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Soluble Markers of Inflammation and Coagulation but Not T-Cell Activation Predict Non–AIDS-Defining Morbid Events During Suppressive Antiretroviral Treatment

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Background. Defining the association of non–AIDS-defining events with inflammation and immune activation among human immunodeficiency virus (HIV)–infected persons with antiretroviral therapy (ART)–associated virological suppression is critical to identifying interventions to decrease the occurrence of these events.

Methods. We conducted a case-control study of HIV-infected subjects who had achieved virological suppression within 1 year after ART initiation. Cases were patients who experienced non–AIDS-defining events, defined as myocardial infarction, stroke, non–AIDS-defining cancer, non–AIDS-defining serious bacterial infection, or death. Controls were matched to cases on the basis of age, sex, pre-ART $CD4^+$ T-cell count, and ART regimen. Peripheral blood mononuclear cells and plasma specimens obtained at the visit before ART initiation (hereafter, baseline), the visit approximately 1 year after ART initiation (hereafter, year 1), and the visit immediately preceding the non–AIDS-defining event (hereafter, pre-event) were analyzed for activated $CD4^+$ and $CD8^+$ T cells, plasma interleukin 6 (IL-6) level, soluble tumor necrosis factor receptor I (sTNFR-I) level, sTNFR-II level, soluble CD14 level, kynurenine-to-tryptophan (KT) ratio, and D-dimer level. Conditional logistic regression analysis was used to study the association between biomarkers and outcomes, with adjustment for potential confounders.

Results. Higher IL-6 level, sTNFR-I level, sTNFR-II level, KT ratio, and D-dimer level at year 1 were associated with the occurrence of a non–AIDS-defining event. Significant associations were also seen between non–AIDS-defining events and values of these biomarkers in specimens obtained at baseline and the pre-event time points. Effects remained significant after control for confounders. T-cell activation was not significantly related to outcomes.

Conclusions. Interventional trials to decrease the incidence of non–AIDS-defining events among HIV-infected persons with virological suppression should consider targeting the pathways represented by these soluble markers.

Clinical Trials Registration. NCT00001137.

Keywords. HIV; ART; Non-AIDS morbidity; inflammation; immune activation.

Despite increased survival with antiretroviral treatment (ART) [1], non–AIDS-defining events such as cardiovascular disease (CVD) and non–AIDS-defining malignancies are more prevalent in ART-treated human immunodeficiency virus (HIV)–infected adults than in uninfected adults [2–5]. While factors such as smoking and antiretroviral drug toxicity [6] explain some of the increased risk for these events, elevated immune activation

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and inflammation [7–16] and poor immune restoration [17] have been shown to predict disease. Characterizing the association of immune activation and inflammation with non–AIDS-defining events, after accounting for the effects of ART and traditional risk factors, is critical to identifying interventions that may reduce excess morbidity and mortality in adult ART recipients.

Chronic immune activation and inflammation are hallmarks of HIV infection and are associated with disease progression [18, 19]. A diverse array of biomarkers [13], including indices of T-cell activation (eg, HLA-DR and CD38 expression) [19–21], soluble tumor necrosis factor receptor I (sTNFR-I), sTNFR-II, and interleukin 6 (IL-6) [18, 22], are elevated in untreated HIV infection and often remain elevated among ART recipients with undetectable viremia [10, 13, 23, 24]. Many mechanisms have been proposed to explain this inflammatory state, including the direct effects of HIV replication, HIV-mediated destruction of mucosal barriers with chronic exposure to gut microbial elements, excess burden of coinfections (eg, those involving cytomegalovirus), and loss of normal immune regulatory responses [25, 26]. Recent studies have shown that morbidity and/or mortality are associated with inflammation biomarkers (IL-6, fibrinogen, cystatin C, and C-reactive protein), soluble CD14 (sCD14), and D-dimer independently of viremia and $CD4^+$ T-cell count [7, 8, 11, 12, 14, 15, 27]. Of note, these studies included individuals with variable levels and duration of virologic control, and the independent role of these biomarkers after controlling for potential confounders was not consistently defined.

Cellular activation is associated with AIDS-defining outcomes in untreated and treated HIV infection [20, 21]. The impact of cellular immune activation on non–AIDS-defining events is less well studied because of limited stored peripheral blood mononuclear cell (PBMC) availability. In a Ugandan cohort, T-cell activation predicted short-term mortality [9]. In a cohort of HIV-infected women, decreased carotid artery distensibility, a marker for future CVD, was associated with T-cell activation (as measured by the percentage of $HLA\text{-}DR^+CD38^+$ cells among $CD4^+$ and $CD8^+$ T cells) and senescence (as measured by the percentage of $CD57^+CD28^-$ cells among $CD4^+$ and $CD8^+$ T cells) [11]. The above studies largely examined the relationship between immune activation and morbidity before ART initiation or the event of interest [27] but do not address whether elevated immune activation after a defined and uniform period of ART-induced viral suppression is associated with outcomes. To our knowledge, no single study has simultaneously evaluated the impact of markers of inflammation (eg, IL-6), monocyte activation/microbial translocation (sCD14), coagulation (D-dimer), and T-cell activation/dysfunction on outcome when values of the markers were measured before initiation of and during ART. Also, few cohorts have adequately controlled for important confounders, such as CVD risk factors, substance abuse, and treatment-mediated $CD4^+$ T-cell changes. Because immunodeficiency is linked to both inflammation and non–AIDS-defining morbidity, defining how inflammation and $CD4^+$ T cells interact over the course of treatment will be critical in prioritizing future interventions.

The AIDS Clinical Trials Group (ACTG) Longitudinal Linked Randomized Trials (ALLRT) is a well-characterized cohort of HIV-infected subjects who were randomized into treatment trials [28]. Standardized visits occurred every 16 weeks, and clinical data, including the occurrence of non–AIDS-defining events, defined here as CVD (ie, stroke and myocardial infarction [MI]), non–AIDS-defining malignancy, non–AIDS-defining serious bacterial infections, and death, were collected during study visits. Plasma specimens and PBMCs were obtained and stored at these visits. We performed a case-control study using stored plasma specimens and PBMCs from ALLRT participants to assess the role of selected biomarkers on disease outcomes.

METHODS

Subjects (or, for minors, their parent or legal guardian) provided written informed consent, and institutional review board approval for ALLRT was obtained by each ACTG site.

All subjects were ART naive when enrolled into an ACTG study, had a plasma HIV-1 RNA load of < 400 copies/mL at week 48 after ART initiation, and maintained a plasma HIV RNA load of < 400 copies/mL at all subsequent time points (isolated values ≥400 copies/mL were allowed if preceding and subsequent values were <400 copies/mL without a change in ART). Cases were defined as patients who died from a nonaccidental non–AIDS-related event, had a MI, had a stroke, or had a non–AIDS-defining malignancy or serious bacterial infection. Events were reviewed by the core team; MI diagnoses accorded with standard ACTG definitions and ALLRT reporting criteria and were reviewed for accuracy by ALLRT investigators [29]; criteria for non–AIDS-defining malignancies have been described elsewhere [30]. For each case, we identified 2–3 controls who had an endpoint-free follow-up time greater than that of the case and matched the case on the basis of age (within 10 years), sex, baseline $CD4^+$ T-cell count (within 50 cells/mm^3), ART regimen at week 48 (whether it contained a protease inhibitor or abacavir), and parent study.

Stored PBMCs and plasma specimens obtained from cases and controls at the following 3 time points were evaluated: before ART initiation (hereafter, baseline); 48–64 weeks after starting ART (hereafter, year 1); and the time point immediately preceding the event, for cases, and a similar time point, for corresponding controls (hereafter, pre-event). The year 1 time point was chosen because by 48 weeks after ART, the slope of decline in activation has stabilized [31].

Biomarker Measurements

At each visit, whole blood was obtained in tubes containing ethylenediaminetetraacetic acid. Specimens were spun at 400 ×*g* for

10 minutes, and plasma was pipetted and spun again at 800 ×*g* for 10 minutes. Plasma was aliquoted, frozen, and stored at −70°C until assayed. Plasma was analyzed for levels of IL-6 (HS600B, R&D Systems, Minneapolis, MN), sCD14 (DC140, R&D Systems), interferon γ (IFN-γ)–inducible protein 10 (IP-10; DIP100, R&D Systems), sTNFR-1 and sTNFR-II (DRT100/200, R&D Systems), and D-dimer (Diagnostica Stago, Parsippany, NJ) per the manufacturer's protocols. Samples were run without dilution for evaluation of IL-6, at a 1:400 dilution for sCD14, at a 1:4 dilution for IP-10, at a 1:10 dilution for sTNFR-I, at a 1:50 dilution for sTNFR-II, and at 1:21 and 1:42 dilutions for D-dimer. The plasma kynurenine-to-tryptophan (KT) ratio was determined at the year 1 time point by using published techniques [32].

The T-cell phenotype was characterized in batch by polychromatic flow cytometry on cryopreserved PBMCs that were removed from liquid nitrogen storage, thawed rapidly at 37°C in a water bath, washed, and rested overnight at 37°C in an incubator. The following day, cells were washed and stained for viability with Aqua Live/Dead cell stain kit (Invitrogen) prior to cell surface staining with fluorochrome-conjugated monoclonal antibodies to CD3, CD8, HLA-DR, CD38, CD28, CD57, and PD-1 (BD Biosciences for all) and to CD4 (Invitrogen). After staining, cells were fixed in 2% formaldehyde and analyzed within 24 hours on a LSR2 flow cytometer (BD), using FACS Diva software, v6.1.1. Analysis of flow cytometry data was performed using FlowJo software (Tree Star). Immune activation ($CD38^+$\HLA DR^+ or PD1+) and senescence ($CD57^+$\$CD28^-$) analyses were performed after stringent gating on singlet live $CD3^+$\$CD4^+$ or $CD3^+$\$CD8^+$ T cells.

Statistical Analysis

Conditional logistic regression analysis incorporating the case-control matching factors was used to study the associations of baseline, year 1, and pre-event values of each biomarker with nonaccidental mortality, MI, stroke, malignancy, and serious bacterial infection and with all events combined. Effects are quantified in terms of the odds ratio (OR) per 1 interquartile range (IQR) on the $\log_{10}$-transformed scale for soluble markers [12]; the IQR was obtained from pooling cases and controls. Adjusted analyses further controlled for concurrent $\log_{10}$ HIV RNA load (at baseline) or $CD4^+$ T-cell counts (at year 1 or pre-event). The following additional potential confounders at the time of the biomarker measurement were also evaluated individually in the conditional logistic model: (1) chronic hepatitis B or C, (2) smoking status, (3) injection drug use, (4) waist-to-hip ratio, (5) history of clinician-diagnosed diabetes or hypertension, (6) use of antihypertensive or lipid-level-lowering agents, (7) family history of MI, and (8) change in $CD4^+$ T-cell count after ART initiation. No adjustments were made for multiple comparisons. A sample size of ≥175 case-control sets with evaluable data was estimated as providing >80% power to detect an OR of ≥2.0 for a specific biomarker between cases and controls (comparing high levels [ie, above the median value] vs low levels). Supplemental analyses evaluated biomarkers in categories of above versus below the median. Changes from baseline to year 1 were assessed using the signed rank test. The correlation between $CD8^+$ T-cell activation measured on cryopreserved cells in this study and available measurements from the same date done on fresh cells was estimated by resampling methods [33], with statistical significance assessed using generalized estimating equations.

RESULTS

Among 143 cases, non–AIDS-defining events occurred at a median of 2.9 years after ART initiation (Figure 1). Three hundred fifteen controls were identified. Cases and controls had similar baseline demographic characteristics (Table 1).

$CD4^+$ T-Cell Changes After ART Initiation

Controls had higher median change in $CD4^+$ T-cell count (an increase of 186 cells/μL) at year 1 than cases (an increase of 127 cells/μL). A greater increase in $CD4^+$ T-cell count at year 1 was associated with a decreased risk for a non–AIDS-related event (OR per increase of 100 cells/μL increase, 0.81; $P = .007$). This effect persisted even after adjustment for biomarker levels at year 1 (ORs per increase of 100 cells/μL, 0.81–0.84; $P \leq .025$).

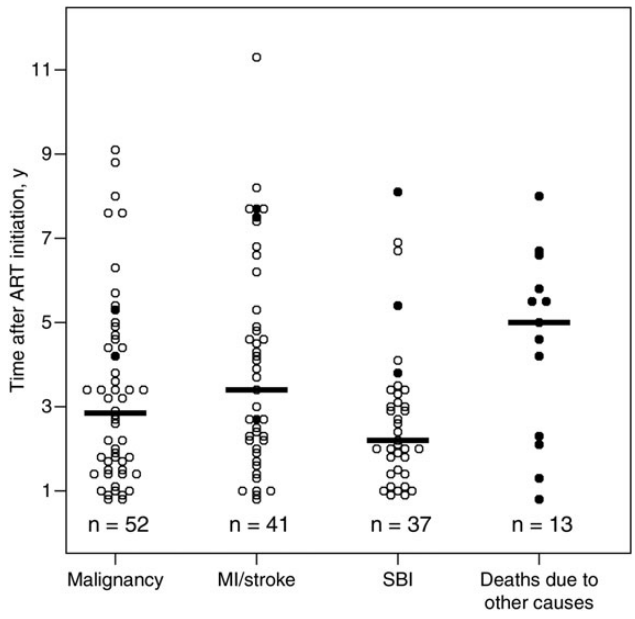

Figure 1. Timing of non–AIDS-defining events in relation to time in years after antiretroviral therapy (ART) initiation. Fatal cases are indicated by solid black circles. Abbreviations: MI, myocardial infarction; SBI, serious bacterial infection.

Table 1. Select Demographic Characteristics of Cases and Controls at Baseline

Characteristic	Cases	Controls	Total
Age at parent study entry, y			
Range	23–73	23–67	23–73
Median (IQR)	46 (40–53)	44 (39–50)	45 (39–51)
Regimens evaluated, by parent study			
A384: (AZT + 3TC vs d4T + ddI) + (EFV vs NFV vs NFV + EFV)	26 (18)	77 (24)	103 (22)
A388: (AZT + 3TC vs d4T + 3TC) + (IDV vs NFV vs IDV + NFV)	16 (11)	15 (5)	31 (7)
A5014: NVP + [LPV/r vs (ABC + 3TC + d4 T)]	3 (2)	8 (3)	11 (2)
A5095: AZT/3TC + (ABC vs EFV vs ABC + EFV)	45 (31)	84 (27)	129 (28)
A5142: (EFV + AZT/d4T + 3TC) vs (LPV/r + AZT/d4 T + 3TC) vs (EFV + LPV/r)	21 (15)	67 (21)	88 (19)
A5202: (ABC/3TC vs TFV/FTC) + (ATV/r vs EFV)	32 (22)	64 (20)	96 (21)
Sex			
Male	121 (85)	267 (85)	388 (85)
Female	22 (15)	48 (15)	70 (15)
Race/ethnicity			
White, non-Hispanic	74 (52)	151 (48)	225 (49)
Black, non-Hispanic	52 (36)	89 (28)	141 (31)
Hispanic (regardless of race)	16 (11)	64 (20)	80 (17)
Other	1 (1)	11 (3)	12 (3)
Baseline $CD4^+$ T-cell count, cells/μL			
Range	2–764	0–756	0–764
Median (IQR)	208 (87–338)	221 (70–331)	215 (76–334)
Baseline $\log_{10}$ plasma HIV-1 RNA load, copies/mL			
Range	2.9–6.9	2.3–7.0	2.3–7.0
Median (IQR)	4.8 (4.4–5.3)	4.8 (4.4–5.4)	4.8 (4.4–5.4)
Chronic hepatitis B or C	37 (27)	34 (11)	71 (16)
Current or previous use injection drug use	20 (13)	30 (10)	50 (11)
Waist-to-hip ratio[a]			
Range	0.72–1.12	0.67–1.08	0.67–1.12
Median (IQR)	0.92 (0.89–0.97)	0.92 (0.88–0.97)	0.92 (0.88–0.97)
History of clinician-diagnosed diabetes	11 (8)	15 (5)	26 (6)
History of hypertension	47 (33)	58 (18)	105 (23)
Use of antihypertensive or lipid-level-lowering agents	32 (22)	42 (13)	64 (16)
Current or past smoker	108 (75)	172 (55)	280 (61)
Family history of MI	30 (21)	47 (15)	77 (17)

Data no. (%) of patients, unless otherwise indicated. Values are for 143 cases, 315 controls, and 458 total patients, unless otherwise indicated.

Abbreviations: ABC, abacavir; ATZ/r, ritonavir-boosted atazanavir; AZT, zidovudine; d4T, stavudine; ddI, didanosine; EFV, efavirenz; FTC, emtricitabine; HIV-1, human immunodeficiency virus type 1; IDV, indinavir; IQR, interquartile range; LPV/r, ritonavir-boosted lopinavir; MI, myocardial infarction; NFV, nelfinavir; NVP, nevirapine; TFV, tenofovir; 3TC, lamivudine.

[a] Data are for 115 cases, 259 controls, and 374 total patients.

Soluble Markers of Inflammation and Coagulation

At year 1, both cases and controls had decreases in levels of all soluble markers ($P < .001$ for all markers; Figure 2). Higher baseline levels of IL-6, sTNFR-I, sTNFR-II, sCD14, and D-dimer were associated with a non–AIDS-related event (ORs per 1 IQR increase, 1.39–1.83; $P \leq .014$; Figure 3). These associations were unchanged after adjustment for confounders. For example, ORs per 1 IQR increase for IL-6 level, adjusted for waist-to-hip ratio, diabetes, hypertension, and smoking, were 1.7 ($P = .001$), 1.6 ($P < .001$), 1.6 ($P < .001$), and 1.6 ($P = .001$), respectively (Supplementary Table 1*A*). Findings were similar when comparing levels of soluble markers above versus those below the median values (Supplementary Figure 1*A*). When considering different types of events separately, baseline IL-6, sTNFR-I, and sTNFR-II levels were associated with increased mortality. No baseline markers were associated with an increased risk of a non–AIDS-defining malignancy (although the statistical power was reduced in analyses of individual types of events), while sTNFR-I and sTNFR-II were associated with a risk of a cardiovascular event.

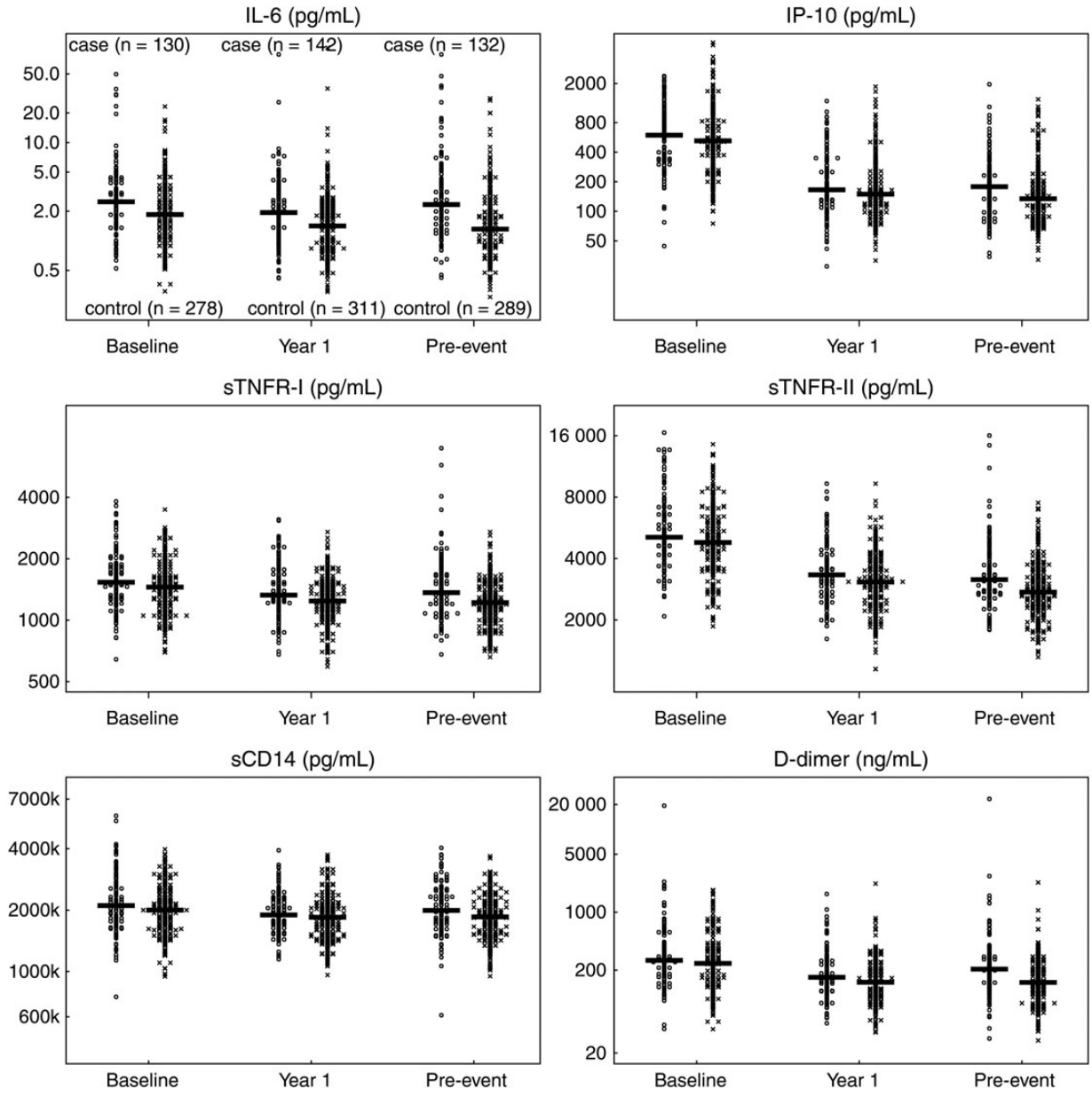

Figure 2. Median levels of soluble markers of inflammation and coagulation between cases (open circle) and controls (x) in specimens obtained at the visit before antiretroviral therapy (ART) initiation (baseline), the visit approximately 1 year after ART initiation (year 1), and the visit immediately preceding the non–AIDS-defining event (pre-event). Abbreviations: IL-6, interleukin 6; IP-10, interferon γ–inducible protein 10; sTNFR, soluble tumor necrosis factor receptor.

At year 1, having a non–AIDS-related event was significantly associated with higher levels of IL-6, sTNFR-I, sTNFR-II, or D-dimer (ORs per 1 IQR increase, 1.58–1.82; $P \leq .001$) but not with higher levels of IP-10 or sCD14 (Figure 4). These associations were unchanged after adjustment for confounders. For example, the ORs per 1 IQR increase for IL-6 level, adjusted for waist-to-hip ratio, diabetes, hypertension, and smoking were 1.6 ($P = .002$), 1.8 ($P < .001$), 1.8 ($P < .001$), and 1.7 ($P < .001$), respectively (Supplementary Table 1*B*). Similar findings comparing soluble markers above versus those below the median values are shown in Supplementary Figure 1*B*. Higher levels of IL-6, sTNFR-I, sTNFR-II and D-dimer were associated with increased mortality. Higher levels of IL-6 and sTNFR-I were significantly associated with the development of a non–AIDS-defining malignancy. Higher levels of sTNFR-I, sTNFR-II, and D-dimer were associated with having a cardiovascular event.

At the pre-event time point, all soluble markers were strongly associated with the occurrence of a non–AIDS-related event (ORs per 1 IQR increase, 1.44–2.56; $P \leq .005$; Figure 5 and

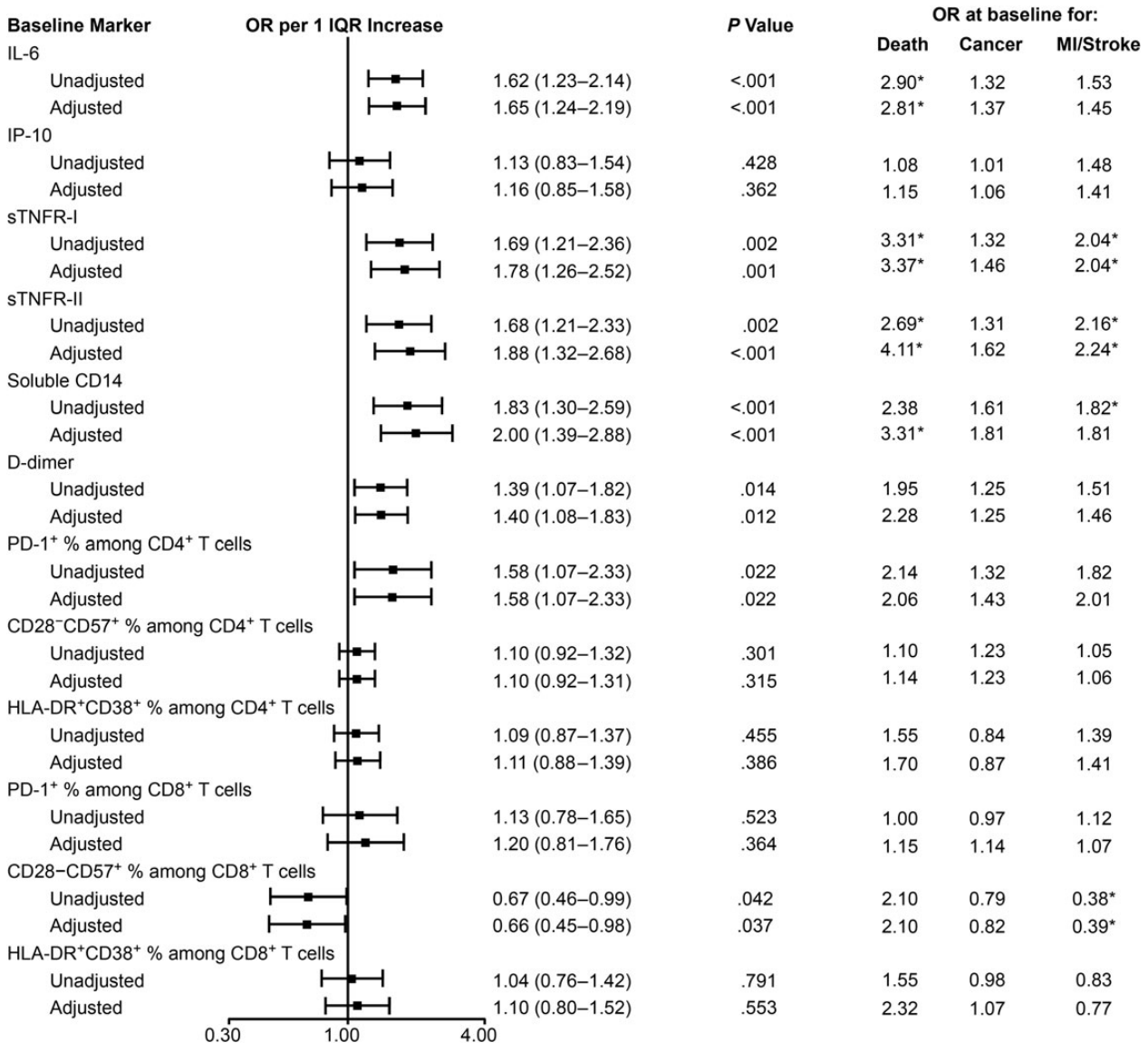

Figure 3. Biomarker levels in specimens obtained at the visit before antiretroviral therapy initiation (baseline) and odds ratios (ORs) of having a non–AIDS-defining event. Adjusted analyses controlled for $\log_{10}$ baseline human immunodeficiency virus RNA load. *P = .01 to <.05; **P < .01. Abbreviations: IL-6, interleukin 6; IP-10, interferon γ–inducible protein 10; IQR, interquartile range; KT, kynurenine to tryptophan; MI, myocardial infarction; sTNFR, soluble tumor necrosis factor receptor.

Supplementary Figure 1*C*). These associations were unchanged after adjustment for confounders (Supplementary Table 1*C*). IL-6, sTNFR-I, sTNFR-II, and D-dimer were also associated with mortality, a non–AIDS-defining malignancy, and a cardiovascular event.

Plasma KT Ratio

In an exploratory analysis, the KT ratio was determined only at the year 1 time point. The median KT ratio was 0.042 (IQR, 0.033–0.055) for cases and 0.038 (IQR, 0.032–0.048) for controls. The KT ratio had a positive correlation with all soluble and cellular markers and a negative correlation with $CD4^+$ T-cell counts. In unadjusted analysis and analysis adjusted for year 1 $CD4^+$ T-cell count, a higher KT ratio was associated with an increased risk of a non–AIDS-related outcome or death (adjusted OR per 1 IQR increase, 1.30 [95% confidence interval, 1.06–1.60]; P = .01].

T-Cell Activation and Senescence

For the cell-based marker analysis, only results obtained from viable cells and from samples with >100 flow cytometry events were included in the analysis. Hence, only 88, 120, and 90 cases (with their corresponding controls) contributed data to the baseline, year 1, and pre-event analyses, respectively. The

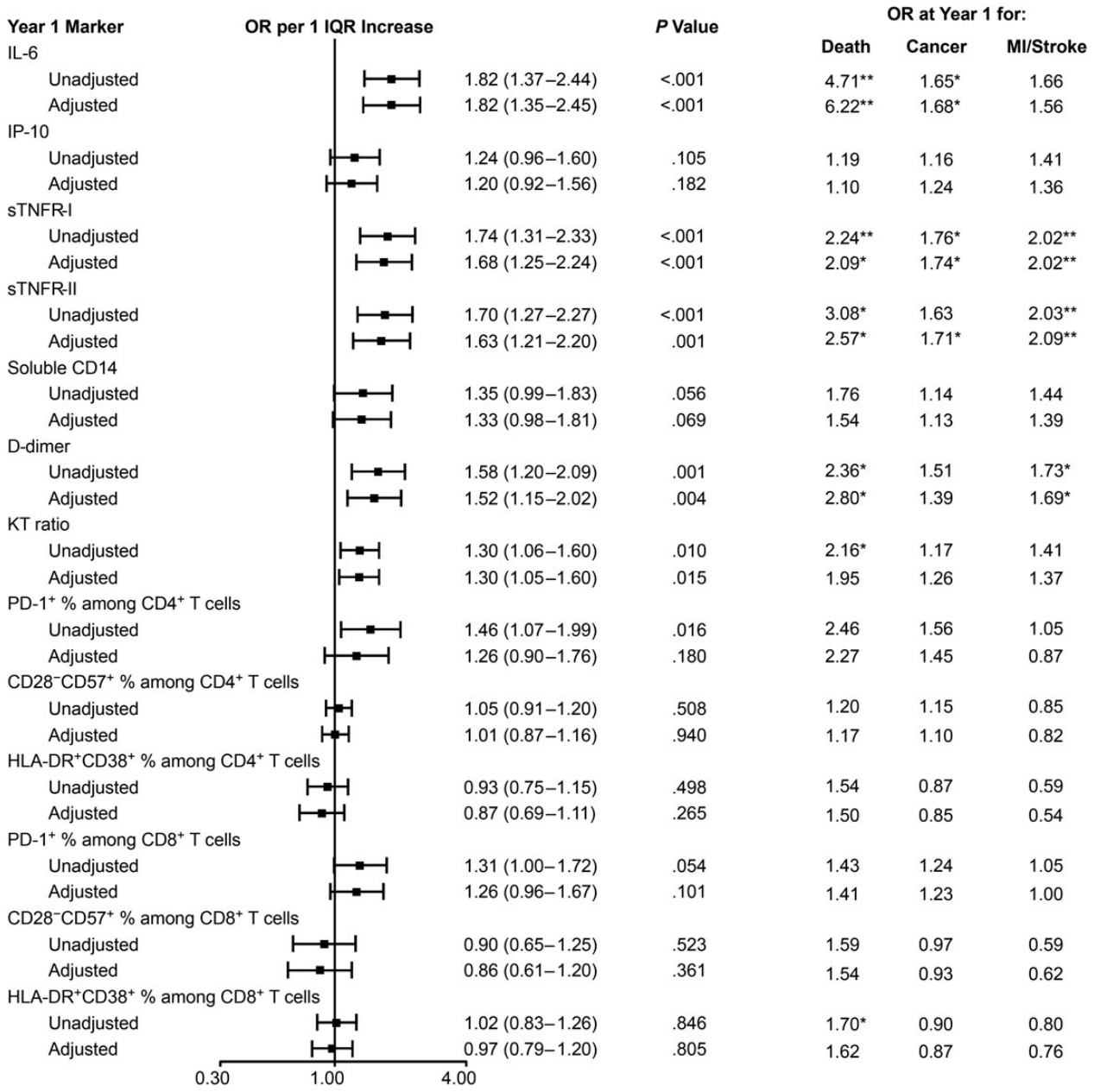

Figure 4. Biomarker levels in specimens obtained at the visit approximately 1 year after ART initiation (year 1) and odds ratios (ORs) of having a non–AIDS-defining event. Adjusted analyses controlled for concurrent $CD4^+$ T-cell count. *P = .01 to <.05; **P < .01. Abbreviations: IL-6, interleukin 6; IP-10, interferon γ–inducible protein 10; IQR, interquartile range; KT, kynurenine to tryptophan; MI, myocardial infarction; sTNFR, soluble tumor necrosis factor receptor.

median lymphocyte viability was 71% (IQR, 20%–91%). Flow cytometry results obtained from cryopreserved samples correlated with historical results that were performed in real time (during the parent study) from samples from the same time points (R = 0.48–0.52; $P \leq .012$).

At year 1, both cases and controls had decreases in the frequency of all cell-based markers (Figure 6; $P < .001$ for all markers except the percentage of $CD28^-CD57^+$ cells among $CD8^+$ T cells from cases; $P = .57$). A higher percentage of cells expressing $CD4^+PD\text{-}1^+$ at baseline was associated with increased risk of a non–AIDS-defining event (OR per 1 IQR increase, 1.58; $P = .022$; Figure 3). A higher percentage of $CD28^-CD57^+$ cells among $CD8^+$ T cells at baseline was associated with a lower risk of a non-AIDS event (OR, 0.67; $P = .042$; Figure 3). None of the other markers, including the percentages of $CD4^+CD38^+DR^+$ or $CD8^+CD38^+DR^+$ T cells, was predictive of a non–AIDS-defining event. These associations were unchanged after adjustment for confounders.

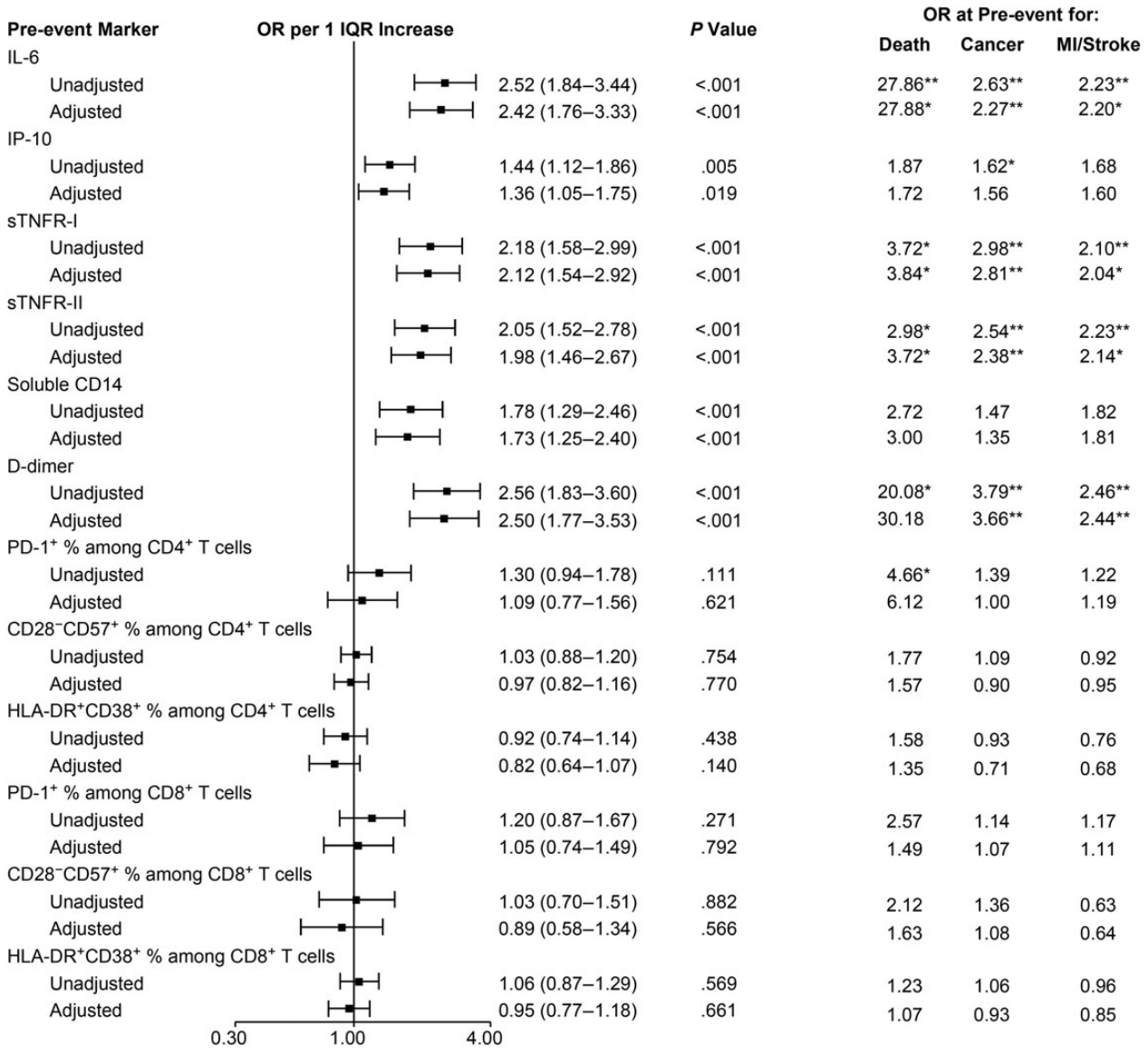

Figure 5. Biomarker levels in specimens obtained at the visit immediately preceding the non–AIDS-defining event (pre-event) and odds ratios (ORs) of having a non–AIDS-defining event. Adjusted analyses controlled for concurrent $CD4^+$ T-cell count. *P = .01 to <.05; **P < .01. Abbreviations: IL-6, interleukin 6; IP-10, interferon γ–inducible protein 10; IQR, interquartile range; KT, kynurenine to tryptophan; MI, myocardial infarction; sTNFR, soluble tumor necrosis factor receptor.

Sensitivity Analyses

A sensitivity analysis limited to subjects with a viral load of <50 copies/mL at year 1 revealed similar results for analyses of both the soluble and cellular biomarkers. We also did sensitivity analysis by only including lymphocyte viability of >10% and of >75%, and the results for the cellular biomarker analysis are similar, although the level of significance decrease because of the reduced sample size.

DISCUSSION

In this study of HIV-infected adults with a durable virologic response to ART, higher levels of IL-6, sTNFR-I, sTNFR-II, and D-dimer at baseline, year 1, and pre-event time points and a higher level of sCD14 at baseline and pre-event time points were associated with the occurrence of non–AIDS-related morbidity or death. These effects were independent of traditional risk factors, other comorbid conditions, age, treatment regimen, and treatment-mediated changes in $CD4^+$ T-cell counts. These effects were not altered when measures of T-cell activation and senescence were considered. Collectively, these data suggest that HIV-associated inflammation improves with therapy, but that abnormalities persist and that pathways that involve IL-6, TNF, and D-dimer predict non–AIDS-defining events even when virus replication is controlled. Moreover, this effect

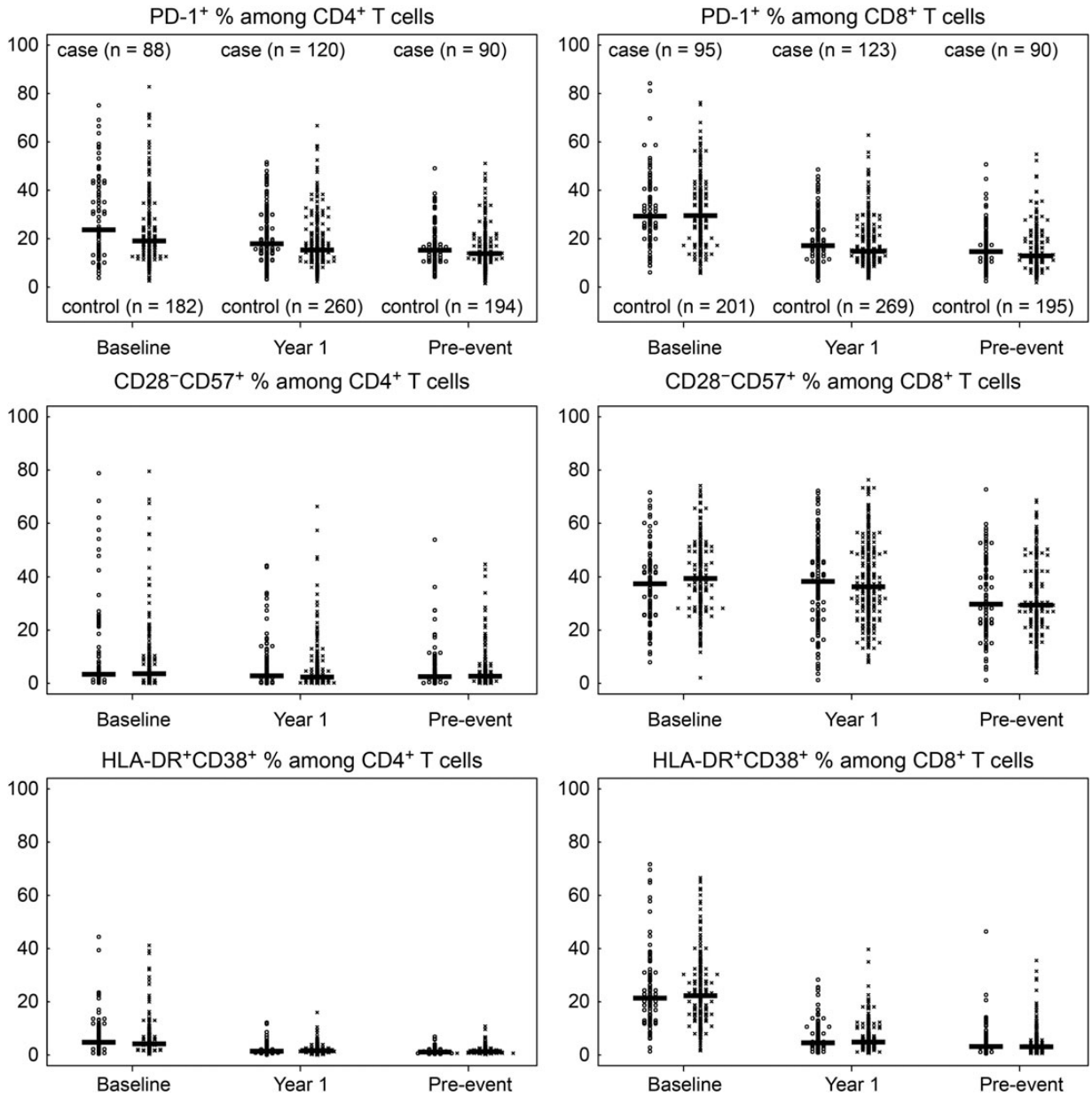

Figure 6. Median levels of T-cell markers of activation and senescence between cases (open circle) and controls (x) in specimens obtained at the visit before antiretroviral therapy (ART) initiation (baseline), the visit approximately 1 year after ART initiation (year 1), and the visit immediately preceding the non–AIDS-defining event (pre-event).

appears to be mediated independently of the classic markers of immunodeficiency (eg, $CD4^+$ T-cell count) and other recognized perturbations in the T-cell phenotype that have been linked to HIV immunopathogenesis (specifically, expression of CD38, HLA-DR, PD-1, CD28, and CD57 on circulating cells). On the other hand, increases in $CD4^+$ T-cell count also predicted morbid outcomes independently of inflammatory indices in this study, indicating that at least 2 pathways determine the risk for ART-era morbidities or that these pathways are linked through mechanisms that are not apparent through this analysis. The effect sizes we observed varied considerably with both the marker examined and the outcome considered, suggesting that some of these markers may be more proximate than others to the pathogenesis of each clinical outcome. Thus, for example, a role for coagulation (D-dimer) is easier to envisage for vascular outcomes than for malignancies. Whether these associations persist with longer duration of virologic suppression and/or immune restoration remains to be clarified.

Our observation that pretherapy values predict disease prior to "removal" of HIV infection as a major determinant argue

that some inflammatory set point is present before therapy initiation that is distinct from current clinical measures of the HIV disease course. This set point may be partly determined by host-related factors or other factors (eg, smoking) that may also be associated with inflammation in HIV-uninfected individuals and that are more prevalent among HIV-infected persons, including those in our cohort. This implies that interventions that target inflammation and/or factors that are associated with inflammation may be needed to decrease the excess morbidity seen despite virologic suppression.

As with AIDS-related events, the extent of restoration of the $CD4^+$ T-cell count with ART has a protective effect on the occurrence of non–AIDS-related events. While this study cannot prove causality, a more robust increase in the $CD4^+$ T-cell count after ART initiation may promote better control of coinfecting pathogens, better restoration of gut defenses, and lower levels of inflammation. Alternatively, lower levels of inflammation may promote more-robust restoration of the $CD4^+$ T-cell count. Untangling these pathways will require interventions that either directly enhance immune restoration or reduce inflammation.

The biomarker most strongly associated with outcomes here and in other studies [12] is IL-6. This suggests a central role for pathways that lead to elevated systemic IL-6 levels in non–AIDS-related diseases among HIV-infected persons with viral suppression during ART. IL-6 has a strong association with both mortality and incident CVD across many disease states [34–36]. The universal nature of this association suggests that the IL-6 pathway may be a final common pathway for many disease processes mediated by inflammation. The role of HIV replication in driving elevated IL-6 levels is not clear because earlier work could not show consistent relationships between plasma IL-6 and HIV levels [37, 38]. Intervention trials with IL-6 inhibitors are warranted to explore this relationship further.

This study also shows an association between non–AIDS-related outcomes and the plasma KT ratio. The KT ratio reflects activity of the indoleamine 2,3-dioxygenase (IDO1) pathway. IDO1 activity is driven by IFN-α and IFN-γ, HIV antigens, and endotoxin; is upregulated in untreated HIV infection; and predicts disease progression [38]. Overactivity of the IDO1 pathway may contribute to the T-cell dysfunction and chronic inflammation that is characteristic of HIV infection. It may not be readily reversed by virally suppressive ART.

The relatively strong associations seen with sTNFR-I and –II, the modest association with sCD14, and the relationship to IDO activity seem to implicate activated monocytes and macrophages in the pathogenesis of inflammation that appears to drive non–AIDS-related disease outcomes. This observation is consistent with findings from other studies that have also shown strong associations between various markers of activated monocytes/macrophages, such as sCD14 and CD163 and CVD surrogates [39–41].

Contrary to our expectations, levels of T-cell activation were not associated with non–AIDS-related events but for expression of PD-1 on $CD4^+$ T cells, which was modestly associated with morbid outcomes in unadjusted analyses but not after adjustment for $CD4^+$ T-cell count. This is unlike the scenario with AIDS-related disease in both untreated and treated HIV infection, in which the level of T-cell activation is strongly associated with outcomes [18, 19, 21]. These findings imply that T-cell activation may not be as important in the pathogenesis of the non–AIDS-related diseases examined in this study. However, others have shown an association between various other T-cell phenotypes and subclinical atherosclerosis [11, 40, 42, 43]. Prospective studies incorporating selected biomarkers may help to clarify the relative contributions of the innate and adaptive immune system to the pathogenesis of non–AIDS-related diseases in HIV-infected persons.

Limitations to this study include a case-control design, which makes it impossible to generate direct estimates of incident morbidity risk related to each of the variables. Also, the possibility of residual confounding not addressed by the matching strategy is a significant risk. It is possible that participants with the highest risk for non–AIDS-related complications are underrepresented in our study population, because of the usually stringent inclusion and exclusion criteria of many clinical trials. In addition, the analysis of cellular markers was limited by decreases in sample availability attributable to decreased cell viability. This introduced several biases, including the underrepresentation of patients with lower $CD4^+$ T cell counts at the outset and, consequently, resulted in a decreased power to detect associations. However, other studies have also shown the absence of an association between markers of T-cell activation and non–AIDS-related disease outcomes among persons with successfully treated HIV infection [27, 44]. Prospective cohort studies of long-term outcomes of ART that incorporate the biomarkers identified in this and other studies [8, 11–15, 27] are needed to address these limitations.

Among HIV-infected subjects receiving virally suppressive ART, soluble markers of inflammation and coagulation, particularly IL-6 level, sTNFR-I level, sTNFR-II level, sCD14 level, plasma KT ratio, and D-dimer level, were associated with a higher risk of non–AIDS-related morbidity and mortality. Levels of T-cell activation were not associated with the occurrence of a non–AIDS-related outcome. Interventions that target the pathways represented by or the factors associated with these biomarkers should be investigated to determine their role in the pathogenesis of non–AIDS-related outcomes in HIV-infected adults receiving virally suppressive ART.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of

data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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