

# Changes in Inflammatory and Coagulation Biomarkers: A Randomized Comparison of Immediate versus Deferred Antiretroviral Therapy in Patients With HIV Infection

Jason V. Baker, MD, MS,\*† Jacqueline Neuhaus, PhD,\* Daniel Duprez, MD, PhD,\* Lewis H. Kuller, MD, PhD,‡ Russell Tracy, PhD,§ Waldo H. Belloso, MD,|| Stephane De Wit, MD, PhD,¶ Fraser Drummond, MBChB,\*\* H. Clifford Lane, MD,†† Bruno Ledergerber, PhD,‡‡ Jens Lundgren, MD,§§ Daniel E. Nixon, MD,||| Nicholas I. Paton, MD¶¶ and James D. Neaton, PhD\* for the INSIGHT SMART Study Group

**Objectives:** Among a subgroup of participants in the Strategies for Management of Antiretroviral Therapy (SMART) Trial that were naïve to antiretroviral therapy (ART) or off ART (6 months or longer) at study entry, risk of AIDS and serious non-AIDS events were increased for participants who deferred ART compared with those randomized to (re)initiate ART immediately. Our objective was to determine whether ART initiation in this group reduced markers of inflammation and coagulation that have been associated with increased mortality risk in SMART. Changes in these

biomarkers have been described after stopping ART, but not after starting ART in SMART.

**Methods:** Stored specimens for 254 participants (126 drug conservation [DC] and 128 viral suppression [VS]) who were naïve to ART or off ART (6 months or longer) were analyzed for interleukin-6, high sensitivity C-reactive protein, and D-dimer at baseline and Months 2 and 6.

**Results:** At Month 6, 62% of the VS group had HIV RNA less than 400 copies/mL and median CD4 count was 190 cells/mm<sup>3</sup> higher than for the DC group (590 versus 400 cells/mm<sup>3</sup>). Compared with DC, the VS group had 32% (95% confidence interval, 19%–43%) lower D-dimer levels at Month 6 ( $P < 0.001$ ); differences were not significant for high sensitivity C-reactive protein or interleukin-6 levels.

**Conclusions:** In this randomized comparison of immediate versus delayed ART initiation, D-dimer, but not interleukin-6 and high sensitivity C-reactive protein, declined significantly after starting ART. Further studies are needed to determine whether improvements in D-dimer are associated with reduced risk of clinical disease and whether adjunct treatments used in combination with ART can reduce inflammation among individuals with HIV infection.

**Key Words:** HIV infection, antiretroviral therapy, inflammation, coagulation, biomarkers, cardiovascular disease, non-AIDS conditions

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From the \*University of Minnesota, Minneapolis, MN; †Hennepin County Medical Center, Minneapolis, MN; ‡University of Pittsburgh, Pittsburgh, PA; §University of Vermont, Burlington, VT; ||Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; ¶Saint-Pierre Hospital, Brussels, Belgium; \*\*National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia; ††National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; ‡‡University Hospital, University of Zurich, Zurich, Switzerland; §§University of Copenhagen, Copenhagen, Denmark; |||Virginia Commonwealth University, Richmond, VA; and ¶¶Medical Research Council Clinical Trials Unit, London, UK.

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Correspondence to: Jason V. Baker, MD, MS, 701 Park Avenue, MC G5, Minneapolis, MN 55415 (e-mail: baker459@umn.edu).

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## INTRODUCTION

In the Strategies for Management of Anti-Retroviral Therapy Study (SMART), episodic use of antiretroviral treatment (ART) guided by CD4 count (drug conservation [DC] group) to maintain the CD4 count greater than 250 cells/mm<sup>3</sup> was compared with the current practice of continuous ART (viral suppression [VS] group). Risk of death was 84% higher for the DC group ( $P < 0.001$ ), and over 90% of deaths were attributed to causes other than traditional opportunistic diseases.<sup>1</sup> In follow-up studies, plasma levels of

inflammatory and coagulation markers, including high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), and D-dimer, were analyzed to better understand the factors associated with the excess mortality. Mortality risk during follow-up was associated with higher levels of hsCRP, IL-6, and D-dimer with odds ratios for the highest versus lowest quartile of 2.0 ( $P = 0.05$ ), 8.3 ( $P < 0.0001$ ), and 12.4 ( $P < 0.0001$ ), respectively. In addition, increases in IL-6 and D-dimer levels 1 month after stopping ART were associated with the degree of viral replication.<sup>2</sup> Whether or not starting ART, with corresponding declines in HIV RNA levels, leads to reciprocal changes in IL-6 and D-dimer levels has not previously been reported in SMART.

A small subgroup of participants in SMART had never taken ART or had not used ART for at least 6 months before randomization (henceforth referred to as the “no-ART” subgroup), and for these participants, the comparison of DC and VS groups was a comparison of immediate versus deferred (until CD4 declined to 250 cells/mm<sup>3</sup>) initiation of ART. For this subgroup, morbidity and mortality, both AIDS- and non-AIDS-related, was significantly lower in those who immediately initiated ART compared with those who deferred ART.<sup>3</sup>

The present study was motivated by the findings described previously. The three biomarkers, hsCRP, IL-6, and D-dimer, evaluated in this investigation were then chosen because they are associated with all-cause mortality in SMART and in studies of the general population,<sup>2,4-7</sup> and they have high laboratory and biologic reproducibility.<sup>8</sup> Declines in D-dimer levels after ART initiation have been reported, although changes with ART in hsCRP or IL-6 in other reports have been inconsistent.<sup>9-12</sup> None of the previously reported studies included a randomized control group not taking ART for comparison. To our knowledge, this is the first evaluation of combination ART versus no ART on these markers in a randomized study.

## METHODS

The methods and results of the SMART trial have been published.<sup>1</sup>

### Study Population

Of the 5472 randomized participants, 477 were either ART-naïve or had not received ART for at least 6 months (the no-ART subgroup; Fig. 1).<sup>3</sup> To reduce the likelihood of recent ART exposure, participants with low HIV RNA levels (less than 10,000 copies/mL) during the 6 months before randomization were excluded. The SMART study, including the consent for stored specimens, was approved by the Institutional Review Board or ethics committee at each clinical site and at the University of Minnesota, which served as the Statistical and Data Management Center. The Institutional Review Board at the University of Minnesota also approved plans for analysis of stored specimens for consenting participants.

### Study Treatments

In SMART, participants were randomized to one of two ART strategies. The VS strategy aimed to maximally suppress viral replication by continuous use of ART. The DC strategy

entailed intermittent use of ART for periods defined by CD4 count thresholds. For the no-ART subgroup, the randomization in SMART corresponded to the immediate initiation of ART (VS group) versus the deferral of ART until the CD4 count declined to less than 250 cells/mm<sup>3</sup> or symptoms of HIV disease developed (DC group). Any licensed ART could be used. A subset of participants enrolled in the United States and Australia were asked to consent to store blood at baseline, 1 month, 2 months, and every 2 months thereafter in the first year. US and Australian participants in the no-ART subgroup who consented to store plasma and who had specimens available at baseline, 2 months of follow-up, and 6 months of follow-up form the basis of analyses in this report. A flow diagram outlining reasons for exclusion from this study sample is presented in Figure 1.

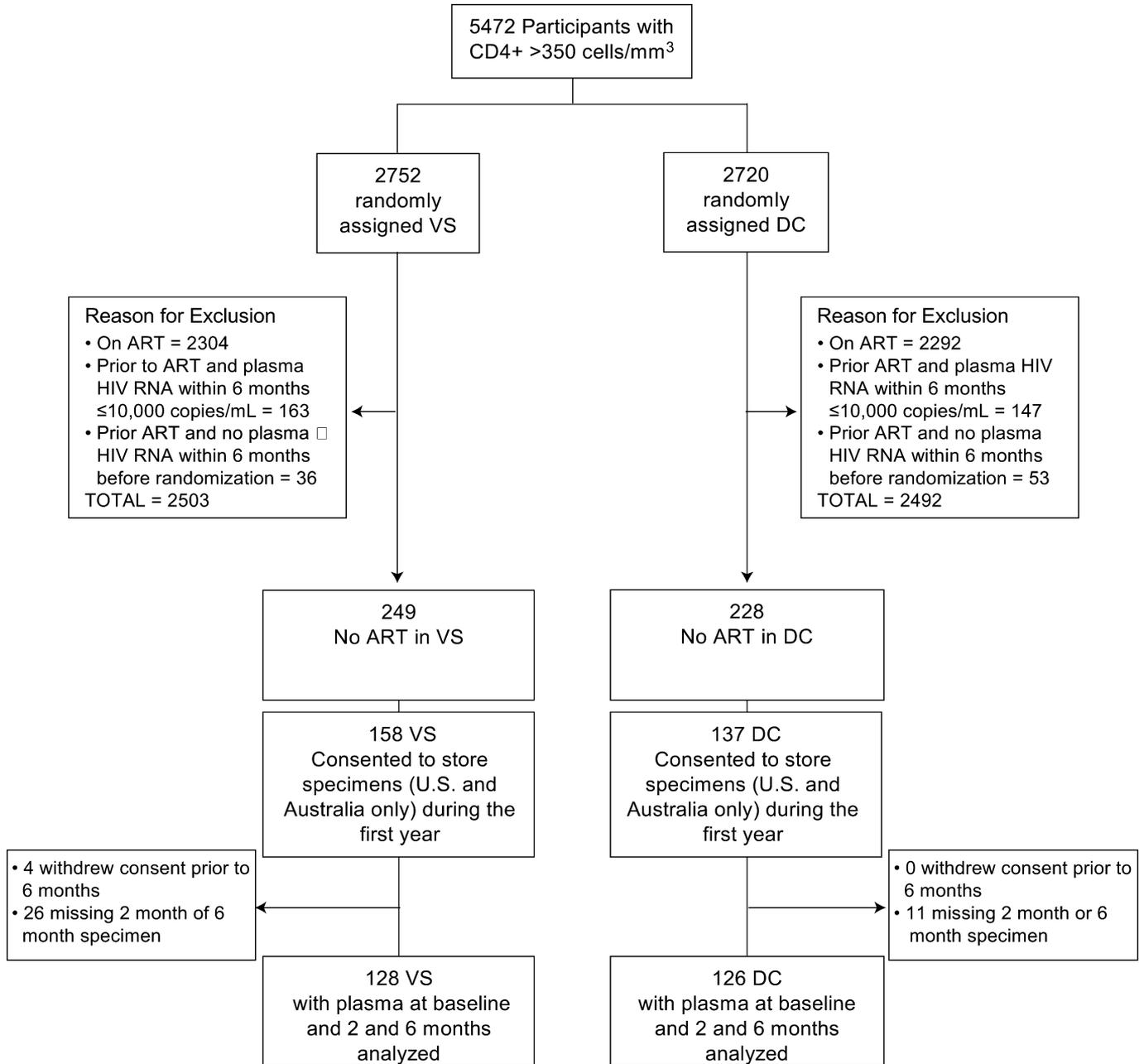
### Biomarkers

For consenting participants in SMART, plasma specimens were collected using EDTA tubes and were shipped frozen to a central repository. Two inflammatory markers, hsCRP and IL-6, and a coagulation marker, D-dimer, were measured by the Laboratory for Clinical Biochemistry Research at the University of Vermont. IL-6 was measured with Chemiluminescent Sandwich enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN); hsCRP with a NB<sup>TM</sup> II nephelometer, N Antiserum to Human CRP (Siemens Diagnostics, Deerfield, IL); and D-dimer levels with immunoturbidometric methods on the Sta-R analyzer, Liatest D-DI (Diagnostica Stago, Parsippany, NJ). The lower level of detection for IL-6, hsCRP, and D-dimer were 0.16 pg/mL, 0.16 µg/mL, and 0.01 µg/mL. Samples were not required to be fasting specimens. All samples were analyzed blinded to treatment group. The assay coefficient of variance using these methods is 5% for hsCRP, 7% for IL-6, and 12% for D-dimer.<sup>5,8</sup> The corresponding biologic coefficient of variance for these markers over the short-term has been previously reported as 50% for hsCRP, 27% for IL-6, and 56% for D-dimer.<sup>8,13</sup>

### Statistical Methods

Comparisons between the VS and DC groups were intent to treat. In addition, supportive analyses were carried out excluding small numbers of VS participants who did not immediately initiate ART and DC participants who initiated ART before 6 months. Separate analyses were carried out for the subset of participants that were naïve to ART and those that were not. Analysis of covariance with the baseline level of the biomarker included as a covariate was used to compare the two treatment groups for change in hsCRP, IL-6, and D-dimer levels at 2 and 6 months. Values of hsCRP, IL-6, and D-dimer were log<sub>e</sub>-transformed before analysis. The log<sub>e</sub> transformed biomarker differences were exponentiated to obtain percent differences. Biomarker levels below assay detection limits (none for IL-6; 2 for D-dimer, one at Month 2 and one at Month 6; and 43 for hsCRP, 10 at baseline, 17 at Month 2, and 16 at Month 6) were set to the lower level of detection.

Multiple regression analysis was used to study factors associated with baseline biomarker levels (while off ART) and to study predictors of biomarker change after 6 months for the



**FIGURE 1.** Design and study population of no-ART subgroup in SMART.

VS group (after starting ART). Six months, rather than 2 months, was chosen to allow more time to achieve virologic suppression. Adjusted models considered the following covariates: age, gender, race, hepatitis coinfection, smoking, total/high-density lipoprotein cholesterol, blood-pressure lowering medication, lipid-lowering medication, body mass index (BMI), CD4 count, HIV RNA level (log<sub>10</sub> transformed), history of ART use (ART-naïve or not), prior AIDS, and baseline biomarker level (when considering biomarker change). For the VS group, biomarker responses were also compared by the class of ART started and HIV RNA level at

Month 6. Statistical analyses were performed using SAS (Version 9.1; SAS Institute Inc, Cary, NC). All reported *P* values are two-sided.

**RESULTS**

Most participants in SMART were asked before randomization to consent to store blood at baseline and at 4- or 12-month intervals. In addition, 2554 participants in the United States and Australia consented to have plasma stored at Month 2 and every 2 months afterward for the first year after randomization. Among these 2554 participants, 295 were

among the no-ART subgroup (62% of the  $n = 477$  in the complete no-ART subgroup). Of these 295 participants, 254 (86%) had plasma available at baseline, 2 months, and 6 months (Fig. 1). These 254 participants form the basis of this report. When compared with the participants in the SMART no-ART subgroup not included in this report ( $n = 223$ ), the cohort analyzed in this report had a greater median age (42 versus 39 years), were more likely to be black (50% versus 21%), have a history of an AIDS event (14% versus 8%), be coinfecting with hepatitis B or C (23% versus 13%), have diabetes (8% versus 3%), and a have a history of cardiovascular disease (CVD) (4% versus 1%). For the participants naïve to ART that reported a prior AIDS event, the diagnoses were: tuberculosis ( $n = 3$ ), chronic herpes simplex ( $n = 3$ ), herpes zoster ( $n = 2$ ), recurrent bacterial pneumonia ( $n = 1$ ), Kaposi sarcoma ( $n = 1$ ), HIV-related encephalopathy ( $n = 1$ ), and one participant with both prior histoplasmosis and coccidioidomycosis.

### Baseline Characteristics of Drug Conservation and Viral Suppression Participants in the No-Antiretroviral Therapy Subgroup

Characteristics of the DC and VS groups are given in Table 1. One hundred thirty (51.2%) of the 254 participants were ART-naïve. For both treatment groups combined, median baseline and nadir CD4 count for ART-naïve participants were 448 and 380 cells/mm<sup>3</sup>, respectively. Corresponding CD4 counts for the 124 participants who had used ART in the past were 447 and 321 cells/mm<sup>3</sup>, respectively.

Overall, median baseline levels of hsCRP, IL-6, and D-dimer were 1.32 µg/mL (interquartile range [IQR], 0.59–3.60), 2.68 pg/mL (IQR, 1.59–4.36), and 0.43 µg/mL (IQR, 0.24–0.73), respectively. Levels for those participants that were ART-naïve were 1.18 µg/mL (IQR, 0.56–3.20), 2.03 pg/mL (IQR, 1.39–3.92), and 0.40 µg/mL (IQR, 0.19–0.76), respectively. Levels for those that were not ART-naïve (and  $P$  values for ART-naïve versus not) were 1.71 µg/mL (IQR, 0.63–3.98) ( $P = 0.29$ ), 3.16 pg/mL (IQR, 2.06–4.51) ( $P = 0.003$ ), and 0.45 µg/mL (IQR, 0.30–0.72) ( $P = 0.16$ ), respectively.

In multiple regression models, older age at baseline was associated with higher levels of hsCRP ( $P = 0.053$ ) and D-dimer ( $P = 0.010$ ) but not IL-6 ( $P = 0.188$ ). Female gender ( $P = 0.001$ ), a history of a prior AIDS event ( $P = 0.006$ ), and a higher BMI ( $P = 0.019$ ) were associated with higher baseline D-dimer levels; hsCRP levels were lower for persons coinfecting with hepatitis B or C ( $P = 0.001$  versus those HIV monoinfected). Higher levels of IL-6 were seen in women (versus men;  $P = 0.013$ ) and participants taking blood pressure-lowering drugs ( $P = 0.013$ ). None of the three biomarker levels were significantly associated with prior ART exposure (versus none), baseline CD4 count, or HIV RNA level. Finally, at baseline, biomarker levels were correlated with one another:  $r = 0.41$  for hsCRP and IL-6 ( $P < 0.001$ );  $r = 0.34$  for hsCRP and D-dimer ( $P < 0.001$ ); and  $r = 0.33$  for IL-6 and D-dimer ( $P < 0.001$ ).

**TABLE 1.** Characteristics of Participants in No-ART Subgroup At Baseline in SMART

	DC Group (N = 126)	VS Group (N = 128)	Total (N = 254)
<b>Demographics</b>			
Age, median (IQR)	43	42	42 (37–48)
Gender (percent female)	23.8	31.3	27.6
Race (percent black)	50.0	50.8	50.4
<b>Clinical Characteristics</b>			
Nadir CD4 count (cells/mm <sup>3</sup> ), median (IQR)	361	362	362 (299–429)
CD4 Count (cells/mm <sup>3</sup> ), median (IQR)	464	431	447 (391–550)
HIV-RNA (log <sub>10</sub> copies/mL), median (IQR)	4.6	4.5	4.6 (4.1–4.9)
HIV-RNA less than 10,000 copies/mL, no. (%)	19 (15)	32 (25)	51 (20)
HIV-RNA 10,000 to less than 25,000 copies/mL, no. (%)	24 (19)	21 (17)	45 (18)
HIV-RNA 25,000 to less than 100,000 copies/mL, no. (%)	54 (43)	53 (42)	107 (42)
HIV-RNA 100,000 copies/mL or greater, no. (%)	29 (23)	21 (17)	50 (20)
Prior AIDS (%)	11.9	16.4	14.2
ART-naïve (%)	51.6	50.8	51.2
Coinfection with hepatitis B/C (%)	21.4	24.2	22.8
Current smoker (%)	50.0	46.9	48.4
Diabetes (%)	9.5	5.5	7.5
Blood pressure-lowering drugs (%)	22.2	18.8	20.5
Lipid lowering drugs (%)	7.9	6.3	7.1
Prior CVD (%)	2.4	4.7	3.5
Body mass index (kg/m <sup>2</sup> ), median (IQR)	25.3	26.2	25.9 (23.1–30.4)
<b>Lipids, median (IQR)</b>			
Total cholesterol (mg/dL)	158	161	161 (140–184)
Triglycerides (mg/dL)	124	139	132 (91–194)
HDL cholesterol (mg/dL)	37	35	36 (28–44)
LDL cholesterol (mg/dL)	94	95	94 (76–116)
Total/HDL cholesterol	4.4	4.7	4.5 (3.5–5.8)

ART, antiretroviral therapy; DC, drug conservation; VS, viral suppression; IQR, interquartile range; CVD, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

### Randomized Comparisons for the Immediate (Viral Suppression) and Deferred (Drug Conservation) Antiretroviral Therapy Groups

One hundred twenty-six of 128 participants in the VS group initiated ART after randomization and most (62%) achieved viral suppression by 6 months (Table 2). The ART regimen initiated for VS participants included a ritonavir-boosted protease inhibitor for 24.2%, a nonnucleoside reverse transcriptase inhibitor for 53.1%, an unboosted protease inhibitor for 5.5%, and a regimen consisting only of nucleoside reverse transcriptase inhibitors for 15.6%. The percent of DC participants who started ART increased from 1.6% at 2 months to 10.3% at 6 months. CD4 count declines in

**TABLE 2.** ART Use, Viral Suppression, and CD4 Change Over Follow-Up

	DC Group (N = 126) Median (IQR) or %	VS Group (N = 128) Median (IQR) or %	P (Difference)
Percent on ART			
Month 2	1.6	93.0	<0.001
Month 6	10.3	92.2	<0.001
Percent HIV-RNA 400 or less (copies/mL)			
Month 2	2.4	52.8	<0.001
Month 6	6.3	61.9	<0.001
Change in CD4 count (cells/mm <sup>3</sup> )			
Month 2	-30 (-109 to 15)	65 (-38 to 165)	<0.001
Month 6	-60 (-128 to -9)	93 (-2 to 233)	<0.001

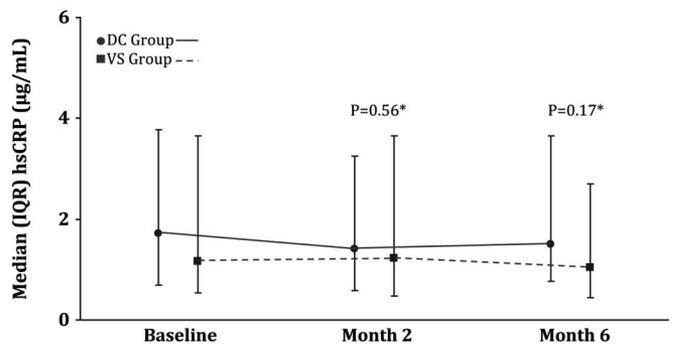
DC, drug conservation; IQR, interquartile range; VS, viral suppression.

the DC group and increases in the VS group resulted in a difference between groups of nearly 100 cells/mm<sup>3</sup> at 2 months and over 150 cells/mm<sup>3</sup> at month 6 (*P* < 0.001; Table 2). For the VS group, HIV RNA declines were rapid with a difference compared with the DC group of -1.75 log<sub>10</sub> copies/mL at 2 months (*P* < 0.001) and -1.89 log<sub>10</sub> copies/mL at 6 months (*P* < 0.001).

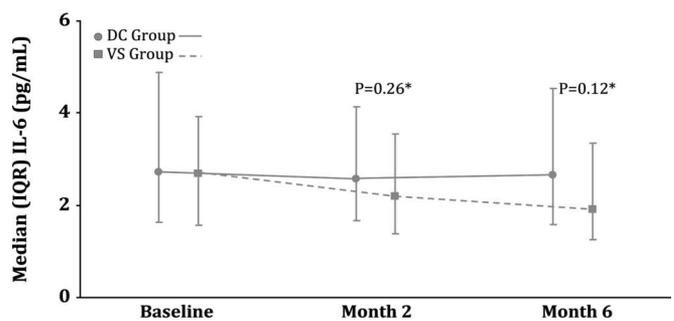
Median (IQR) biomarker levels over follow-up by treatment group are presented in Figure 2. Significant differences between the DC and VS groups were evident for D-dimer at Month 2 (21.7% lower for VS compared with DC; 95% confidence interval [CI], 8.8–32.8) and the difference was greater by Month 6 (32.2% lower for VS than DC; 95% CI, 19.2–43.0). Median levels of D-dimer for VS participants declined from 0.40 μg/mL at baseline to 0.27 and 0.24 μg/mL at 2 and 6 months (Fig. 2). Differences between treatment groups (VS versus DC) in levels of hsCRP (-17%; 95% CI, -36% to 9%, *P* = 0.17) and IL-6 (-13%; 95% CI, -27% to 4%; *P* = 0.12) were not significant by Month 6. Percent differences in biomarker levels between the treatment groups did not vary according to prior ART use (versus naïve to ART; *P* = 0.52, 0.59, and 0.68 for treatment-by-subgroup interactions, respectively; data not shown). Among DC participants who stayed off ART, baseline biomarker levels were highly correlated with measures of the same marker at month 2 (*r* = 0.63 for hsCRP, *r* = 0.63 for IL-6, *r* = 0.74 for D-dimer; *P* < 0.001 for all) and at Month 6 (*r* = 0.54 for hsCRP, *r* = 0.62 for IL-6, *r* = 0.68 for D-dimer; *P* < 0.001 for all).

Analyses excluding VS participants that did not initiate ART (*n* = 2), and DC participants that initiated ART before 6 months (*n* = 14), were carried out. Reasons for starting ART in the excluded participants from the DC group were drop in CD4 count or percent (eight participants), clinical symptoms or disease progression (two participants), high HIV RNA (two participants), participant preference (one participant), and after modification of the SMART protocol (1 participant).<sup>14</sup> After these exclusions, the percent differences between VS and DC groups at Month 6 were -12% (95% CI, -33% to 17%) for hsCRP (*P* = 0.38), -8% (95% CI, -23% to 10%) for IL-6 (*P* = 0.37), and -33% (95% CI, -45% to -20%) for D-dimer (*P* < 0.001).

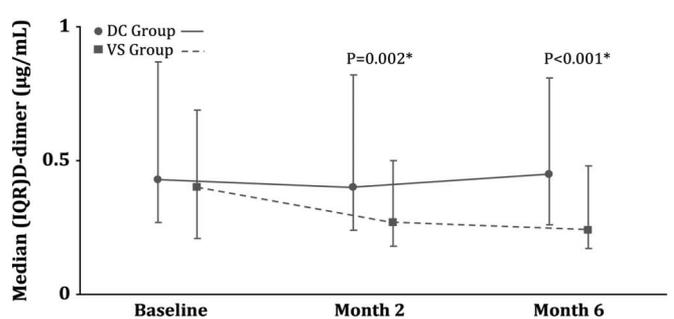
**A: hsCRP**



**B: IL-6**



**C: D-dimer**



**FIGURE 2.** Median levels of hsCRP (A), IL-6 (B), and D-dimer (C) are presented for VS and DC groups at each visit. Error bars represent the interquartile range (IQR). The reduction in D-dimer levels was greater after 2 and 6 months of ART (VS) when compared with participants randomized to defer ART (DC). The change in inflammatory biomarkers (hsCRP and IL-6) was not significantly different between treatment groups at the two follow-up visits. \**P* values represent the difference between treatment groups in the change from baseline (on loge scale) and are adjusted for baseline biomarker level. VS, viral suppression; DC, drug conservation; ART, antiretroviral therapy; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6.

**Baseline Predictors of Biomarker Change for the Viral Suppression Group**

Among VS participants, baseline CD4 count, HIV RNA level, a history of AIDS, prior ART exposure, and type of ART started were not associated with changes in any of the three biomarkers at 6 months. Nonsmokers demonstrated greater improvement in hsCRP (-36%; *P* = 0.04) and IL-6 levels (-42%; *P* < 0.001) after 6 months compared when compared

with smokers. A lower BMI was associated with a greater decline in hsCRP levels ( $-28\%$  per  $5\text{-kg/m}^2$  lower BMI;  $P = 0.003$ ). The decline in IL-6 levels was greater for blacks ( $-28\%$  versus nonblack race;  $P = 0.02$ ) and less for participants using a blood pressure-lowering medication ( $53\%$  versus not;  $P = 0.04$ ). Gender was the only traditional CVD risk factor associated with changes in D-dimer levels ( $-34\%$  for males versus females;  $P = 0.04$ ).

After starting ART, correlations of change in D-dimer levels with change in hsCRP and IL-6 were  $0.17$  ( $P = 0.08$ ) and  $0.09$  ( $P = 0.35$ ), respectively (correlations calculated using  $\log_e$ -transformed values). Among those with a decline in hsCRP (below the median change of zero), the median change in D-dimer was  $-0.09 \mu\text{g/mL}$ ; for those with an increase in hsCRP (above the median the change of zero), the change in D-dimer was  $-0.06 \mu\text{g/mL}$ . Corresponding changes for D-dimer for those below and above the median change for IL-6 were  $-0.07$  and  $-0.06 \mu\text{g/mL}$ , respectively.

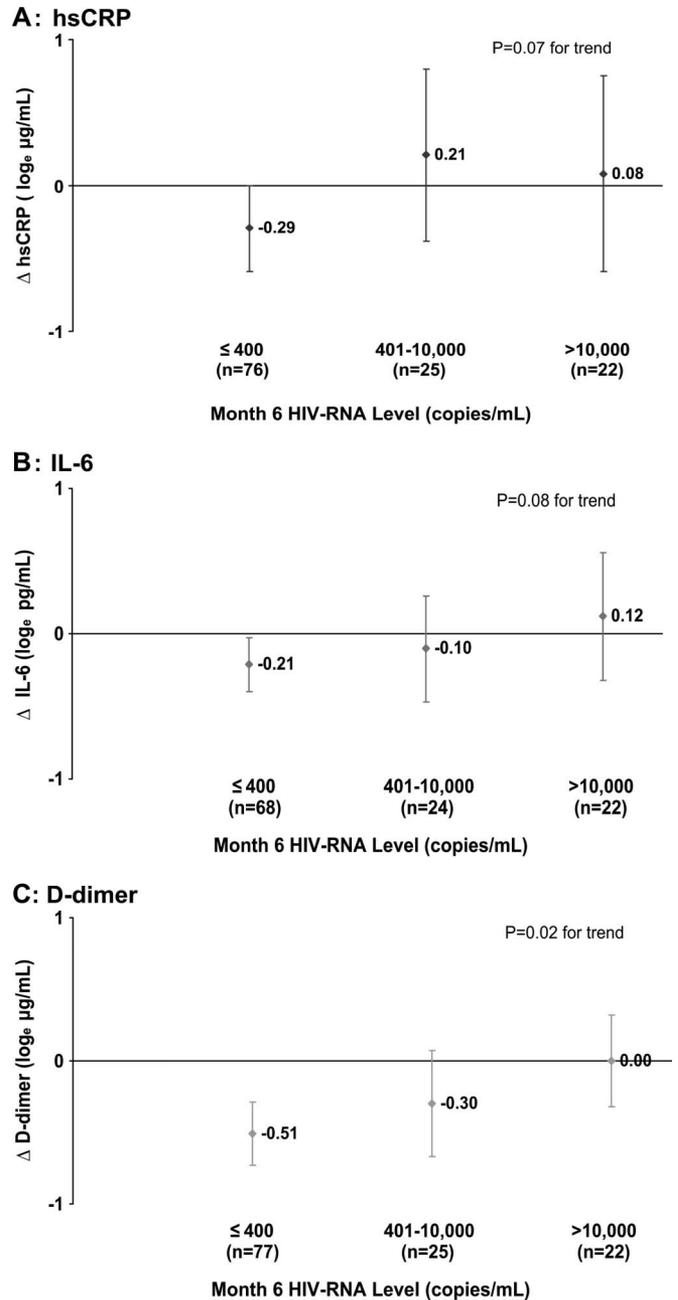
### Changes in Biomarkers and HIV RNA Levels After Starting Antiretroviral Therapy in the Viral Suppression Group

Baseline HIV RNA levels were not associated with changes in HIV RNA levels, achieving an undetectable viral load specifically or changes in biomarker levels at Month 6. The relationship between HIV RNA level and biomarker change 6 months after starting ART (VS group only) are presented in Figure 3. The degree of improvement (ie, decrease) in D-dimer, but not hsCRP and IL-6, levels were inversely associated with HIV RNA levels at 6 months (Fig. 3).

Among participants in VS who achieved HIV RNA levels 400 copies/mL or less, median (IQR) and mean percent (on  $\log_e$  scale) change in biomarker levels at month 6 were:  $0.00$  ( $-0.47$  to  $0.70$ )  $\mu\text{g/mL}$  and  $-28\%$  for hsCRP;  $-0.34$  ( $-1.41$  to  $0.32$ )  $\text{pg/mL}$  and  $-26\%$  for IL-6; and  $-0.10$  ( $-0.31$  to  $0.00$ )  $\mu\text{g/mL}$  and  $-51\%$  for D-dimer. The corresponding estimates for VS participants with RNA greater than 400 copies/mL at 6 months are:  $-0.07$  ( $-0.61$  to  $1.34$ )  $\mu\text{g/mL}$  and  $60\%$ ;  $-0.06$  ( $-0.83$  to  $0.65$ )  $\text{pg/mL}$  and  $2.5\%$ ; and  $-0.05$  ( $-0.42$  to  $0.13$ )  $\mu\text{g/mL}$  and  $-20\%$ . These changes represent a significant within participant decline in all three biomarkers from baseline to Month 6 among VS participants who achieved an undetectable viral load at Month 6 (Fig. 3), but these changes cannot be compared with a group randomized to defer ART. Finally, among the subgroup with undetectable viral loads at Month 6, levels of inflammatory markers hsCRP and IL-6 were correlated at Month 6 ( $r = 0.38$ ;  $P < 0.001$ ), whereas levels of D-dimer were not correlated with either hsCRP ( $r = 0.07$ ;  $P = 0.53$ ) or IL-6 ( $r = 0.15$ ;  $P = 0.20$ ).

### DISCUSSION

We analyzed stored specimens in a unique subset of SMART trial participants to carry out a randomized comparison of immediate versus delayed initiation of ART on inflammatory and coagulation biomarkers among a small subgroup of participants and found that initiating ART reduces levels of D-dimer. For those who initiated ART after randomization (VS group), D-dimer levels declined by 2



**FIGURE 3.** The 6-month change in biomarker levels (after natural log transformation) for participants randomized to start ART (VS group) are presented for hsCRP (A), IL-6 (B), and D-dimer (C). The mean change (error bars representing 95% confidence intervals) in biomarkers at Month 6 are shown stratified by HIV RNA level at Month 6. The  $P$ -values reported for the association between 6-month biomarker change and HIV RNA level as a continuous variable. The figure demonstrates that significant biomarker changes at Month 6 are only apparent for those participants who achieve HIV RNA levels less than 400 copies/mL. ART, antiretroviral therapy; VS, viral suppression; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6.

months, the decline persisted through 6 months, and the decline was greater for participants who achieved viral suppression. Differences between the DC and VS groups for changes in hsCRP and IL-6 were more modest and did not reach statistical significance in this randomized comparison.

A large body of epidemiologic data support the importance of inflammation and thrombotic activity for CVD risk and mortality from any cause.<sup>15</sup> In the general population, higher levels of hsCRP, IL-6, and D-dimer have all been associated with risk for CVD.<sup>4-7,16-18</sup> In SMART, levels of all three biomarkers in HIV-infected individuals at baseline predicted risk for both short- and long-term mortality and were more strongly related to risk for non-AIDS-related conditions than for AIDS.<sup>2,19,20</sup> Non-AIDS-related conditions are now more common than AIDS events for persons with HIV infection receiving ART at higher CD4 counts.<sup>1,21-25</sup> Thus, reducing inflammation and thrombotic activity may represent an additional therapeutic goal in the clinical management of HIV infection in the current era.

HIV replication has been shown to be an important factor in the upregulation of coagulation pathways and thrombotic activity.<sup>2,10,12,26-28</sup> In the Swiss-Thai-Australia Treatment Interruption Trial (STACCATO), D-dimer levels were associated with HIV RNA levels before and after (median 8 months) starting ART.<sup>10</sup> In another study of 41 participants, D-dimer levels improved after 5 to 13 months of treatment with ART.<sup>12</sup> In SMART, D-dimer increased markedly 1 month after stopping ART among persons with suppressed virus at baseline, and the increase was strongly correlated with HIV RNA levels.<sup>2</sup> Here we show that the D-dimer levels declined 6 months after starting ART by an amount (–32%) similar to the increase 1 month after ART interruption that we previously reported.<sup>2</sup> However, D-dimer levels among SMART participants with suppressed HIV viral loads are still 49% higher than uninfected controls, suggesting residual thrombotic activity persists despite effective HIV treatment with ART.<sup>29</sup>

Chronic inflammation among persons with HIV infection may be, in part, a consequence of activation of lymphocytes and dendritic cells, damage to the mucosal barrier, injury to endothelial surfaces, and other factors related to HIV replication.<sup>12,30-34</sup> In STACCATO, initiating ART was associated with declines in markers of endothelial activation (P-selectin and soluble vascular cell adhesion molecule-1).<sup>10</sup> However, in this and another report, hsCRP levels did not decline after treatment with ART.<sup>10,11</sup> Similarly, we did not observe significant improvement in hsCRP or IL-6 levels after ART initiation when compared with those who deferred ART. Given that not all participants achieved an HIV RNA level less than 400 copies/mL by Month 6, it is possible that ART associated declines in hsCRP and IL-6 may have been apparent with a greater degree of viral suppression. However, baseline levels of IL-6 and hsCRP were not associated with baseline HIV RNA levels, and ART-related changes in D-dimer levels were poorly correlated with changes in the two inflammatory markers in this study. The findings for IL-6 were surprising given the rapid increase in IL-6 that we saw after ART interruption in SMART and the relationship of that increase with loss of virologic control.<sup>2</sup> One other explanation may be that an improvement in inflammatory markers

associated with starting ART takes longer than 6 months. However, among SMART participants with viral suppression on ART, hsCRP levels were 38% to 40% higher and IL-6 levels were 39% to 60% higher, respectively, when compared with HIV-uninfected control subjects from the Coronary Artery Risk Development In Young Adults (CARDIA) study and the Multi-Ethnic Study of Atherosclerosis (MESA).<sup>29</sup>

Findings from additional studies support that inflammatory markers remain upregulated despite treatment with ART. Fibrinogen (men and women) and CRP (men only) levels were elevated in 1131 HIV-infected participants from the Study of Fat Redistribution and Metabolic Change in HIV infection (FRAM) when compared with 281 general population control subjects (from CARDIA).<sup>35,36</sup> In another cross-sectional comparison of 494 persons, hsCRP levels were higher among persons with HIV infection, compared with HIV-negative control subjects, even among “HIV controllers” with undetectable HIV RNA levels in the absence of ART.<sup>37</sup>

The cause(s) of persistent inflammation despite HIV viral suppression is an area of active research. One explanation is residual immune activation, whether related to low-level HIV replication or other mechanisms.<sup>30,38,39</sup> In addition, adverse lipid and metabolic changes associated with HIV infection or ART exposure may be important proinflammatory factors.<sup>36,40,41</sup> The potential benefits of ART with respect to reducing inflammation and thrombotic activity may also differ by specific class or drug.<sup>42-45</sup> Cumulatively, these data suggest ART-associated viral suppression is not going to normalize inflammation, and studies that evaluate anti-inflammatory treatments used in addition to ART should be an active research priority.

Limitations of this study include the small sample size, short follow-up duration, and that not all participants in the VS group achieved an undetectable viral load. The variability in biomarker levels, and CRP in particular, over time in this cohort also limits the ability to detect significant changes. The ongoing Strategic Timing of Antiretroviral Therapy (START) trial will be able to explore the influence of ART-related HIV suppression on inflammatory markers in a much larger data set. Another limitation is that current data are lacking to quantify the clinical event risk associated with absolute changes in these biomarkers among HIV-infected persons. START will also provide valuable insight into whether these biomarkers are useful for risk stratification by assessing the effects of treatment-related changes on AIDS and non-AIDS-related events. Finally, the ART treatment used in SMART was chosen by the investigator and patient and was not randomized. The effects of different treatments on these biomarkers will require larger randomized studies of different ART regimens.

In summary, the initiation of ART resulted in a rapid decline in D-dimer levels that are associated with suppression of HIV replication. Larger studies, with longer follow-up, are needed to determine if these treatment-related changes are clinically relevant.

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