Abnormal Levels of Some Biomarkers of Immune Activation Despite Very Early Treatment of Human Immunodeficiency Virus

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Background: Despite early antiretroviral therapy (ART), ART-suppressed people with human immunodeficiency virus (HIV) (PWH) remain at higher risk for infections and infection-related cancers than the general population. The immunologic pathways that remain abnormal in this setting, potentially contributing to these complications, are unclear.

Methods: ART-suppressed PWH and HIV-negative controls, all cytomegalovirus seropositive and enriched for HIV risk factors, were sampled from an influenza vaccine responsiveness study. PWH were stratified by timing of ART initiation (within 6 months of infection [early ART] vs later) and nadir CD4+ T-cell count among later initiators. Between-group differences in kynurenine-tryptophan (KT) ratio, interferon-inducible protein 10, soluble CD14 and CD163, soluble tumor necrosis factor receptor 2, interleukin 6, and soluble urokinase plasminogen activator receptor were assessed after confounder adjustment.

Results: Most participants (92%) were male, reflecting the demographics of early-ART initiators in San Francisco. Most biomarkers were higher among later-ART initiators. Participants in the early-ART group achieved near-normal soluble tumor necrosis factor receptor 2, interleukin 6, and soluble urokinase plasminogen activator receptor levels, but substantially higher KT ratio than those without HIV after confounder adjustment ($P = .008$). Soluble CD14, soluble CD163, and interferon-inducible protein 10 trended similarly.

Conclusions: While early-ART initiators restore near-normal levels of many inflammatory markers, the kynurenine pathway of tryptophan catabolism remains abnormally high. Because this pathway confers adaptive immune defects and predicts tuberculosis and cancer progression, this it may contribute to persistent risks of these complications in this setting.

Keywords. immune activation; inflammation; kynurenine; tryptophan; indoleamine 2,3-dioxygenase-1; HIV; antiretroviral therapy; sCD14; IP-10; sCD163.

Antiretroviral therapy (ART) strongly reduces morbidity and mortality rates in people with human immunodeficiency virus (HIV) (PWH) regardless of CD4+ T-cell count [1, 2]. Nevertheless, ART-suppressed PWH—even those with high nadir CD4+ T-cell counts (ie, >500/µL)—remain at higher risk for several morbid conditions compared with the general population [1–4]. While health-related behaviors no doubt contribute to some of this increased morbidity risk, systemic inflammation persists despite ART and strongly predicts subsequent morbidity and mortality risks. Levels of multiple biomarkers of innate and adaptive immune activation remain abnormally elevated despite suppressive ART and also strongly predict morbidity and mortality risks [5–13].

While this elevated inflammatory state predicts a host of morbid outcomes, not all of these pathways and morbidity risks remain abnormally elevated among PWH who start ART at very early disease stages [14]. Indeed, while individuals starting ART at higher CD4+ T-cell counts remain at higher risk than the general population for tuberculosis and infection-related cancers, there is very little evidence for an increased risk of cardiovascular disease, pulmonary disease, and neurocognitive dysfunction in this setting [1–4, 15–18]. Similarly, levels of some inflammatory biomarkers, but not all of them, remain abnormal in individuals who start ART early [19, 20]. For example, among Thai PWH who initiated extremely early ART within the first 2 weeks of their infection, soluble CD14 (sCD14) levels remained abnormal compared with a control group without HIV, while other markers like interleukin 6 (IL-6) appeared to normalize [19].

It remained unclear whether the apparently persistent sCD14 elevations may have been due to confounding by health-related

behaviors or efavirenz use (which has been associated with higher sCD14 levels in several clinical trials [21–24]), but these data suggested that some immune defects may remain abnormal despite ART, while others may normalize. Collectively, these observations raise the possibility that some of the root drivers of the inflammatory state (eg, HIV reservoirs, microbial translocation, and coinfections such as cytomegalovirus [CMV]) may be less active among PWH who initiated ART early, driving a more limited number of inflammatory pathways and disease manifestations [14].

To address these issues, we leveraged a study of influenza vaccine responsiveness to compare several soluble markers of immune activation between ART-suppressed PWH stratified by timing of ART initiation and CD4^{+} T-cell nadir and people without HIV matched for age, sex, and lifestyle. Sampling was enriched for PWH who initiated ART within the first 6 months of their infection, and all participants were required to be CMV seropositive, given the influence of this copathogen on several immune activation pathways. We also made sure to assess the kynurenine pathway of tryptophan catabolism, because it confers adaptive immune defects, predicts mortality risk, and has yet to be assessed in a cohort of durably suppressed PWH who started ART at early disease stages.

METHODS

Study Design and Participants
This prospective cohort study included all patients in an ongoing study assessing influenza vaccine responsiveness from 1 June 2014 through 31 December 2018. Enrollment of PWH was facilitated by the University of California, San Francisco, SCOPE cohort and Options project, described elsewhere [25]. Age- and sex-matched HIV-uninfected participants enriched for HIV risk factors were also enrolled.

Inclusion criteria for those with or without HIV included age between 40–65 years and CMV seropositivity. PWH were also required to have plasma HIV RNA levels <40 copies/mL for the past 12 months. Detectable "blips" <500 copies were allowed if flanked by undetectable values. Exclusion criteria included active viral hepatitis, injection drug use within the past 12 months, treatment with potent anti-inflammatory drugs within the past 4 months, acute or chronic infection requiring antibiotics or any hospitalization within the past 4 months.

PWH were stratified by timing of ART initiation. Early ART was considered within 6 months of HIV infection (using a published algorithm for estimating the date of initial HIV infection [26]) and later-ART initiators were further stratified by nadir CD4^{+} T-cell count (>350/μL, 200–350/μL, or <200/μL).

Biomarker Measurements
We selected biomarkers for analysis that have been shown to be persistently elevated in PWH receiving suppressive ART that predict morbidity and mortality risks and span overlapping but discrete inflammatory pathways. The kynurenine-tryptophan (KT) ratio reflects systemic activity of the kynurenine pathway of tryptophan catabolism, which confers adaptive immune defects and predicts incident tuberculosis and mortality risk in treated HIV infection [8–10, 27–29]. Interferon-inducible protein 10 (IP-10 or CXCL10) is an interferon response marker that predicts morbidity and mortality risks in treated HIV infection [10, 20]. Soluble tumor necrosis factor receptor 2 (sTNFR2) is one of the strongest predictors of cardiovascular events in treated HIV and predicts morbidity and mortality risk [10]. IL-6 is an inflammatory cytokine that strongly predicts subsequent morbidity and mortality risks [5, 8, 10]. sCD14 is associated with both microbial translocation and monocyte/macrophage activation and also predicts increased morbidity and mortality risks in treated HIV [8–10, 12]. Soluble urokinase plasminogen activator receptor (suPAR) and soluble CD163 (sCD163) are also associated with monocyte/macrophage activation and predict mortality risk and cardiovascular events [13, 30–32].

Plasma was cryopreserved at −80ºC. Previously unthawed aliquots were thawed for subaliquoting and refrozen just once before thawing for each assay. KT ratios were assessed on cryopreserved plasma obtained in the fasting state via a high-performance liquid chromatography tandem mass spectrometry assay [33]. The remaining biomarkers were measured with enzyme-linked immunosorbent assay (ELISA) using commercial immunoassay kits in duplicate: Quantikine ELISA sTNFR2, sCD14, sCD163, IP-10, suPAR and Quantikine HS ELISA IL-6, all from R&D Systems. The median coefficients of variation for each biomarker assessed in duplicate (ie, from the same plasma aliquot) were <5%. For all analytes except KT ratio, 2 plasma specimens from each participant were assessed approximately 1 month apart and before (or ≥1 month after) vaccination to establish a more stable within-participant average. Median within-participant standard deviations for each biomarker were as follows: sTNFR2, 0.03 $\log_{10}$ pg/mL; sCD14, 0.02 $\log_{10}$ ng/mL; sCD163, 0.03 $\log_{10}$ ng/mL; IP-10, 0.05 $\log_{10}$ ng/mL; IL-6, 0.1 $\log_{10}$ pg/mL; and suPAR, 0.02 $\log_{10}$ pg/mL.

Statistical Analysis
For participants in the early-ART group, the estimated date of detectable HIV infection was derived by a published algorithm that integrates clinical testing history and quantitative plasma HIV RNA level data, using data available from the time of Options cohort enrollment [26]. Nonparametric tests for trend were used to assess trends across ordered groups (ie, from HIV negative through to the group with nadir CD4^{+} T-cell counts <200/μL). Differences in biomarkers between early-ART and HIV-uninfected groups were assessed with Wilcoxon rank sum tests. Multivariate linear regression analysis was used to assess adjusted differences in immunologic biomarkers among the 5 groups. Biomarkers were $\log_{10}$-transformed and standard errors were calculated with heteroscedasticity-consistent

covariance matrix estimators when necessary to satisfy model assumptions.

Adjusted tests for trend were performed by modeling the ordinal groups from HIV-uninfected participants to those with a CD4$^+$ T-cell nadir <200/µL as a continuous variable (ie, linear increase across ordered groups). Age, natal sex, and men who have sex with men (MSM) status were included as potential confounders in all models. Additional covariates associated with the outcome at $P < .20$ in univariate analyses were considered potential confounders; these covariates included race, ethnicity, history of injection drug use, current smoker, and efavirenz-containing regimen. A covariate was maintained in the multivariate model if it affected the β coefficient of the primary predictor by≥10%. Differences between groups compared with HIV-uninfected participants were also normalized to the interquartile range (IQR) in the HIV-uninfected group (or those with HIV for analyses restricted to ART-suppressed participants) to facilitate comparisons between biomarkers with different dynamic ranges.

All statistical tests were 2-tailed, with an α level of 0.05 considered statistically significant. All statistical analyses were performed with SAS software (version 9.4).

Ethical Considerations

This study received approval of the University of California, San Francisco, Committee on Human Research. All participants provided written informed consent.

RESULTS

Patient Demographics and Characteristics

Of the 205 participants, 41 were HIV negative, and of the 164 PWH, 34 had initiated early ART within the first 6 months of their infection (median, 3 months; IQR, 1–4 months). The remaining PWH who initiated ART during chronic HIV infection were stratified by nadir CD4$^+$ T-cell count: >350/µL (n = 32), 200–350/µL (n = 43), or <200/µL (n = 55). Most participants (92%) were male, with a median age of 54 years (IQR, 49–60 years), 40% were people of color, and 81% were MSM (Table 1). The HIV-uninfected participants were notably enriched for HIV risk factors and health-related behaviors associated with inflammation: 56% were MSM, 41% had >100 lifetime male sexual partners, 15% had ever injected drugs, and 34% were current smokers. Among PWH, the median duration of virologic suppression was 8 years (IQR, 5–11 years), and those with lower nadir CD4$^+$ T-cell counts tended to be ART suppressed for longer periods.

Table 1. Characteristics of Antiretroviral Therapy–Suppressed Participants and Those Without Human Immunodeficiency Virus

Characteristic	HIV-Negative Participants (n = 41), No. (%)[a]	HIV-Positive Participants, No. (%)[a]			
			Later ART by CD4$^+$ T-Cell Nadir		
		Early ART (n = 34)	>350/µL (n = 32)	200–350/µL (n = 43)	<200/µL (n = 55)
Age, median (IQR), y	57 (51–60)	51 (47–53)	51 (46–56)	55 (49–61)	57 (53–62)
Male sex	36 (88)	34 (100)	32 (100)	37 (86)	50 (91)
Race/ethnicity					
White	20 (49)	28 (82)	18 (56)	27 (63)	32 (58)
Black	11 (27)	1 (3)	5 (16)	10 (23)	9 (16)
Latino	1 (2)	1 (3)	4 (13)	4 (9)	6 (11)
Other	9 (22)	4 (12)	5 (16)	2 (5)	8 (15)
MSM	23 (56)	33 (97)	29 (91)	34 (79)	47 (85)
Lifetime male sex partners					
0–10	18 (44)	1 (3)	4 (13)	4 (11)	6 (12)
11–100	6 (15)	6 (18)	6 (19)	9 (24)	17 (33)
>100	17 (41)	26 (79)	22 (68)	25 (66)	28 (55)
IDU ever	6 (15)	3 (9)	2 (6)	7 (17)	19 (35)
Current smoker	13 (34)	2 (6)	2 (6)	6 (14)	12 (22)
EFV regimen	…	4 (12)	3 (9)	9 (27)	17 (52)
Duration of viral suppression, median (IQR), y[b]	…	7 (4–9)	6 (4–7)	8 (5–11)	9 (7–13)
Nadir CD4$^+$ T-cell count, median (IQR), cells/µL	…	361 (277–501)	433 (373–499)	264 (221–309)	79 (28–138)
Current CD4$^+$ T-cell count, median (IQR), cells/µL	760 (644–976)	741 (610–846)	763 (659–956)	632 (553–841)	523 (328–686)

Abbreviations: ART, antiretroviral therapy; EFV, efavirenz; HIV, human immunodeficiency virus; IDU, injection drug use; IQR, interquartile range; MSM, men who have sex with men.

[a]Data represent no. (%) of participants unless otherwise specified.

[b]Viral suppression defined as viral load (plasma HIV RNA level) <40 copies/mL.

Higher Levels of Most Biomarkers Among Later-ART Initiators

As we stratified our sampling of ART-suppressed individuals across a broad range of ART initiation timing (ie, within the first 6 months of infection or later across a range of nadir CD4^{+} T-cell counts), we were able to assess the degree to which timing of ART initiation and CD4^{+} T-cell nadir were associated with each biomarker of interest. Most biomarkers tended to increase with later timing of ART initiation in comparison with HIV-uninfected individuals (Figure 1 and Supplementary Table 1). Notably, there is substantial overlap in most biomarkers across
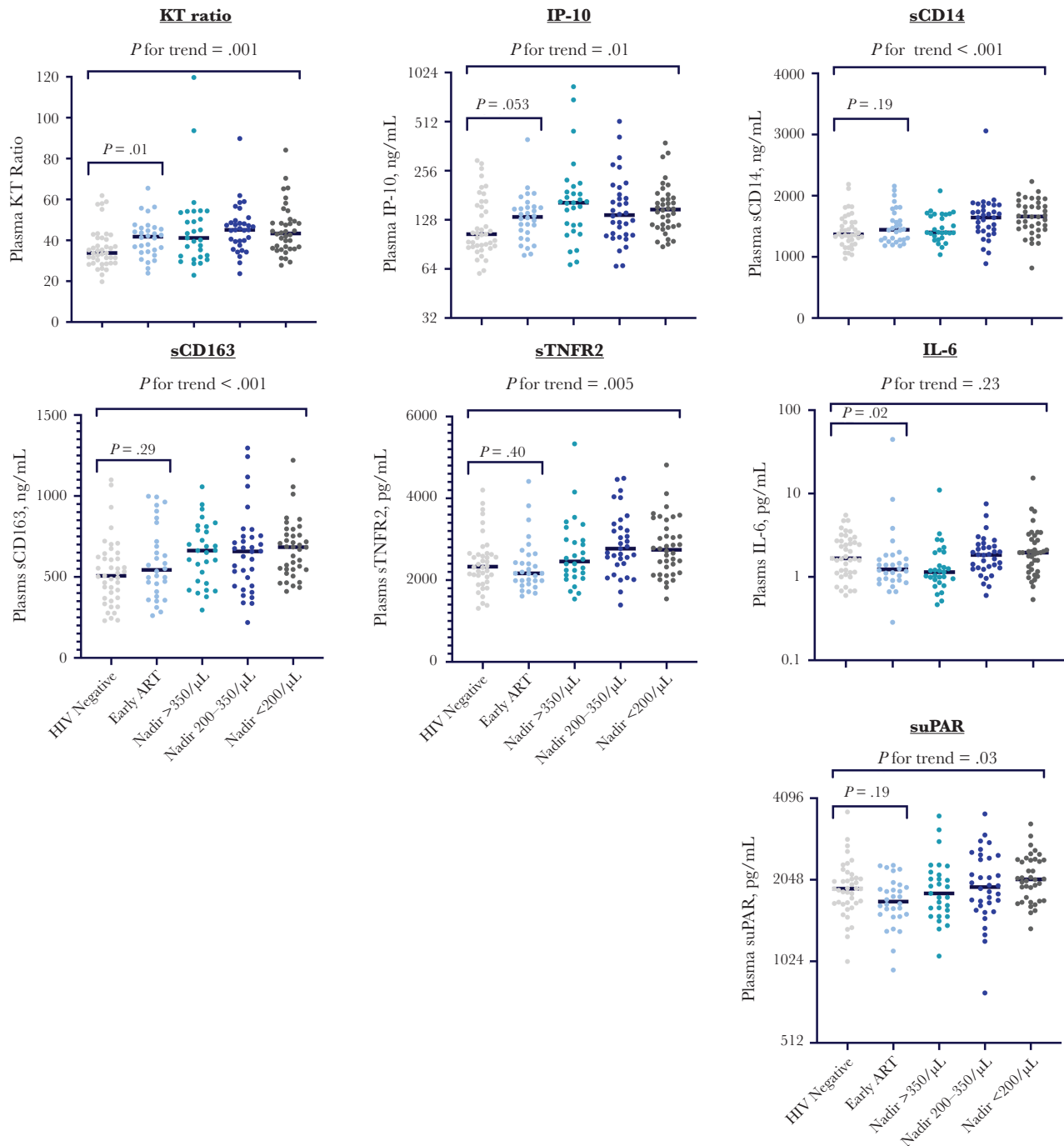

Figure 1. Inflammatory biomarkers by human immunodeficiency virus (HIV) status and timing of prior antiretroviral therapy (ART) initiation. Plasma biomarker levels are compared between HIV-uninfected controls (n = 41) and ART-suppressed people with HIV, stratified by timing of prior ART initiation: early ART (within 6 months of infection; n = 34) or later ART stratified by nadir CD4^{+} T-cell count (>350/μL [n = 32], 200–350/μL [n = 43], or <200/μL [n = 55]). The kynurenine-tryptophan (KT) ratio is the ratio of the kynurenine level (in nanomoles per liter) to the tryptophan level (in micromoles per liter). *P* for trend reflects an unadjusted nonparametric test of trend across all ordered groups from HIV negative to CD4^{+} T-cell nadir <200/μL, and the *P* value between early-ART participants and HIV-uninfected controls reflects a Wilcoxon rank sum test. Abbreviations: IL-6, interleukin 6; IP-10, interferon-inducible protein 10; sCD14, soluble CD14; sCD163, soluble CD163; sTNFR2, soluble tumor necrosis factor receptor 2; suPAR, soluble urokinase plasminogen activation receptor.

the range of ART initiation timing, suggesting that other factors besides the timing of ART contribute to the "inflammatory set point." For each biomarker, we next assessed additional potential confounders in adjusted analyses (Table 2). After adjustment for confounders, the trends for increasing biomarkers with delayed ART initiation remained significant for KT ratio ($P = .03$), sCD163 ($P < .001$), IP-10 ($P = .04$), sTNFR2 ($P = .047$), and suPAR ($P = .03$). While sCD14 and IL-6 demonstrated no evidence for an association with delayed ART initiation when modeling across ordered groups, when restricting to later-ART participants (n = 130) and modeling nadir CD4+ T-cell count as a continuous variable, lower CD4+ T-cell nadir was associated with both higher IL-6 ($\rho = -0.22$; $P = .01$) and sCD14 ($\rho = -0.22$; $P = .01$) levels.

Association of Efavirenz Use with Selective Increase in sCD14 Levels

Given prior randomized controlled trials suggesting that efavirenz-based regimens result in less robust declines in sCD14 or intestinal fatty acid binding protein than comparator regimens [21–24], we assessed whether efavirenz-containing regimens might be associated with the biomarkers in this study. Among all ART-suppressed participants, and after multivariate adjustment, those taking efavirenz-containing regimens (n = 33) had a mean 3.9-fold IQR greater sCD14 level than those taking efavirenz-sparing regimens (95% confidence interval [CI], 2.2–6.8; $P < .001$) (Table 3 and Supplementary Figure 1). There was little evidence for an association between efavirenz use and any of the other measured biomarkers.

Abnormal Levels of Certain Biomarkers in Early-ART Participants Compared with HIV-Uninfected Controls

We next assessed the degree to which biomarker levels remained abnormal among PWH who initiated ART in the first 6 months of their infection compared with participants without HIV. After adjustment for age, sex, and other health-related behaviors, KT ratio and sCD14 and sCD163 levels remained significantly higher in early-ART participants than those without HIV (Table 2 and Figure 2). After normalizing to the IQR of each biomarker in those without HIV to facilitate comparisons between biomarkers, early-ART participants had a mean 2.9-fold IQR higher plasma KT ratio (95% CI, 1.3–6.2) than those without HIV after adjustment for age, sex at birth, and MSM status ($P = .008$). Similar trends were observed when excluding early-ART participants who had CD4+ T-cell counts below the group median (ie, <361/µL) during acute/early HIV infection. After adjustment for confounders, sCD163 and sCD14 levels were a mean 2.6-fold and 2.1-fold IQRs higher than HIV-negative controls, but both CIs bordered closely on 1 (1.02–6.9 and 1.05–4.1, respectively). IP-10 levels tended to be higher in early-ART participants than in those without HIV, with a mean 2.2-fold IQRs higher (95% CI, .8–5.7-fold), but this difference did not reach statistical

significance ($P = .11$). There was no evidence for a difference in sTNFR2, IL-6, or suPAR levels between early-ART participants and HIV-uninfected controls after adjustment for confounders ($P > .65$ for all).

DISCUSSION

While it is now widely acknowledged that immune activation persists in many PWH despite ART-mediated viral suppression, few studies to date have carefully discriminated biomarkers that remain abnormally elevated between those who started ART at early versus late disease stages. Using a robust control group of people without HIV who were enriched for HIV risk factors and health-related behaviors associated with the inflammatory state, we were able to address this gap in the literature.

We made several important observations. First, delayed ART initiation (and lower CD4+ T-cell nadir) was associated with greater persistent immune activation for most analytes assessed. Second, we identified a striking association between efavirenz use and sCD14 (but not other biomarkers), which may have confounded earlier studies addressing the degree to which immune activation normalizes sCD14 during very early ART [19]. Finally, while many biomarkers seemed to normalize in PWH who started ART in the first 6 months of their infection, the KT ratio, a biomarker of the immunoregulatory kynurenine pathway of tryptophan catabolism, remained strikingly high. Other associated pathways (sCD163, sCD14, and perhaps IP-10) remained elevated but to a lesser degree. While the clinical consequences of these differences remain unclear, these data may provide clues as to the discrete immunologic pathways that mediate the persistently increased risk of infections and infection-related cancers even among PWH who initiate ART at very early disease stages.

As in many prior studies, we confirmed that later ART initiation is associated with a greater immune activation set point in PWH maintaining ART-mediated viral suppression. Indeed, very early observational studies identified a link between lower nadir CD4+ T-cell counts and T-cell activation during suppressive ART [34]. Many subsequent observational studies confirmed that markers of innate immune activation and inflammation tended to remain more abnormal in those initiating ART at lower nadir CD4+ T-cell counts, and particularly those with poor CD4+ T cell recovery [35, 36]. Most recently, the START trial provided more definitive evidence that PWH with relatively early-stage infection (ie, CD4+ T-cell counts >500/ µL) randomized to immediate ART initiation achieved a lower inflammatory set point (defined by IL-6 and D-dimer levels) during viral suppression than those randomized to delayed ART initiation [37].

Our findings are broadly consistent with this prior literature and highlight how biomarkers spanning several immunologic pathways that predict morbidity and mortality risks remain abnormal in PWH who initiate ART at advanced disease stages.

Table 2. Independent Associations Between Timing of Antiretroviral Therapy (ART) Initiation and Inflammatory Biomarkers During ART-Mediated Viral Suppression

Biomarkers and Covariates	Mean Fold Change per IQR in HIV-Negative Participants (95% CI)	P Value
KT ratio		
HIV negative	Reference	…
Early ART	2.9 (1.3–6.2)	.008
Later ART: CD4+ T-cell nadir		
>350/µL	3.6 (1.7–7.9)	.001
200–350/µL	3.0 (1.5–6.0)	.003
<200/µL	2.4 (1.2–4.7)	.01
Age, per year	1.1 (1.03–1.1)	.002
Female sex	1.6 (.6–4.5)	.38
Male sex, not MSM	Reference	…
Male sex, MSM	1.1 (.5–2.3)	.76
sCD163		
HIV negative	Reference	…
Early ART	2.6 (1.0–6.9)	.046
Later ART: CD4+ T-cell nadir		
>350/µL	5.0 (1.9–12.9)	.001
200–350/µL	3.8 (1.6–8.8)	.003
<200/µL	4.6 (2.0–10.4)	<.001
Age, per year	1.03 (.99–1.1)	.13
Female sex	1.1 (.3–3.9)	.84
Male sex, not MSM	Reference	…
Male sex, MSM	0.5 (.2–1.2)	.14
Current smoking	0.6 (.3–1.3)	.20
IP-10		
HIV negative	Reference	…
Early ART	2.2 (.8–5.7)	.11
Later ART: CD4+ T-cell nadir		
>350/µL	5.1 (2.0–13.2)	<.001
200–350/µL	2.6 (1.1–6.0)	.03
<200/µL	2.6 (1.2–6.0)	.02
Age, per year	1.04 (1.0–1.1)	.05
Female sex	0.6 (.2–2.0)	.39
Male sex, not MSM	Reference	…
Male sex, MSM	0.7 (.3–1.8)	.52
Current smoking	0.5 (.3–1.1)	.09
sCD14		
HIV negative	Reference	…
Early ART	2.1 (1.1–4.06)	.04
Later ART: CD4+ T-cell nadir		
>350/µL	1.7 (.9–3.4)	.11
200–350/µL	2.0 (1.1–3.7)	.03
<200/µL	1.8 (1.0–3.3)	.07
Age, per year	1.05 (1.02–1.1)	<.001
Female sex	10.3 (4.2–25.5)	<.001
Male sex, not MSM	Reference	…
Male sex, MSM	1.8 (.97–3.5)	.06
EFV	3.8 (2.2–6.5)	<.001
sTNFR2		
HIV negative	Reference	…
Early ART	1.2 (.5–2.9)	.66
Later ART: CD4+ T-cell nadir		
>350/µL	2.1 (.9–5.0)	.08
200–350/µL	2.6 (1.2–5.6)	.02
<200/µL	1.7 (.8–3.7)	.14
Age, per year	1.1 (1.03–1.1)	<.001
Female sex	2.8 (.9–9.0)	.08
Male sex, not MSM	Reference	…
Male sex, MSM	1.1 (.5–2.5)	.79
suPAR		
HIV negative	Reference	…
Early ART	1.0 (.4–2.4)	.98
Later ART: CD4+ T-cell nadir		
>350/µL	2.2 (.9–5.2)	.07
200–350/µL	2.0 (.9–4.3)	.08
<200/µL	1.8 (.9–3.8)	.11
Age, per year	1.05 (1.02–1.1)	.004
Female sex	2.4 (.8–7.4	.12
Male sex, not MSM	Reference	…
Male sex, MSM	0.4 (.2–0.8)	.02
Current smoking	2.8 (1.5–5.3)	.002
IL-6		
HIV negative	Reference	…
Early ART	1.0 (.4–2.2)	.97
Later ART: CD4+ T-cell nadir		
>350/µL	0.7 (.3–1.4)	.28
200–350/µL	1.1 (.5–2.2)	.80
<200/µL	1.2 (.6–2.4)	.58
Age, per year	1.04 (1.01–1.08)	.006
Female sex	2.7 (.9–7.9)	.06
Male sex, not MSM	Reference	…
Male sex, MSM	0.6 (.3–1.2)	.12

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HIV, human immunodeficiency virus; IL-6, interleukin 6; IP-10, interferon-inducible protein 10; IQR, interquartile range; KT, kynurenine-tryptophan; MSM, men who have sex with men; sCD14, soluble CD14; sCD163, soluble CD163; sTNFR2, soluble tumor necrosis factor receptor 2; suPAR, soluble urokinase plasminogen activation receptor.

This is particularly true for biomarkers that strongly predict cardiovascular events (sTNFR2, sCD163, and suPAR [10, 32, 38, 39]), neurocognitive decline (sCD14 and sCD163 [40–42]), and pulmonary disease (sCD14, sCD163, and IL-6 [43–46]). These relationships may also help explain why lower CD4+ T-cell nadirs have been consistently associated with a greater risk of these particular morbid conditions.

Our finding that efavirenz use is associated with substantially higher sCD14 levels (but not other biomarkers) is also important. Indeed, several prior trials established that efavirenz-based regimens result in less robust declines in sCD14 or greater increases in intestinal fatty acid binding protein than comparator regimens [21–24]. These observations may suggest a direct effect of efavirenz on microbial translocation, oxidized low-density lipoproteins (another Toll-like receptor 4 ligand) [47, 48], or some other mechanism that specifically increases sCD14 levels. Arguing against a direct impact of efavirenz on microbial translocation is the trend toward lower KT ratio (another biomarker

Table 3. Association Between Efavirenz Use and Inflammatory Biomarkers in Antiretroviral Therapy–Suppressed Participants

Biomarker	Adjusted Relative Difference in EFV- vs Non-EFV Regimen Normalized to IQR of ART-Suppressed Participants (95% CI)[a]	P Value
KT ratio	0.53 (.27–1.1)	.07
sCD163	0.56 (.29–1.1)	.09
IP-10	0.89 (.42–1.9)	.75
sCD14	3.9 (2.2–6.8)	<.001[b]
sTNFR2	0.67 (.36–1.2)	.20
suPAR	0.88 (.46–1.7)	.69
IL-6	0.75 (.43–1.32)	.32

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; EFV, efavirenz; IL-6, interleukin 6; IP-10, interferon-inducible protein 10; IQR, interquartile range; KT, kynurenine-tryptophan; sCD14, soluble CD14; sCD163, soluble CD163; sTNFR2, soluble tumor necrosis factor receptor 2; suPAR; soluble urokinase plasminogen activation receptor.

[a]Relative differences were adjusted with covariates listed in Table 2.

[b]Significant at $P < .05$.

associated with microbial translocation [29]) in efavirenz-treated participants, though efavirenz may suppress kynurenine levels directly by inhibiting hepatic tryptophan 2,3-dioxygenase [49], an observation supported by another clinical study [50].

Regardless of the mechanism, these findings are important to consider in assessing the degree to which biomarkers normalize during ART, because HIV-uninfected comparator groups are always efavirenz naive. For example, the RV254 study from Thailand previously reported that sCD14 levels fail to normalize in PWH starting ART in the first 2 weeks of their

	Covariates adjusted for:
KT ratio — 2.9	Age, sex, MSM
sCD163 — 2.6	Age, sex, MSM, smoking
IP-10 — 2.2	Age, sex, MSM, smoking
sCD14 — 2.1	Age, sex, MSM, EFV
sTNFR2 — 2	Age, sex, MSM
suPAR — 1.0	Age, sex, MSM, smoking
IL-6 — 1.0	Age, sex, MSM

Adjusted Mean Fold Difference
Early ART vs HIV Negative
(per HIV-Negative IQR)

Figure 2. Adjusted difference between early antiretroviral therapy (ART) and human immunodeficiency virus (HIV)–uninfected participants. The mean fold difference in plasma biomarkers between early-ART and HIV-uninfected participants is plotted after normalization to the interquartile range (IQR; in the HIV-uninfected group) and after adjustment for the indicated confounders. Mean fold differences are denoted by circles, with bars spanning 95% confidence intervals. The vertical dotted line reflects a relative fold change of 1.0 (ie, no difference between early-ART and HIV-uninfected groups). Abbreviations: EFV, efavirenz; IL-6, interleukin 6; IP-10, interferon-inducible protein 10; KT, kynurenine-tryptophan; MSM, men who have sex with men; sCD14, soluble CD14; sCD163, soluble CD163; sTNFR2, soluble tumor necrosis factor receptor 2; suPAR, soluble urokinase plasminogen activation receptor.

infection, but because all of these individuals were receiving an efavirenz-based regimen, it is impossible to know how much of this persistent elevation is explained by efavirenz versus an irreversible effect of HIV [19]. Our study demonstrates that PWH who started ART within the first 6 months of their infection do in fact have higher sCD14 levels than HIV-uninfected individuals but that this difference is significantly attenuated after accounting for efavirenz use.

Finally, we found that only some biomarkers (KT ratio and to a lesser degree sCD163 and sCD14) remained abnormal in PWH who initiated ART in the first 6 months of their infection. The higher levels of sCD14 (and trend toward higher IP-10) in the early-ART compared with HIV-uninfected participants observed in our study is consistent with the findings from the RV254 study of Thai HIV-infected individuals who initiated ART in the first 2 weeks of their infection [19, 20]. Two smaller studies also suggested that KT ratio might not normalize in PWH initiating ART early, but our study is the first to report that durably suppressed early-ART initiators continue to have abnormally high plasma KT ratios when adjusted for confounders, compared with HIV-negative controls [51, 52]. Plasma KT ratio reflects the immunoregulatory kynurenine pathway of tryptophan catabolism, which has been implicated in the pathogenesis of HIV, tuberculosis, and cancer [27, 29, 53].

Tryptophan catabolism to kynurenine normally occurs at low levels by the constitutively active tryptophan deoxygenase enzyme in the liver, but it can also be induced at high levels by myeloid cell expression of indoleamine 2,3-dioxygenase (IDO) 1 in response to inflammatory stimuli, including type I and II interferons (or by similar enzymes in gut-resident bacteria) [29, 54]. IDO-1 activity is strongly induced by HIV, tuberculosis, and several cancers, and downstream catabolites of the kynurenine pathway confer adaptive immune defects by suppressing proliferation of T, B, and natural killer cells, suppressing T-helper 17 cells, and expanding regulatory T cells [29, 55, 56].

The KT ratio is also one of the strongest immunologic predictors of mortality risk in ART-suppressed Africans with HIV [9, 28]. Thus, the abnormal increase in the kynurenine pathway among early-ART participants might provide a plausible mechanistic explanation for the persistently increased risk of tuberculosis and infection-related cancers in early-ART initiators. Indeed, the kynurenine pathway is induced even in latent tuberculosis infection, appears to play a critical role in containing tuberculosis in granulomas in nonhuman primate models of tuberculosis, and predicts incident tuberculosis in a South African cohort [27, 57, 58]. The kynurenine pathway is also mechanistically linked to the other biomarkers that tended to be abnormal in early-ART initiators (sCD14, sCD163, and IP-10). IP-10, like IDO, is expressed in response to type I and II interferons, and the kynurenine pathway–mediated suppression of T-helper 17 cells may also contribute to microbial translocation, increasing sCD14 directly and macrophage activation more generally (sCD163).
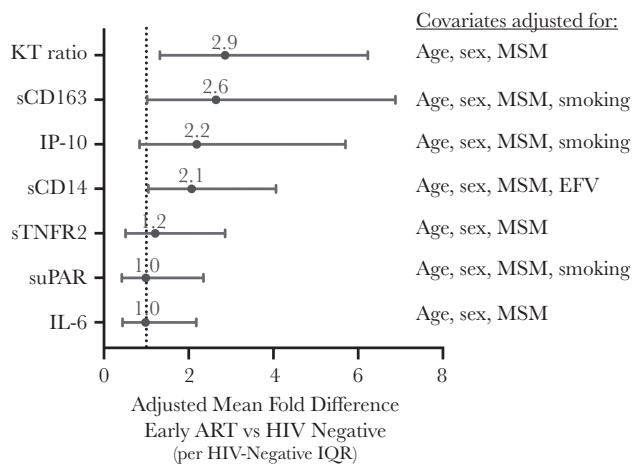
Our study did have some important limitations. The relatively small sample size may have limited our ability to detect clinically significant differences in some biomarkers between early-ART initiators and HIV-uninfected participants. Indeed, the nonsignificant trend for IP-10 was nevertheless substantial in effect size relative to the dynamic range observed in HIV-uninfected individuals. The small sample size may have also limited our ability to adjust for multiple confounders simultaneously (particularly in the early-ART group). Nonetheless, we adjusted for a substantial number of confounders that typically have been neglected in the prior literature, including CMV serostatus (by excluding seronegative participants), sexual risk behavior, smoking, and injection drug use (largely by excluding active users), all of which are known to affect immune activation. In addition, our study was too small to evaluate the association between biomarkers and clinical outcomes, particularly in the early-ART group, so we cannot establish the clinical relevance of these findings in the current study. Finally, because we had a small number of female participants in this cohort, constrained by the low prevalence of female early-ART initiators in our setting [59], we may not have been able to adequately assess the influence of sex and sex-related hormones.

In summary, we found that the inflammatory set point for most biomarkers of immune activation during ART-mediated viral suppression tends to be higher in individuals who started ART at more advanced disease stages. These data further support current guidelines suggesting ART initiation as soon as possible after HIV diagnosis. Nevertheless, only a subset of biomarkers of immune activation—particularly those reflecting the kynurenine pathway of tryptophan catabolism—remain abnormal in individuals who started ART within the first 6 months of their infection. This finding may plausibly explain why the risk of tuberculosis and infection-related cancers remains elevated even among individuals who start ART early and may highlight the kynurenine pathway of tryptophan catabolism as an important interventional target in future studies.

Supplementary Data

Supplementary materials are available at The *Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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