MAJOR ARTICLE

Factors Associated With Plasma IL-6 Levels During HIV Infection

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Background. Elevated interleukin 6 (IL-6) levels have been linked to cardiovascular disease, cancer and death. Persons with human immunodeficiency virus (HIV) infection receiving treatment have higher IL-6 levels, but few data are available on factors associated with circulating IL-6.

Methods. Participants in 3 trials with IL-6 measured at baseline were included (N = 9864). Factors associated with IL-6 were identified by linear regression. Demographic and HIV variables (nadir/entry $CD4^+$ cell count, HIV RNA level, antiretroviral therapy regimen) were investigated in all 3 trials. In the SMART (Strategies for Management of Anti-Retroviral Therapy) trial, CD4/CD8 ratio, smoking, comorbid conditions, serum lipids, renal function (estimated glomerular filtration rate [eGFR]), and educational level were assessed.

Results. Demographics associated with higher IL-6 levels were older age and lower education, whereas black race was associated with lower IL-6. Higher HIV RNA levels were associated with higher IL-6 levels, and higher nadir $CD4^+$ cell counts with lower IL-6 levels. Compared with efavirenz, protease inhibitors were associated with higher and nevirapine with lower IL-6 levels. Smoking and all comorbid conditions were related to higher IL-6. IL-6 levels increased with decreasing eGFR and decreasing serum lipids.

Conclusions. Higher levels of IL-6 were associated with older age, nonblack race, higher body mass index, lower serum lipid levels, HIV replication, low nadir $CD4^+$ cell count, protease inhibitor use, comorbid conditions, and decreased eGFR. Multiple factors affect inflammation in HIV and should be considered in studies of IL-6 as a biomarker of clinical outcomes.

Keywords. HIV; IL-6; inflammation; cancer; cardiovascular disease.

Interleukin 6 (IL-6) is a proinflammatory cytokine that regulates various physiological processes [1, 2]. It plays a key role in the acute phase response [1] and in the transition from acute to chronic inflammation [3]. Evidence has accrued to suggest that dysregulation of IL-6 production is a major contributor to the pathogenesis of chronic inflammatory and autoimmune diseases [2, 4].

Human immunodeficiency virus (HIV) infection has long been shown to induce expression and secretion of IL-6 [5] by monocytes and macrophages [6]. Even when virologically suppressed, treated HIV-infected persons have significantly higher plasma levels of IL-6 than well-matched uninfected controls [7, 8]. Activated inflammation, as demonstrated by persistently higher IL-6 levels, may have profound and far-reaching clinical implications. Recent reports involving both HIV-infected and HIV-uninfected persons have linked increased

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plasma IL-6 levels to a variety of adverse clinical outcomes, including anemia [9, 10], cancer [11, 12], cardiovascular disease [13–15], and death [16, 17].

The reasons HIV infection is associated with a chronic inflammatory state are not entirely understood. In a random sample of 499 individuals participating in the Strategies for Management of Anti-Retroviral Therapy (SMART) trial [17], older age and higher body mass index (BMI) were independently associated with elevated IL-6 levels. Among SMART participants coinfected with hepatitis C virus (HCV), IL-6 levels were particularly higher in those with preexisting hepatic fibrosis, as defined by abnormally elevated levels of the liver fibrosis marker hyaluronic acid [18]. Identifying additional factors associated with higher IL-6 levels could elucidate the mechanisms driving inflammation during HIV infection. The main purpose of the present study was to identify factors independently associated with plasma levels of IL-6 among a large number of HIV-infected individuals participating in 3 international HIV trials.

MATERIAL AND METHODS

This is a cross-sectional study involving HIV-infected persons participating in 3 randomized controlled trials conducted by the International Network for Strategic Initiatives in Global HIV Trials: the SMART (NCT00027352) [19], ESPRIT (Evaluation of Subcutaneous Proleukin in a Randomized International Trial; NCT00004978), and SILCAAT (Subcutaneous Recombinant, Human Interleukin-2 in HIV-Infected Patients With Low $CD4^+$ Counts Under Active Antiretroviral Therapy; NCT00013611) [20] trials, which have been described in detail elsewhere. The SMART, ESPRIT, and SILCAAT studies, including the consent for stored specimens, were approved by the institutional review board or ethics committee of each clinical site and the University of Minnesota. Written informed consent was obtained from all participants involved in the 3 trials.

Biomarker Measurements

Biomarkers were measured at baseline, before randomization, in all participants who consented to storing blood samples for future research (N = 9864). For SMART participants, measurements were performed at the Laboratory for Clinical Biochemistry Research at the University of Vermont in Burlington. In ESPRIT and SILCAAT participants, laboratory measurements were performed by SAIC-Frederick. IL-6 was measured with the same method at both laboratories (Chemiluminescent Sandwich ELISA; R&D Systems).

In the SMART trial, D-dimer levels were measured using immunoturbidometric methods with the Sta-R analyzer (Liatest D-DI; Diagnostic Stago) and high-sensitivity C-reactive protein (hsCRP) was measured with an NBTMII nephelometer, N Antiserum to Human CRP (Siemens Diagnostics). In the ESPRIT and SILCAAT trials, D-dimer and hsCRP were both measured using an enzyme-linked fluorescent assay (VIDAS instrument [bioMerieux] for D-dimer and ELISA system [R&D Systems] for hsCRP). As explained in detail elsewhere [15, 21], the assays used to measure D-dimer and hsCRP, while different, compared well in duplicates. The median plasma levels measured at each laboratory were comparable and well correlated [15].

SMART and ESPRIT participants were screened for coinfection with hepatitis B virus (HBV) or HCV, as described elsewhere [22, 23]. Briefly, baseline plasma samples obtained from individuals with antibody tests positive for HBV and HCV were analyzed for levels of HCV RNA and HBV DNA using branched DNA assays (Versant HCV RNA 3.0 and Versant HBV DNA 3.0, respectively; Bayer Diagnostics). Participants with positive HBV/HCV antibody and viral load test results were considered to have hepatitis virus coinfection. Baseline levels of hyaluronic acid, a validated marker of hepatic fibrosis, were measured in coinfected patients with an enzyme-linked binding protein assay (Corgenix) [23]. The estimated glomerular filtration rate (eGFR) was calculated using the chronic kidney disease-epidemiology collaboration equation [24] in ESPRIT and SMART participants. Leevels of triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured in the SMART trial by Quest Diagnostics, using standard enzymatic methods.

Statistical Analyses

Factors independently associated with IL-6 levels were identified by multivariable linear regression models. Because the IL-6 distribution was skewed to the right, $\log_2$-transformed IL-6 levels were used as the dependent variable. Back-transformed exponentiated estimates (ie, corresponding to fold differences in IL-6 levels per unit or category difference in the covariates) with 95% confidence intervals (CIs) were calculated to assess the contribution of baseline variables to the variance of IL-6. To account for interlaboratory variability and inherent differences among participants in each trial, all models were adjusted for study type. Goodness of fit was assessed using the adjusted R^2 coefficient, and multicollinearity was assessed using variance inflation factors.

The variables investigated were chosen on the basis of their epidemiological importance and biological plausibility. Because the variables of interest were not routinely collected in all 3 trials, models were fitted to 3 data sets: (1) SMART, ESPRIT, and SILCAAT participants (N = 9864): age, sex, ethnicity, BMI, $CD4^+$ cell counts (nadir and baseline), markers of inflammation (hsCRP) and activated coagulation (D-dimer), antiretroviral therapy (ART) use, and ART regimens; (2) SMART and ESPRIT participants (n = 6938): comorbid conditions (HBV or HCV infection, diabetes mellitus, cardiovascular disease) and renal function (eGFR); and (3) SMART participants only (n = 4498): smoking, level of education (less than high school, high school, and bachelor's degree or above), triglyceride levels, and cholesterol levels (LDL-C and HDL-C). Among

the SMART participants with $CD8^+$ T-cell counts available (n = 2399), we also investigated the association between the CD4/CD8 ratio and IL-6 levels.

Because the 3 trials involved participants with different baseline characteristics, we investigated interactions between studies (SMART, ESPRIT, and SILCAAT) and demographic characteristics (sex, race, and age). We also investigated interactions between age and HIV RNA, between age and biomarkers (IL-6, hsCRP, and D-dimer), and between sex and BMI.

Additional preplanned analyses were performed, stratified by sex, HIV RNA (<500, 501–30 000; 30 001–100 000, or >100 000 copies/mL), and current ART use (yes or no) and regimen. These analyses were done to compare factors associated with IL-6 levels in men and women, in virologically suppressed and unsuppressed participants, and in those treated with different ART regimens. ART regimens were categorized into nonnucleoside reverse-transcriptase inhibitor (NNRTI)– and protease inhibitor (PI)–based regimens. PI-based regimens were further split into ritonavir-boosted and unboosted regimens. The relationship between IL-6 levels and specific NNRTI and PI drugs (ritonavir boosted or unboosted) was also analyzed. In these analyses, 2 covariates were fitted to model a participant's regimen: (1) the nucleoside/nucleotide reverse-transcriptase inhibitor (NRTI) backbone drugs and (2) the third drug (ie, NNRTI or PI) in the regimen. Only participants receiving NNRTI- or PI-based ART regimens (but not both drug classes together) were included in these analyses of specific drugs (n = 7291). Because of collinearity between ART use and HIV RNA, these variables could not be entered into the same models.

Although the impact of liver fibrosis on circulating IL-6 had been characterized in SMART participants with HCV coinfection [18], the relative contribution of replication of hepatitis viruses to variance in IL-6 levels had not been previously investigated among HBV- and HCV-coinfected persons, to our knowledge. We thus further adjusted models restricted to HBV- and HCV-coinfected persons for hyaluronic acid (measured at baseline in 245 study participants coinfected with HBV and 860 coinfected with HCV), HBV DNA, and HCV RNA levels.

RESULTS

Baseline characteristics of study participants are summarized separately for each of the 3 data sets included in the analyses and are presented in Table 1. There were marginally significant differences in terms of sex distribution across the 3 studies ($P = .06$ for interaction between study and sex), although it is unlikely that these differences were large enough to constitute clinical significance. There were significant interactions between study and race ($P < .001$) and between study and age ($P < .001$). The addition of an increasing number of covariates in multiple regression models on different data sets did not substantially change the power to estimate the variance of IL-6 levels (adjusted R^2 values ranged from 0.23 to 0.27).

Demographics

The demographic factors found to be independently correlated with higher IL-6 levels were older age, higher BMI, and lower educational level (Figure 1). Overall, similar results were seen consistently across multiple models using different data sets and after adjustment for an increasing range of covariates. Black race, on the other hand, was found to be associated with higher IL-6 levels only in univariable analysis. In adjusted models, black race became associated with lower IL-6 levels. Men and women had similar IL-6 levels.

In analyses stratified by sex, higher BMI was associated with higher IL-6 levels in women but not in men (P for interaction, <.001).

HIV-Specific Variables

HIV replication was associated with significantly increased IL-6 levels. There was apparently a dose-response effect with increasing fold differences of IL-6 with higher HIV RNA plasma levels (Figure 2). No significant associations were seen between baseline $CD4^+$ cell counts and IL-6 levels. We did find, however, a negative and independent correlation between nadir $CD4^+$ cell counts and IL-6 levels: higher nadir $CD4^+$ cell counts were associated with lower IL-6 levels. There was no evidence of an interaction between age and plasma HIV RNA levels ($P = .89$). Higher CD4/CD8 ratios ere associated with lower IL-6 levels in univariable analyses only, but there was no significant association after adjustment (Figure 2).

ART Regimens

Among participants receiving ART, PI-based regimens, ritonavir boosted or unboosted, were independently related to higher IL-6 levels when compared with NNRTI-based regimens (Figure 3). This was a drug class effect, and all individual PI drugs were associated with higher IL-6 levels when compared with efavirenz, although this association was not significant for atazanavir. Nevirapine, on the other hand, was associated with significantly lower IL-6 levels. Drugs composing the NRTI backbone were not associated with IL-6 levels. Participants not receiving ART at baseline had significantly increased IL-6 levels.

Biomarkers of Inflammation and Activated Coagulation

Levels of hsCRP and D-dimer were independently and positively correlated with IL-6 levels. In multivariable models involving participants from all 3 trials, higher levels of hsCRP (adjusted fold difference [95% CI], 1.16 [1.11–1.13] per 1 $\log_2$ μg/mL; $P < .001$) and D-dimer (1.12 [1.11–1.13] per 1 $\log_2$ μg/mL; $P < .001$) were associated with higher IL-6 levels. There was no evidence of an interaction between the effects of age and these biomarkers on IL-6 ($P = .69$ for hsCRP and $P = .89$ for D-dimer).

Table 1. Baseline Characteristics by Data Set in ESPRIT, SILCAAT, and SMART Trial Participants

Characteristic	SMART, ESPRIT, and SILCAAT (N = 9864)	SMART and ESPRIT (n = 6938)	SMART (n = 4498)
IL-6, median (IQR), pg/mL	1.80 (1.20–2.89)	1.81 (1.17–2.90)	1.72 (1.07–2.93)
Demographics			
Age, median (IQR), y	42 (36–49)	42 (36–49)	44 (38–50)
Female sex, No. (%)	2150 (21.8)	1609 (23.2)	1147 (25.5)
Black race, No. (%)	1913 (19.4)	1478 (21.3)	1250 (27.8)
BMI, median (IQR), kg/m^2	24.34 (22.12–27.00)	24.45 (22.15–27.30)	24.99 (22.50–28.09)
Educational level, No. (%)[a]			
Less than high school	NA	NA	1138 (25.3)
High school or some college/university	NA	NA	2550 (56.7)
Bachelor's degree or above	NA	NA	810 (18.0)
HIV-specific variables			
Baseline CD4$^+$ cell count, median (IQR), cells/mm^3	490 (368–671)	540 (422–722)	601 (470–799)
Nadir CD4$^+$ cell count, median (IQR), cells/mm^3	200 (84–316)	229 (121–335)	250 (154–358)
Plasma HIV RNA ≤500 copies/mL, No. (%)	7536 (76.4)	5293 (76.3)	3297 (73.3)
CD4/CD8 ratio, median (IQR)[b]	NA	NA	0.69 (0.48–0.98)
ART regimen, No. %			
None	824 (8.4)	701 (10.1)	683 (15.2)
Other	1749 (17.7)	1200 (17.3)	683 (15.2)
PI/r based	1261 (12.8)	1020 (14.7)	841 (18.7)
PI based[c]	2430 (24.6)	1311 (18.9)	589 (13.1)
Amprenavir-fosamprenavir	137 (1.9)	91 (1.8)	60 (1.9)
Atazanavir	267 (3.7)	244 (4.9)	240 (7.7)
Indinavir	920 (12.6)	485 (9.6)	170 (5.4)
Lopinavir	721 (9.9)	574 (11.4)	436 (13.9)
Nelfinavir	1006 (13.8)	571 (11.3)	354 (11.3)
Saquinavir	352 (4.8)	188 (3.7)	103 (3.3)
Other/multiple PIs	288 (4.0)	179 (3.6)	65 (2.1)
NNRTI based	3600 (36.5)	2699 (38.9)	1704 (37.9)
Nevirapine	1505 (20.6)	1116 (22.2)	688 (22.0)
Efavirenz	2082 (28.6)	1578 (31.4)	1011 (32.3)
Other NNRTI	13 (0.2)	9 (0.2)	5 (0.2)
NRTI backbone[c]			
Zidovudine + TTC	2841 (39.0)	1958 (38.9)	1203 (38.4)
Abacavir + TTC	414 (5.7)	292 (5.8)	202 (6.5)
Tenofovir + TTC	471 (6.5)	437 (8.7)	414 (13.2)
Didanosine + TTC	240 (3.3)	171 (3.4)	114 (3.6)
Stavudine + TTC	1667 (22.9)	1037 (20.6)	496 (15.8)
Zidovudine + lamivudine + abacavir	181 (2.5)	167 (3.3)	147 (4.7)
Other NRTI drugs	1451 (19.9)	955 (19.0)	543 (17.3)
No NRTI	26 (0.4)	18 (0.4)	13 (0.4)
Biomarker of inflammation or activated coagulation, median (IQR), μg/mL[d]			
hsCRP	1.59 (0.70–3.67)	1.61 (0.71–3.76)	1.70 (0.71–4.07)
D-dimer	0.24 (0.15–0.38)	0.22 (0.15–0.37)	0.20 (0.13–0.36)
Comorbid conditions, No. (%)			
Cardiovascular disease[e]	NA	187 (2.7)	162 (3.6)
Diabetes mellitus[e]	NA	368 (5.3)	301 (6.7)
Hepatitis B[e]	NA	257 (3.7)	99 (2.2)
Hyaluronic acid, median (IQR), ng/mL[f]	NA	23.80 (14.63–43.69)	18.15 (12.33–32.18)
HBV DNA, median (IQR), IU/mL[f]	NA	71 704 (2000–100 000 000)	5251 (357–119 226 071)

Table 1 continued.

Characteristic	SMART, ESPRIT, and SILCAAT (N = 9864)	SMART and ESPRIT (n = 6938)	SMART (n = 4498)
Hepatitis C[e]	NA	999 (14.4)	603 (13.4)
Hyaluronic acid, median (IQR), ng/mL[g]	NA	33.17 (18.75–59.82)	32.33 (19.95–59.82)
HCV RNA, median (IQR), IU/mL[g]	NA	2 576 804 (583 936–7 610 964)	579 088 (217 236–1 538 882)
Smoking[e]	NA	NA	1822 (40.5)
Renal function, median (IQR)			
eGFR, mL/min per 1.73 m^2[e]	NA	111.56 (100.66–121.03)	110.82 (100.25–120.56)
Cystatin C, mg/dL[a]	NA	NA	0.81(0.71–0.92)
Serum lipids, median (IQR), mg/dL[a]			
Cholesterol			
Total cholesterol	NA	NA	192 (164–222)
LDL-C	NA	NA	112 (90–137)
HDL-C	NA	NA	40 (33–51)
Triglycerides	NA	NA	166 (107–265)

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; eGFR, estimated glomerular filtration rate; ESPRIT, Evaluation of Subcutaneous Proleukin in a Randomized International Trial; HBV, hepatitis B virus; HCV, hepatitis C virus; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; NA, not available; NNRTI, nonnucleoside reverse-transcriptase inhibitors; NRTI, nucleoside/nucleotide reverse-transcriptase inhibitor; PI, protease inhibitor; PI/r, ritonavir-boosted PI; SILCAAT, Subcutaneous Recombinant, Human Interleukin-2 in HIV-Infected Patients With Low $CD4^+$ Counts Under Active Antiretroviral Therapy; SMART, Strategies for Management of Anti-Retroviral Therapy; TTC, lamivudine or emtricitabine.

[a] Not ascertained for SILCAAT or ESPRIT participants.

[b] SMART participants with CD8 data (n = 2339).

[c] Including only those receiving a nucleoside/nucleotide backbone plus a third drug (PI or NNRTI). Percentages for the drug in question are for this subset of participants in each data set.

[d] hsCRP is the biomarker of inflammation, and D-dimer the biomarker of activated coagulation.

[e] Not ascertained for SILCAAT participants.

[f] Data available for 245 participants.

[g] Data available for 860 participants.

Comorbid Conditions and Smoking

Smoking and all the comorbid conditions investigated, namely, prior cardiovascular disease, diabetes mellitus, and HBV and HCV infection, were found to be associated with higher IL-6 levels in univariable and adjusted analyses (Figure 4). Comorbid conditions were associated with larger fold differences in IL-6 levels among virologically suppressed persons (HIV RNA, ≤500 copies/mL) than among those with HIV RNA levels >500 copies/mL (data not shown).

Among HCV-coinfected participants, the degree of liver damage, as demonstrated by higher hyaluronic acid levels (fold difference [95% CI], 1.14 [1.11–1.18] per 1 $\log_2$ ng/mL; $P < .001$), but not HCV RNA plasma levels (1.00 [0.99–1.01] per 1 $\log_2$ IU/mL; $P = .56$), was found to be positively correlated with IL-6 levels. Among participants with HBV coinfection, on the other hand, neither hyaluronic acid levels (0.99 [0.97–1.01] per 1 $\log_2$ ng/mL; $P = .45$), nor HBV DNA (1.01 [1.00–1.02] per 1 $\log_2$ IU/mL; $P = .18$) were associated with lower IL-6 levels.

Renal Function and Serum Lipids

In adjusted analyses, higher eGFR values at baseline were found to be significantly associated with lower IL-6 levels (0.98 [0.97–1.00] per 10 mL/min per 1.73 m^2; $P = .01$). We also observed a negative correlation between serum cholesterol levels and IL-6 levels: both higher LDL-C levels (0.99 [0.99–0.99] per 10 mg/dL, $P < .001$) and higher HDL-C levels (0.98 [0.96–0.99] per 10 mg/dL; $P < .001$) were associated with lower IL-6 levels. We found no association between triglyceride levels (1.01 [1.00–1.02] per 100 mg/dL; $P = .24$) and IL-6 levels in multivariable analyses.

DISCUSSION

In this study of almost 10 000 HIV-infected persons from across the globe, we identified demographic, viroimmunological, and clinical factors associated with plasma levels of IL-6. The factors associated with plasma IL-6 levels had been partially characterized in a small subset of randomly selected SMART participants [17] and among those with HCV coinfection [18], but there was still a need for systematic studies with adequate information on potential confounders involving large numbers of HIV-infected persons. We found that demographic factors, HIV RNA plasma levels, markers of inflammation and coagulation, smoking, comorbid conditions, renal function, and serum lipid levels were independently associated with IL-6 levels. A possible effect of

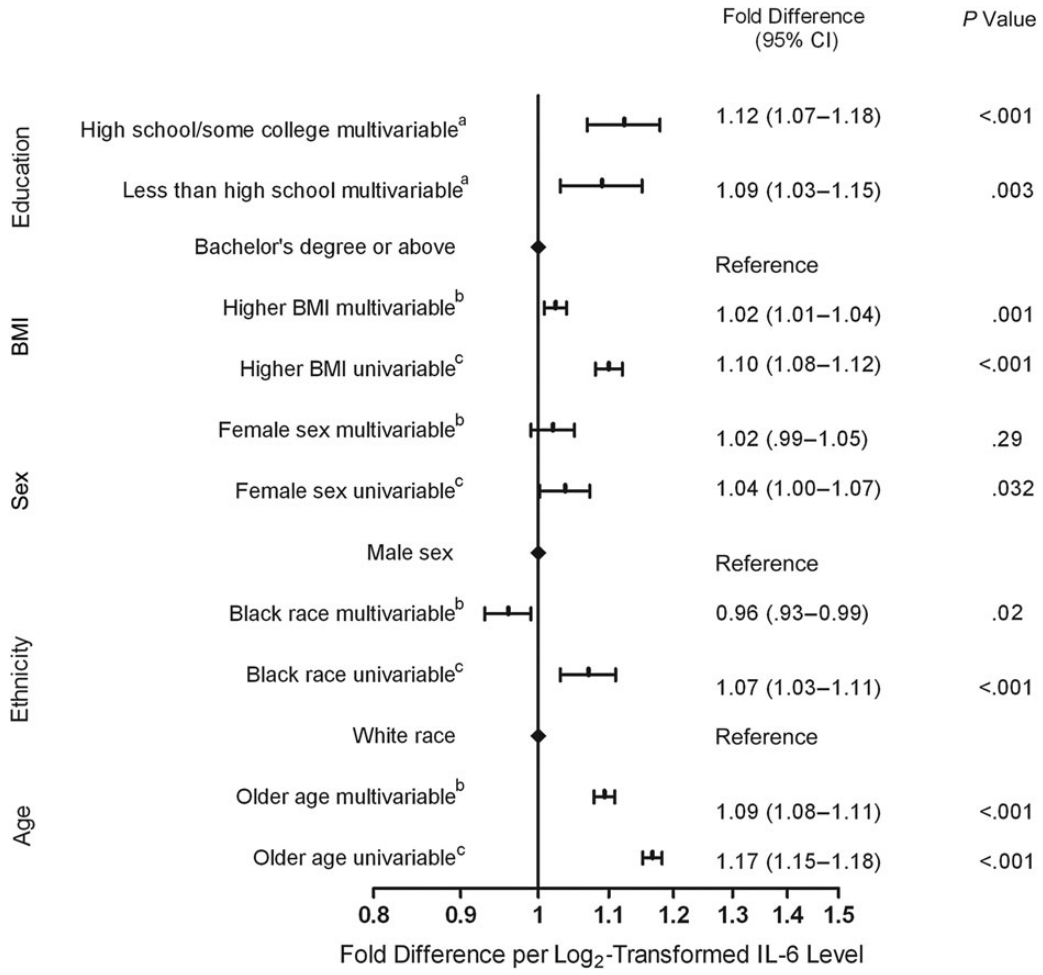

Figure 1. Fold differences in interleukin 6 (IL-6) levels associated with demographic factors. Fold differences associated with older age are expressed per 10 years older and fold differences associated with higher body mass index (BMI) are expressed per 5 kg/m^2 higher. [a]SMART (Strategies for Management of Anti-Retroviral Therapy) participants only (n = 4498): adjusted as described in footnote *b* and also for comorbid conditions (hepatitis B and hepatitis C virus infection, diabetes mellitus, cardiovascular disease), renal function (estimated glomerular filtration rate), smoking status, educational level (less than high school, high school/some college, or bachelor's degree or above), and cholesterol levels (low- and high-density lipoprotein cholesterol); [b]SMART, ESPRIT (Evaluation of Subcutaneous Proleukin in a Randomized International Trial), and SILCAAT (Subcutaneous Recombinant, Human Interleukin-2 in HIV-Infected Patients With Low CD4$^+$ Counts Under Active Antiretroviral Therapy) participants (N = 9864): models adjusted for age, sex, ethnicity, BMI, CD4$^+$ cell counts (nadir and baseline), markers of inflammation (high-sensitivity C-reactive protein) and activated coagulation (D-dimer), and antiretroviral therapy (ART) use and regimens; [c]SMART, ESPRIT, and SILCAAT participants (N = 9864): univariable analysis. Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus.

ART regimen on plasma IL-6 levels was also observed. Taken together, these findings suggest that the inflammatory state seen during HIV infection has several possible causes. This enhanced inflammation, as demonstrated by elevated IL-6 plasma levels, is probably determined by a synergistically deleterious interaction of HIV-related and HIV-unrelated morbidity.

Congruent with previous reports [8, 17, 25–27], we found that older age is associated with higher IL-6 levels, consistent with the view that the aging process is tightly integrated with inflammation [28]. The lower IL-6 levels that we observed among black participants may reflect, in part, the fact that circulating IL-6 levels are genetically determined [13, 29], although interactions between race and other contributing factors we were unable to capture and hence adjust for may play a role as well. The association we observed between lower education levels and increased IL-6 levels has not been reported before. Lower education levels may be associated with increased inflammation through indirect mechanisms involving poorer socioeconomic status and health status. Similarly to studies involving both HIV-infected persons [17, 30] and the general population [25, 31], we found BMI to be positively correlated with IL-6. Indeed, the subcutaneous adipose tissue can produce, in some instances, up to 25% of circulating IL-6 [32, 33]. The effect of BMI on IL-6 was significantly influenced by sex, which can be

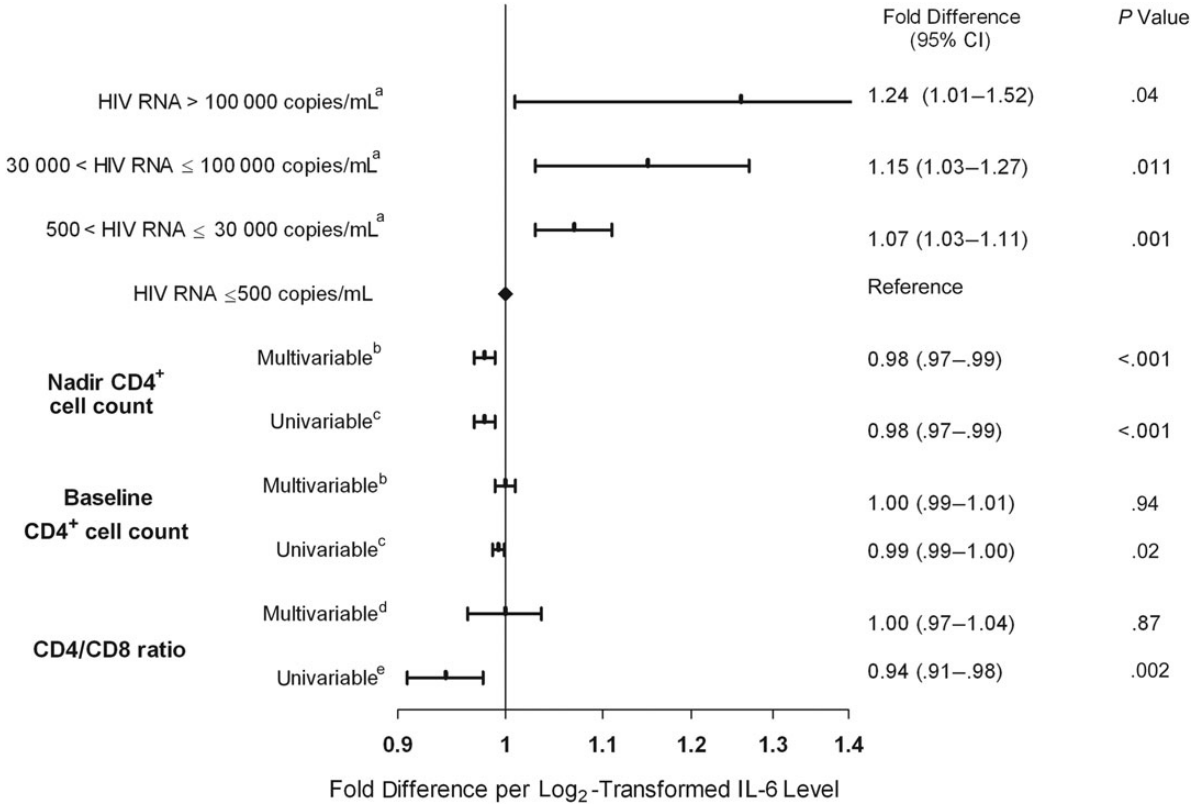

Figure 2. Fold differences in interleukin 6 (IL-6) levels associated with human immunodeficiency virus (HIV)–specific variables. Fold differences associated with the CD4/CD8 ratio are expressed per 1 $\log_2$ higher ratio; fold differences associated with higher baseline and nadir $CD4^+$ cell counts are expressed per 100 cells/mm^3 higher. [a]SMART (Strategies for Management of Anti-Retroviral Therapy), ESPRIT (Evaluation of Subcutaneous Proleukin in a Randomized International Trial), and SILCAAT (Subcutaneous Recombinant, Human Interleukin-2 in HIV-Infected Patients With Low $CD4^+$ Counts Under Active Antiretroviral Therapy) participants receiving antiretroviral therapy (ART) at baseline (n = 9027): models adjusted for age, sex, ethnicity, body mass index (BMI), $CD4^+$ cell counts (nadir and baseline), and markers of inflammation (high-sensitivity C-reactive protein [hsCRP]) and activated coagulation (D-dimer). Because of collinearity between ART use and HIV RNA, these variables could not be entered into the same models; [b]SMART, ESPRIT, and SILCAAT participants (N = 9864): models adjusted for age, sex, ethnicity, BMI, $CD4^+$ cell counts (nadir and baseline), markers of inflammation (hsCRP) and activated coagulation (D-dimer), and ART use and regimens; [c]SMART, ESPRIT, and SILCAAT participants (N = 9864): univariable; [d]SMART participants with CD8 data (n = 2339): adjusted for age, sex, ethnicity, BMI, $CD4^+$ cell counts (nadir and baseline), markers of inflammation (hsCRP) and activated coagulation (D-dimer), ART use and regimens, comorbid conditions (hepatitis B and hepatitis C virus infection, diabetes mellitus, cardiovascular disease), renal function (estimated glomerular filtration rate), smoking, and cholesterol levels (low- and high-density lipoprotein cholesterol); [e]SMART participants with CD8 data (n = 2339): univariable. Abbreviation: CI, confidence interval.

attributable to sexual dimorphism in body fat distribution [34] and percentage [35] that is not captured by BMI.

Among the HIV-specific variables that we investigated, we found that lower nadir $CD4^+$ cell count and HIV RNA plasma levels, but not baseline $CD4^+$ cell count or CD4/CD8 ratio, were related to higher IL-6 levels. This suggests that $CD4^+$ cell depletion before ART initiation and ongoing HIV replication are more important drivers of inflammation than immunodeficiency. Other studies, however, have observed increased IL-6 levels in those with lower $CD4^+$ cell counts [8, 26]. Inherent differences in patient cohorts and differences in variables adjusted for may explain these discrepant findings. Our study, however, is the largest investigation into factors associated with IL-6 published so far, and we had extensive information on confounding factors.

The lack of association between CD4/CD8 ratio and IL-6 levels suggests that this ratio is a poor surrogate for ongoing inflammation among the HIV-infected persons with relatively high $CD4^+$ cells enrolled in SMART. This is in agreement with the report by Serrano-Villar and colleagues [36], in which no correlation between CD4/CD8 ratio and IL-6 was observed among individuals with $CD4^+$ cell counts $\geq 500/mm^3$.

Among participants receiving ART, we found that PI-based regimens were independently associated with higher IL-6 levels, whereas nevirapine use was associated with lower IL-6 levels, compared with efavirenz. These findings raise the possibility that

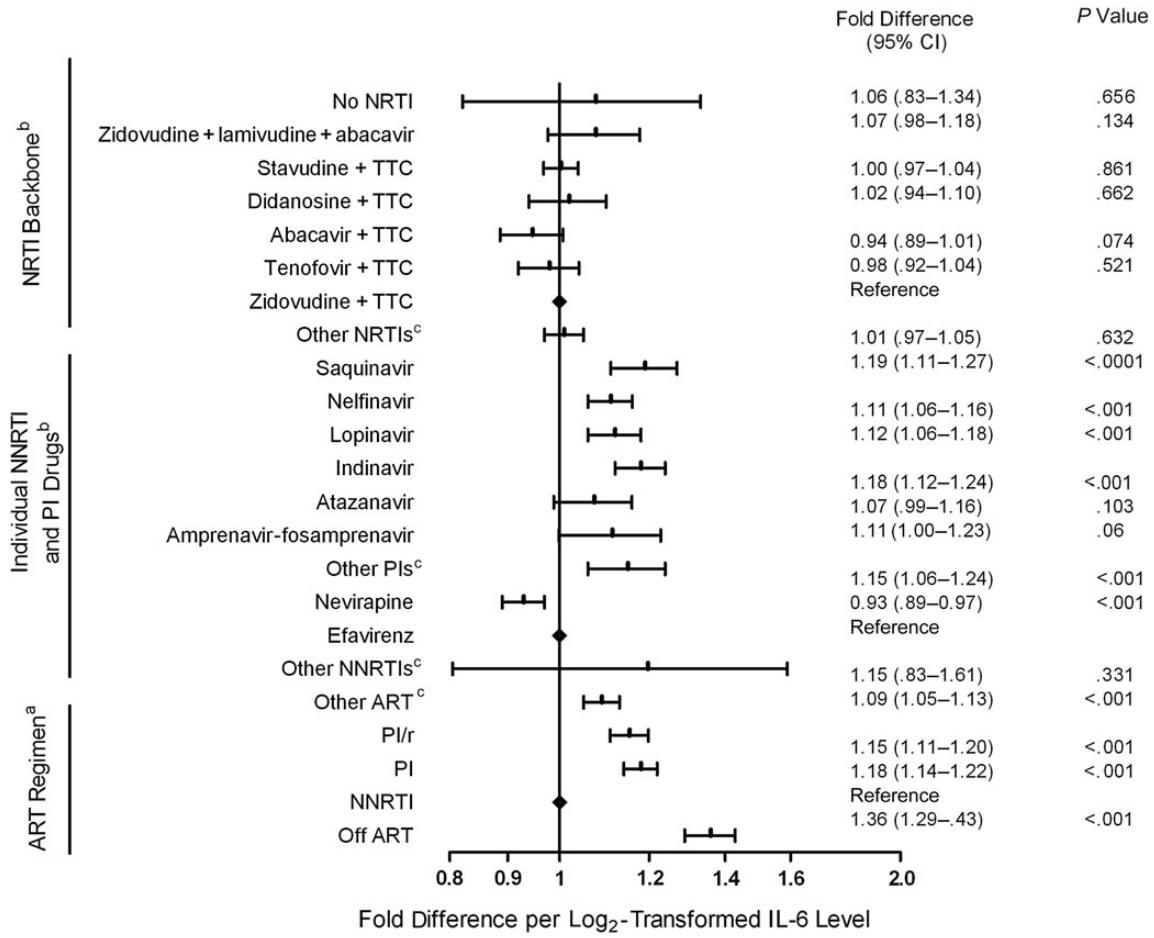

Figure 3. Fold differences in interleukin 6 (IL-6) levels associated with antiretroviral therapy (ART) and regimens and individual antiretroviral drugs. [a]SMART (Strategies for Management of Anti-Retroviral Therapy), ESPRIT (Evaluation of Subcutaneous Proleukin in a Randomized International Trial), and SILCAAT (Subcutaneous Recombinant, Human Interleukin-2 in HIV-Infected Patients With Low CD4$^+$ Counts Under Active Antiretroviral Therapy) participants (N = 9864): models adjusted for age, sex, ethnicity, body mass index (BMI), CD4$^+$ cell counts (nadir and baseline), markers of inflammation (high-sensitivity C-reactive protein) and activated coagulation (D-dimer); ART regimens were categorized as off ART, protease inhibitor (PI) based, ritonavir-boosted PI (PI/r) based, nonnucleoside reverse-transcriptase inhibitor (NNRTI) based, or other; [b]Adjusted as described in footnote *a* but including only participants receiving an NNRTI- or PI-based ART regimen (but not both) (SMART, ESPRIT, and SILCAAT participants; n = 7291); ART regimens were categorized as off ART, PI based (boosted and unboosted), NNRTI based, or other; [c]Other nucleoside/nucleotide reverse-transcriptase inhibitors (NRTIs) include adefovir (used in some hepatitis B virus–coinfected participants), zalcitabine, any of the drugs in our chosen NRTI pairs without lamivudine or emtricitabine, or multiple NRTIs, including a pair of NRTIs that are not among our chosen NRTI pairs. Other PIs include tipranavir, darunavir, and multiple PI drugs; other NNRTIs, delavirdine; other ART, ART regimens including NRTI drugs only. Abbreviations: CI, confidence interval; TTC, lamivudine or emtricitabine.

specific ART regimens can influence inflammation, which may have important clinical implications. Although levels of inflammatory biomarkers were similar between persons initiating NNRTI- and PI-based ART in small subsets of randomized trials [37], switching away from PI-based regimens led to significant reductions in plasma levels of IL-6 [38]. Nevirapine-based regimens, on the other hand, were associated with significantly lower IL-6 levels in this study. NNRTI drugs, particularly nevirapine, have better penetration into body compartments than PIs [39], and the use of nevirapine is associated with lower plasma HIV RNA levels than the use of efavirenz or lopinavir-ritonavir in patients with HIV RNA levels <50 copies/mL when an assay with a lower limit of detection is used [40]. Our findings, however, should be interpreted with caution. Because participants were not randomized to PI- or NNRTI-based regimes, we cannot rule out the possibility that the choice of a PI regimen could be a marker for certain participant characteristics, such as increased visceral adiposity [41], that may be associated with higher IL-6 levels. Future studies should address the question as to whether some ART regimens may suppress HIV-associated inflammation more than others.

In accordance with other studies, we found that smoking [8, 25, 34, 42], diabetes mellitus [43, 44], prior cardiovascular disease [13, 45], and hepatitis virus coinfection [26] were associated

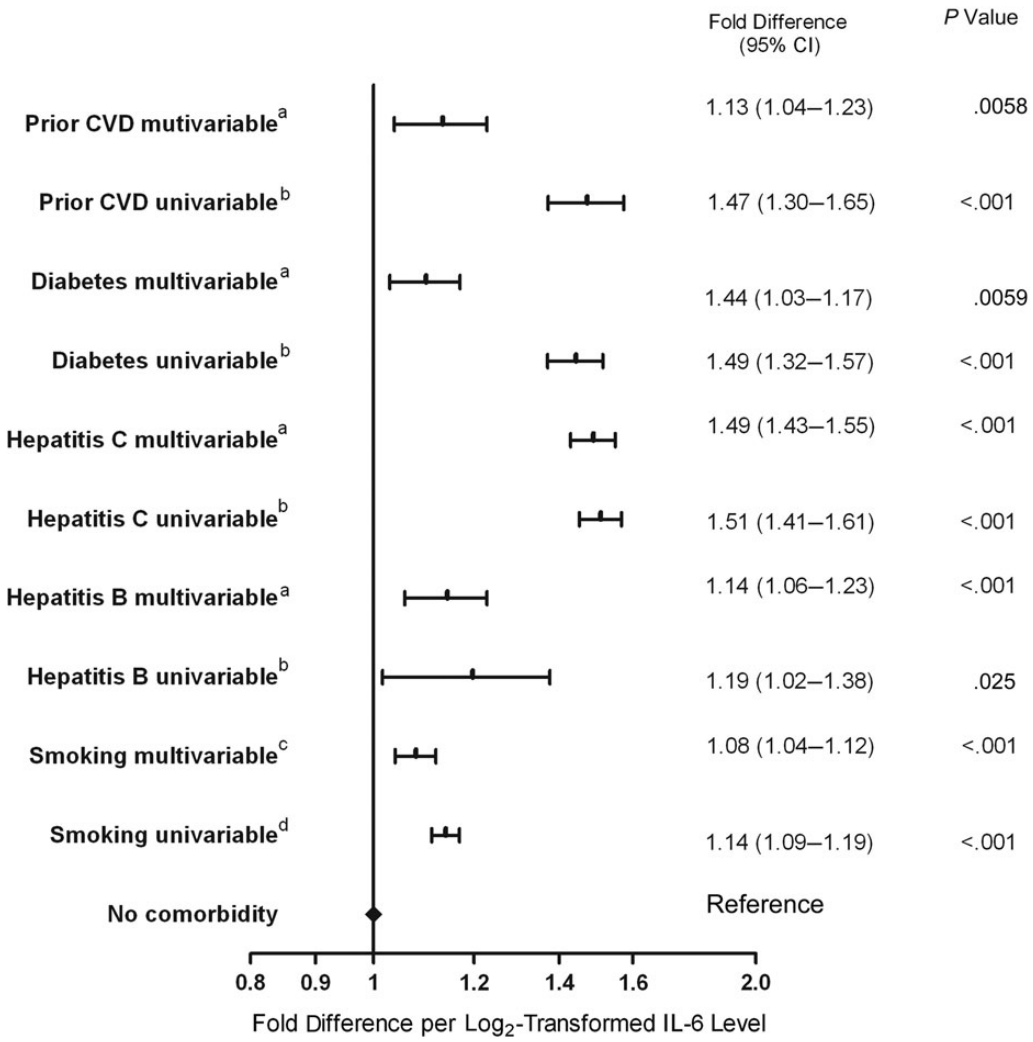

Figure 4. Fold differences in interleukin 6 (IL-6) levels associated with comorbid conditions and smoking. [a]SMART (Strategies for Management of Anti-Retroviral Therapy) and ESPRIT (Evaluation of Subcutaneous Proleukin in a Randomized International Trial) participants (n = 6938): models adjusted for age, sex, ethnicity, body mass index (BMI), $CD4^+$ cell counts (nadir and baseline), markers of inflammation (high-sensitivity C-reactive protein [hsCRP]) and activated coagulation (D-dimer), antiretroviral therapy (ART) use and regimens, comorbid conditions (hepatitis B virus [HBV] and hepatitis C virus [HCV] infection, diabetes mellitus, cardiovascular disease), and renal function (estimated glomerular filtration rate [eGFR]); [b]SMART and ESPRIT participants (n = 6938): univariable; [c]SMART participants (n = 4498): models adjusted for age, sex, ethnicity, BMI, $CD4^+$ cell counts (nadir and baseline), markers of inflammation (hsCRP) and activated coagulation (D-dimer), ART use and regimens, comorbid conditions (HBV and HCV infection, diabetes mellitus, cardiovascular disease), renal function (eGFR), smoking, educational level (less than high school, high school/some college and bachelor's degree or above), and cholesterol levels (low- and high-density lipoprotein cholesterol); [d]SMART participants (n = 4498): univariable. Abbreviation: CI, confidence interval.

with increased IL-6. At least for cardiovascular disease, mendelian randomization studies suggest that the link may be causal [13]. Therefore, HIV-unrelated morbidity may contribute to enhanced inflammation during HIV infection. This effect was stronger among virologically suppressed individuals, which indicates that once HIV replication is suppressed by ART, the relative association of non-HIV comorbid conditions with inflammation becomes more prominent. Among participants with HCV coinfection, we observed that hepatic fibrosis, as demonstrated by elevated hyaluronic levels but not HCV RNA plasma levels, was independently associated with higher IL-6 levels. As others have already pointed out [18], this suggests that the enhanced proinflammatory state associated with HCV coinfection is mainly mediated by liver fibrosis. Hyaluronic acid, however, is not a perfect marker of hepatic fibrosis, and information on other measures of liver fibrosis found to be associated with IL-6 by other investigators (eg, fibrosis 4 score [46]) would have been helpful.

There was a negative correlation between eGFR and IL-6, indicating that poorer renal function is associated with increased inflammation, which may just reflect a proinflammatory state inherently associated with renal dysfunction [47]. We also

observed a negative correlation between IL-6 and serum lipid levels. IL-6 infusions have been shown to reduce cholesterol levels [48], and a polymorphism of the IL-6 gene associated with higher IL-6 circulating levels and lower serum lipids has been described [29].

Our study had several limitations. First, its cross-sectional design and the lack of HIV-uninfected controls hampered our ability to study the temporal direction of associations and to infer causality. Second, although the assays used to measure biomarkers compared well and the main associations that we observed held within large sub–data sets using the same assay, there may have been residual confounding due to different assays used. Third, serum levels of inflammatory markers may not accurately reflect end-organ inflammation and damage. Fourth, only a single biomarker was measured; however, other studies of SMART participants have shown that no other cytokine or chemokine predicts mortality better than IL-6 [49]. Fifth, we did not have information on hormonal replacement therapy, physical exercise, and alcohol use, which were shown to be significantly associated with IL-6 levels in HIV-uninfected women [25]. Finally, structural equation modeling (SEM) can test models with multiple dependent variables using several regression equations simultaneously [50] and is a more robust technique than multivariable linear regression, which we used in this study. Through causal modeling or path-analysis methods, SEM can deal with nonnormally distributed and autocorrelated data and would have allowed us to make more causal inferences from our data set. A reanalysis of factors associated with IL-6 levels among HIV-infected persons using SEM is therefore warranted.

To conclude, higher IL-6 levels in HIV-infected persons were associated with older age, nonblack race, higher BMI, lower serum lipid levels, ongoing HIV replication, low nadir $CD4^+$ cell counts, comorbid conditions, and decreased renal function. Furthermore, PI-based ART regimens were associated with higher IL-6 levels, whereas nevirapine-based regimens were associated with lower IL-6 levels. These findings suggest that there are multiple determinants of inflammation and/or IL-6 production during HIV infection. These determinants should be considered when investigating IL-6 as a biomarker of clinical outcomes in HIV-infected persons.

Notes

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Author contributions. A. H. B., J. L. O., A. N. P., and J. D. L. conceived the study; J. L. O. and A. N. P. performed all statistical analyses; and A. H. B. drafted the manuscript. All authors contributed to data interpretation, critically revised the manuscript, and approved the final version.

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Potential conflicts of interest. All authors: No potential conflicts of interest.

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