial artery diameter, age, and selected traditional CVD risk factors in multivariable model, SAP remained significantly associated with brachial artery FMD (beta =  $-1.094$ ,  $P=0.030$ ), while CRP was not (beta =  $-0.391$ ,  $P=0.181$ ).

**Discussion**—Serum amyloid P was independently associated with impaired brachial artery FMD and may potentially relate to ED and increased CVD risk in HIV-infected patients on stable ART.

### Keywords

Serum amyloid P; Pentraxin; Inflammatory biomarkers; Endothelial dysfunction; Brachial artery flow-mediated dilation; HIV infection

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## Introduction

Human immunodeficiency virus (HIV) infection has been associated with increased risk of atherosclerotic cardiovascular diseases (ASCVD), even among patients with well-suppressed HIV infection on ART.<sup>1,2</sup> Endothelial dysfunction (ED) is considered to play an important role in the development of atherosclerosis<sup>3,4</sup> and when assessed by brachial artery flow-mediated dilation (FMD) has been shown to independently predict future ASCVDs.<sup>5-7</sup> Paralleling clinical observation, studies assessing ED by brachial artery FMD in HIV-infected patients have consistently demonstrated ED relative to patients without HIV infection.<sup>8-10</sup>

Human immunodeficiency virus-infected patients, including those with well-suppressed HIV infection on ART, are in the state of chronic inflammation as evidenced by elevated levels of inflammatory biomarkers, such as C-reactive protein (CRP) and interleukin (IL)-6.<sup>11</sup> Although the role of these inflammatory biomarkers as markers of cardiovascular risk remains uncertain in HIV-infected patients, several studies have reported the association of these inflammatory biomarkers with cardiovascular disease (CVD) events.<sup>12-14</sup> It has, therefore, been hypothesized that persistent immune activation and inflammation contribute to atherosclerosis in HIV-infected patients.<sup>15,16</sup> However, studies previously assessing inflammatory biomarkers, such as CRP, for their association with brachial artery FMD yielded inconsistent results.<sup>9,17-22</sup> Thus, the relationship between inflammation and ED in HIV-infected patients remains uncertain.

In this study, we hypothesized that persistent inflammation is related to ED in HIV-infected patients. We examine biomarkers reflecting several aspects of inflammation, including cytokines: IL-1b, IL-6, IL-8, IL-10, tumor necrosis factor (TNF)-alpha, and interferon (IFN)-gamma; acute phase proteins such as CRP and serum amyloid A (SAA); soluble

adhesion molecules such as soluble E-selectin (sE-selectin), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble intercellular adhesion molecule-1 (sICAM-1); chemokine monocyte chemoattractant protein (MCP)-1; and other inflammatory markers including serum amyloid P (SAP), matrix metalloproteinase (MMP)-9, and myeloperoxidase (MPO), for their association with ED as measured by brachial artery FMD in HIV-infected participants on stable ART.

## Materials and Methods

### Study participants

This is a cross sectional analysis utilizing the baseline data of participants from the Hawaii Aging with HIV-Cardiovascular (HAHC-CVD) study. We included only participants who had available baseline inflammatory biomarkers and brachial artery FMD measurements. The details of study design and enrollment have been published previously.<sup>23</sup> Briefly, the study enrolled adults, age  $\geq 40$  years with documented HIV infection who were on stable ART for  $\geq 3$  months, from 2009 to 2012. The study was approved by the Committee on Human Subjects at our institution and was performed in accordance with the Declaration of Helsinki, all International Conference on Harmonization Good Clinical Practice guidelines, and applicable local regulatory requirements and laws. Written informed consents were obtained from all participants.

### Clinical assessment

General medical history with special emphasis on CVD and HIV infection was obtained. Clinical parameters including blood pressure (BP), height, weight, body mass index (BMI), and waist to hip ratio were measured. Smoking history was defined as a dichotomous variable of ever smoked or never smoked. Routine HIV blood tests, including CD4zT-cell count and HIV RNA, as well as CVD blood tests, including fasting blood tests for total, high-density lipoprotein (HDL), directly measured low-density lipoprotein (LDL) cholesterol, triglycerides, and glucose were performed.

Hypertension was defined as systolic BP  $\geq 140$  mmHg or diastolic BP  $\geq 90$  mmHg on entry visit, self-reported history of hypertension, or use of anti-hypertensive medications. Diabetes mellitus was defined by FBS  $\geq 126$  mg/dl, 2-hour OGTT  $\geq 200$  mg/dl, or self-reported history of diabetes mellitus. Ten-year coronary heart disease (CHD) risk was calculated by Framingham Risk Score (FRS) based on a model comprised of age, gender, total cholesterol, HDL cholesterol, systolic BP, treatment of hypertension, and any cigarette smoking in the past month using the National Cholesterol Education Program website (<http://hp2010.nhlbihin.net/atp/iii/calculator.asp>).<sup>24</sup> Participants with diabetes (as a CVD equivalent) or clinical CVD (history of myocardial infarction, angina, coronary disease-related cardiac surgery, or ischemic stroke) were automatically classified as having 10-year CHD risk by FRS of 20%. Clinical CVD events were adjudicated by two physician-researchers (CMS and DCC). Undetectable plasma HIV RNA was defined as HIV RNA  $<50$  copies/ml.

## Brachial artery FMD

Flow-mediated dilation testing was performed in the morning following a 12-hour fast utilizing previously published methodology.<sup>18,23,25</sup> Those who regularly used tobacco products refrained for at least 8 hours prior to the test. The test was conducted by forearm occlusion using a high-resolution linear array vascular ultrasound transducer (Siemens ACUSON Cypress) after a 10-minute rest in a temperature-controlled room (70–76°F). Each study was recorded digitally and measured in triplicate by a single blinded reader at the core FMD laboratory at the University of Wisconsin at Madison. Flow-mediated dilation testing was obtained between 2 and 14 days of the biomarker assessments.

## Biomarkers assessment

Plasma was assayed for inflammatory biomarkers, including sE-selectin, sVCAM-1, sICAM-1, MMP-9, MPO, CRP, SAA, SAP, IL-1b, IL-6, IL-8, IL-10, TNF-alpha, MCP-1, and IFN-gamma, using anti-body-coated beads in a high sensitivity Milliplex Human CVD biomarker panel (Millipore, Billerica, MA, USA) as previously described.<sup>26</sup> The minimum detectable concentration of CRP of this assay is 0.001 ng/ml. Standard curves and samples were tested in duplicate. Samples were acquired on a Labscan 200 analyzer (Luminex, Austin, TX, USA) using Bio-Plex manager software (Bio-Rad, Hercules, CA, USA). The average coefficient of variation of all biomarker measurements was <10%.

## Statistical analyses

Demographic, cardiovascular, and HIV-related characteristics, as well as inflammatory biomarkers and brachial artery FMD measurements were described using the median, first quartile (Q1), and third quartile (Q3) for continuous variables and frequency and percent for categorical variables.

Univariable linear regression analyses were performed to assess inflammatory biomarkers as well as demographic, cardiovascular, and HIV-related characteristics for their associations with brachial artery FMD. Significant unadjusted associations between inflammatory biomarkers and brachial artery FMD were further evaluated in multivariable linear regression analyses adjusting for participants' characteristics that were significantly associated with brachial artery FMD and selected traditional CVD risk factors that were previously reported to be associated with brachial artery FMD.<sup>27</sup> Final model included adjustment for baseline brachial artery diameter, age, systolic BP, LDL cholesterol, waist to hip ratio, diabetes mellitus, and smoking history. All inflammatory biomarkers were log transformed to normalization for analyses. All statistical analyses were conducted using SPSS (IBM, Version 21, Armonk, NY, USA). A two-sided probability of *P*-value <0.05 was considered statistically significant.

## Results

### Participant characteristics

Of all participants enrolled into the HAHC-CVD cohort, a total of 135 participants had available baseline inflammatory biomarkers and brachial artery FMD measurements and were included in this analysis. The demographic, cardiovascular, HIV-related, and brachial

artery FMD characteristics of the participants are presented in the Table 1. Participants were predominantly white (58.5%) and male (88.1%), with median age of 50 years. History of CVD events was presented in 7.4% of participants. The median 10-year CHD risk estimated by FRS was 6%. Most participants (85.9%) had undetectable HIV RNA and the median CD4zT-cell count of all participants was 480 cells/mm<sup>3</sup>. Nucleoside Reverse Transcriptase Inhibitors (NRTI), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI), Protease Inhibitors (PI), and Integrase Inhibitor were currently used by 94.8, 48.9, 47.4, and 12.6% of participants, respectively. The median (Q1, Q3) of brachial artery FMD in this cohort was 3.72% (2.36%, 6.01%). The median (Q1, Q3) of sE-selectin, sVCAM-1, sICAM-1, MMP-9, MPO, CRP, SAA, SAP, IL-1b, IL-6, IL-8, IL-10, TNF-alpha, MCP-1, and IFN-gamma are shown in Table 1.

### **Association between Participant characteristics, Inflammatory biomarkers, and Brachial artery FMD**

In univariable linear regression analysis (Table 2), larger baseline brachial artery diameter (beta = -20.777,  $P < 0.001$ ), older age (beta = -0.064,  $P = 0.033$ ), male gender (beta = -1.640,  $P = 0.025$ ), higher BMI (beta = -0.128,  $P = 0.017$ ), higher waist to hip ratio (beta = -11.358,  $P = 0.012$ ), hypertension (beta = -1.674,  $P = 0.001$ ), higher systolic (beta = -0.048,  $P = 0.001$ ) and diastolic BP (beta = -0.084,  $P = 0.001$ ), higher LDL cholesterol (beta = -0.015,  $P = 0.032$ ), and higher 10-year CHD risk estimated by FRS (beta = -6.595,  $P = 0.049$ ) were significantly associated with lower brachial artery FMD (reflecting worse ED). However, HIV-related factors (CD4zT-cell count, CD4zT-cell nadir, and undetectable HIV RNA), current use of cardiovascular medications (angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers, beta blockers, aspirin, and statins) and antiretroviral medications (NRTIs, NNRTIs, PIs, and integrase inhibitor) were not associated with brachial artery FMD.

Higher CRP (beta = -0.695,  $P = 0.030$ ) and SAP (beta = -1.318,  $P = 0.021$ ) levels were significantly associated with lower brachial artery FMD in univariable linear regression analysis (Table 2). While sE-selectin, sVCAM-1, sICAM-1, MMP-9, MPO, SAA, IL-1b, IL-6, IL-8, IL-10, TNF-alpha, MCP-1, and IFN-gamma were not significantly associated with brachial artery FMD.

The association between inflammatory biomarkers, CRP and SAP, and brachial artery FMD was further explored in multivariable linear regression analysis (Table 3). In the model adjusting for baseline brachial artery diameter, age, systolic BP, LDL cholesterol, waist to hip ratio, diabetes mellitus, and smoking history, higher level of SAP remained significantly associated with lower brachial artery FMD (beta = -1.094,  $P = 0.030$ ), while CRP was not (beta = -0.391,  $P = 0.181$ ). Exclusion of 10 participants with history of CVD did not affect the significance of these associations (data not shown).

## **Discussion**

Endothelial dysfunction has been proposed to be a precursor in development of atherosclerosis and an integrative marker of the net effect of all cardiovascular risk factors, both traditional and emerging.<sup>3,4</sup> In this cohort of HIV-infected patients on stable ART for

3 months, we found significant associations between higher levels of inflammatory biomarkers, CRP and SAP, and lower brachial artery FMD, a marker of ED. After adjusting for baseline brachial artery diameter, age, and traditional CVD risk factors, SAP remained significantly associated with brachial artery FMD.

Both CRP and SAP are members of pentraxin, a family of serum proteins that serve as pattern recognition molecules and can activate an immune system and modulate inflammatory response.<sup>28</sup> C-reactive protein is widely utilized as a marker of acute and chronic inflammation. It is considered to be an acute phase protein as its serum level varies widely in response to inflammatory cytokines IL-1 and IL-6,<sup>28,29</sup> especially in acute inflammatory processes. Serum amyloid P is a lesser-known inflammatory biomarker that has been associated with subclinical atherosclerosis and clinical ASCVDs in non-HIV patients.<sup>30</sup> Although its expression is influenced by the inflammatory cytokines IL-1 and IL-6, it is not considered to be an acute phase protein because its serum level is relatively stable and only weakly influenced by acute inflammatory processes.<sup>28,29</sup>

C-reactive protein was previously reported to be associated with impaired brachial artery FMD in one study in HIV-infected children.<sup>20</sup> Most studies previously assessing CRP and brachial artery FMD in patients with HIV failed to demonstrate this association.<sup>9,17–19,22,31,32</sup> One study enrolled 331 ART-naïve HIV-infected participants also failed to demonstrate the association between CRP and brachial artery FMD.<sup>21</sup> The difference in the results of this study and our study may be due to different participants' demographic as well as ART utilization. To our knowledge, the association between SAP and brachial artery FMD has never been demonstrated. This association might not be entirely unexpected given that pentraxin 3 (PTX3), another member of pentraxin, was also reported to be associated with brachial artery FMD.<sup>33</sup>

Overall, the association of CRP and SAP with brachial artery FMD demonstrated in our study is consistent with the hypothesis that inflammation is related to ED in HIV-infected patients. However, the association between CRP and brachial artery FMD is not independent of traditional CVD risk factors given attenuation of the association in the multivariable regression model. This finding is consistent with the result of a large cohort of non-HIV patients<sup>34</sup> and suggests that, similar to non-HIV patients, traditional CVD risk factors remain important driving force of inflammation in HIV-infected patients on stable ART.

Higher levels of SAP remained significantly associated with lower brachial artery FMD after adjustment for traditional CVD risk factors. This suggests that SAP may reflect different pathological processes from CRP. The difference in ligand binding and functional response between CRP and SAP<sup>28,29</sup> may partly explain this observed difference. For example, CRP has been demonstrated to promote native and oxidized LDL uptake by macrophages,<sup>35,36</sup> while SAP was reported to prevent oxidized LDL uptake by macrophages.<sup>37</sup> Thus, CRP and SAP may have different effects on foam cell formation and early development of atherosclerosis. Serum amyloid P was also reported to bind to HDL and inhibit HDL-mediated neutralization of lipopolysaccharide (LPS).<sup>38</sup> This issue may be especially important as higher LPS levels has been associated with lower brachial artery FMD in HIV-

infected on ART.<sup>39</sup> In addition, SAP may modulate the degradation of and inflammatory response to amyloid fibrils,<sup>29,40</sup> which can be found in atherosclerosis.

Although the role and incremental value of brachial artery FMD in cardiovascular risk stratification remains uncertain, lower brachial artery FMD has been shown to independently predict future ASCVDs in a number of studies.<sup>5-7</sup> Thus, given the independent association between SAP and brachial artery FMD, SAP may provide additional predictive power to traditional CVD risk factors for ED and, potentially, ASCVDs in HIV-infected patients. Given different kinetics of CRP and SAP in relation to acute inflammatory processes,<sup>28,29,41</sup> CRP and SAP may be more suitably utilized at different stages of atherosclerosis. C-reactive protein is sensitive to acute inflammatory processes and may be more useful as a marker of acute ASCVD events. Serum amyloid P may be more useful as a marker of ASCVD risk during the chronic asymptomatic stage of atherosclerosis as its level is less likely to be confounded by other acute inflammatory processes.

This study is limited by its cross-sectional design and observational nature of surrogate markers. The association between the inflammatory markers, CRP and SAP, and ED cannot imply causation. Both CRP and SAP have been shown to accumulate in atherosclerotic plaques.<sup>28,42,43</sup> However, it remains uncertain if they are markers or active mediators of the pathologic process leading to the development of ED. The potential for chance findings on multiple comparisons of the different biomarkers is a possibility. However, the study initially analyzed only pentraxin biomarkers such as CRP and SAP *a priori* in influencing FMD. The limited comparison between these two biomarkers was consistent with one another in their overall effect on FMD. The significant findings document an opportunity to further explore pentraxin biomarkers as a marker of arterial injury. This study was limited by its ability to assess affects from other infections such as cytomegalovirus (CMV) co-infection, as well as translocation of gut microbial products. Host genetics were also not examined in this study, as well as comparison to a comparable HIV negative group. Additionally, FMD can be a technically challenging test that requires locating consistent vascular landmarks. Our sonographers underwent training through the University of Wisconsin at Madison and had regular quality assurance evaluations to insure consistency in repeated measures. The core FMD laboratory at the University of Wisconsin at Madison oversaw the FMD acquisitions and measures at the University of Hawaii. Despite these limitations, the strength of this study is the careful clinical characterization and brachial artery FMD measurement performed in the cohort in association with detailed biomarker assays performed in plasma using in a sensitivity CVD biomarker panel.

## Conclusion

Serum amyloid P was independently associated with brachial artery FMD and may potentially relate to ED and increased ASCVD risk in HIV-infected patients on stable ART. This association warrants validation in future studies.

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## References

1. Freiberg MS, Chang CC, Kuller LH, Skanderson M, Lowy E, Kraemer KL, et al. HIV infection and the risk of acute myocardial infarction. *JAMA Intern Med.* 2013; 173(8):614–622. [PubMed: 23459863]
2. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab.* 2007; 92(7):2506–2512. [PubMed: 17456578]
3. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation.* 2007; 115(10):1285–1295. [PubMed: 17353456]
4. Gutierrez E, Flammer AJ, Lerman LO, Elizaga J, Lerman A, Fernandez-Aviles F. Endothelial dysfunction over the course of coronary artery disease. *Eur Heart J.* 2013; 34(41):3175–3181. [PubMed: 24014385]
5. Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. *Circulation.* 2007; 115(18):2390–2397. [PubMed: 17452608]
6. Yeboah J, Folsom AR, Burke GL, Johnson C, Polak JF, Post W, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. *Circulation.* 2009; 120(6):502–509. [PubMed: 19635967]
7. Rossi R, Nuzzo A, Origliani G, Modena MG. Prognostic role of flow-mediated dilation and cardiac risk factors in post-menopausal women. *J Am Coll Cardiol.* 2008; 51(10):997–1002. [PubMed: 18325438]
8. Stein JH, Currier JS, Hsue PY. Arterial disease in patients with human immunodeficiency virus infection: what has imaging taught us? *JACC Cardiovasc Imaging.* 2014; 7(5):515–525. [PubMed: 24831212]
9. van Wijk JP, de Koning EJ, Cabezas MC, Joven J, op't Roodt J, Rabelink TJ, et al. Functional and structural markers of atherosclerosis in human immunodeficiency virus-infected patients. *J Am Coll Cardiol.* 2006; 47(6):1117–1123. [PubMed: 16545639]
10. Solages A, Vita JA, Thornton DJ, Murray J, Heeren T, Craven DE, et al. Endothelial function in HIV-infected persons. *Clin Infect Dis.* 2006; 42(9):1325–1332. [PubMed: 16586393]
11. Neuhaus J, Jacobs DR Jr, Baker JV, Calmy A, Duprez D, La Rosa A, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J Infect Dis.* 2010; 201(12):1788–1795. [PubMed: 20446848]
12. Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. *J Acquir Immune Defic Syndr.* 2009; 51(3):268–273. [PubMed: 19387353]
13. Duprez DA, Neuhaus J, Kuller LH, Tracy R, Belloso W, De Wit S, et al. Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. *PloS One.* 2012; 7(9):e44454. [PubMed: 22970224]
14. Nordell AD, McKenna M, Borges ÁH, Duprez D, Neuhaus J, Neaton JD, 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]
15. Fisher SD, Miller TL, Lipshultz SE. Impact of HIV and highly active antiretroviral therapy on leukocyte adhesion molecules, arterial inflammation, dyslipidemia, and atherosclerosis. *Atherosclerosis.* 2006; 185(1):1–11. [PubMed: 16297390]
16. Maniar A, Ellis C, Asmuth D, Pollard R, Rutledge J. HIV infection and atherosclerosis: evaluating the drivers of inflammation. *Eur J Prev Cardiol.* 2013; 20(5):720–728. [PubMed: 22556374]
17. Nolan D, Watts GF, Herrmann SE, French MA, John M, Mallal S. Endothelial function in HIV-infected patients receiving protease inhibitor therapy: does immune competence affect cardiovascular risk. *QJM.* 2003; 96(11):825–832. [PubMed: 14566037]
18. Torriani FJ, Komarow L, Parker RA, Cotter BR, Currier JS, Dubé MP, et al. Endothelial function in human immunodeficiency virus-infected antiretroviral-naive subjects before and after starting

- potent antiretroviral therapy: the ACTG (AIDS Clinical Trials Group) Study 5152s. *J Am Coll Cardiol.* 2008; 52(7):569–576. [PubMed: 18687253]
19. Ho JE, Scherzer R, Hecht FM, Maka K, Selby V, Martin JN, et al. The association of CD4+T-cell counts and cardiovascular risk in treated HIV disease. *AIDS.* 2012; 26(9):1115–1120. [PubMed: 22382147]
  20. Charakida M, Donald AE, Green H, Storry C, Clapson M, Caslake M, et al. Early structural and functional changes of the vasculature in HIV-infected children: impact of disease and antiretroviral therapy. *Circulation.* 2005; 112(1):103–109. [PubMed: 15983247]
  21. Stein JH, Brown TT, Ribaud HJ, Chen Y, Yan M, Lauer-Brodell E, et al. Ultrasonographic measures of cardiovascular disease risk in antiretroviral treatment-naïve individuals with HIV infection. *AIDS.* 2013; 27(6):929–937. [PubMed: 23196938]
  22. Dube MP, Shen C, Mather KJ, Waltz J, Greenwald M, Gupta SK. Relationship of body composition, metabolic status, antiretroviral use, and HIV disease factors to endothelial dysfunction in HIV-infected subjects. *AIDS Res Hum Retroviruses.* 2010; 26(8):847–854. [PubMed: 20673142]
  23. Shikuma CM, Seto T, Liang CY, Bennett K, DeGruttola V, Gerschenson M, et al. Vitamin D levels and markers of arterial dysfunction in HIV. *AIDS Res Hum Retroviruses.* 2012; 28(8):793–797. [PubMed: 21978287]
  24. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation.* 2002; 106(25):3143–3121. [PubMed: 12485966]
  25. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol.* 2002; 39(2):257–265. [PubMed: 11788217]
  26. Shikuma CM, Barbour JD, Ndhlovu LC, Keating SM, Norris PJ, Budoff M, et al. Plasma monocyte chemoattractant protein-1 and tumor necrosis factor-alpha levels predict the presence of coronary artery calcium in HIV-infected individuals independent of traditional cardiovascular risk factors. *AIDS Res Hum Retroviruses.* 2014; 30(2):142–146. [PubMed: 23984974]
  27. Moens AL, Goovaerts I, Claeys MJ, Vrints CJ. Flow-mediated vasodilation: a diagnostic instrument, or an experimental tool. *Chest.* 2005; 127(6):2254–2263. [PubMed: 15947345]
  28. Du Clos TW. Pentraxins: structure, function, and role in inflammation. *ISRN Inflamm.* 2013; 2013:379040. [PubMed: 24167754]
  29. Bassi N, Zampieri S, Ghirardello A, Tonon M, Zen M, Cozzi F, et al. Pentraxins, anti-pentaxin antibodies, and atherosclerosis. *Clin Rev Allergy Immunol.* 2009; 37(1):36–43. [PubMed: 19016000]
  30. Jenny NS, Arnold AM, Kuller LH, Tracy RP, Psaty BM. Serum amyloid P and cardiovascular disease in older men and women: results from the Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol.* 2007; 27(2):352–358. [PubMed: 17138933]
  31. Gleason RL Jr, Caulk AW, Seifu D, Parker I, Vidakovic B, Getenet H, et al. Current Efavirenz (EFV) or ritonavir-boosted lopinavir (LPV/r) use correlates with elevated markers of atherosclerosis in HIV-infected subjects in Addis Ababa, Ethiopia. *PloS One.* 2015; 10(4):e0117125. [PubMed: 25915208]
  32. Wohl DA, Arnoczky G, Fichtenbaum CJ, Campbell T, Taiwo B, Hicks C, et al. Comparison of cardiovascular disease risk markers in HIV-infected patients receiving abacavir and tenofovir: the nucleoside inflammation, coagulation and endothelial function (NICE) study. *Antivir Ther.* 2014; 19(2):141–147. [PubMed: 23985706]
  33. Yasunaga T, Ikeda S, Koga S, Nakata T, Yoshida T, Masuda N, et al. Plasma pentraxin 3 is a more potent predictor of endothelial dysfunction than high-sensitive C-reactive protein. *Int Heart J.* 2014; 55(2):160–164. [PubMed: 24632958]

34. Vita JA, Keaney JF Jr, Larson MG, Keyes MJ, Massaro JM, Lipinska I, et al. Brachial artery vasodilator function and systemic inflammation in the Framingham Offspring Study. *Circulation*. 2004; 110(23):3604–3609. [PubMed: 15569842]
35. Zwaka TP, Hombach V, Torzewski J. C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. *Circulation*. 2001; 103(9):1194–1197. [PubMed: 11238260]
36. Singh U, Dasu MR, Yancey PG, Afify A, Devaraj S, Jialal I. Human C-reactive protein promotes oxidized low density lipoprotein uptake and matrix metalloproteinase-9 release in Wistar rats. *J Lipid Res*. 2008; 49(5):1015–1023. [PubMed: 18245817]
37. Stewart CR, Tseng AA, Mok YF, Staples MK, Schiesser CH, Lawrence LJ, et al. Oxidation of low-density lipoproteins induces amyloid-like structures that are recognized by macrophages. *Biochemistry*. 2005; 44(25):9108–9116. [PubMed: 15966734]
38. de Haas CJ, Poppelier MJ, van Kessel KP, van Strijp JA. Serum amyloid P component prevents high-density lipoprotein-mediated neutralization of lipopolysaccharide. *Infect Immun*. 2000; 68(9):4954–4960. [PubMed: 10948110]
39. Blodget E, Shen C, Aldrovandi G, Rollie A, Gupta SK, Stein JH, et al. Relationship between microbial translocation and endothelial function in HIV infected patients. *PloS One*. 2012; 7(8):e42624. [PubMed: 22952600]
40. Stewart CR, Haw A 3rd, Lopez R, McDonald TO, Callaghan JM, McConville MJ, et al. Serum amyloid P colocalizes with apolipoproteins in human atheroma: functional implications. *J Lipid Res*. 2007; 48(10):2162–2171. [PubMed: 17630380]
41. Steel DM, Whitehead AS. The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunol Today*. 1994; 15(2):81–88. [PubMed: 8155266]
42. Song Z, Cai L, Guo L, Tsukamoto Y, Yutani C, Li XA. Accumulation and expression of serum amyloid P component in human atherosclerotic lesions. *Atherosclerosis*. 2010; 211(1):90–95. [PubMed: 20189569]
43. Bisioendial RJ, Kastelein JJ, Stroes ES. C-reactive protein and atherogenesis: from fatty streak to clinical event. *Atherosclerosis*. 2007; 195(2):e10–e18. [PubMed: 17669411]

**Table 1**

Clinical, laboratory, and brachial artery FMD characteristics of study participants

<i>N</i>	135
Age, years [median (Q1, Q3)]	50 (45, 57)
Male gender, <i>n</i> (%)	119 (88.1)
Ethnicity	
Caucasian, <i>n</i> (%)	79 (58.5)
African American, <i>n</i> (%)	4 (3.0)
Native Hawaiian/Pacific Islander, <i>n</i> (%)	16 (11.8)
Asian, <i>n</i> (%)	12 (8.9)
Others, <i>n</i> (%)	24 (17.8)
Systolic blood pressure (BP), mmHg [median (Q1, Q3)]	120 (111,129)
Diastolic BP, mmHg [median (Q1, Q3)]	74 (68, 81)
Hypertension, <i>n</i> (%)	49 (36.3)
Diabetes mellitus, <i>n</i> (%)	11 (8.1)
LDL cholesterol, mg/dl [median (Q1, Q3)]	109 (89, 132)
Total/HDL cholesterol ratio [median (Q1, Q3)]	4.18 (3.35, 5.11)
Body mass index (BMI), kg/m <sup>2</sup> [median (Q1, Q3)]	25.9 (24.0, 28.3)
Waist to hip ratio [median (Q1, Q3)]	0.94 (0.91, 0.98)
Smoking history, <i>n</i> (%)	86 (63.7)
History of cardiovascular disease (CVD), <i>n</i> (%)	10 (7.4)
10-Year CHD risk estimated by Framingham risk score (FRS),% [median (Q1, Q3)]	6 (2, 13)
Current cardiovascular medications	
ACEI/ARB, <i>n</i> (%)	24 (17.8)
Beta blocker, <i>n</i> (%)	11 (8.1)
Aspirin, <i>n</i> (%)	21 (15.6)
Statin, <i>n</i> (%)	41 (30.4)
CD4+T-cell count, cells/mm <sup>3</sup> [median (Q1, Q3)]	480 (340, 638)
CD4+T-cell nadir, cells/mm <sup>3</sup> [median (Q1, Q3)] <sup>a</sup>	152 (30, 251)
Undetectable HIV RNA (<50 copies/ml), <i>n</i> (%)	116 (85.9)
Current antiretroviral medications	
Nucleoside reverse transcriptase inhibitor (NRTI), <i>n</i> (%)	128 (94.8)
Abacavir, <i>n</i> (%)	25 (18.5)
Non-nucleoside reverse transcriptase inhibitor (NNRTI), <i>n</i> (%)	66 (48.9)
Protease inhibitor, <i>n</i> (%)	64 (47.4)
Integrase Inhibitor, <i>n</i> (%)	17 (12.6)
Hepatitis C infection, <i>n</i> (%)	11 (8.1)
Inflammatory biomarkers	
sE-Selectin, ng/ml [median (Q1, Q3)]	33.93 (23.07, 48.21)
sVCAM-1, ng/ml [median (Q1, Q3)]	1174.89 (936.33, 1336.60)
sICAM-1, ng/ml [median (Q1, Q3)]	138.68 (108.15, 171.45)
MMP-9, ng/ml [median (Q1, Q3)]	55.44 (36.87, 89.35)

MPO, ng/ml [median (Q1, Q3)]	16.37 (11.53, 22.66)
CRP, ng/ml [median (Q1, Q3)]	10 886.30 (3563.68, 48 305.40)
SAA, ng/ml [median (Q1, Q3)]	15 766.30 (3938.55, 46 054.10)
SAP, ng/ml [median (Q1, Q3)]	87 364.20 (51 077.10, 158 399.00)
IL-1b, pg/ml [median (Q1, Q3)]	0.305 (0.275, 0.315)
IL-6, pg/ml [median (Q1, Q3)]	1.44 (0.85, 2.51)
IL-8, pg/ml [median (Q1, Q3)]	3.53 (2.74, 4.46)
IL-10, pg/ml [median (Q1, Q3)]	2.05 (0.70, 4.69)
TNF-alpha, pg/ml [median (Q1, Q3)]	2.97 (1.75, 4.45)
MCP-1, pg/ml [median (Q1, Q3)]	145.19 (118.45, 176.51)
Interferon (IFN)-gamma, pg/ml [median (Q1, Q3)]	0.66 (0.37, 1.16)
Baseline brachial artery diameter, cm [median (Q1, Q3)]	0.443 (0.409, 0.490)
Baseline brachial flow, ml/min [median (Q1, Q3)]	131.53 (92.11, 196.64)
Brachial artery FMD,% [median (Q1, Q3)]	3.72 (2.36, 6.01)

ACEI: Angiotensin-converting enzyme inhibitors; ARB: angiotensin II receptor blockers; CHD: coronary heart disease; CRP: C-reactive protein; FMD: flow-mediated dilation; LDL: low-density lipoprotein; HDL: high-density lipoprotein; IFN-gamma: interferon-gamma; IL: interleukin; MCP-1: monocyte chemoattractant protein-1; MMP-9: matrix metalloproteinase-9; MPO: myeloperoxidase; SAA: serum amyloid A; SAP: serum amyloid P; sE-selectin: soluble E-selectin; sICAM-1: soluble intercellular adhesion molecule-1; sVCAM-1: soluble vascular cell adhesion molecule-1; TNF-alpha: tumor necrosis factor-alpha.

<sup>a</sup>N= 126.

**Table 2**

Univariable linear regression for brachial artery FMD

Independent variable	beta Coefficient	Std. Error	t	P value
<b>Age, years</b>	<b>-0.064</b>	<b>0.030</b>	<b>-2.156</b>	<b>0.033</b>
<b>Male gender</b>	<b>-1.640</b>	<b>0.724</b>	<b>-2.264</b>	<b>0.025</b>
Caucasian	0.226	0.484	0.467	0.641
<b>Systolic blood pressure (BP), mmHg</b>	<b>-0.048</b>	<b>0.014</b>	<b>-3.309</b>	<b>0.001</b>
<b>Diastolic BP, mmHg</b>	<b>-0.084</b>	<b>0.024</b>	<b>-3.523</b>	<b>0.001</b>
<b>Hypertension</b>	<b>-1.674</b>	<b>0.474</b>	<b>-3.529</b>	<b>0.001</b>
Diabetes mellitus	0.591	0.870	0.679	0.498
<b>LDL Cholesterol, mg/dl</b>	<b>-0.015</b>	<b>0.007</b>	<b>-2.169</b>	<b>0.032</b>
Total/HDL cholesterol ratio	-0.019	0.173	-0.109	0.913
<b>Body mass index (BMI), kg/m<sup>2</sup></b>	<b>-0.128</b>	<b>0.053</b>	<b>-2.428</b>	<b>0.017</b>
<b>Waist to hip ratio</b>	<b>-11.358</b>	<b>4.431</b>	<b>-2.563</b>	<b>0.012</b>
Positive smoking history	-0.762	0.492	-1.550	0.124
Positive history of cardiovascular disease (CVD)	-0.585	0.909	-0.644	0.521
<b>10-year CHD risk estimated by Framingham risk score (FRS) (%)</b>	<b>-6.595</b>	<b>3.319</b>	<b>-1.987</b>	<b>0.049</b>
Current Cardiovascular Medications				
ACEI/ARB	-1.087	0.617	-1.763	0.080
Beta blocker	-1.522	0.862	-1.766	0.080
Aspirin	-0.210	0.658	-0.319	0.750
Statin	-0.070	0.519	-0.134	0.894
CD4+T-cell count, cells/mm <sup>3</sup>	-0.001	0.001	-0.929	0.355
CD4+T-cell nadir, cells/mm <sup>3</sup> <sup>a</sup>	0.001	0.002	0.541	0.589
Undetectable HIV RNA (< 50 copies/ml)	-0.602	0.684	-0.880	0.381
Current Antiretroviral Medications				
Nucleoside reverse transcriptase inhibitor (NRTI)	0.318	1.075	0.296	0.768
Abacavir	-0.695	0.611	-1.138	0.257
Non-nucleoside reverse transcriptase inhibitor (NNRTI)	-0.288	0.476	-0.605	0.547
Protease inhibitor	0.322	0.477	0.675	0.501
Integrase inhibitor	0.028	0.719	0.039	0.969
Hepatitis C infection	-0.055	0.875	-0.063	0.950
<b>Baseline brachial artery diameter, cm</b>	<b>-20.777</b>	<b>3.071</b>	<b>-6.764</b>	<b>&lt;0.001</b>
Inflammatory biomarkers <sup>b</sup>				
sE-Selectin, ng/ml	-0.930	0.909	-1.023	0.308
sVCAM-1, ng/ml	-1.564	1.785	-0.877	0.382
sICAM-1, ng/ml	0.198	1.157	0.171	0.864
MMP-9, ng/ml	-0.826	0.853	-0.969	0.334
MPO, ng/ml	-0.485	0.751	-0.645	0.520
<b>CRP, ng/ml</b>	<b>-0.695</b>	<b>0.317</b>	<b>-2.191</b>	<b>0.030</b>
SAA, ng/ml	-0.550	0.288	-1.913	0.058

Independent variable	beta Coefficient	Std. Error	t	P value
SAP, ng/ml	<b>-1.318</b>	<b>0.562</b>	<b>-2.345</b>	<b>0.021</b>
IL-1b, pg/ml	-1.855	1.467	-1.265	0.208
IL-6, pg/ml	-0.093	0.540	-0.172	0.864
IL-8, pg/ml	-1.759	1.204	-1.461	0.146
IL-10, pg/ml	0.131	0.345	0.379	0.705
TNF-alpha, pg/ml	0.490	0.628	0.780	0.437
MCP-1, pg/ml	0.515	1.408	0.366	0.715
IFN-gamma, pg/ml	-0.099	0.567	-0.175	0.861

ACEI: Angiotensin-converting enzyme inhibitors; ARB: angiotensin II receptor blockers; CHD: coronary heart disease; CRP: C-reactive protein; FMD: flow-mediated dilation; LDL: low-density lipoprotein; HDL: high-density lipoprotein; IFN-gamma: interferon-gamma; IL: interleukin; MCP-1: monocyte chemoattractant protein-1; MMP-9: matrix metalloproteinase-9; MPO: myeloperoxidase; SAA: serum amyloid A; SAP: serum amyloid P; sE-selectin: soluble E-selectin; sICAM-1: soluble intercellular adhesion molecule-1; sVCAM-1: soluble vascular cell adhesion molecule-1; TNF-alpha: tumor necrosis factor-alpha.

Significant associations (P-value < 0.05) are bolded.

<sup>a</sup>N = 126.

<sup>b</sup>All inflammatory biomarkers are log-transformed for analysis.

**Table 3**

Multivariable linear regression for brachial artery FMD (%)

	Unstandardized coefficients		Standardized coefficients		P-value
	B	Std. error	Beta	t	
<b>CRP</b>					
Baseline brachial artery diameter, cm	-19.696	3.256	-0.462	-6.050	0.000
Age, years	-0.077	0.027	-0.215	-2.886	0.005
Systolic blood pressure (BP), mmHg	-0.019	0.014	-0.108	-1.389	0.167
LDL cholesterol, mg/dl	-0.012	0.007	-0.135	-1.792	0.076
Waist to hip ratio	-2.700	4.074	-0.053	-0.663	0.509
Diabetes mellitus	1.194	0.793	0.115	1.506	0.135
Positive smoking history	-0.727	0.437	-0.125	-1.665	0.099
CRP, ng/ml <sup>a</sup>	-0.391	0.291	-0.104	-1.345	0.181
<b>Serum amyloid P (SAP)</b>					
Baseline brachial artery diameter, cm	-19.989	3.216	-0.469	-6.215	0.000
Age, years	-0.078	0.026	-0.217	-2.959	0.004
Systolic BP, mmHg	-0.019	0.013	-0.110	-1.448	0.150
LDL cholesterol, mg/dl	-0.010	0.007	-0.118	-1.559	0.122
Waist to hip ratio	-2.445	3.965	-0.048	-0.617	0.539
Diabetes mellitus	1.404	0.787	0.135	1.785	0.077
Positive smoking history	-0.741	0.430	-0.127	-1.722	0.088
SAP, ng/ml <sup>a</sup>	-1.094	0.498	-0.165	-2.198	0.030

LDL, low-density lipoprotein. In the two multivariable linear regression models, the dependent variable was brachial artery FMD (%) and the independent variables that were sequentially added were the baseline brachial artery diameter, age, systolic BP, LDL cholesterol, waist to hip ratio, diabetes mellitus, smoking history, and either CRP or SAP. The standardized beta coefficient is based on a one standard deviation of change in the exposure on the outcome and provides context to each variable's contribution to brachial artery FMD variance.

<sup>a</sup>C-reactive protein (CRP) and SAP are log-transformed for analysis.