

## MAJOR ARTICLE

# Markers of Inflammation, Coagulation, and Renal Function Are Elevated in Adults with HIV Infection

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(See the article by Kalayjian et al, on pages 1796–1805, and the editorial commentary by Dubé and Sattler, on pages 1783–1785.)

**Background.** Human immunodeficiency virus (HIV) replication and immune activation may increase inflammation and coagulation biomarkers. Limited data exist comparing such biomarkers in persons with and without HIV infection.

**Methods.** For persons 45–76 years of age, levels of high-sensitivity C-reactive protein (hsCRP), interleukin (IL)-6, D-dimer, and cystatin C were compared in 494 HIV-infected individuals in the Strategies for Management of Anti-Retroviral Therapy (SMART) study and 5386 participants in the Multi-Ethnic Study of Atherosclerosis (MESA) study. For persons 33–44 years of age, hsCRP and IL-6 levels were compared in 287 participants in the SMART study and 3231 participants in the Coronary Artery Development in Young Adults (CARDIA) study.

**Results.** hsCRP and IL-6 levels were 55% ( $P < .001$ ) and 62% ( $P < .001$ ) higher among HIV-infected participants than among CARDIA study participants. Compared with levels noted in MESA study participants, hsCRP, IL-6, D-dimer, and cystatin C levels were 50%, 152%, 94%, and 27% higher, respectively ( $P < .001$ , for each), among HIV-infected participants. HIV-infected participants receiving antiretroviral therapy who had HIV RNA levels  $\leq 400$  copies/mL had levels higher (by 21% to 60%) ( $P < .001$ ) than those in the general population, for all biomarkers.

**Conclusions.** hsCRP, IL-6, D-dimer, and cystatin C levels are elevated in persons with HIV infection and remain so even after HIV RNA levels are suppressed with antiretroviral therapy. Additional research is needed on the pathophysiology of HIV-induced activation of inflammatory and coagulation pathways, to guide potential interventions.

There is a growing body of data indicating that the risk of serious non-AIDS conditions, such as cardiovascular disease, kidney disease, liver disease, and non-AIDS-defining malignancies, is increased in individuals with human immunodeficiency virus (HIV) infection, compared with the general population [1]. The reasons for

this increase are not clear. HIV-induced activation of inflammatory and coagulation pathways could explain the increased risk.

In the Strategies for Management of Anti-Retroviral Therapy (SMART) trial, episodic use of antiretroviral

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treatment (ART) guided by the CD4<sup>+</sup> cell count was compared with the current practice of continuous ART. The episodic ART strategy was also associated with an 84% ( $P = .007$ ) increased risk of all-cause mortality. Most of the deaths were attributed to serious non-AIDS diseases [2]. A study using stored plasma specimens for SMART study participants was undertaken to explore the reasons for the greater risk of death from non-AIDS diseases in the episodic ART group. Interleukin (IL)-6 and D-dimer levels were found to increase significantly in the first month after the interruption of ART. Furthermore, levels of high-sensitivity C-reactive protein (hsCRP), IL-6, and D-dimer measured at study entry were strongly associated with mortality, and the association with mortality was evident in participants who were assigned to both the episodic and continuous ART groups [3]. Taken together, the findings from these 3 reports support the hypothesis that HIV induces activation of inflammatory and coagulation pathways, and they suggest that ART might dampen the effects.

To further understand these findings, we compared the levels of hsCRP, IL-6, D-dimer, and cystatin C in SMART study participants with levels for participants of similar age in 2 large population-based studies: the Multi-Ethnic Study of Atherosclerosis (MESA) study [4] and The Coronary Artery Risk Development in Young Adults (CARDIA) study [5]. Our hypothesis was that SMART study participants, all of whom were HIV infected, would have higher values of each of these markers than either the CARDIA or MESA study participants, who were recruited from the general population and were unlikely to be HIV infected.

## METHODS

The design, methods, and results of the SMART trial and the MESA and CARDIA studies have been published elsewhere [2, 4–6].

**SMART study sample.** In the SMART study, 5472 men and women >13 years of age who had CD4<sup>+</sup> cell counts >350 cells/mm<sup>3</sup> were enrolled at 318 sites in 33 countries between January 2002 and January 2006 [2]. Fifty percent of participants were 33–76 years of age and were enrolled at sites in the United States. hsCRP, IL-6, D-dimer, and cystatin C levels were measured at baseline in a subset of the randomized participants [3, 7]. SMART participants with prior cardiovascular disease and participants enrolled at sites outside of the United States were excluded from the analyses. A total of 287 black and non-Hispanic, white SMART study participants 33–44 years of age had hsCRP, IL-6, or D-dimer measurements, and 172 of these participants also had cystatin C measurements. A total of 494 non-Hispanic white, black, or Hispanic SMART study participants 45–76 years of age had levels of hsCRP, IL-6, or D-dimer, and 231 of these participants also had cystatin C measurements.

The SMART study, including consent for stored specimens,

was approved by the institutional review board at each 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.

**MESA study sample.** In the MESA study, 6814 men and women 45–84 years of age who were from 4 American racial/ethnic groups (non-Hispanic white, black, Hispanic, and Chinese) and were without clinical cardiovascular disease were enrolled from the general population in 6 US communities between July 2000 and August 2002 [4]. hsCRP, IL-6, D-dimer, and cystatin C levels were measured from samples collected at the time of enrollment, and findings for 5386 participants of non-Hispanic white, black, or Hispanic race/ethnicity who were 45–76 years of age are used in analyses in this report. HIV infection was not assessed, but there have been few deaths attributed to HIV/AIDS, and HIV infection was assumed to be rare.

**CARDIA study sample.** In the CARDIA study, 5115 black and non-Hispanic white men and women were enrolled in 4 US communities between March 1985 and June 1986 (year 0) [5]. After enrollment, 6 additional examinations were conducted. hsCRP and IL-6 were measured at year 15 (in years 2000 and 2001); all covariate values were also taken from the examination done at year 15. Findings from 3231 participants 33–44 years of age are included in this analysis. Only 2 of the 4 sites measured IL-6 levels. Data comparing the cystatin C levels in CARDIA study participants and HIV-infected participants have already been reported, and CARDIA data in that report are used to make comparisons with SMART study participants (raw data were not available) [8]. As in MESA, HIV infection status was not assessed and was assumed to be rare.

**Biomarker methods.** In the SMART and MESA studies, for consenting participants, specimens stored at baseline were used to measure the biomarkers. In the CARDIA study, samples obtained during follow-up were used. Ethylenediaminetetraacetic acid plasma specimens were collected and were shipped frozen to a central repository, where they were stored at  $-70^{\circ}\text{C}$ .

The biomarkers for the SMART, MESA, and CARDIA studies were measured by the Laboratory for Clinical Biochemistry Research at the University of Vermont. hsCRP was measured using the BNII nephelometer (N High Sensitivity CRP; Siemens Healthcare Diagnostics). The lower limit of detection was 0.16  $\mu\text{g}/\text{mL}$ . IL-6 was measured by ultrasensitive enzyme-linked immunosorbent assay (ELISA) (Quantikine HS Human IL-6 Immunoassay; R&D Systems). The lower limit of detection was 0.16 pg/mL. For D-dimer, an immunoturbidimetric assay (Liatest D-DI; Diagnostica Stago) was used on a Sta-R analyzer (Diagnostica Stago). The lower limit of detection of the assay was 0.01  $\mu\text{g}/\text{mL}$ . A BNII nephelometer (Siemens Healthcare Diagnostics) that utilized a particle-enhanced immunonephe-

lometric assay (N Latex Cystatin-C) was used to assay cystatin C. The assay range is 0.195–7.330 mg/dL. The biological and laboratory variability of these assays has been described. Intra-assay coefficients of variation for IL-6, hsCRP, and D-dimer are 6.3%, 8.9%, and 12.2%, respectively. For cystatin C, the coefficients of variation range from 2.0% to 2.8% [9–13]. Based on 3–4 controls per assay, interassay coefficients of variation range from 7% to 15% for IL-6, 3% to 6% for hsCRP, 5% to 14% for D-dimer, and 2% to 6% for cystatin C (Elaine Cornell, personal communication).

**Statistical methods.** This study uses baseline measurements of hsCRP, IL-6, D-dimer, and cystatin C in 2 age groups from the SMART study. hsCRP and IL-6 levels in SMART study participants 33–44 years of age were compared with the levels for participants in the same age range in the CARDIA study. Levels of hsCRP, IL-6, D-dimer, and cystatin C for those 45–76 years of age in the SMART study were compared with those for MESA study participants in the same age range. Comparisons were made for all SMART study participants, for those not receiving ART, and for those receiving ART who had an HIV RNA level  $\leq 400$  copies/mL. Median values and interquartile ranges for biomarker levels are cited. Biomarker differences between HIV-infected participants in the SMART study and participants in the MESA and CARDIA studies were compared using analysis of variance after  $\log_e$  transformation. Other characteristics between participants in the different studies are compared using Student's *t* test and Fisher's exact test.

Log-transformed biomarker levels were compared using 3 models with (1) no covariates; (2) covariates corresponding to age, race, and sex; and (3) covariates corresponding to age, race, sex, body mass index, smoking status, ratio of total/high-density lipoprotein (HDL) cholesterol, diabetes history, use of lipid-lowering drugs, and use of blood pressure-lowering drugs. The percentage differences between biomarkers levels in participants in the SMART and CARDIA studies and between participants in the SMART and MESA studies were obtained using the  $\log_e$ -transformed biomarker level by exponentiating the difference in the mean values. The adjusted percentage differences derived from the 3 models described above are displayed graphically.

To assess the effects of HIV infection and the use of HIV treatment at study entry on the biomarkers, SMART study participants were further divided according to their HIV RNA level and/or whether they were using ART. Within the SMART study, Pearson correlation coefficients between  $\log_{10}$  HIV RNA and  $\log_e$  biomarker levels were calculated for participants with an HIV RNA level  $>400$  copies/mL, according to the ART status at baseline. In a multiple regression model for each biomarker, we assessed whether levels varied by class of ART (nonnucleoside reverse-transcriptase inhibitors [NNRTIs] with or without a protease inhibitor [PI], PI [no NNRTI], or nucleoside-reverse

transcriptase inhibitors [NRTIs; no PI or NNRTI]) and by type of NRTI used (abacavir, no didanosine [ddI], ddI, other NRTI, and no abacavir or ddI). These analyses were explored because we previously reported that participants receiving abacavir had higher inflammatory marker levels than those using NRTIs other than abacavir or ddI [14]. Comparisons by class of drug and type of NRTI are not protected by randomization. In addition to the ART indicators, the aforementioned baseline covariates for comparisons with the MESA and CARDIA studies were included in the regression models.

Coinfection with hepatitis C virus was common in the SMART study [15]. Thus, separate analyses for SMART study participants who were not coinfecting with hepatitis C virus were also performed. An earlier study that compared hsCRP levels for HIV-infected participants with those for CARDIA participants found that HIV infection without hepatitis C virus coinfection was associated with higher hsCRP levels in men but not in women [16]. Thus, we explored possible interactions with sex.

Statistical analyses were performed using SAS software (version 9.1; SAS Institute). *P* values are 2-sided, and 95% confidence intervals (CIs) are cited.

## RESULTS

HIV-infected participants in the SMART study were more likely to be male and black than were participants of similar age in the CARDIA and MESA studies. HIV-infected participants were also more likely to smoke cigarettes and take lipid and blood pressure-lowering drugs; they had higher total cholesterol/HDL ratios and a lower body mass index than participants in the CARDIA and MESA studies (Table 1).

Fifty-one percent of SMART participants 33–44 years of age and 62% of those 45–76 years of age had HIV RNA levels  $\leq 400$  copies/mL (Table 1). The majority of participants in the SMART study were receiving ART. Of these participants receiving ART, 68% of those 33–44 years of age and 72% of those 45–76 years of age had HIV RNA levels  $\leq 400$  copies/mL. Of those not receiving ART in the younger group, 29% were ART naive, and 32% had not used ART for 6 months. Corresponding percentages for those 45–76 years of age were 20% and 28%, respectively. CD4<sup>+</sup> cell counts averaged  $>600$  cells/mm<sup>3</sup> at study entry.

Unadjusted levels of hsCRP and IL-6 were 42% ( $P = .002$ ) and 59% ( $P < .001$ ) higher in HIV-infected participants than in participants in the CARDIA study (Figure 1A). hsCRP, IL-6, D-dimer, and cystatin C levels were 18% ( $P = .003$ ), 118% ( $P < .001$ ), 52% ( $P < .001$ ), and 25% ( $P < .001$ ) higher for HIV-infected participants in the SMART study than for participants in the MESA study (Figure 1B). With adjustment, the percentage differences increased, and all were significant ( $P < .001$ ) (Table 2 and Figure 1A and 1B).

**Table 1. Characteristics of SMART, CARDIA, and MESA Study Participants**

Characteristic	Participants 33–44 years of age			Participants 45–76 years of age		
	SMART study (n = 287)	CARDIA study (n = 3231)	P <sup>a</sup>	SMART study (n = 494)	MESA study (n = 5386)	P <sup>a</sup>
Age, years	40.0 ± 3.1	39.5 ± 3.3	.04	53.0 ± 6.6	60.3 ± 8.9	<.001
Female sex, % of participants	27.5	55.5	<.001	26.5	53.0	<.001
Race/ethnicity, % of participants			<.001			<.001
Black	55.4	47.6		49.2	31.9	
Latino	...	...		12.3	24.9	
White	44.6	52.4		38.5	43.3	
BMI, kg/m <sup>2</sup>	27.4 ± 5.7	28.7 ± 6.9	.002	27.4 ± 5.7	29.1 ± 5.5	<.001
Ratio of total cholesterol:HDL cholesterol	4.7 ± 2.0	3.9 ± 1.4	<.001	5.1 ± 2.7	4.1 ± 1.3	.004
Total cholesterol level, mg/dL	190.7 ± 41.7	184.2 ± 35.7	.004	197.4 ± 47.4	194.7 ± 36.4	.12
LDL cholesterol level, mg/dL	111.7 ± 32.5	112.6 ± 32.4	.62	111.1 ± 34.7	117.8 ± 31.9	<.001
HDL cholesterol level, mg/dL	45.0 ± 15.9	50.7 ± 14.5	<.001	43.9 ± 15.7	51.0 ± 15.1	<.001
Triglyceride level, mg/dL	179.6 ± 138.4	104.1 ± 86.6	<.001	244.8 ± 229.3	131.1 ± 91.1	<.001
Smoker, % of participants	42.5	22.2	<.001	36.4	15.0	<.001
Diabetes, % of participants	5.6	4.2	.26	13.6	9.7	.46
Receiving BP-lowering drugs,	13.6	7.1	<.001	38.9	32.6	.006
Receiving lipid-lowering drugs, % of participants	11.1	2.1	<.001	24.9	16.0	<.001
Hepatitis C, % of participants	14.3			24.3		
CD4 <sup>+</sup> cell count, cells/mm <sup>3</sup>	623 ± 235			657 ± 260		
HIV RNA level ≤400 copies/mL, % of participants	51.4			62.5		
Receiving ART, % of participants	72.5			82.4		

**NOTE.** Data are the mean value ± standard deviation, unless otherwise indicated. ART, antiretroviral therapy; BMI, body mass index; BP, blood pressure; CARDIA, Coronary Artery Development in Young Adults; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; LDL, low-density lipoprotein; MESA, Multi-Ethnic Study of Atherosclerosis; SMART, Strategies for Management of Anti-Retroviral Therapy.

<sup>a</sup> For the difference.

**Differences according to the use of ART and the HIV RNA level.** With the exception of D-dimer, the biomarkers did not vary for HIV-infected participants, according to use of ART. Among HIV-infected participants 33–44 years of age, D-dimer was 62% (95% CI, 27%–110%) ( $P < .001$ ) higher for participants not receiving ART, compared with those receiving ART. For those 45–76 years of age, D-dimer levels were 63% (95% CI, 31%–101%) ( $P < .001$ ) higher for those not receiving ART than for those receiving ART. For both groups, study participants not receiving ART and those receiving ART, D-dimer levels were significantly higher, compared with levels noted in MESA study participants; these levels were 127% higher for those not receiving ART and 39% higher for those receiving ART ( $P < .001$ , for both).

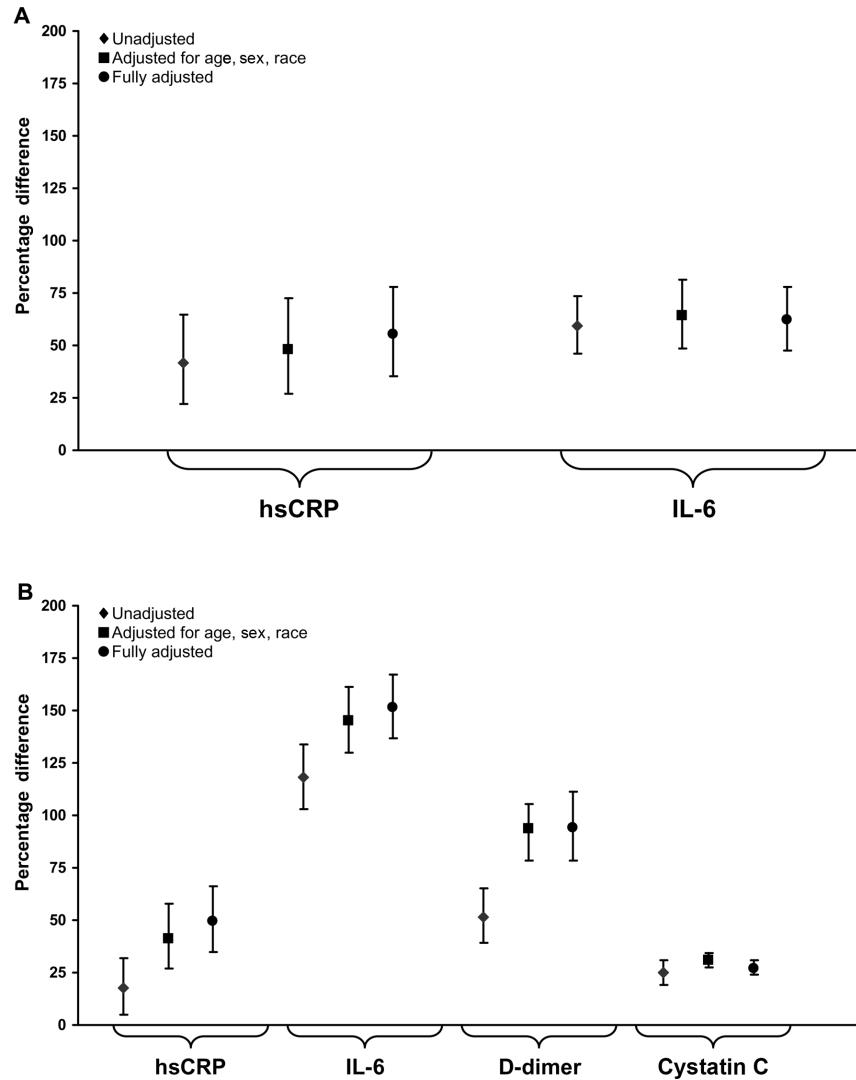
Table 3 presents median biomarker levels for study participants receiving ART who had an HIV RNA level ≤400 copies/mL. These levels were also higher than those in the 2 general population cohorts (Table 2). For HIV-infected participants 33–44 years of age, the adjusted percentage differences in hsCRP and IL-6 levels were 40% and 39% higher than those in the general population ( $P < .001$ , for both comparisons). For those 45–76 years of age, hsCRP, IL-6, D-dimer, and cystatin C levels

were 38%, 60%, 49%, and 21% higher, respectively, than in the general population ( $P < .001$ , for all comparisons).

SMART study participants were further subdivided according to use of ART and the HIV RNA level (see Table A1 in the appendix, which appears only in the electronic version of the *Journal*).  $P$  values corresponding to adjusted differences between those receiving ART who had an HIV RNA level ≤400 copies/mL and those receiving ART who had an HIV RNA level >400 copies/mL for log<sub>e</sub>-transformed levels of hsCRP, IL-6, D-dimer, and cystatin-C are  $P = .27$ ,  $P = .63$ ,  $P = .93$ , and  $P = .27$ , respectively.

We also explored the correlation of log<sub>10</sub> HIV RNA levels and log<sub>e</sub> biomarker levels for those receiving ART who had an HIV RNA level >400 copies/mL. A significant positive correlation was found between the HIV RNA level and the D-dimer level, but not the other markers. The correlation coefficients ( $P$  values) were  $-0.03$  ( $P = .68$ ),  $0.10$  ( $P = .18$ ),  $0.25$  ( $P < .001$ ), and  $-0.01$  ( $P = .96$ ), for hsCRP, IL-6, D-dimer, and cystatin C, respectively.

**Differences according to type and duration of ART.** Some differences were noted in biomarker levels according to type of ART received in SMART participants. In all cases, however,



**Figure 1.** A, Percentage difference in the levels of high-sensitivity C-reactive protein (hsCRP) and interleukin (IL)-6 in human immunodeficiency virus (HIV)-infected study participants 33–44 years of age vs. the general population. B, Percentage difference in the levels of hsCRP, IL-6, D-dimer, and cystatin C in HIV-infected study participants 45–76 years of age vs. the general population.

median levels were higher for SMART participants than for participants in the MESA and CARDIA studies. The median levels of hsCRP were greater for those receiving an NNRTI (2.81  $\mu\text{g/mL}$ ) versus those receiving a PI (2.14  $\mu\text{g/mL}$ ). In a regression model that adjusted for baseline covariates, hsCRP levels were 48% higher for those receiving an NNRTI (with or without a PI) versus a PI alone ( $P < .001$ ). Levels of IL-6 ( $P = .46$ ), D-dimer ( $P = .12$ ), and cystatin C ( $P = .13$ ) did not differ significantly between these classes of drugs. Median levels of hsCRP and IL-6 were higher for those receiving abacavir (3.07  $\mu\text{g/mL}$  and 2.70 pg/mL, respectively) versus those receiving other NRTIs other than ddI (2.39  $\mu\text{g/mL}$  and 2.36 pg/mL, respectively). After adjustment, the hsCRP level was 28% higher for those receiving abacavir, compared with those

receiving other NRTIs other than ddI ( $P = .05$ ), and the IL-6 level was 19% higher ( $P = .03$ ). Also, although median levels of D-dimer were similar for those taking abacavir and other NRTIs other than ddI (0.27  $\mu\text{g/mL}$  and 0.27  $\mu\text{g/mL}$ , respectively), the adjusted percentage difference was 21% ( $P = .054$ ). Cystatin C levels were similar for those receiving abacavir and other NRTIs (1% difference,  $P = .81$ ).

We also examined whether biomarker levels varied according to the total number of years of ART use, the number of years of PI use, and the number of years of NNRTI use, and these associations were not significant (data not shown).

**Differences for smokers and nonsmokers.** Because of the large differences in smoking status between HIV-infected participants and participants in the CARDIA and MESA studies,

**Table 2. Median Levels and Interquartile Ranges (IQRs) of Biomarkers for SMART, CARDIA, and MESA Study Participants**

Biomarker	Participants 33–44 years of age			Participants 45–76 years of age		
	SMART (n = 287) <sup>a</sup>	CARDIA (n = 3231) <sup>b</sup>	% Diff. <sup>c</sup> (P)	SMART (n = 494) <sup>d</sup>	MESA (n = 5386)	% Diff. <sup>c</sup> (P)
hsCRP, $\mu\text{g/mL}$	2.12 (0.80–4.77)	1.36 (0.53–3.78)	55.2 (<.001)	2.68 (1.07–6.01)	2.17 (0.94–4.70)	49.6 (<.001)
IL-6, pg/mL	2.09 (1.26–3.49)	1.29 (0.80–2.07)	62.1 (<.001)	2.63 (1.63–4.18)	1.23 (0.79–1.92)	151.6 (<.001)
D-dimer, $\mu\text{g/mL}$	0.29 (0.17–0.55)	NA (NA)	NA	0.34 (0.20–0.61)	0.20 (0.13–0.35)	94.3 (<.001)
Cystatin C, mg/dL	0.94 (0.82–1.07)	NA (NA)	NA	1.00 (0.88–1.17)	0.85 (0.76–0.96)	27.2 (<.001)

**NOTE.** CARDIA, Coronary Artery Development in Young Adults; Diff., difference; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; MESA, Multi-Ethnic Study of Atherosclerosis; NA, not available; SMART, Strategies for Management of Anti-Retroviral Therapy.

<sup>a</sup> A total of 172 participants had cystatin C levels measured.

<sup>b</sup> A total of 689 participants had IL-6 levels measured.

<sup>c</sup> The percentage difference in the means of the log-transformed value, adjusted for age, race, sex, body mass index, smoking, ratio of total cholesterol: high-density lipoprotein cholesterol, diabetes, lipid-lowering therapy, and blood pressure-lowering therapy.

<sup>d</sup> A total of 231 participants had cystatin C levels measured.

separate analyses were conducted for nonsmokers. Adjusted hsCRP and IL-6 levels were 50% (95% CI, 26%–79%) and 63% (95% CI, 46%–83%) higher in nonsmoking HIV-infected participants than in nonsmoking participants in the CARDIA study. For nonsmokers in the SMART study, compared with nonsmokers in the MESA study, levels of hsCRP, IL-6, D-dimer, and cystatin C were 60% (95% CI, 41%–81%), 164% (95% CI, 146%–184%), 88% (95% CI, 70%–108%), and 29% (95% CI, 25%–34%) higher, respectively.

#### Differences according to sex and hepatitis C virus infection.

In all 3 studies, hsCRP levels were higher in women than men. In the SMART study, median (IQR) levels were 3.81  $\mu\text{g/mL}$  (1.23–7.50  $\mu\text{g/mL}$ ) for women and 2.08  $\mu\text{g/mL}$  (0.92–4.66  $\mu\text{g/mL}$ ) for men. There was evidence of an interaction between sex and HIV for hsCRP ( $P < .001$ , for comparison with the CARDIA study;  $P = .01$ , for comparison with the MESA study) but not for the other biomarkers. Both men and women with HIV infection had higher adjusted levels of hsCRP than participants of the same sex in the CARDIA and MESA studies, but relative differences were larger for men (77% [ $P < .001$ ] higher than in the CARDIA study and 60% [ $P < .001$ ] higher

than in the MESA study) than for women (9% [ $P = .48$ ] higher than in the CARDIA study and 26% [ $P = .02$ ] higher than in the MESA study).

Differences in hsCRP levels between HIV-infected study participants and those in the CARDIA and MESA study cohorts were greater when SMART study participants with hepatitis C virus coinfection were excluded (22% of men and 18% of women in the SMART study) (information to make a similar exclusion was not available for the CARDIA and MESA studies, but the prevalence of hepatitis C virus infection was assumed to be low). hsCRP levels were 97% higher ( $P < .001$ ) for HIV-infected men in both age groups (the SMART study vs. the CARDIA study and the SMART study vs. the MESA study). The corresponding percentage differences for women were 25% ( $P = .11$ ) (the SMART study vs. the CARDIA study) and 62% ( $P < .001$ ) (the SMART study vs. the MESA study). For both age groups, the interaction with sex persisted ( $P = .001$ , for comparison with the CARDIA study;  $P = .05$ , for comparison with the MESA study) after the exclusion of participants in the SMART study who had hepatitis C virus infection.

**Table 3. Biomarkers Levels in SMART Study Participants Receiving Antiretroviral Therapy (ART) Who Had an HIV RNA Level  $\leq 400$  Copies/mL and Percentage Differences in Levels Versus CARDIA and MESA Study Participants, as Cited in Table 2**

Biomarker	Participants 33–44 years of age			Participants 45–76 years of age		
	No.	Median level (IQR)	% Diff. (P)	No.	Median level (IQR)	% Diff. (P)
hsCRP, $\mu\text{g/mL}$	140	2.13 (0.77–5.20)	40.2 (<.001)	293	2.83 (1.07–6.80)	37.8 (<.001)
IL-6, pg/mL	139	1.89 (1.15–3.42)	39.0 (<.001)	291	2.64 (1.55–4.14)	60.1 (<.001)
D-dimer, $\mu\text{g/mL}$	140	0.21 (0.15–0.46)	NA	293	0.29 (0.17–0.57)	49.1 (<.001)
Cystatin C, mg/dL	86	0.90 (0.78–0.97)	NA	130	1.00 (0.86–1.16)	20.9 (<.001)

**NOTE.** Data are the median level and (interquartile range [IQR]). CARDIA, Coronary Artery Development in Young Adults; Diff., difference; MESA, Multi-Ethnic Study of Atherosclerosis; NA, not available; SMART, Strategies for Management of Anti-Retroviral Therapy.

## DISCUSSION

We compared the levels of 4 biomarkers between HIV-infected study participants and participants in 2 large population-based studies. Each of the biomarkers has been associated with cardiovascular disease in the general population [17–24], and 3 of the biomarkers (hsCRP, IL-6, and D-dimer) have recently been associated with all-cause mortality in the SMART study [3]. Compared with participants in the general population cohorts, CARDIA and MESA HIV-infected individuals in the SMART study had higher levels of inflammatory markers, as measured by hsCRP and IL-6; coagulation and fibrinolysis activity, as measured by D-dimer; and impaired renal function, as measured by cystatin C. Higher levels of these markers were evident even among HIV-infected study participants who were receiving ART and had HIV RNA levels  $\leq 400$  copies/mL.

The elevation of these biomarker levels among HIV-infected persons receiving effective ART as well as among those not receiving ART may reflect ongoing immune activation even with successful suppression of HIV replication [25]. Given the magnitude of the elevations and their association with cardiovascular disease, renal disease, and all-cause mortality in the general population, as well as with all-cause mortality in the SMART study, treatments that target inflammatory and coagulation pathways and decrease hsCRP, IL-6, and D-dimer levels may warrant investigation among patients with HIV infection [26, 27]. Among SMART study participants, some differences were noted in biomarker levels according to NNRTI and PI use and according to NRTI type. For the latter, we found higher levels of hsCRP and IL-6 among participants taking abacavir, compared with those taking other NRTIs other than ddI. This finding is consistent with a previous report on the SMART study cohort [14]. Differences among ART drugs are best explored in randomized studies, and these findings indicate that these markers should be considered in future trials of ART regimens.

Only a few studies have compared these biomarkers between participants with HIV infection and uninfected controls. hsCRP has been the focus of several reports [16, 28, 29]. In the largest study, the Fat Redistribution and Metabolic Change in HIV Infection (FRAM) study, hsCRP levels were elevated in HIV-infected men but not women, compared with HIV-uninfected participants in the CARDIA study, and the differences between HIV-infected and HIV-negative participants varied according to coinfection with hepatitis C virus [16]. Like the FRAM study, our study found that HIV infection was associated with greater differences in hsCRP levels for men, compared with women. The reasons for this finding are unclear. We also found that differences in hsCRP levels between those with HIV infection and those in the general population were smaller for those with hepatitis C virus coinfection, compared with those without hepatitis C virus coinfection. Reasons for differences in hsCRP levels according to hepatitis C status are likely multifactorial,

although hsCRP is primarily synthesized by hepatocytes, and differences in hepatic function among patients coinfecting with HIV and hepatitis C virus, compared with patients with hepatitis C virus mono-infection, may be a contributing factor to the hsCRP difference between coinfecting and mono-infected participants [30].

In the FRAM study, cystatin C levels were also compared with those in participants in the CARDIA study [8]. Levels were higher in HIV-infected participants, compared with participants in the CARDIA study, after adjustment for demographic and clinical factors. Cystatin C may reflect, in part, ongoing inflammation as well as loss of kidney function. We recently showed that cystatin C levels increased rapidly after ART interruption and that cystatin C levels were correlated with inflammatory and coagulation markers [7].

The strengths of this investigation include the use of a single laboratory for the measurement of biomarkers in the SMART, CARDIA, and MESA studies. The age range and sex-ethnic distribution of SMART permitted comparisons across a broad spectrum of people with HIV infection. A weakness is that the SMART study did not exclude participants with more advanced HIV infection and included few ART-naïve participants. Furthermore, there were many differences between the participants in the SMART study and those in the CARDIA and MESA studies. Although many factors were considered in the multivariate analyses and they did not have a substantial effect on the results, and although several subgroup analyses were considered, it is possible that there are other differences that explain our findings. Finally, because these were cross-sectional comparisons, the temporal relationship between the biomarkers and some of the predictors considered is uncertain. In summary, we found that markers of inflammation, coagulation, and renal function were elevated in HIV-infected study participants receiving or not receiving ART, compared with participants in 2 large population-based studies. Additional research on the reasons for these elevations and the interventions required to lower them is needed.

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