Associations of Inflammatory Markers With AIDS and Non-AIDS Clinical Events After Initiation of Antiretroviral Therapy: AIDS Clinical Trials Group A5224s, a Substudy of ACTG A5202

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Background: The association of inflammatory biomarkers with clinical events after antiretroviral therapy initiation is unclear.

Methods: A5202 randomized 1857 treatment-naive subjects to abacavir/lamivudine or tenofovir-DF/emtricitabine with efavirenz or atazanavir/ritonavir. Substudy A5224s measured inflammatory biomarkers on subjects with available plasma from baseline and week 24 or 96. An exploratory analysis of the association of high-sensitivity C-reactive protein, interleukin-6 (IL-6), soluble receptors of tumor necrosis factor α (sTNF)-RI, sTNF-RII, TNF-α, soluble vascular cellular adhesion molecules (sVCAM-1), and soluble intercellular adhesion molecules (sICAM-1) with times to AIDS and to non-AIDS events used Cox proportional hazards models.

Results: Analysis included 244 subjects; 85% men and 48% white non-Hispanic with median age 39 years, HIV-1 RNA of 4.6 $\log_{10}$ copies per milliliter, and CD4 of 240 cells per microliter. Overall, 13 AIDS events (9 opportunistic infections, 3 AIDS-cancers, and 1 recurrent bacterial pneumonia) and 18 non-AIDS events (6 diabetes, 4 cancers, 3 cardiovascular, and 5 pneumonias) occurred. Higher baseline IL-6, sTNF-RI, sTNF-RII, and sICAM-1 were significantly associated with increased risk of AIDS-defining events. Adjustment for baseline HIV-1 RNA did not change results, whereas adjusting for baseline CD4 count left only sTNF-RI and sICAM-1 significantly associated with increased risk. Time-updated values of IL-6, sTNFR-I and II, and sICAM-1 were also associated with an increased risk. For non-AIDS events, only higher baseline high-sensitivity C-reactive protein was significantly associated with increased risk, whereas higher IL-6 was marginally associated with higher risk. Analyses of time-updated biomarker values showed tumor necrosis factor α to be significantly associated with increased risk, even after adjustment for antiretroviral therapy, and CD4 count or HIV-1 RNA.

Conclusions: Higher levels of several inflammatory biomarkers were independently associated with increased risk of AIDS and non-AIDS events.

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INTRODUCTION

In the current era of potent antiretroviral therapy (ART), cross-sectional measurements of inflammation markers, notably interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hsCRP) are linked to higher risk of subsequent mortality.[1] Recently the International Network for Strategic Initiatives in Global HIV Trials study group reported that higher pre-ART levels of IL-6 and hsCRP were associated with increased risk of AIDS events and mortality on ART.[2] Detailed measurements of markers of tumor necrosis factor α (TNF-α) and adhesion molecules were not performed in these aforementioned studies. Moreover, in past cross-sectional studies (not necessarily pre-ART measurements), the results have been conflicting in terms of the association between TNF-α levels and higher mortality or AIDS progression.[3–5] No study has yet investigated the association between time-updated inflammatory marker levels after ART initiation and the occurrence of AIDS and non-AIDS events in the setting of a prospective randomized ART-initiation study.

METHODS

AIDS Clinical Trials Group (ACTG) A5224s was a metabolic substudy of ACTG A5202. In A5202, ART-naive subjects aged ≥16 years with HIV-1 RNA of >1000 copies per milliliter were randomized in a double-blinded fashion to co-formulated tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) or abacavir/lamivudine (ABC/3TC), along with open-labeled efavirenz or atazanavir/ritonavir. Randomization was stratified by screening HIV-1 RNA (< vs. ≥100,000 copies/mL) and by intent to participate in A5224s. A secondary biomarker substudy of A5224s included all subjects with available stored plasma at baseline and week 24 and/or 96 and was designed with the primary objective to compare the effect of ART initiation on inflammation markers, results that were already published.[6] A secondary objective was to assess the association between baseline and on-ART markers of inflammation and clinical events, separately categorized as AIDS-defining event (CDC category C clinical events) or non–AIDS-defining events (including cardiovascular disease, non–AIDS-defining malignancies, diabetes, liver disease, serious infection that does not fit CDC category C clinical event) and bone fractures. The classification of non-AIDS events was similar to that used in a recently published analysis conducted within the ACTG,[7] except that a priori, we selected to consider bone fractures as a separate events category and not lump them within the non-AIDS events category because all bone fractures that occurred within A5224s were the result of a trauma. A5224s' major exclusion criteria were endocrine diseases including diabetes mellitus. The duration of the substudy was 96 weeks after the last subject enrolled into A5202, and subjects were followed regardless of their antiretroviral treatment status. Each subject signed an informed consent that was approved by participating site's local Institutional Review Board.

Biomarker Assays

Plasma samples were stored at −80°C without previous thawing until analysis. Assays were performed at Johns Hopkins Bayview Advanced Chemistry Laboratory, Baltimore, MD. We measured 7 markers, hsCRP (μg/mL), IL-6 (pg/mL), TNF-α (pg/mL), and the soluble receptors of TNF-α (sTNFR-I and -II, both in pg/mL), along with the endothelial activation markers soluble vascular cellular and intercellular adhesion molecules (sVCAM-1 and sICAM-1, both in ng/mL). High-sensitivity C-reactive protein was measured using a highly sensitive enzyme-linked immunosorbent assay (ALPCO Diagnostics, Windham, NH). Other markers were measured using an enzyme-linked immunosorbent assay (R&D Systems, Minnesota, WI). Markers were measured in duplicate, and values averaged for analysis. The intra-assay and inter-assay precisions of these assays were 1.3%–7.6% coefficient of variation (average 3.3%) and 1.8%–9.0% coefficient of variation (average 6.9%), respectively.

Statistical Analysis

This exploratory and secondary analysis of the association between baseline and time-updated biomarker levels and times to first AIDS-defining event, non–AIDS-defining event, and bone fracture used Cox proportional hazards regression. Multivariable models adjusted for the following prespecified covariates that could affect inflammation: ART assignment, then ART assignment plus each of the following individually; baseline CD4, baseline HIV-1 RNA, time-updated CD4, time-updated HIV-1 RNA suppression <50 copies per milliliter, and time-updated HIV-1 RNA suppression <200 copies per milliliter.

In time-updated analysis, for subjects with missing week 24 or 96 biomarker levels, their last observed value, including the baseline value, was carried forward. In addition, to account for the potential confounding effect on the time-updated analysis of the early events that occurred before week 24, we performed a 24-week landmark analysis that excluded subjects with early events before week 24, subjects with missing week 24 sample and subjects with HIV-1 RNA level ≥50 copies per milliliter at week 24. In all models, CD4 was modeled with a linear and a quadratic term. *P* values below 0.05 were considered statistically significant; no adjustments were made for multiple comparisons. Analyses were performed using SAS, version 9.2 (SAS Institute). Because of skewed distributions, biomarkers were $\log_e$ transformed before analysis.

RESULTS

Subject Characteristics

As previously detailed,[8,9] 269 subjects were enrolled in A5224s. Of these 269 subjects, 244 (91%) with available stored plasma from baseline and week 24 and/or 96 were

included in this biomarker substudy. Baseline characteristics were previously reported.[6] Table S1 is added to show baseline characteristics by study population (see **Supplemental Digital Content**, http://links.lww.com/QAI/A462). Overall, 85% were men, 48% white non-Hispanics, and among 205 subjects with available data, 41% were smokers. Median age was 39 years, CD4 240 cells per microliter, and HIV-1 RNA 4.64 $\log_{10}$ copies per milliliter. None of the subjects had a previous history of myocardial infarction and 1 had a history of stroke. Baseline characteristics were similar between the 244 included in the biomarker substudy, and the 25 A5224s subjects not included (data not shown).

At week 24, 171 (70%) had HIV-1 RNA<50 copies per milliliter (70% on TDF/FTC; 70% on ABC/3TC). Among subjects who had screening HIV-1 RNA ≥100,000 copies per milliliter, 56% on TDF/FTC and 60% on ABC/3TC had HIV-1 RNA <50 copies per milliliter.

Characteristics of Clinical Events

Only the first clinical event was considered for each subject in time to event analyses. There were a total of 13 AIDS events that occurred during the study: recurrent bacterial pneumonia (1), CMV retinitis (1), cryptosporidiosis (1), esophageal candidiasis (1), non-Hodgkin's lymphoma (2), Kaposi sarcoma (1), *Mycobacterium avium* complex (4), and *Pneumocystis jirovecii* pneumonia (2). These AIDS events happened after a median (range) of 15.6 (2.0–132.6) weeks on study, with 7 events occurring before week 24. A total of 18 subjects had at least 1 non-AIDS event that occurred during the study: acute myocardial infarction (2), pulmonary embolism (1), cancers (4: Hodgkin's disease 1, hypopharyngeal cancer 1, and prostate cancer 2), diabetes (6), isolated episode of non-pneumocystis pneumonia (5). These non-AIDS events happened after a median (range) of 81.4 (3.6–165.1) weeks on study, with 4 events occurring before week 24. When considering time to first AIDS or non-AIDS event, a total of 28 subjects had at least 1 event that occurred during study, 11 of which occurring before week 24. In addition, a total of 15 bone fractures occurred during the study, all of which were associated with a trauma.

Deaths were not included in the AIDS event or in the non-AIDS events. A total of 2 deaths were reported in the analysis sample. One subject was diagnosed with diabetes at week 24 then at week 106 was diagnosed with septic shock, non-Hodgkin's lymphoma, and a pulmonary embolism followed by death. The second subject died without a previous event at week 25 with the cause of death reported as substance abuse.

The week 24 landmark analysis included 5 AIDS-defining events (3 that occurred between weeks 24 and 96, and 2 that occurred after week 96), 12 non–AIDS-defining events (6 between weeks 24 and 96 and 6 after week 96), and for the combined event analysis, 14 AIDS or non-AIDS events (9 between 24 and 96 weeks and 5 after week 96).

Biomarker Associations With CD4 Counts

At baseline, CD4 count was inversely but not strongly correlated with levels of IL-6 (Spearman rank correlation $r = -0.20$, $P = 0.002$), sTNF-RI ($r = -0.25$, $P < 0.001$), and sTNF-RII ($r = -0.30$, $P < 0.001$) (see **Table S2, Supplemental Digital Content**, http://links.lww.com/QAI/A462). Also, the change in CD4 count from baseline to week 24 was inversely correlated with baseline to week 24 changes in levels of sVCAM-1 ($r = -0.40$, $P < 0.001$), sICAM-1 ($r = -0.22$, $P = 0.001$), sTNF-RII ($r = -0.36$, $P < 0.001$), sTNF-RI ($r = -0.16$, $P = 0.015$), and TNF-α ($r = -0.29$, $P < 0.001$). Similarly, the change in CD4 count from baseline to week 96 was correlated with baseline to week 96 changes in levels of sVCAM-1 ($r = -0.36$, $P < 0.001$), sTNF-RII ($r = -0.23$, $P = 0.001$), sTNF-RI ($r = -0.14$, $P = 0.046$), and TNF-α ($r = -0.22$, $P = 0.001$). Notably, neither baseline nor changes in CD4 count correlated with baseline or changes in hsCRP levels ($r \leq 0.07$, $P \geq 0.31$).

Biomarker Associations With HIV-1 RNA Levels

At baseline, HIV-1 RNA level correlated with levels of IL-6 ($r = 0.17$, $P = 0.008$), sVCAM-1 ($r = 0.45$, $P < 0.001$), sICAM-1 ($r = 0.26$, $P < 0.001$), sTNF-RII ($r = 0.52$, $P < 0.001$), sTNF-RI ($r = 0.43$, $P < 0.001$), and TNF-α ($r = 0.38$, $P < 0.001$), but not with hsCRP ($r = 0.04$, $P = 0.49$). Only for sTNFR-I was mean change from baseline to week 24 significantly different between subjects who at week 24 were virologically suppressed (<50 copies/mL) or not [estimated mean (standard deviation) −0.18 (0.23) vs. −0.12 (0.17) pg/mL; $P = 0.018$]. The mean (standard deviation) change from baseline to week 96 in sTNFR-II, sVCAM-1, and TNF-α were statistically significantly different between subjects who were virologically suppressed (<50 copies/mL) or not [−0.73 (0.43) vs. −0.52 (0.56) pg/mL; $P = 0.019$ and −0.53 (0.33) vs. −0.25 (0.42) ng/mL; $P < 0.001$, and −0.75 (0.42) vs. −0.37 (0.52) pg/mL; $P < 0.001$, respectively].

Biomarker Association With AIDS-Defining Event

We first examined for each of the 7 biomarkers the association between baseline biomarker value and time to first AIDS-defining event (Table 1). Higher values in baseline IL-6, sTNF-RI, sTNF-RII, and sICAM-1 were significantly associated with an increased risk of AIDS-defining event. Adjustment for ART assignment and for baseline HIV-1 RNA did not change the results (data not shown). Adjusting for baseline CD4 count attenuated the association of higher values with increased risk with these biomarkers, with baseline sICAM-1 remaining significantly associated with increased risk of events.

Table 1 also shows the associations between time-updated biomarker values and time to first AIDS event. The time-updated values in IL-6, sTNF-RI, sTNF-RI, and sICAM-1 were significantly associated with increased risk of AIDS-defining events. Adjustment for ART assignment and for baseline or time-updated CD4 counts attenuated the relationship (Table 1). Also, adjustment for ART assignment and for baseline HIV-1 RNA levels or for achieving virological suppression did not change the results. Results were similar

TABLE 1. Baseline and Time-Updated Biomarker Association With AIDS-Defining Events

	Unadjusted		Baseline CD4, NRTI, and NNRTI/PI Adjusted		Time-Updated CD4, NRTI, and NNRTI/PI Adjusted	
Biomarker	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Baseline hsCRP (per 0.5 $\log_e$ μg/mL higher)	1.10 (0.89 to 1.35)	0.36	1.14 (0.94 to 1.39)	0.19	—	—
Time-updated hsCRP (per 0.5 $\log_e$ μg/mL higher)	1.08 (0.88 to 1.33)	0.44	1.10 (0.90 to 1.34)	0.37	1.10 (0.91 to 1.35)	0.33
Baseline IL-6 (per 0.5 $\log_e$ pg/mL higher)	1.41 (1.03 to 1.92)	**0.032**	1.26 (0.91 to 1.75)	0.16	—	—
Time-updated IL-6 (per 0.5 $\log_e$ pg/mL higher)	1.43 (1.06 to 1.94)	**0.020**	1.33 (0.98 to 1.82)	0.068	1.30 (0.95 to 1.77)	0.10
Baseline sICAM-1 (per 0.5 $\log_e$ ng/mL higher)	2.88 (1.39 to 5.97)	**0.004**	2.28 (1.17 to 4.42)	**0.015**	—	—
Time-updated sICAM-1 (per 0.5 $\log_e$ ng/mL higher)	2.11 (1.09 to 4.08)	**0.027**	1.85 (0.99 to 3.47)	0.055	1.80 (0.95 to 3.39)	0.071
Baseline sTNF-RI (per 0.5 $\log_e$ pg/mL higher)	3.20 (1.44 to 7.09)	**0.004**	2.04 (0.88 to 4.74)	0.096	—	—
Time-updated sTNF-RI (per 0.5 $\log_e$ pg/mL higher)	4.26 (1.71 to 10.58)	**0.002**	2.95 (1.15 to 7.59)	**0.024**	2.90 (1.14 to 7.35)	**0.025**
Baseline sTNF-RII (per 0.5 $\log_e$ pg/mL higher)	1.86 (1.13 to 3.05)	**0.015**	1.64 (0.96 to 2.82)	0.071	—	—
Time-updated sTNF-RII (per 0.5 $\log_e$ pg/mL higher)	1.87 (1.13 to 3.11)	**0.015**	1.69 (0.98 to 2.90)	0.059	1.59 (0.92 to 2.75)	0.099
Baseline sVCAM-1 (per 0.5 $\log_e$ ng/mL higher)	1.51 (0.77 to 2.99)	0.23	1.49 (0.74 to 3.01)	0.26	—	—
Time-updated sVCAM-1 (per 0.5 $\log_e$ ng/mL higher)	1.33 (0.64 to 2.76)	0.45	1.33 (0.64 to 2.77)	0.45	1.22 (0.59 to 2.52)	0.58
Baseline TNF-α (per 0.5 $\log_e$ pg/mL higher)	1.51 (0.82 to 2.79)	0.19	1.60 (0.86 to 2.95)	0.14	—	—
Time-updated TNF-α (per 0.5 $\log_e$ pg/mL higher)	1.39 (0.73 to 2.64)	0.31	1.39 (0.72 to 2.68)	0.33	1.38 (0.71 to 2.69)	0.35

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

whether virological suppression was defined as HIV-1 RNA <50 copies per milliliter or <200 copies per milliliter.

Biomarker Association With Non–AIDS-Defining Event

We also examined the associations between biomarker levels at baseline and time to first non–AIDS-defining event (Table 2). Baseline hsCRP was significantly associated with developing a non-AIDS event, and there was a trend for an association with IL-6. Adjustment for ART assignment and for baseline HIV-1 RNA levels did not change either of the results. Adjustment for baseline CD4 did not change the hsCRP results.

Table 2 shows the associations between time-updated biomarkers and non-AIDS events. Only the time-updated TNF-α values were statistically significantly associated with an increased risk of non–AIDS-defining events. Adjustment for ART assignment, and for baseline or time-updated CD4 count, or baseline HIV-1 level or for achieving virological suppression (defined at <50 copies/mL or <200 copies/mL) did not change the results.

We considered fracture events as a separate category because of its unclear association with inflammation and the other categories of non-AIDS events. Neither baseline nor time-updated biomarker values were significantly associated with time to first fracture (data not shown).

TABLE 2. Baseline and Time-Updated Biomarker Association With Non–AIDS-Defining Events

	Unadjusted		Baseline CD4, NRTI, and NNRTI/PI Adjusted		Time-Updated CD4, NRTI, and NNRTI/PI Adjusted	
Biomarker	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Baseline hsCRP (per 0.5 $\log_e$ μg/mL higher)	1.29 (1.07 to 1.55)	**0.007**	1.29 (1.07 to 1.55)	**0.009**	—	—
Time-updated hsCRP (per 0.5 $\log_e$ μg/mL higher)	1.07 (0.90 to 1.28)	0.44	1.08 (0.90 to 1.30)	0.40	1.08 (0.90 to 1.29)	0.42
Baseline IL-6 (per 0.5 $\log_e$ pg/mL higher)	1.30 (0.98 to 1.71)	0.068	1.30 (0.96 to 1.76)	0.087	—	—
Time-updated IL-6 (per 0.5 $\log_e$ pg/mL higher)	0.98 (0.71 to 1.35)	0.91	1.01 (0.74 to 1.39)	0.94	0.99 (0.72 to 1.37)	0.97
Baseline sICAM-1 (per 0.5 $\log_e$ ng/mL higher)	0.90 (0.73 to 1.12)	0.33	0.87 (0.70 to 1.08)	0.21	—	—
Time-updated sICAM-1 (per 0.5 $\log_e$ ng/mL higher)	0.95 (0.74 to 1.20)	0.64	0.93 (0.73 to 1.17)	0.52	0.95 (0.74 to 1.20)	0.64
Baseline sTNF-RI (per 0.5 $\log_e$ pg/mL higher)	1.15 (0.49 to 2.69)	0.75	1.07 (0.41 to 2.77)	0.89	—	—
Time-updated sTNF-RI (per 0.5 $\log_e$ pg/mL higher)	1.61 (0.58 to 4.45)	0.36	1.63 (0.56 to 4.75)	0.37	1.60 (0.56 to 4.62)	0.38
Baseline sTNF-RII (per 0.5 $\log_e$ pg/mL higher)	1.29 (0.83 to 2.00)	0.26	1.34 (0.85 to 2.11)	0.21	—	—
Time-updated sTNF-RII (per 0.5 $\log_e$ pg/mL higher)	1.44 (0.87 to 2.37)	0.15	1.50 (0.90 to 2.51)	0.12	1.47 (0.88 to 2.47)	0.14
Baseline sVCAM-1 (per 0.5 $\log_e$ ng/mL higher)	1.02 (0.57 to 1.82)	0.95	1.13 (0.62 to 2.07)	0.69	—	—
Time-updated sVCAM-1 (per 0.5 $\log_e$ ng/mL higher)	1.46 (0.78 to 2.75)	0.24	1.56 (0.82 to 2.96)	0.18	1.46 (0.76 to 2.80)	0.25
Baseline TNF-α (per 0.5 $\log_e$ pg/mL higher)	1.53 (0.90 to 2.60)	0.12	1.49 (0.90 to 2.48)	0.12	—	—
Time-updated TNF-α (per 0.5 $\log_e$ pg/mL higher)	1.94 (1.14 to 3.28)	**0.014**	2.00 (1.17 to 3.40)	**0.011**	1.97 (1.16 to 3.34)	**0.012**

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

TABLE 3. Baseline and Time-Updated Biomarker Association With AIDS and Non–AIDS-Defining Events

	Unadjusted		Baseline CD4, NRTI, and NNRTI/PI Adjusted		Time-Updated CD4, NRTI, and NNRTI/PI Adjusted	
Biomarker	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Baseline hsCRP (per 0.5 $\log_e$ μg/mL higher)	1.21 (1.05 to 1.40)	**0.008**	1.22 (1.06 to 1.40)	**0.005**	—	—
Time-updated hsCRP (per 0.5 $\log_e$ μg/mL higher)	1.05 (0.92 to 1.21)	0.46	1.06 (0.92 to 1.22)	0.40	1.06 (0.93 to 1.22)	0.37
Baseline IL-6 (per 0.5 $\log_e$ pg/mL higher)	1.38 (1.10 to 1.71)	**0.004**	1.29 (1.02 to 1.62)	**0.032**	—	—
Time-updated IL-6 (per 0.5 $\log_e$ pg/mL higher)	1.13 (0.89 to 1.44)	0.31	1.11 (0.87 to 1.41)	0.42	1.08 (0.84 to 1.38)	0.54
Baseline sICAM-1 (per 0.5 $\log_e$ ng/mL higher)	1.03 (0.77 to 1.38)	0.83	1.01 (0.76 to 1.35)	0.95	—	—
Time-updated sICAM-1 (per 0.5 $\log_e$ ng/mL higher)	1.04 (0.79 to 1.37)	0.77	1.03 (0.77 to 1.36)	0.86	1.03 (0.79 to 1.35)	0.83
Baseline sTNF-RI (per 0.5 $\log_e$ pg/mL higher)	2.26 (1.25 to 4.07)	**0.007**	1.77 (0.93 to 3.34)	0.080	—	—
Time-updated sTNF-RI (per 0.5 $\log_e$ pg/mL higher)	2.77 (1.37 to 5.62)	**0.005**	2.32 (1.12 to 4.80)	**0.024**	2.35 (1.13 to 4.89)	**0.022**
Baseline sTNF-RII (per 0.5 $\log_e$ pg/mL higher)	1.59 (1.13 to 2.24)	**0.008**	1.48 (1.04 to 2.13)	**0.031**	—	—
Time-updated sTNF-RII (per 0.5 $\log_e$ pg/mL higher)	1.53 (1.06 to 2.21)	**0.024**	1.46 (1.00 to 2.15)	0.051	1.42 (0.96 to 2.10)	0.078
Baseline sVCAM-1 (per 0.5 $\log_e$ ng/mL higher)	1.14 (0.72 to 1.82)	0.57	1.15 (0.72 to 1.85)	0.55	—	—
Time-updated sVCAM-1 (per 0.5 $\log_e$ ng/mL higher)	1.18 (0.71 to 1.97)	0.52	1.20 (0.73 to 1.99)	0.47	1.12 (0.67 to 1.85)	0.67
Baseline TNF-α (per 0.5 $\log_e$ pg/mL higher)	1.46 (0.96 to 2.23)	0.076	1.48 (0.99 to 2.24)	0.059	—	—
Time-updated TNF-α (per 0.5 $\log_e$ pg/mL higher)	1.53 (1.00 to 2.36)	0.052	1.54 (1.00 to 2.38)	0.051	1.53 (0.99 to 2.37)	0.058

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Biomarker Association With First Clinical Event (AIDS or Non-AIDS Events)

A total of 28 first clinical events (AIDS or non-AIDS) were combined (Table 3). Higher values in baseline hsCRP, IL-6, sTNF-RI, and sTNF-RII were significantly associated with an increased risk of time to first AIDS- or non-AIDS clinical event. The Kaplan–Meier plots are shown in Figure 1, stratified by tertiles for these 4 markers. There was also a trend toward higher baseline TNF-α being associated with increased risk. Adjustment for ART assignment and for baseline HIV-1 RNA or CD4 count did not change the results for any of the baseline biomarkers associations with clinical events with the exception of sTNF-RI after adjustment for baseline CD4 which was no longer statistically significant.

The association between time-updated biomarker value and the composite endpoint of clinical events was also assessed. Only the time-updated values for sTNF-RI and sTNF-RII were significantly associated with an increased risk

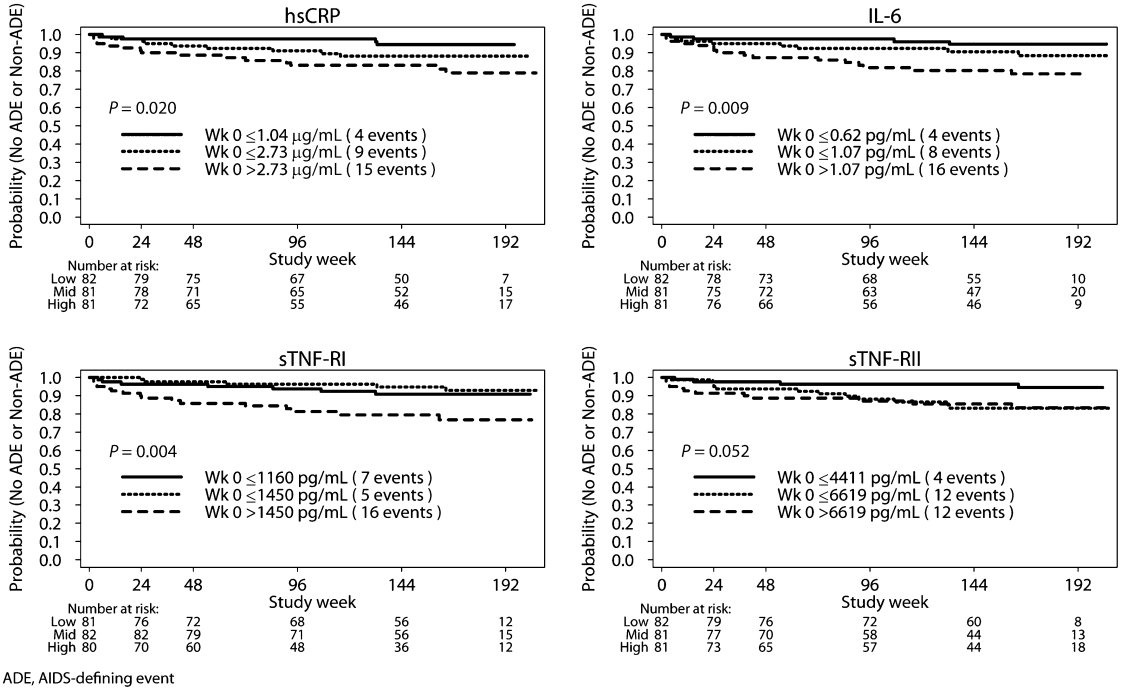

FIGURE 1. Kaplan–Meier plots stratified by tertiles for hsCRP, IL-6, sTNFR-I, and sTNF-RII.

of clinical events. In addition, time-updated biomarker value for TNF-α trended toward being associated with increased risk. Adjustment for ART assignment, for baseline or time-updated CD4 count, or baseline HIV-1 level, or for achieving virological suppression (defined at <50 copies/mL or <200 copies/mL) did not change the results for sTNF-RI but sTNF-RII was no longer statistically significant. Adjusting for ART assignment and for achieving virological suppression (HIV-1 RNA <200 copies/mL) did strengthen the TNF-α association.

Week 24 Landmark Analysis

Among virologically suppressed subjects (HIV-1 RNA <50 copies/mL at week 24), additional analyses were undertaken to explore the association between week 24 biomarker level and time to first event occurring after week 24 (Table 4).

For time to first AIDS or non–AIDS defining event, 171 subjects were virologically suppressed and an additional 8 subjects were removed because of having an early event within the first 24 weeks (5 subjects) or having their baseline biomarker level carried forward to week 24 (3 subjects). Among the 163 subjects analyzed, there were 14 events (9 between week 24 and 96 and 5 after week 96). Week 24 sTNF-RI, sTNFR-II, TNF-α, and sVCAM-1 (per 0.5 $\log_e$ higher) were significantly associated with developing an AIDS or non-AIDS event [hazard ratio (HR) = 3.53, 95% confidence interval (CI): 1.02 to 12.18, $P = 0.046$; HR = 1.80, 95% CI: 1.05 to 3.08, $P = 0.033$; HR = 1.75, 95% CI: 1.04 to 2.96, $P = 0.035$; and HR = 1.96, 95% CI: 1.02 to 3.76, $P = 0.044$, respectively]. After adjustment for ART assignment and week 24 CD4 count, only sTNF-RI remained statistically significant (HR = 4.20, 95% CI: 1.10 to 16.03, $P = 0.036$).

DISCUSSION

The absolute CD4 cell count has been extensively used as a tool to predict HIV disease progression and morbidities. A more recent focus has been on the association between immune activation and inflammatory biomarkers and HIV mortality and co-morbidities on ART. Thus far, several studies have linked a single measurement of an inflammatory marker to subsequent mortality,[10–12] myocardial infarction risk,[13] and increased intima media thickness[14,15] (a marker of vascular disease). In contrast, very limited data exist on the association between either pre-ART or on-ART biomarker values to clinical events. To our knowledge, our study is the largest and longest study to use such an approach to assess this association in a randomized ART-initiation clinical trial. We showed that higher pre-ART and on-ART levels of several inflammatory biomarkers were associated independently of CD4 count with increased risk of progression to AIDS and/or non-AIDS events.

TABLE 4. Landmark Analyses, Week 24 Biomarker Associations Among Subjects Without a Previous Event and HIV RNA Level <50 Copies/mL at Week 24

	Unadjusted		Week 24 CD4, NRTI, and NNRTI/PI Adjusted	
Week 24 Biomarker	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Biomarker association with AIDS-defining events				
hsCRP (per 0.5 $\log_e$ μg/mL higher)	0.89 (0.64 to 1.24)	0.48	0.78 (0.49 to 1.24)	0.29
L-6 (per 0.5 $\log_e$ pg/mL higher)	0.94 (0.50 to 1.78)	0.85	0.83 (0.37 to 1.83)	0.64
sICAM-1 (per 0.5 $\log_e$ ng/mL higher)	2.82 (0.84 to 9.49)	0.094	2.57 (0.56 to 11.88)	0.23
sTNF-RI (per 0.5 $\log_e$ pg/mL higher)	1.69 (0.21 to 13.44)	0.62	2.26 (0.14 to 37.02)	0.57
sTNF-RII (per 0.5 $\log_e$ pg/mL higher)	1.60 (0.58 to 4.39)	0.36	2.07 (0.26 to 16.41)	0.49
sVCAM-1 (per 0.5 $\log_e$ ng/mL higher)	2.69 (1.01 to 7.13)	**0.047**	1.88 (0.51 to 7.00)	0.34
TNF-α (per 0.5 $\log_e$ pg/mL higher)	1.60 (0.61 to 4.19)	0.33	1.20 (0.23 to 6.31)	0.83
Biomarker association with non–AIDS-defining events				
hsCRP (per 0.5 $\log_e$ μg/mL higher)	0.97 (0.78 to 1.19)	0.76	0.96 (0.76 to 1.22)	0.74
L-6 (per 0.5 $\log_e$ pg/mL higher)	1.07 (0.75 to 1.53)	0.73	1.00 (0.68 to 1.46)	0.98
sICAM-1 (per 0.5 $\log_e$ ng/mL higher)	1.60 (0.75 to 3.43)	0.22	1.26 (0.57 to 2.81)	0.57
sTNF-RI (per 0.5 $\log_e$ pg/mL higher)	2.56 (0.69 to 9.60)	0.16	2.94 (0.70 to 12.41)	0.14
sTNF-RII (per 0.5 $\log_e$ pg/mL higher)	1.76 (0.98 to 3.16)	0.060	1.72 (0.89 to 3.35)	0.11
sVCAM-1 (per 0.5 $\log_e$ ng/mL higher)	1.60 (0.74 to 3.42)	0.23	1.43 (0.64 to 3.18)	0.38
TNF-α (per 0.5 $\log_e$ pg/mL higher)	1.66 (0.93 to 2.96)	0.085	1.63 (0.86 to 3.08)	0.13
Biomarker association with AIDS and non-AIDS events				
hsCRP (per 0.5 $\log_e$ μg/mL higher)	0.96 (0.79 to 1.17)	0.69	0.94 (0.76 to 1.17)	0.59
L-6 (per 0.5 $\log_e$ pg/mL higher)	1.04 (0.74 to 1.47)	0.82	1.01 (0.71 to 1.44)	0.95
sICAM-1 (per 0.5 $\log_e$ ng/mL higher)	1.74 (0.85 to 3.54)	0.13	1.42 (0.68 to 2.99)	0.35
sTNF-RI (per 0.5 $\log_e$ pg/mL higher)	3.53 (1.02 to 12.18)	**0.046**	4.20 (1.10 to 16.03)	**0.036**
sTNF-RII (per 0.5 $\log_e$ pg/mL higher)	1.80 (1.05 to 3.08)	**0.033**	1.83 (0.98 to 3.41)	0.059
sVCAM-1 (per 0.5 $\log_e$ ng/mL higher)	1.96 (1.02 to 3.76)	**0.044**	1.76 (0.90 to 3.44)	0.10
TNF-α (per 0.5 $\log_e$ pg/mL higher)	1.75 (1.04 to 2.96)	**0.035**	1.76 (0.98 to 3.16)	0.060

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

The association between pre-ART inflammatory marker levels and the risk of progression to clinical events on suppressive ART has been investigated in few studies. Our findings that higher pre-ART IL-6 is associated with increased risk of AIDS-defining event is consistent with the results of 2 case–control studies.[2,16] One of these studies also found that pre-ART sTNF-RI (but not sTNF-RII) was associated with such a risk.[16] However, in contrast to one of these studies,[2] we did not find an association with baseline hsCRP. Rather, we found that higher baseline hsCRP is associated with increased rate of progression to a non-AIDS event. When we combined progression to AIDS and non-AIDS events, baseline hsCRP and IL-6 were associated with increased risk of clinical progression.

Limited data exist on serial measurements of inflammatory markers in HIV-infected subjects starting their first ART regimen. It was notable in our study that overall, in both unadjusted and CD4-adjusted or HIV-1 RNA–adjusted analyses, time-updated levels in markers of TNF-α levels (levels of TNF-α or its soluble receptors) were associated with clinical events, whereas this was not observed for hsCRP, and less consistently so with IL-6. Importantly, our analyses were also adjusted for ART assignment. The lack of association between hsCRP and events is notable because of our previously described findings of more favorable changes in hsCRP after initiation of TDF/FTC versus ABC/3TC-containing regimens.[6] However, we cannot rule out the possibility that hsCRP could be specifically associated with cardiovascular events as shown by others[12] because only 3 cardiovascular events occurred during study. Also notable is that the findings of an association between markers of TNF-α levels with events is consistent with a previous case–control study in which 48 weeks after initiation of ART, levels of sTNFR-I and sTNFR-II but not CRP or IL-6 were independently associated with incident diabetes.[17]

The lack of association between any baseline or time-updated inflammatory markers and bone fractures is not too surprising because a relationship between HIV-associated heightened inflammation and changes in bone health remains speculative and not proven. Indeed, in 1 previous study, we showed that changes in the inflammatory markers sTNFR-I and -II were not associated with changes in whole-body bone mineral density.[18] Also, all bone fractures that occurred in A5224s were associated with a traumatic event, and thus may not represent pathologic bone health.

Our study has some limitations, including relatively small number of events that limits the precision on the hazard ratio estimates, resulting in wide CIs, possible selection bias of A5224s subjects who have available week 24 and/or 96 samples (possibly favoring healthier subjects with lower inflammation and maybe lower event rates) and the large number of analyses performed without adjustment for multiple comparisons, which may increase the probability of erroneously declaring an association. Also, because we had a relatively small number of events, the adjusted analyses should be interpreted with caution, as the number of events per covariate in some of these models was <10.[19] The small number of events also limited our ability to adjust for all potential confounders such as smoking or hepatitis. The time-updated analyses need to be interpreted with caution because 7 of 13 AIDS events and 4 of 18 non-AIDS occurred before the first postbaseline biomarker measurement time point at week 24, where subjects had to remain event free before through that time point before the baseline value was updated. Nonetheless, this study is the longest study to date that investigated biomarker associations with clinical events in a group of subjects who recently initiated ART with regimens that are still current today. Also, an additional strength of the study is the randomization to the ART regimen, which provides balance in unmeasured covariates.

In summary, we showed that higher levels of several inflammatory biomarkers were associated independently of CD4 with increased risk of AIDS or non-AIDS events. Larger and longer studies should investigate the use of these markers as predictors of clinical end points, especially during long-term viral suppression on ART.

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APPENDIX. AIDS Clinical Trials Group A5224s

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