JAIDS Journal of Acquired Immune Deficiency Syndromes Publish Ahead of Print
DOI: 10.1097/QAI.0b013e318252f99f

HIV Replication, Inflammation, and the Effect of Starting Antiretroviral Therapy on Plasma Asymmetric Dimethylarginine, a Novel Marker of Endothelial Dysfunction

Jason V. Baker[1,2], Jacqueline Neuhaus[1], Daniel Duprez[1], Matthew Freiberg[3], Jose I. Bernardino[4], Andrew D. Badley[5], Daniel E. Nixon[6,] Jens D. Lundgren[7], Russell P. Tracy[8] and James D. Neaton[1] for the INSIGHT SMART Study Group

[1]University of Minnesota, Minneapolis, MN, USA
[2]Hennepin County Medical Center, Minneapolis, MN, USA
[3]University of Pittsburgh, Pittsburgh, PA, USA
[4]Hospital La Paz, IdiPAZ, Madrid, Spain
[5]Mayo Clinic, Rochester, MN, USA
[6]Virginia Commonwealth University, Richmond, Virginia, USA
[7]University of Copenhagen & Copenhagen University Hospital, Copenhagen, Denmark
[8]University of Vermont, Burlington, VT, USA

Key words: *HIV infection, antiretroviral therapy, inflammation, endothelial dysfunction, asymmetric dimethylarginine (ADMA), CD40 ligand, P-selectin*

Abstract: 210 words (currently)
Manuscript text: 2967 words (currently)

Corresponding author:
Jason Baker, MD, MS
University of Minnesota
Hennepin County Medical Center
Address: 701 Park Ave; MC G5
Minneapolis, MN, 55415, USA
Email: baker459@umn.edu
Fax: 612-904-4299

Support provided by: NIH/NIAID grants U01AI042170, U01AI46362, U01AI068641, U01AI068641

Clinical Trials.gov identifier: NCT00027352
Acknowledgments: We would like to sincerely thank the SMART study participants, the SMART study team (see N Engl J Med, 2006:355:2294-2295 for list of investigators), and the INSIGHT Executive Committee.

Role of the Funding Source: The study was funded in part by the National Institute of Allergy and Infectious Disease (NIAID). The funding sources had no role in data collection, data analysis, or the decision to publish the results.

Conflicts of Interest: J. Baker reported research support from Gilead, Tibotec and ViiV. J. Lundgren reported honoraria and research grants from Boehringer-Ingelheim, Roche, Abbott, Bristol-Myers Squibb, Merck, Sharp & Dohme, GlaxoSmithKline, Tibotec, Pfizer, and Gilead. R. Tracy reported the following activities: Honoraria or grant support from Aviir, Abbott, Merck, GlaxoSmithKline/diaDexus, Celera Diagnostics; external advisory board for Wake Forest University Pepper Center on Aging, Johns Hopkins University Pepper Center on Aging, University of Florida Pepper Center on Aging; Haematologic Technologies—owner; thrombosis and fibrinolysis biochemical reagents and blood collection tubes; contract research in this area; and Ashcraft & Gerel Attorneys at Law—consulting on mechanisms in inflammation, atherosclerosis and thrombosis.

Abstract

Background: HIV infection is associated with premature development of cardiovascular disease (CVD). Understanding the effects of HIV replication on endothelial dysfunction and platelet activation may identify treatment targets to reduce CVD risk.

Methods: A subgroup of HIV-infected participants in the Strategies for Management of Antiretroviral Therapy (SMART) study off antiretroviral therapy (ART) at entry enabled a randomized comparison of immediate versus deferred ART initiation of changes in asymmetric dimethylarginine (ADMA), soluble CD40L and P-selectin levels.

Results: At study entry, median (IQR) levels of ADMA, sCD40L, and P-selectin were 0.57 (0.49-0.66) μg/mL, 251 (135-696) μmol/L, and 34 (28-44) pg/mL. Compared to those randomized to deferral of ART (n=114), participants randomized to immediate ART (n=134) had 10.3% lower ADMA levels (p=0.003) at 12 months; treatment differences in sCD40L (95% CI: -17 to 44%; p=0.53) and P-selectin (95% CI: -10 to 10%; p=0.95) were not significant. The difference in ADMA for those assigned immediate ART compared to those assigned ART deferral was greater among younger patients and those with higher levels of hsCRP and D-dimer (p≤0.05 for interaction for both), but not HIV RNA level at baseline (p=0.51).

Discussion: ART initiation leads to declines in ADMA levels, a marker of nitric-oxide-mediated endothelial dysfunction. Improvement in ADMA levels was related to the degree of inflammation and coagulation, suggesting that up-regulation of these pathways contributes to premature vascular disease among individuals with HIV infection. Whether declines in ADMA levels impact risk of disease requires further research.

Introduction

Findings from the Strategies for Management of AntiRetroviral Therapy (SMART) trial identified key markers of inflammation (e.g. high-sensitivity C-reactive protein [hsCRP] and interleukin-6 [IL-6]), and coagulation (D-dimer) that are elevated with HIV infection and associated with risk for CVD and all-cause mortality (1, 2). Furthermore, in SMART, D-dimer changes were correlated with changes in HIV RNA levels resulting from starting or stopping antiretroviral therapy (ART) (2, 3). Anti-thrombotic properties of endothelial surfaces are integral to coagulation homeostasis, and may be influenced by HIV replication itself and/or exposure to inflammatory cytokines (4-7). Biomarkers of endothelial activation (e.g., intercellular and vascular cell adhesion molecules), are consistently elevated in HIV-infected versus uninfected individuals, and have been shown to be associated with HIV replication, inflammation and subclinical atherosclerosis among HIV-infected patients (6-11). The goals of this study were to build on this literature and explore the effect of HIV-related inflammation, resulting from untreated HIV, on nitric-oxide (NO) mediated endothelial dysfunction and endothelial-platelet activation.

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of the NO synthase pathway that reflects endothelial dysfunction (12); soluble CD40 ligand (sCD40L) is expressed by lymphocytes, the vascular wall, and activated platelets (13); and P-selectin is expressed by both endothelial cells and platelets (14). Plasma levels of ADMA, sCD40L and P-selectin have been associated with risk for CVD in the general

population (12-14), and represent inter-related factors that contribute to atherogenesis (15).

We hypothesized that these biomarkers would be associated with HIV replication prior to starting ART, and would improve after starting ART. Therefore, using stored samples from the SMART study, we measured ADMA, sCD40L and P-selectin levels at baseline and 12 months for a subgroup of SMART participants who were not taking ART at study entry that represented a randomized comparison of immediate versus deferred ART.

Methods

The methods and results of the SMART trial, including the subgroup not taking ART at entry, have been reported (3, 16, 17).

Study Population

Of the 5,472 randomized participants in SMART, 477 had never taken ART or had not used ART for at least 6 months prior to randomization (referred to as the subgroup 'off ART'). To reduce the likelihood of recent ART exposure, participants with low HIV RNA levels (<10,000 copies/mL) during the 6 months before randomization were not included in the subgroup off ART. For these participants the randomized intervention in SMART of a DC (drug conservation) or VS (viral suppression) strategy represented a comparison of immediate versus deferred (until CD4 counts declined to 250 cells/mm^3) initiation of ART, respectively (17). Among the 477 participants in the subgroup off ART, 248 participants had specimens from both baseline and month 12 visits available for

analysis and, thus, form the basis of this report. A single follow-up time point of 12 months was chosen based on specimen availability and with consideration of time to achieve viral suppression. Among the 229 SMART participants off ART at entry who were excluded, 30 did not consent to have specimens stored, 10 were lost to follow-up, 25 missed the month 12 visit, and 164 did not have sufficient specimen remaining for use at either baseline and/or month 12 visit.

The SMART study protocol was approved by the institutional review board (IRB) or ethics committee (EC) at each clinical site and at the University of Minnesota, which served as the Statistical and Data Management Center. The IRB at the University of Minnesota also approved plans for analysis of stored specimens for consenting participants.

Biomarker Measures

CD4+ cell count and HIV RNA levels were measured at clinical sites. For consenting participants in SMART, plasma specimens were collected using EDTA tubes, processed within 4 hours of collection, frozen at -70°C, and shipped to a central repository where they continued to be stored at -70°C. Samples were not required to be fasting specimens.

Plasma levels of hsCRP and IL-6 were measured from baseline specimens, and levels of D-dimer ADMA, sCD40L, and P-selectin were measured from baseline and month 12 specimens, by the Laboratory for Clinical Biochemistry Research at the University of

Vermont. The analytic methods and coefficient of variance (CV) for measuring hsCRP, IL-6, and D-dimer levels from SMART specimens have been previously reported (2, 3). ADMA levels were measured with a competitive ELISA method (Euroimmun, Lübeck, Germany), and sCD40L and P-selectin levels with quantitative sandwich immunoassays (R&D Systems, Minneapolis, USA). The lower level of detection for ADMA, sCD40L and P-selectin were 0.1 μmol/L, 62.5 pg/mL, and 20.2 μg/mL, respectively. The inter-assay CV using these methods was 12.7-15.6% for ADMA, 6.7-10.5% for sCD40L, and 6.0-8.1% for P-selectin, respectively. All samples were analyzed blinded to treatment group.

Statistical Methods

Unless otherwise stated, comparisons between the VS and DC groups are intent-to-treat (all randomized participants in the study sample are included in the analysis). Analyses were also carried out in which 2 VS participants who did not initiate ART and 39 DC participants who initiated ART before 12 months are excluded. Simple descriptive statistics are used for baseline cross-sectional analyses. Biomarker values were $\log_e$-transformed prior to analysis. Analysis of covariance with the baseline level of the biomarker included as a covariate was used to compare treatment groups (VS-DC) for changes in biomarker levels at 12 months. Estimates of the percentage difference between treatment groups at the 12 month visit were estimated by exponentiating the $\log_e$-transformed treatment differences. Analysis of covariance models that included interaction terms between treatment group and baseline variables were used for subgroup analyses. Multiple regression analysis was used to study

baseline associations and predictors of change after 12 months for VS participants. The following predictors were included: baseline biomarker level of interest, age, gender, race, hepatitis (B or C) co-infection, smoking, blood-pressure lowering medication, lipid-lowering medication, body mass index (BMI), diabetes, CD4+ cell count, HIV RNA level (log_{10} transformed), naïve to all ART (or not), prior AIDS, and hsCRP, IL-6, and D-dimer levels. Analyses were performed using SAS (Version 9.1). All reported p-values are 2-sided. A significance level of <0.05 was used for treatment comparisons.

Results

Study Sample and Baseline Associations

When compared with the participants in the SMART no ART subgroup (n=477) not included in this report (n=229), the cohort analyzed in this report (n=248) had a lower median age (40 vs. 42 years), had a higher prevalence of persons previously naïve to ART (57 vs. 48%), and were less likely to be black (30 vs. 43%), be co-infected with hepatitis B or C (9 vs. 28%), or be prescribed BP lowering medication (11 vs. 21%). Among the participants in this report, 2 of participants deferring ART (DC) and 1 of those randomized to start ART (VS) had a CVD event (i.e., myocardial infarction, stroke, coronary artery disease requiring surgery or CVD death) during follow-up.

Baseline characteristics of the DC and VS groups were similar and are given in Table 1. Median CD4+ count was 433 cells/mm3, 56% of participants had no prior ART exposure, and the percent of patients with a prior AIDS and CVD event were low at

8.5% and 2.8%, respectively. The use of blood pressure and lipid-lowering medication at study entry was 10.9% and 4.4%, respectively.

Median (IQR) levels of ADMA, sCD40L, and P-selectin were 0.57 (0.49-0.66) μmol/L, 251 (135-696) pg/mL, and 34 (28-44) μg/mL, respectively. ADMA levels, but not sCD40L or P-selectin, were weakly correlated with levels of IL-6 (r=0.16; p=0.02) and D-dimer (r=0.16; p=0.01). Soluble CD40L and P-selectin levels were significantly correlated with each other (r=0.63; p<0.001), but not with ADMA levels (r=0.02 and r=0.06, respectively). These 3 biomarkers were not correlated with HIV RNA levels (r=0.08 for ADMA, r=0.00 for sCD40L, r=-0.02 for P-selectin) or CD4+ counts (r=0.06 for ADMA, r=0.02 for sCD40L, r=0.08 for P-selectin) at baseline. (p>0.20 for all comparisons)

In univariate associations with traditional CVD risk factors, ADMA levels were 6% higher among current smokers (p=0.05), 7% lower among males (p=0.04), and 11% lower among those of black race compared to other ethnic groups which were similar to one another (p<0.001); the correlation with age did not reach significance (r=0.10; p=0.11). At baseline, P-selectin levels were 14% higher for males (p=0.02), and were positively correlated with age (r=0.14; p=0.03) and inversely correlated with eGFR (r=-0.17; p=0.007). Soluble CD40L levels were also inversely correlated with eGFR (r=-0.14; p=0.03), but with none of the other traditional CVD risk factors. In multivariate regression analyses associations between biomarker levels with age, gender and race/ethnicity were similar. Finally, serum platelet counts were available for n=112

participants (n=55 from DC and n=57 from VS group) and were significantly correlated with levels of sCD40L (r=0.32; p<0.001), but not P-selectin (r=0.17; p=0.07).

Biomarker Changes after 12 Months: Immediate (VS) versus Deferred (DC) ART

One hundred and thirty-two of 134 participants in the VS group initiated ART following randomization, and at 12 months 116 (88%) remained on ART. Most participants (56%) initiated ART with an NNRTI (efavirenz in 84% of these patients) plus combination nucleoside treatment. The most frequent nucleoside reverse transcriptase inhibitor combination used was zidovudine plus lamivudine. The most common reason given for the 12% of the immediate ART participants (VS group) who stopped ART during follow-up was toxicity (44%). Thirty-nine of 114 deferred ART participants (DC group) started ART at some point prior to the month 12 visit; 1 (3%) due to disease progression and 15 (38%) due to a CD4+ count <250 cells/mm^3 (the protocol deferral strategy). At 12 months, 25 (23%) participants in the deferred ART group (SMART DC group) and 85 (66%) participants in the immediate ART group (SMART VS group) had an HIV RNA level <400 copies/mL (p<0.001). CD4+ count declines in the DC group and increases in the VS group resulted in a difference between groups of 173 cells/mm^3 at 12 months (p<0.001).

Median biomarker levels over follow-up by treatment group are presented in table 2, and the difference in biomarker level between groups (VS-DC) at 12 months for intention to treat comparisons are presented in Figure 1. In the immediate ART (VS) group, those randomized to start ART immediately had a -10.3% (95% CI: -16.3 to -3.7;

p=0.003) greater decline in ADMA levels at month 12. The median changes (IQR) in ADMA levels from baseline-to-month 12 were -0.02 μmol/L (-0.15-0.13) for the immediate ART (VS) arm (p = 0.12) and 0.02 μmol/L (-0.12-0.18) for the deferred ART (DC) arm (p = 0.17). Soluble CD40L and P-selectin did not differ between VS and DC groups at 12 months, and neither marker changed significantly from baseline-to-month 12 when VS and DC groups were examined separately. D-dimer levels declined significantly among VS (p < 0.001) participants, but the differences by treatment groups at 12 months did not reach significance in the randomized intention to treat comparison (figure 1).

When VS participants that did not initiate ART (n=2) and DC participants that initiated ART before 6 months (n=39) are excluded, the corresponding on-treatment comparison for the effect of starting ART on ADMA levels at month 12 was similar (-12.8%; 95% CI: -19.3 to -5.8; p<0.001) and became more pronounced for D-dimer (-21.4%; 95% CI: -35.8 to -3.8; p=0.02). When participants with known CVD (n=7) were excluded, the corresponding difference for ADMA was -11.4% (-17.4 to -5.0; p<0.001).

Predictors of Change in ADMA Levels after Starting ART in the VS Group

Baseline predictors of change in ADMA levels at 12 months for VS participants were also explored. In univariate models, smaller improvements (i.e., less decline) in ADMA levels were seen for those of older age (6% less decline per 10 years older; p=0.04), whereas greater declines were seen for those with higher baseline hsCRP (6% greater decline per log-e unit higher; p=0.003), IL-6 (7%; p=0.06) and D-dimer (6%; p=0.04)

levels. Baseline CD4+ count (p=0.36) and HIV RNA level (p=0.64) did not predict changes in ADMA levels. In mulitivariate models, only older age (p<0.001) and higher hsCRP levels (p=0.02) at baseline remained independently associated with the degree of change in ADMA levels following ART initiation for the VS group. None of the other HIV-related or traditional CVD risk factors examined at baseline predicted change in ADMA levels in the VS group.

We compared biomarker declines for those who achieved an HIV viral load <400 copies/mL at 12 months versus those who did not and no differences were observed except for D-dimer (p<0.001). Among the VS participants, the percentage decline in ADMA was 3% for those with HIV viral load <400 copies/mL at 12 months and 6% for participants with viral load ≥400 copies/mL at 12 months.

Subgroup Findings

To further explore the above findings on the influence of age, inflammation, coagulation, and HIV replication on ART-related improvements in ADMA, we examined treatment differences in ADMA according to these baseline factors (Figure 2). Significant treatment x baseline subgroup interactions were found for age, hsCRP and D-dimer, but not HIV RNA or IL-6. For younger participants and those with higher levels of hsCRP or D-dimer at entry, the effects of immediate versus deferred ART treatment on ADMA levels are greater.

Discussion

In this randomized comparison of immediate versus delayed initiation of ART, we demonstrated improvements in a novel biomarker of NO-mediated endothelial dysfunction, ADMA, but not in plasma markers of endothelial-platelet activation (sCD40L and P-selectin). Improvements in AMDA were greater in younger patients and in patients with higher levels of baseline hsCRP and D-dimer. These findings expand our understanding of HIV-related endothelial injury and dysfunction, and suggest that up-regulation of inflammatory and coagulation pathways contribute to premature vascular dysfunction and disease among individuals with HIV infection.

ADMA is an endogenous inhibitor of NO synthase that is inversely associated with flow mediated enthothelium-dependent vasodilation in healthy people (18), and can be used as a biomarker of vascular dysfunction. Reference ranges for ADMA have been obtained using Framingham participants that were free of clinical CVD, hypertension, diabetes or obesity, and did not smoke.(19) Median (IQR) ADMA levels were 0.51 (0.45-0.59) μmol/L (results similar by gender) and the 97.5 percentile was 0.732 μmol/L (19). Median (IQR) ADMA levels at baseline in our study were 0.57 (0.49-0.66) μmol/L; 11.3% had levels > 0.732 μmol/L. We describe modest improvements in ADMA levels after 12 months of ART, and the percent of patients with ADMA >0.732 μmol/L was 25.4% for the deferred group (DC) and 16.4% for the immediate ART group (VS). Our finding that ADMA levels were lower for black participants is consistent with reports from general population studies (20, 21), while our finding that women had higher ADMA levels than men has not been consistently reported (19-22). However, one study of 500 participants found women had lower ADMA levels than men at ages <50 years but

higher ADMA levels at ages >50 years (22). Whether HIV-infection has an effect similar to advancing age on gender differences in ADMA levels is not clear, and should be explored in larger epidemiologic studies.

ADMA levels were independently associated with risk for all-cause mortality (HR 1.21 per SD higher ADMA level) in the Framingham Offspring Study, and with risk for CVD events (HR 1.29 per SD higher ADMA level) in the Population Study of Women in Gothenburg (23, 24). Although SMART participants differ from those in Framingham and the Population Study of Women both by HIV-specific and traditional CVD risk factors, the degree of ART-related change in ADMA we observed corresponds to approximately a 5% lower risk of death and 7% lower risk of CVD, respectively, in these studies (23, 24). If morbidity from other non-AIDS-related end organ diseases were considered (e.g., renal), the potential net clinical benefit associated with declines in AMDA levels of this degree could be greater. Currently, these assertions require validation in larger clinical event studies where the longer-term consequences of ART exposure are also assessed.

Brachial artery flow-mediated dilation (FMD), a functional measure of NO-mediated endothelial responsiveness, was shown to improve with ART initiation in a non-randomized comparison conducted by the AIDS Clinical Trial Group (25). Our randomized comparison expands on the biological relevance of these findings by describing ART-related improvements in ADMA levels that varies by the degree of inflammation and coagulation activity present prior to starting HIV treatment.

Endothelial dysfunction, assessed either by FMD or ADMA levels, has been shown to be impaired among HIV-infected compared to uninfected persons (26, 27). ADMA levels were associated with levels of the macrophage inflammatory marker neopterin, but not CRP, in a study of 112 HIV-infected participants (28). In a proof of concept study of 70 HIV negative patients with the metabolic syndrome, aspirin therapy led to declines in ADMA levels across a wide range of doses (81-1300mg) (29). Thus, it may be possible to modify biomarkers such as ADMA with simple adjunctive therapies. Whether any resulting changes influence risk of disease will require larger trials.

The mechanisms underlying the interaction between ART-related improvements in ADMA levels and advancing age described in our subgroup analyses are not entirely clear. At baseline the positive correlation between ADMA levels and advancing age did not reach significance. Our study had limited power to detect modest associations, and the correlation between ADMA levels and advancing age was of similar magnitude between our study ($r=0.10$; $p=0.11$) and a subset of 1126 Framingham participants that were free of clinical CVD, hypertension, diabetes or obesity, and did not smoke ($r=0.14$; $p<0.001$) (19). We describe smaller ART-related declines in ADMA levels with advancing age that suggests a greater burden of vascular disease among older persons may, to some extent, limit the capacity for improvement in endothelial dysfunction (e.g., ADMA levels). Understanding the interaction between HIV-infection, advancing age and vascular disease should be a focus of future research.

Several studies have demonstrated greater platelet activation or higher levels of platelet microparticles among HIV-infected versus uninfected participants (30-32). Among participants in SMART with viral suppression, stopping ART led to significant declines in serum platelet counts that correlated inversely with the rise in HIV RNA and D-dimer levels (33). In our study, sCD40L and P-selectin levels at baseline were tightly correlated with one another and inversely with platelet counts, but neither marker changed significantly after starting ART. Prior data are inconsistent with respect to changes in P-selectin levels after starting or stopping ART (7, 34), and sCD40L has not been extensively studied in this context. In normal physiology, platelets account for the main source of circulating sCD40L and P-selectin levels (14, 35-37). However, in the context of HIV infection, the CD40 receptor-ligand system is also integral to the host anti-viral response and CD40L expression may be down-regulated directly by HIV proteins (38). Thus, interpreting these markers may be difficult in the context of suppressing HIV replication due to competing forces, including the potential contribution of ART-related toxicity (39). Cumulatively, these data support the need for additional research on platelet activation and platelet function among HIV-infected patients.

Strengths of this study include use of a randomized design to estimate ART-related changes in endothelial biomarkers. A limitation is that specific ART regimens were chosen by patients and providers (i.e., not randomized in SMART) and several different regimens were used, limiting any comparisons of specific antiretroviral combinations. The lack of platelet-free plasma or cell-based specimens in SMART also prevented a more comprehensive assessment of platelet activation and function. Finally, the sample

size may lack power to detect more modest associations between ADMA levels and traditional CVD risk factors.

In summary, ART reduces ADMA levels. Reductions with ART are greater for those with higher levels of inflammatory and coagulation markers at entry. Futures studies are warranted to determine whether treatments that improve NO-mediated endothelial function, as measured by ADMA, leads to a reduced risk for morbidity and mortality among individuals with HIV infection.

References:

1. 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):1788-95.
2. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med. 2008;5(10):e203.
3. Baker JV, Neuhaus J, Duprez D, Kuller LH, Tracy R, Belloso W, et al. Changes in inflammatory and coagulation biomarkers: a randomized comparison of immediate versus deferred antiretroviral therapy in patients with HIV infection. JAIDS. 2011;56(1):36-43.
4. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood. 1998;91(10):3527-61.
5. Blann AD. Endothelial cell activation, injury, damage and dysfunction: separate entities or mutual terms? Blood Coagul Fibrinolysis. 2000;11(7):623-30.
6. Papasavvas E, Azzoni L, Pistilli M, Hancock A, Reynolds G, Gallo C, et al. Increased soluble vascular cell adhesion molecule-1 plasma levels and soluble intercellular adhesion molecule-1 during antiretroviral therapy interruption and retention of elevated soluble vascular cellular adhesion molecule-1 levels following resumption of antiretroviral therapy. AIDS. 2008;22(10):1153-61.
7. Calmy A, Gayet-Ageron A, Montecucco F, Nguyen A, Mach F, Burger F, et al. HIV increases markers of cardiovascular risk: results from a randomized, treatment interruption trial. AIDS. 2009;23(8):929-39.
8. de Gaetano Donati K, Rabagliati R, Iacoviello L, Cauda R. HIV infection, HAART, and endothelial adhesion molecules: current perspectives. Lancet Infect Dis. 2004;4(4):213-22.
9. Baker J, Ayenew W, Quick H, Hullsiek KH, Tracy R, Henry K, et al. High-density lipoprotein particles and markers of inflammation and thrombotic activity in patients with untreated HIV infection. J Infect Dis. 2010;201(2):285-92.
10. Melendez MM, McNurlan MA, Mynarcik DC, Khan S, Gelato MC. Endothelial adhesion molecules are associated with inflammation in subjects with HIV disease. CID. 2008;46(5):775-80.
11. Ross AC, Rizk N, O'Riordan MA, Dogra V, El-Bejjani D, Storer N, et al. Relationship between inflammatory markers, endothelial activation markers, and carotid intima-media thickness in HIV-infected patients receiving antiretroviral therapy. Clin Infect Dis. 2009;49(7):1119-27.
12. Cooke JP. ADMA: its role in vascular disease. Vasc Med. 2005;10 Suppl 1:S11-7.
13. Antoniades C, Bakogiannis C, Tousoulis D, Antonopoulos AS, Stefanadis C. The CD40/CD40 ligand system: linking inflammation with atherothrombosis. J Am Coll Cardiol. 2009;54(8):669-77.
14. Blann AD, Nadar SK, Lip GY. The adhesion molecule P-selectin and cardiovascular disease. European heart journal. 2003;24(24):2166-79.

15. Schafer A, Bauersachs J. Endothelial dysfunction, impaired endogenous platelet inhibition and platelet activation in diabetes and atherosclerosis. Current vascular pharmacology. 2008;6(1):52-60.
16. El-Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, Arduino RC, et al. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med. 2006;355(22):2283-96.
17. Emery S, Neuhaus J, Phillips A, Babiker A, Cohen CJ, Gatell JM, et al. Major clinical outcomes in antiretroviral therapy (ART)-naive participants and in those not receiving ART at baseline in the SMART study. JID. 2008;197:1133-44.
18. Ardigo D, Stuehlinger M, Franzini L, Valtuena S, Piatti PM, Pachinger O, et al. ADMA is independently related to flow-mediated vasodilation in subjects at low cardiovascular risk. European journal of clinical investigation. 2007;37(4):263-9.
19. Schwedhelm E, Xanthakis V, Maas R, Sullivan LM, Schulze F, Riederer U, et al. Asymmetric dimethylarginine reference intervals determined with liquid chromatography-tandem mass spectrometry: results from the Framingham offspring cohort. Clinical chemistry. 2009;55(8):1539-45.
20. Sydow K, Fortmann SP, Fair JM, Varady A, Hlatky MA, Go AS, et al. Distribution of asymmetric dimethylarginine among 980 healthy, older adults of different ethnicities. Clinical chemistry. 2010;56(1):111-20.
21. Iribarren C, Husson G, Sydow K, Wang BY, Sidney S, Cooke JP. Asymmetric dimethyl-arginine and coronary artery calcification in young adults entering middle age: the CARDIA Study. European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology. 2007;14(2):222-9.
22. Schulze F, Maas R, Freese R, Schwedhelm E, Silberhorn E, Boger RH. Determination of a reference value for N(G), N(G)-dimethyl-L-arginine in 500 subjects. European journal of clinical investigation. 2005;35(10):622-6.
23. Boger RH, Sullivan LM, Schwedhelm E, Wang TJ, Maas R, Benjamin EJ, et al. Plasma asymmetric dimethylarginine and incidence of cardiovascular disease and death in the community. Circulation. 2009;119(12):1592-600.
24. Leong T, Zylberstein D, Graham I, Lissner L, Ward D, Fogarty J, et al. Asymmetric dimethylarginine independently predicts fatal and nonfatal myocardial infarction and stroke in women: 24-year follow-up of the population study of women in Gothenburg. Arteriosclerosis, thrombosis, and vascular biology. 2008;28(5):961-7.
25. Torriani FJ, Komarow L, Parker RA, Cotter BR, Currier JS, Dube 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):569-76.
26. 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):1325-32.
27. Jang JJ, Berkheimer SB, Merchant M, Krishnaswami A. Asymmetric dimethylarginine and coronary artery calcium scores are increased in patients infected with human immunodeficiency virus. Atherosclerosis. 2011.
28. Kurz K, Teerlink T, Sarcletti M, Weiss G, Zangerle R, Fuchs D. Plasma concentrations of the cardiovascular risk factor asymmetric dimethylarginine (ADMA) are

increased in patients with HIV-1 infection and correlate with immune activation markers. Pharmacological research : the official journal of the Italian Pharmacological Society. 2009;60(6):508-14.
29. Hennekens CH, Schneider WR, Pokov A, Hetzel S, Demets D, Serebruany V, et al. A randomized trial of aspirin at clinically relevant doses and nitric oxide formation in humans. Journal of cardiovascular pharmacology and therapeutics. 2010;15(4):344-8.
30. Satchell CS, Cotter AG, O'Connor EF, Peace AJ, Tedesco AF, Clare A, et al. Platelet function and HIV: a case-control study. AIDS. 2010;24(5):649-57.
31. Holme PA, Muller F, Solum NO, Brosstad F, Froland SS, Aukrust P. Enhanced activation of platelets with abnormal release of RANTES in human immunodeficiency virus type 1 infection. FASEB J. 1998;12(1):79-89.
32. Corrales-Medina VF, Simkins J, Chirinos JA, Serpa JA, Horstman LL, Jy W, et al. Increased levels of platelet microparticles in HIV-infected patients with good response to highly active antiretroviral therapy. J Acquir Immune Defic Syndr. 2010;54(2):217-8.
33. Zetterberg E, Neuhaus J, Baker JV, Lundgren JD. Kinetics of platelet counts following interruption of ART: results from the SMART Study. 18th Conference on Retroviruses and Opportunistic Infections (CROI). Boston, MA, USA; 2011.
34. Wolf K, Tsakiris DA, Weber R, Erb P, Battegay M. Antiretroviral therapy reduces markers of endothelial and coagulation activation in patients infected with human immunodeficiency virus type 1. J Infect Dis. 2002;185(4):456-62.
35. Heeschen C, Dimmeler S, Hamm CW, van den Brand MJ, Boersma E, Zeiher AM, et al. Soluble CD40 ligand in acute coronary syndromes. The New England journal of medicine. 2003;348(12):1104-11.
36. Antoniades C, Bakogiannis C, Tousoulis D, Antonopoulos AS, Stefanadis C. The CD40/CD40 ligand system: linking inflammation with atherothrombosis. Journal of the American College of Cardiology. 2009;54(8):669-77.
37. McEver RP. Adhesive interactions of leukocytes, platelets, and the vessel wall during hemostasis and inflammation. Thrombosis and haemostasis. 2001;86(3):746-56.
38. Chougnet C. Role of CD40 ligand dysregulation in HIV-associated dysfunction of antigen-presenting cells. Journal of leukocyte biology. 2003;74(5):702-9.
39. Baum PD, Kosikova G, Galkina S, Stoddart C, Weiss E, Sullam P, et al. Abacavir, a competitive inhibitor of soluble guanylyl cyclase, increases platelet reactivity. 17th Conference on Retroviruses and Opportunistic Infections (CROI). 2010:Abstract #717.

Table 1: Baseline Characteristics for SMART Participants in Subgroup Not Taking ART at Study Entry

	Deferred ART (N = 114)	Immediate ART (N = 134)	Total (N = 248)
Demographics			
Age, median years (IQR)	40	40	40 (34,45)
Gender (% female)	20.2	29.1	25.0
Race (% black)	27.2	32.8	30.2
Clinical characteristics			
Nadir CD4 Count, median cells/mm^3) (IQR)	364	364	364 (300, 415)
CD4 Count, median cells/mm^3 (IQR)	444	430	433 (385, 526)
HIV RNA Level, mean log_{10}copies/mL (IQR)	4.6	4.4	4.5 (4.1, 4.9)
Prior AIDS (%)	6.1	10.4	8.5
ART Naive (%)	55.3	57.5	56.5
Co-infection with hepatitis B or C (%)	8.8	9.7	9.3
eGFR (IQR)	111	116	114 (103, 123)
Current smoker (%)	43.0	45.5	44.4
Diabetes (%)	3.5	3.7	3.6
Blood pressure lowering drugs (%)	9.6	11.9	10.9
Lipid lowering drugs (%)	4.4	4.5	4.4
Prior CVD (%)	1.8	3.7	2.8
Body Mass Index (BMI), median kg/m^2 (IQR)	24.7	25.6	25.1 (22.6, 28.4)
Started NNRTI-based ART (%)*	--	56.0	--
Started PI-based ART (%)*	--	29.1	--
Started other ART (%)*	--	13.4	--
Traditional serum lipid measures, median (IQR)			
Total cholesterol (mg/dL)	164	164	164 (146, 191)
Triglycerides (mg/dL)	124	126	125 (85, 181)
LDL cholesterol (mg/dL)	100	100	100 (81, 124)
HDL cholesterol (mg/dL)	36	36	36 (28, 43)
Total/HDL cholesterol ratio	4.6	4.6	4.6 (3.7, 6.0)

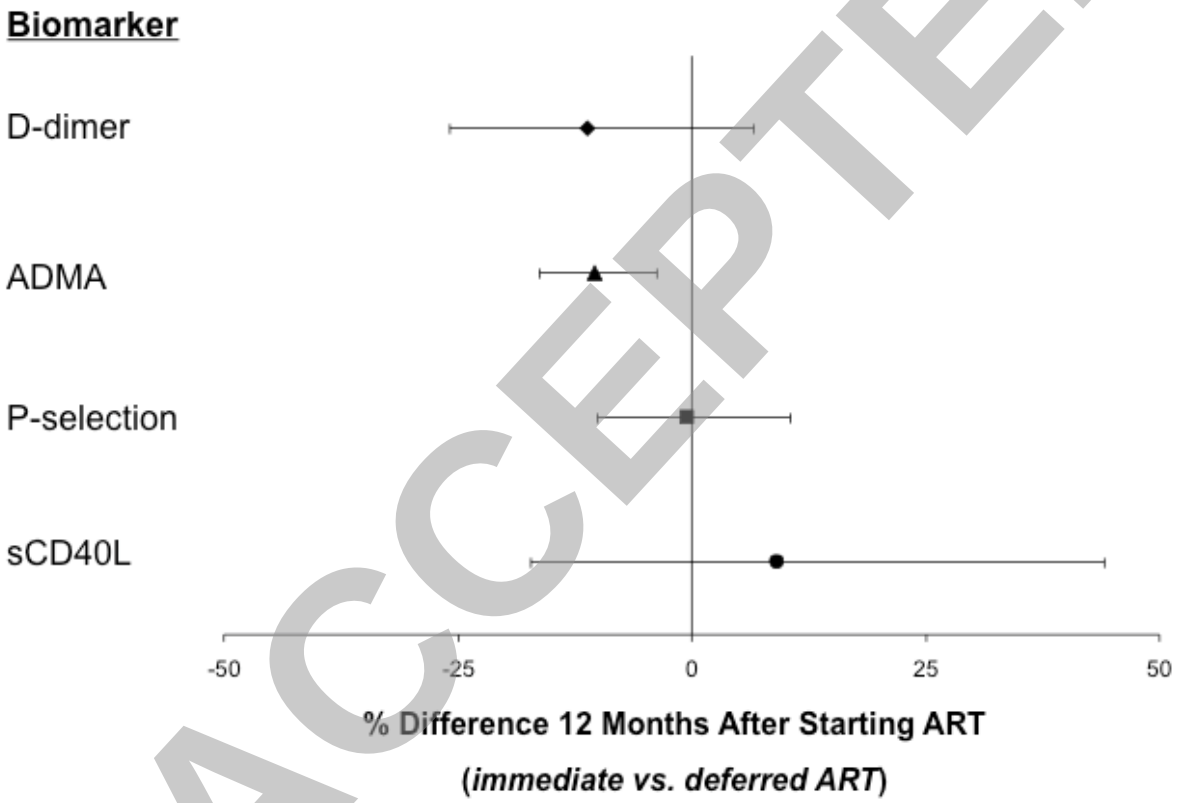
**2 participants in VS group did not start ART and were not included in the ART groups*
'Deferred ART' corresponds to the SMART DC groups and 'Immediate ART' is SMART VS group

Table 2: Median Biomarker Levels at Baseline and 12-Month Follow-up

Biomarker	Median (IQR) at Baseline		Median (IQR) at Month 12 Visit	
	Deferred ART	Immediate ART	Deferred ART	Immediate ART
hsCRP (μg/mL)	1.67 (0.68, 3.25)	1.41 (0.61, 3.26)	--	--
IL-6 (pg/mL)	1.97 (1.34, 3.15)	2.16 (1.56, 3.30)	--	--
D-dimer (μg/mL)	0.32 (0.20, 0.57))	0.32 (0.20, 0.62)	0.25 (0.17, 0.48)	0.25 (0.15, 0.41)
ADMA (μmol/L)	0.59 (0.50, 0.67)	0.56 (0.48, 0.65)	0.60 (0.48, 0.74)	0.53 (0.42, 0.67)
sCD40L (pg/mL)	248 (140, 838)	257 (118, 553)	334 (124, 991)	350 (145, 946)
P-selectin (μg/mL)	34 (28, 50)	33 (28, 41)	40 (30, 56)	39 (30, 51)

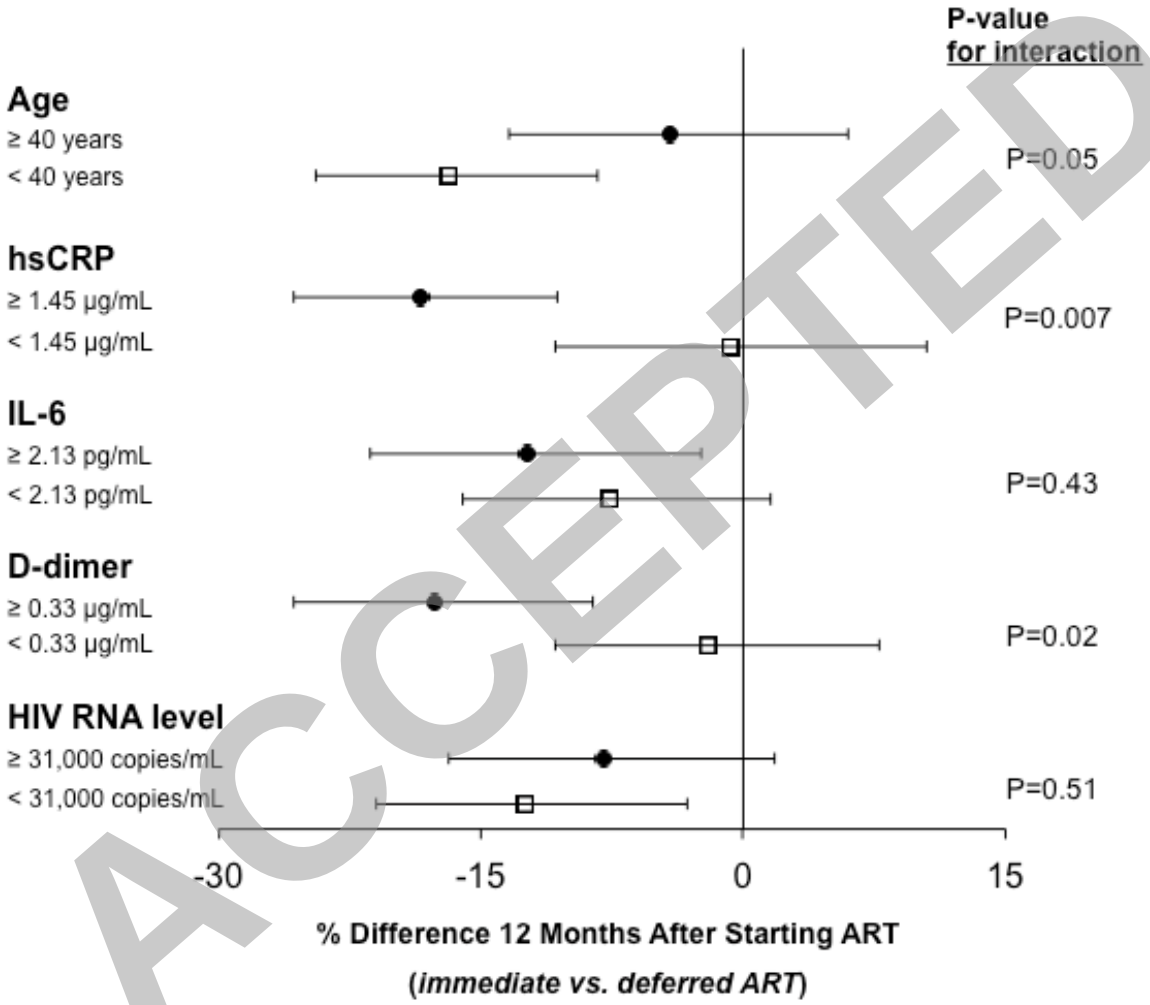
'Deferred ART' corresponds to the SMART DC groups and 'Immediate ART' is SMART VS group

Figure 1: Treatment (Immediate versus Deferred ART Groups) Differences in Biomarker Levels



Legend: The percent difference (immediate vs. deferred ART groups) at 12 months in levels of the 4 biomarkers (sCD40L, P-selectin, ADMA and D-dimer) from intention-to-treat comparisons is presented. The percent difference is adjusted for baseline biomarker level, and thus represents the change attributable to starting ART in randomized comparisons. For on-treatment comparisons excluding DC participants who started ART (n=39) and VS participants who did not start ART (n=2), differences at month 12 were -12.8% for ADMA ($p<0.001$) and -21.4% for D-dimer ($p=0.02$), but remained non-significant for sCD40L and P-selectin levels.

Figure 2: Treatment Effect of Starting versus Deferring ART on ADMA Levels Based on Age, hsCRP, IL-6, D-dimer and HIV RNA level at Entry



Legend: The percent difference (immediate vs. deferred ART) at 12 months in ADMA levels is presented separately for subgroups defined by age and biomarker levels above and below median at entry. The percent difference is adjusted for baseline level of ADMA, and, thus, represents the change attributable to starting ART. P-values represent the interaction term of baseline biomarker level and the effect of starting ART. The degree of improvement in ADMA was dependent on the degree of inflammation and coagulation (reflected in hsCRP and D-dimer) at baseline.