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Unchanged Levels of Soluble CD14 and IL-6 Over Time Predict Serious Non-AIDS Events in HIV-1-Infected People

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Abstract

HIV-1-infected persons have increased risk of serious non-AIDS events (SNAEs) despite suppressive antiretroviral therapy. Increased circulating levels of soluble CD14 (sCD14), soluble CD163 (sCD163), and interleukin-6 (IL-6) at a single time point have been associated with SNAEs. However, whether changes in these biomarker levels predict SNAEs in HIV-1-infected persons is unknown. We hypothesized that greater decreases in inflammatory biomarkers would be associated with fewer SNAEs. We identified 39 patients with SNAEs, including major cardiovascular events, end stage renal disease, decompensated cirrhosis, non-AIDS-defining malignancies, and death of unknown cause, and age- and sex-matched HIV-1-infected controls. sCD14, sCD163, and IL-6 were measured at study enrollment (T1) and proximal to the event (T2) or equivalent duration in matched controls. Over ~34 months, unchanged rather than decreasing levels of sCD14 and IL-6 predicted SNAEs. Older age and current illicit substance abuse, but not HCV coinfection, were associated with SNAEs. In a multivariate analysis, older age, illicit substance use, and unchanged IL-6 levels remained significantly associated with SNAEs. Thus, the trajectories of sCD14 and IL-6 levels predict SNAEs. Interventions to decrease illicit substance use may decrease the risk of SNAEs in HIV-1-infected persons.

Keywords: HIV, sCD14, IL-6, cardiovascular disease, drug abuse

Introduction

Serious non-AIDS events (SNAEs), including cardiovascular disease (CVD), hepatic and pulmonary disease, and non-AIDS malignancies, have emerged as leading causes of morbidity and mortality with the advent of combination antiretroviral therapy (ART).[1] Metabolic abnormalities from ART, endothelial dysfunction, and chronic inflammation and monocyte/macrophage activation, due, in part, to increased microbial translocation, likely contribute to SNAEs.[2] Key monocyte/macrophage activation markers include soluble CD163 (sCD163), soluble CD14 (sCD14), and interleukin 6 (IL-6). Activated monocytes/macrophages shed sCD163, a hemoglobin scavenger receptor,[3] and shed or secrete sCD14 upon lipopolysaccharide (LPS) stimulation. Thus, sCD14 serves as a surrogate marker for microbial translocation.[4] Monocytes and other cell types produce IL-6, which initiates the acute phase reaction.[5] Increased circulating levels of sCD14, sCD163, and IL-6 at a single time point have been associated with SNAEs.[4,6,7] However, levels of these biomarkers vary widely among study participants and are influenced by many factors. We hypothesized that unchanged or smaller decreases in systemic inflammation in HIV-1-infected persons would predict SNAEs.

Materials and Methods

Study design

The Institutional Review Board at the University of Texas Health Science Center approved the study. All participants signed informed consent. The study cohort included HIV-1-infected persons from a single institution enrolled in a plasma bank study with stored study enrollment (T1) and prospectively collected samples. We retrospectively reviewed charts to identify 39 cases with SNAEs, including cardiovascular events [coronary artery disease (CAD) requiring surgery or intervention, myocardial infarction (MI), cerebrovascular accident

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or transient ischemic attack (CVA/TIA), pulmonary embolism (PE), and cardiac arrest], end stage renal disease (ESRD), decompensated cirrhosis, and non-AIDS malignancies. Patients with SNAEs between January 2003 and August 2014 were designated as cases and patients without SNAEs between January 2003 and August 2014 as controls. We obtained cryopreserved plasma samples from time of enrollment in the plasma bank study (T1) and time proximal to SNAE (T2). We collected descriptive data by chart review, including age, sex, race, $CD4^+$ T-cell count, HIV RNA levels, $CD8^+$ T-cell count, CD4:CD8 ratio, HCV coinfection status, ART status, comorbidities, including diabetes mellitus, hypertension, hyperlipidemia, and their treatment, history of cardiovascular disease (CVD), family history of CVD, smoking, and alcohol and illicit drug use. Aspirin use was not recorded consistently in the electronic medical record.

We identified 39 controls matched for age (± 5 years), sex, and the length of time between the two samples used for analysis of biomarkers. Samples were obtained between 2003 and 2014.

Biomarker analysis

Levels of sCD14, sCD163, and IL-6 were measured on cryopreserved plasma samples from study enrollment (T1) and proximal (T2) to the SNAE (or matched duration in controls). sCD14 and IL-6 were quantified using R&D Systems Quantikine ELISA Kits, Minneapolis, Minnesota, and sCD163 by Trillium Diagnostics' ELISA assay, Bangor, Maine, according to manufacturers' instructions. Assays were performed blinded and in duplicate.

Statistical analysis

Descriptive statistics, including median and interquartile range (25th, 75th percentile) for the continuous variables and frequency and proportion for the categorical variables, were reported for the cases (with events) and matched controls (no events). Logarithmic transformation of the HIV RNA levels was performed for further analysis. Biomarker changes between T1 and T2 (T2-T1) for each subject were calculated. The changes within each group and the difference in changes within each matched pair between two groups were tested using Wilcoxon signed rank test. For other variables, including demographic and clinical characteristics, Wilcoxon signed rank test for the continuous variables and McNemar's test/univariate conditional logistic regression for categorical variables were used to test their associations with the events. Multivariate analysis included variables with $p < .20$ from univariate analysis. Conditional logistic regression was used to test the association between the variables and event adjusting these variables. $p < .05$ were considered statistically significant. SAS software version 9.4 (SAS Institute, Cary, NC) was used for all analyses.

Results

We identified 50 patients with SNAEs from 284 HIV-1-infected persons enrolled in the plasma bank study. Thirty-nine had stored samples from two time points (study enrollment [T1] and proximal to event [T2]). Patient characteristics are shown in Table 1. The median age at T1 was 48 years for cases and 47 for controls ($p = .002$); 31 (79%) were male in both groups. Current illicit drug use at T1 was significantly more common among cases compared to controls (36% vs. 10%, $p = .01$). Illicit drug use included cocaine ($N = 16$), opiates ($N = 12$), cannabinoids ($N = 9$), benzodiazepines ($N = 4$), amphetamine ($N = 2$), phencyclidine (PCP; $N = 2$), and barbiturates ($N = 1$). Eleven participants used more than one substance. HCV antibody positivity rates were similar: 72% ($N = 28$) in cases and 67% ($N = 26$) in controls. Four cases and four controls had cleared HCV spontaneously. Of 12 participants treated with anti-HCV treatment (without direct acting antivirals), two achieved SVR (both cases); thus, 22 in each group had active HCV infection. There were no other statistically significant differences between cases and controls in characteristics at T1, including $CD4^+$ T-cell count nadir, hypertension, diabetes mellitus, hyperlipidemia, statin treatment, and smoking or alcohol use. The median time between T1 and T2 was 34 months in cases and 35 months in controls; the median time between T2 and event was 18 months. Cases and controls did not differ significantly at T1 or T2 in the proportion taking ART, the percentage with undetectable plasma HIV RNA levels, or $CD4^+$ T-cell count (Table 1). However, the distribution of HIV RNA levels at T2 was significantly higher in cases than controls, driven by the minority with detectable viremia (2.60 vs. 2.60, $p = .0001$). Thus, age and current drug use, and HIV RNA level at T2, differed significantly between cases and controls.

We characterized the frequencies of SNAEs. The most prevalent SNAE was CVD ($N = 14$), which included six patients with MI & CAD requiring surgery or procedure, four patients with CVA/TIA, three patients with cardiac arrest, and one patient with PE. Twelve patients had non-AIDS malignancies ($N = 12$), including lung cancer ($N = 3$), Hodgkin's lymphoma ($N = 2$), hepatocellular carcinoma ($N = 1$), colon cancer ($N = 1$), renal-cell carcinoma ($N = 1$), urethral squamous cell carcinoma ($N = 1$), vaginal squamous cell carcinoma ($N = 1$), Burkitt's lymphoma ($N = 1$), and myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) ($N = 1$). Four patients developed ESRD and three patients had decompensated cirrhosis or acute liver failure. Non-AIDS-related deaths included one patient that died from septic shock and five with unknown causes of death, likely sudden cardiac events. In sum, most SNAEs were cardiovascular events and malignancies.

To determine the change over time in biomarkers associated with SNAEs, we measured sCD14, sCD163, and IL-6 in cases and controls at T1 and T2. All biomarkers decreased significantly between T1 and T2 in controls: sCD14 (median $\Delta = -0.5$ μg/mL, $p < .0001$), sCD163 ($\Delta = -359$ pg/mL, $p = .002$), and IL-6 ($\Delta = -0.2$ pg/mL, $p = .04$) (Table 2). However, only sCD163 decreased significantly in the cases ($\Delta = -286$ pg/mL, $p = .02$). Levels of sCD14, sCD163, and IL-6 at T1 or T2 were not predictive of events, likely because of the small sample size, and the average of biomarker levels at T1 and T2 was not predictive of events. Biomarker levels did not differ between viremic and aviremic participants. However, patients were more likely to have SNAEs if they had a smaller decrease or no change in sCD14 (odds ratio [OR] 2.4 per 1 μg/mL difference; 95% CI 1.1–5.4) or IL-6 (OR 1.2 per 1 pg/mL difference; 95% CI 1.0–1.4) levels, but the rate of decline in sCD163 levels was not associated with SNAEs. Thus, unchanged or slow decreases of sCD14 and IL-6 levels were associated with SNAEs.

Next, we evaluated the association of patient characteristics with events. In univariate analysis, older age at T1 or T2 was associated with increased risk of events (OR 1.4 [1.1–1.9] and 1.4 [1.1–1.7] per year of age, respectively). Patients of white/

Table 1. Patient Characteristics

Characteristic	*Cases* N = *39 (%)*	*Controls* N = *39 (%)*	p
Median age at T1 in years (25th, 75th)	48 (41,53)	47 (41,51)	.002
Median age at T2 in years (25th, 75th)	51 (44,56)	50 (44,54)	.006
Median time T2-T1 in months (25th,75th)	34 (15,49)	35 (20,49)	.52
Median time event-T2 in months (25th, 75th)	18 (5,42)	N/A	N/A
Male	31 (79)	31 (79)	Exactly matched
Race			
White/Caucasian	11 (28)	6 (15)	ref
African American	23 (59)	16 (41)	.52
White/Hispanic	5 (13)	17 (44)	.02
Median CD4 (T1) (25th,75th) (cells/mm^3)	378 (229,574)	376 (96,536)	.23
Median CD4 (T2) (25th,75th) (cells/mm^3)	392 (260,589)	435 (312,506)	.79
Median HIV RNA (T1) (25th, 75th) ($\log_{10}$ copies/mL)	3.94 (2.60,4.64)	3.90 (2.60,5.46)	.38
Median HIV RNA (T2) (25th, 75th) ($\log_{10}$ copies/mL)	2.60 (2.60,4.36)	2.60 (2.60,3.20)	.0001
Subjects on ART (T1)	34 (87)	38 (97)	.22
Subjects on ART (T2)	32 (82)	35 (90)	.52
Undetectable HIV RNA (T1)	12 (31)	11 (28)	1.00
Undetectable HIV RNA (T2)	22 (56)	23 (59)	1.00
HCV Antibody + (T1)	28 (72)	26 (67)	.59
Statin treatment (T1)	11 (28)	8 (21)	.62
Diabetes (T1)	13 (33)	11 (28)	.81
Hypertension (T1)	28 (72)	24 (62)	.45
Dyslipidemia (T1)	27 (69)	23 (59)	.50
Smoking (T1)			
Current	20 (51)	12 (31)	.18
Past	6 (15)	9 (23)	1.00
Never	13 (33)	18 (46)	ref
Alcohol (T1)			
Current	18 (46)	12 (31)	.23
Past	2 (5)	1 (3)	.85
Never	19 (49)	26 (67)	ref
Illegal drug use (T1)			
Current	14 (36)	4 (10)	.01
Past	5 (13)	4 (10)	.42
Never	20 (51)	31 (80)	ref

ref, reference group for comparison.
ART, antiretroviral therapy.

Hispanic race were less likely to have events than patients of white/non-Hispanic race (OR 0.05, 95% CI 0.004–0.59). African Americans were more likely to have events than patients of white/Hispanic race (OR 11.4, 95% CI 1.44–89.5). No significant differences between African Americans and white/non-Hispanic groups were identified. Median $CD4^+$ T-cell counts and HIV-1 RNA levels at T1 or T2 were not associated with increased risk of events. Current illicit substance users were more likely to have events than nonusers (OR 5.3; 95% CI 1.3–31.6). Of 18 illicit substance users, eight had HIV RNA levels greater than 400 copies/mL, but there were no significant differences in biomarker levels or in rates of change in biomarkers between current users and nonusers (data not shown). Former illicit substance users did not have a statistically significant increased likelihood of having an SNAE compared to nonusers (OR 2.8; 95% CI 0.4–26.9). There was no significant association of HCV coinfection status (OR 1.3; 95% CI 0.4–4.7), dyslipidemia (OR 1.4; 95% CI 0.5–4.4), or statin use (OR 1.5; 95% CI 0.6–4.2) with SNAEs. In sum, older age, white/non-Hispanic race, and current substance abuse were associated with increased SNAEs.

Multivariate analysis was performed as age, substance use, severity of HIV disease, and other factors may increase levels of inflammatory biomarkers. Change in sCD14 was not significantly associated with SNAEs after adjusting for change in IL-6, age at T2, and substance use. However, patients with smaller or absent decreases in IL-6 levels were more likely to develop SNAEs while controlling for age at T2 and illicit drug use (OR 1.5 per 1 pg/mL difference; 95% CI 1.1–2.0). Older patients also had increased likelihood for events while controlling for IL-6 change and illicit drug use (OR 1.6 per 1 year greater age; 95% CI 1.1–2.4). Controlling for IL-6 change and age at T2, current illicit drug users were more likely to develop an event compared to never users (OR 12.2; 95% CI 1.7–86.7). Past users had a nonsignificantly increased risk for events compared to never users (OR 7.3; 95% CI 0.7–73.2). These findings remained significant when including sCD14 in the model. Thus, older age, current substance use at T1, and rate of IL-6 decrease independently predict SNAEs.

Discussion

High levels of inflammatory markers at any single time point predict SNAEs in HIV-1-infected persons,[4,6,7] but whether the rate of change in biomarker levels predicts SNAEs has not been established. We found that levels of

Table 2. Biomarker Levels at Initial Time Point (T1), Time Proximal to Event (T2), and Change in Biomarkers Between T1 and T2

	Cases				*Controls*				*Difference in T2-T1 (Case–Control)*	
Marker	*T1 median (IQR)*	*T2 median (IQR)*	*T2-T1 median (IQR)*	p	*T1 median (IQR)*	*T2 median (IQR)*	*T2-T1 median (IQR)*	p		p
sCD14 (μg/mL)	2.1 (1.7, 2.3)	1.8 (1.4, 2.4)	−0.1 (−0.5, 0.2)	.14	1.9 (1.4, 2.3)	1.5 (1.2, 1.9)	−0.5 (−1.1, 0.0)	<.0001	0.2 (−0.1, 1.1)	.01
sCD163 (ng/mL)	1978 (1084, 3011)	1671 (947, 2638)	−286 (−884, 203)	.02	1763 (1171, 2535)	1157 (765, 1799)	−359 (−1055, 47)	.002	428 (−507, 829)	.26
IL-6 (pg/mL)	2.8 (1.8, 5.6)	3.8 (1.7, 4.8)	−0.2 (−1.6, 2.0)	.68	2.6 (1.7, 5.3)	1.9, (1.5, 3.0)	−0.2, (−3.7, 0.6)	.04	1.1, (−0.8, 4.8)	.02

sCD14 and IL-6, but not sCD163, were unchanged in patients who developed SNAEs but decreased in controls. The reason for this difference is unclear, as both groups had similar rates of ART use, undetectable HIV RNA levels, $CD4^+$ T-cell counts, and active HCV infection. The degree to which HIV RNA levels influence these changes warrants further study with a larger number of viremic participants. Monocytes/macrophages from the controls may respond more quickly to the decreased viral stimulus and, consequently, decrease sCD14 and IL-6 production. As LPS induces sCD14 and IL-6 production but not sCD163,[8,9] the data suggest that slower decline in LPS-mediated inflammation specifically is associated with SNAEs. However, the consistent decrease in sCD163 may reflect shifts in monocyte populations (e.g., $CD14^+CD16^+$ monocytes) rather than decreased microbial translocation.[3] Alternatively, the underlying comorbidities that contribute to SNAEs may have continued to keep inflammatory markers elevated in the cases, reflecting association rather than causation.

Whereas previous studies have identified cigarette smoking as a possible contributor to acute coronary syndrome, we did not observe that in our cohort.[10] Our small study had fewer CVD events compared to others and the cases tended to be smokers more often than controls. We also could not capture the amount of smoking in our retrospective study. However, current illicit drug use at study enrollment emerged as a key factor that was associated with SNAEs. Illicit drug use, especially cocaine, is associated with increased acute coronary syndrome risk among young adults.[11] Drug use likely affected adherence, based on the number with detectable HIV RNA levels, and intermittent stopping and starting ART can increase SNAEs.[12] Data are conflicting on whether cocaine and/or heroin increase sCD14 and IL-6 levels in persons with or without HIV infection.[13–17] The effects of illicit substances on inflammatory markers in HIV-1-infected persons on ART are unknown. Regardless, these data suggest that aggressive drug rehabilitation may be an additional intervention to lower SNAE risk in HIV-1-infected persons.

Our study was limited by small sample size and few time points measured, both of which may have limited detection of conventional risk factors, including $CD4^+$ T-cell counts and initial biomarker levels as risk factors for SNAEs, and may limit the generalizability. The high HCV infection rate in our population may also limit generalizability as HCV can increase microbial translocation and monocyte activation.[18] Nonetheless, the small sample size and consistent presence of HCV coinfection in most participants did not limit our ability to detect the association of changes in biomarkers over time with end-organ events.

Taken together, our data show that unchanged or slow rates of decrease of sCD14 and IL-6, but not sCD163, levels predict SNAEs. These patients may have less of a decline in microbial translocation with ART or different monocyte/macrophage phenotypes. Thus, changes in biomarker levels over time in addition to absolute values may facilitate risk stratification of HIV-1-infected persons and identify persons who need further immunomodulatory interventions to avoid SNAEs.

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Author Disclosure

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References

1. Palella FJ, Baker RK, Moorman AC, *et al.*: Mortality in the highly active antiretroviral therapy era: Changing causes of death and disease in the HIV outpatient study. J Acquir Immune Defic Syndr 2006;43:27–34.
2. Islam FM, Wu J, Jansson J, *et al.*: Relative risk of cardiovascular disease among people living with HIV: A systematic review and meta-analysis. HIV Med 2012;13: 453–468.
3. Burdo TH, Lentz MR, Autissier P, *et al.*: Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after anti-retroviral therapy. J Infect Dis 2011;204:154–163.
4. Sandler NG, Wand H, Roque A, *et al.*: Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis 2011;203:780–790.
5. Fonseca JE, Santos MJ, Canhão H, *et al.*: Interleukin-6 as a key player in systemic inflammation and joint destruction. Autoimmun Rev 2009;8:538–542.
6. Tenorio AR, Zheng Y, Bosch RJ, *et al.*: Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect Dis 2014;210:1248–1259.
7. Nordell AD, McKenna M, Borges ÁH, *et al.*: Severity of cardiovascular disease outcomes among patients with HIV is related to markers of inflammation and coagulation. J Am Heart Assoc 2014;3:e000844.
8. Sandler NG, Douek DC. Microbial translocation in HIV infection: Causes, consequences and treatment opportunities. Nat Rev Microbiol 2012;10:655–666.
9. Etzerodt A, Rasmussen MR, Svendsen P, *et al.*: Structural basis for inflammation-driven shedding of CD163 ectodomain and tumor necrosis factor-alpha in macrophages. J Biol Chem 2014;289:778–788.
10. Calvo-Sánchez M, Perelló R, Pérez I, *et al.*: Differences between HIV-infected and uninfected adults in the contributions of smoking, diabetes and hypertension to acute coronary syndrome: Two parallel case-control studies. HIV Med 2013;14:40–48.
11. Carrillo X, Curós A, Muga R, *et al.*: Acute coronary syndrome and cocaine use: 8-year prevalence and inhospital outcomes. Eur Heart J 2011;32:1244–1250.
12. El-Sadr WM, Lundgren J, Neaton JD, *et al.*: CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med 2006;355:2283–2296.
13. Ancuta P, Kamat A, Kunstman KJ, *et al.*: Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. PLoS One 2008;3:e2516.
14. Pedraz M, Araos P, Garcia-Marchena N, *et al.*: Sex differences in psychiatric comorbidity and plasma biomarkers for cocaine addiction in abstinent cocaine-addicted subjects in outpatient settings. Front Psychiatry 2015;6:17.
15. Meijerink H, Indrati A, Utami F, *et al.*: Heroin use is associated with suppressed pro-inflammatory cytokine response after LPS exposure in HIV-infected individuals. PLoS One 2015;10:e0122822.
16. Chan YY, Yang SN, Lin JC, *et al.*: Inflammatory response in heroin addicts undergoing methadone maintenance treatment. Psychiatry Res 2015;226:230–234.
17. Neri M, Panata L, Bacci M, *et al.*: Cytokines, chaperones and neuroinflammatory responses in heroin-related death: What can we learn from different patterns of cellular expression? Int J Mol Sci 2013;14:19831–19845.
18. Sandler NG, Koh C, Roque A, *et al.*: Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. Gastroenterology 2011;141:1220–1230.

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