Title

Do Biomarkers of Inflammation, Monocyte Activation, and Altered Coagulation Explain Excess Mortality Between HIV Infected and Uninfected People?

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Title: Do Biomarkers Of Inflammation, Monocyte Activation And Altered Coagulation Explain Excess Mortality Between HIV Infected and Uninfected People?

Short title: Innate immunity, HIV status and mortality

Key points: HIV infection is associated with elevated IL-6, sCD14 and D-dimer, which are in turn are associated with mortality. Baseline measures of these biomarkers partially explain the excess risk of mortality among HIV infected compared to uninfected people.

Keywords: HIV; mortality; inflammation; monocyte activation; coagulation

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Abstract

Background: HIV infection and biomarkers of inflammation (measured by interleukin-6 [IL-6]), monocyte activation (soluble CD14 [sCD14]), and coagulation (D-dimer) are associated with morbidity and mortality. We hypothesized that these immunologic processes mediate (explain) some of the excess risk of mortality among HIV infected (HIV+) versus uninfected people independently of co-morbid diseases.

Methods: Among 2350 (1521 HIV+) participants from the Veterans Aging Cohort Study Biomarker Cohort (VACS BC) we investigated whether the association between HIV and mortality was altered by adjustment for IL-6, sCD14 and D-dimer, accounting for confounders. Participants were followed from date of blood draw for biomarker assays

(baseline) until death or 7/25/2013. Analyses included ordered logistic regression and Cox Proportional Hazards regression.

Results: During 6·9 years (median), 414 deaths occurred. The proportional odds of being in a higher quartile of IL-6, sCD14 or D-dimer was 2-3 fold higher for viremic HIV+ versus uninfected people. Mortality rates were higher among HIV+ compared to uninfected people (incidence rate ratio (95% CI): 1·31 (1·06-1·62). Mortality risk increased with increasing quartiles of IL-6, sCD14 and D-dimer regardless of HIV status. Adjustment for IL-6, sCD14 and D-dimer partially attenuated mortality risk among HIV+ people with unsuppressed viremia (HIV-1 RNA≥10000 copies/mL) compared to uninfected people – hazard ratio (95% CI) decreased from 2·18 (1·60-2·99) to 2·00 (1·45-2·76).

Conclusions: HIV infection is associated with elevated IL-6, sCD14 and D-dimer, which are in turn associated with mortality. Baseline measures of these biomarkers partially mediate excess mortality risk among HIV+ versus uninfected people.

Introduction

_ENREF_1Biomarkers of inflammation (measured by interleukin-6 [IL-6]), monocyte activation (soluble CD14 [sCD14]), and coagulation (D-dimer) are associated with morbidity and increased mortality among HIV infected (HIV+) and uninfected people. [1-5] However, it is unclear whether these immunologic processes explain the excess risk of mortality among HIV+ versus uninfected people [6,7] independently of co-morbid diseases_ENREF_9. There are sparse data comparing HIV+ to uninfected people who have similar demographic and behavior characteristics (i.e., prevalence of smoking and alcohol consumption) while also accounting for HIV-specific biomarkers, comorbidities, substance

use, biomarkers of inflammation, monocyte activation and coagulation, and complete capture of mortality events.

Our objective, therefore, was to determine whether increased inflammation, monocyte activation and coagulation explain the excess mortality risk among HIV+ compared to uninfected people. Data for these analyses were from the Veterans Aging Cohort Study Biomarker Cohort (VACS BC), an observational, longitudinal cohort of HIV+ and uninfected Veterans in care with detailed phenotypic data, biomarkers of immune function, and thorough capture of mortality outcomes. We assessed whether the association between HIV status and mortality persisted after adjusting for multiple potentially confounding co-morbid conditions alone and when combined with IL-6, sCD14 and D-dimer.

Methods

Cohort

_ENREF_12The VACS BC is a subset of VACS 8,[8] a prospectively enrolled observational longitudinal study of HIV+ and uninfected veterans matched on age, race-ethnicity, sex and geographic region.[8] In 2005-2006, 1,525 HIV+ and 843 uninfected VACS 8 participants consented to provide blood samples forming the VACS BC as previously described.[9] These specimens were collected using serum separator and EDTA blood collection tubes and shipped to a central repository at the Massachusetts Veterans Epidemiology Research and Information Center in Boston, Massachusetts. The date of the blood draw was used as the baseline date for each participant in the VACS BC. Those with available measurements of IL-6, sCD14, D-dimer and HIV-1 RNA (for HIV+) were included in analyses. Participants were followed from their baseline date until death or censored on 7/25/2013.

Independent, dependent, and potentially mediating variables

HIV status was the primary independent variable. We collected data on HIV-1 RNA, CD4+ T-cell (CD4) count, and antiretroviral therapy use at baseline. We used HIV-1 RNA measurements obtained as part of clinical care at baseline (±180 days). Death was the primary outcome. It was determined from the VHA vital status file, which uses inputs from the social security administration death master file, the Beneficiary Identification and Records Locator Subsystem, and the VHA Medical Statistical Analysis Systems inpatient datasets. We assessed whether biomarkers of inflammation (IL-6), monocyte activation (sCD14) and altered coagulation (D-dimer) altered the association between HIV and mortality (see description of mediation in statistical analysis below). IL-6, sCD14 and D-dimer were assessed as categorical values (quartiles) or as a composite inflammatory burden score (number of elevated biomarkers i.e. ≥75th percentile threshold among those who died).[10] Measurement of these biomarkers has been previously described._ENREF_13[9]

Covariates

Baseline covariate data, obtained closest to the time of blood draw, have been previously described.[9]_ENREF_13 Briefly, sociodemographic data included age, sex, and race-ethnicity. Cardiovascular disease (CVD) was defined by myocardial infarction,[11,12] and diagnostic or procedural codes for congestive heart failure, coronary artery bypass graft, percutaneous coronary intervention, or ischemic stroke. Cancer was determined using VA Central Cancer Registry data.[13] Chronic obstructive pulmonary disease (COPD) was defined by ICD-9 code.[14] Hypertension was categorized as no hypertension (untreated BP <120/80 mmHg); pre-hypertension (untreated BP 120-139/80-89 mmHg); controlled hypertension (treated

BP <140/90 mmHg); or uncontrolled hypertension (BP ≥140/90 mmHg).[15] Diabetes was diagnosed using a combination of glucose measurements, use of insulin or oral hypoglycemic agents, and/or ICD-9 codes.[16] Smoking was self-reported and obesity was defined as body mass index (BMI) >30 kg/m^2. _ENREF_18 _ENREF_17Cholesterol lowering medication use (HMG CoA reductase inhibitor (statins) or gemfibrozil) was assessed using patient pharmacy data. Total cholesterol measurements were obtained from the VA Decision Support System (DSS) and categorized as <200 mg/dL untreated, <200 mg/dL treated, or ≥200 mg/dL.[17] Medication data were from the VA Pharmacy Benefits Management database.

Cocaine and alcohol use at baseline were determined by self-report. We categorized alcohol use with data from the Alcohol Use Disorders Identification Test (AUDIT-C) and alcohol abuse and dependence diagnoses using ICD-9 codes based on prior work in VACS as_ENREF_8: 1) Low risk current drinking, 2) no current drinking, 3) at-risk or heavy current drinking, and 4) alcohol abuse or dependence diagnosis and current drinking. Current drinking was defined as any drinking reported in the prior 12 months. VACS index was calculated as previously described.[18,19] Hepatitis C virus (HCV) infection was defined as a positive HCV antibody test or at least 1 inpatient and/or 2 outpatient ICD-9 codes.[20]_ENREF_8 Liver fibrosis was estimated using FIB-4 scores.[21] Hemoglobin was dichotomized at 12g/dL. Renal disease was defined as an estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73m^2.[22]

Statistical analysis

We compared continuous variables (t-test or median test) and categorical variables (chi-squared test) by HIV status overall and among participants who died. Kaplan-Meier curves were used to describe time to death by HIV status and/or elevations in IL-6, D-dimer, sCD14 and inflammatory burden (number of elevated biomarkers i.e. ≥75th percentile threshold among those who died).

We adapted the method described by Baron and Kearny [23] and MacKinnon et al [24] to assess whether these immunological biomarkers mediate (explain) the relationship between HIV and mortality. This approach _ENREF_8requires fulfillment of four conditions: 1) a significant relation between the independent and dependent variables, 2) a significant relation between the independent and mediating variables, 3) a significant relation between the mediating and dependent variables after adjustment for the independent variable, 4) given 1-3 hold, an attenuation (in absolute value) of the association between the independent and dependent variables following adjustment for the mediating variable.

Proportional odds models were used to estimate the association between HIV (stratified by HIV-1 RNA <500, 500-9999, ≥10000 copies/mL) and elevated IL-6, sCD14 and D-dimer. The proportional odds model estimates the proportional odds of being above the Nth quartile of the biomarker distribution versus being in the N^{th} quartile or lower based on an assumption of proportional odds. To illustrate: the model assumes that coefficients that describe the relationship between the 3rd and 4th quartiles versus 1st and 2nd quartiles of IL-6 are the same as those that describe the relationship between the 2nd, 3rd, and 4th quartiles versus the 1st quartile. We selected this model because it is more parsimonious than a set of

logistic regression models for each pair of quartiles while still incorporating all levels of the different outcome variables. This assumption was assessed using the Brant Test (Stata Spost package)[25] and found to be valid for all final models except sCD14. Sensitivity analyses using multinomial logistic regression for sCD14 showed consistent results.

Cox proportional hazards models were used to estimate the associations between HIV (stratified by HIV-1 RNA) and mortality adjusting for multiple confounders. All analyses were performed using Stata 13 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP). P-values<0.05 were considered statistically significant.

Results

Of 2389 participants who provided blood specimens, 35 did not have IL-6, sCD14, and D-dimer measured, 4 HIV+ participants had missing HIV-1 RNA and 1 patient subsequently withdrew consent. Of the remainder, 829 were HIV uninfected and 1521 were HIV+. During a median of 6.9 (interquartile range 6·2-7·4) years from baseline (i.e. date of blood draw), 414 deaths occurred (15% of uninfected and 19% of HIV+). Compared to uninfected participants, HIV+ participants were younger and less likely to be female (Table 1). They also had less prevalent cardiovascular disease (14 vs. 25%), diabetes (20 vs. 30%), BMI>30 kg/m^2 (16 vs. 46) and alcohol abuse/dependence (28 vs. 24%), and more hepatitis C (47 vs. 31%), FIB-4 greater than 3.25 i.e. suggestive of advanced fibrosis (9 vs. 4%) and hemoglobin <12g/dL (12 vs. 7%) at baseline (Table 1).

HIV and mortality

Mortality rates per 100 person years were higher among HIV+ versus uninfected people (incidence rate ratio (95% CI): 1·31 (1·06-1·62). Compared to uninfected participants, HIV

infection with HIV-1 RNA≥500-9999 and ≥10000 copies/mL was associated with a higher risk of mortality in age and race-ethnicity adjusted models (Hazard ratio (95% CI): 1·55 (1·09-2·19) and 2·94 (2·22-3·91) respectively). This increased risk remained for both HIV groups after further adjusting for comorbid diseases, substance use, and VACS Index components but was only statistically significant among those with HIV-1 RNA≥10000 copies/mL (1·34 (0·93-1·92) and 2·18 (1·60-2·99) respectively). HIV infected people with CD4+ T-cell count below 350 cells/mm^3 had increased mortality risk relative to uninfected people (1.67 (1.28-2.18)). The association of HIV status stratified by ART receipt at baseline and mortality did not reach statistical significance (data not shown).

HIV and IL-6, sCD14 and D-dimer

After adjustment for demographics, comorbidities and substance use, HIV+ participants with viremia ≥10000 copies/mL had greater proportional odds of elevated IL-6, sCD14, and D-dimer relative to uninfected participants (Table 2). The proportional odds (95% CI) of being in a higher quartile of IL-6, sCD14 or D-dimer was 2-3 fold higher for HIV+ (HIV-1 RNA≥ 10000 copies/mL) versus uninfected people (Table 2).

IL-6, sCD14, D-dimer and mortality

Mortality rates increased with elevations in IL-6, sCD14 and D-dimer (Table 3) and inflammatory burden (Figure 1) among HIV+ and uninfected participants. In Cox proportional hazards models, IL-6, sCD14, and D-dimer elevations (highest quartile) were significantly associated with mortality risk independently of HIV infection or viral suppression (Table 3). These associations were attenuated but persisted after adjustment for comorbid disease, substance use and VACS Index components and the remaining two inflammatory biomarkers (Table 3). The association of elevated sCD14 and mortality

persisted after comorbidity adjustment (Supplemental Digital Content Table 1) but was no longer statistically significant after adjustment for IL-6 and D-dimer (Table 3). These results were consistent in analyses excluding HIV infected people with unsuppressed viral replication (HIV-1 RNA≥500 copies/mL; Supplemental Digital Content Table 2).

Association of HIV and mortality adjusting for IL-6, sCD14 and D-dimer

The association between HIV (with HIV-1 RNA ≥10000 copies/mL or CD4+ T-cell count<350 cells/mm^3) and mortality was partially attenuated after further adjusting the Cox models for IL-6, sCD14, and D-dimer (Table 4). The degree of attenuation was greatest when all three biomarkers were considered simultaneously as quartiles within a single model (Table 4). The risk of death among those with HIV-1 RNA ≥10000 copies/mL went from 2·18 (1·60-2·99) to 2·00 (1·45-2·76) when IL-6, sCD14 and D-dimer were included in the model (Table 4). Similar attenuation was not observed among those with HIV-1 RNA<10000 copies/mL. Relative to uninfected people, the risk of death for those with CD4+ T-cell counts<350 cells/mm3 went from 1.67 (1.28-2.18) to 1.63 (1.25-2.14) after adjustment for IL-6, sCD14 and D-dimer.

We did not find significant interactions between HIV status and biomarker elevations on mortality risk (p≥0.1).

Discussion

We report that HIV infection is associated with biomarkers of inflammation, monocyte activation, and altered coagulation and an increased risk of death compared to those without HIV infection. These biomarkers are also associated with an increased risk of

mortality, independently of HIV status or viremia. After adjustment for comorbid diseases and substance use, biomarkers of inflammation, monocyte activation, and altered coagulation partially explain the excess risk of mortality among viremic HIV infected people compared to uninfected people.

Our results are consistent with prior work linking elevated biomarkers of immune function to an increased risk of mortality among HIV infected people.[4,5] The lack of uninfected comparators in prior work makes it challenging to assess if these biomarkers contribute to excess mortality among HIV infected people. With our cohort of HIV infected and demographically and behaviorally similar uninfected participants, we have extended these findings. Our results show that some of the excess risk of mortality among viremic HIV infected people is explained by biomarkers of inflammation, monocyte activation, and altered coagulation.

This study brings together a number of important findings within a single, well-phenotyped cohort of HIV infected and uninfected people with thorough capture of mortality outcomes. The fact that 1) mortality decreases with viral suppression (HIV-1 RNA<500 copies/mL in this study), 2) these biomarkers do not attenuate the association between HIV infection and mortality among those with lower HIV-1 RNA, and 3) HIV viremia is associated with higher levels of these biomarkers all support the hypothesis that HIV viremia increases the levels of inflammation, monocyte activation, and altered coagulation, which drive increased mortality. Importantly, our results also demonstrate that the three biomarkers we studied do not explain the majority of the excess risk of mortality associated with HIV infection in our cohort. If immune system activation drives this excess risk, our finding may be explained by the fact that three biomarkers, when measured only at baseline, cannot fully

capture the complexity of immune system activation. Additionally, mechanisms beyond inflammation, monocyte activation, and altered coagulation may contribute to the excess risk of mortality associated with viremic HIV infection. Further, these biomarkers can change for reasons unrelated to HIV e.g. comorbid conditions. Lastly, while these specific biomarkers do not explain most of the excess total mortality risk associated with HIV infection, they may explain more cause-specific mortality.

This study has limitations that warrant discussion. First, as the majority of our cohort is men, our results may not be generalizable to women. Second, our analysis did not have multiple longitudinal measures of inflammatory biomarkers to assess the impact of changes in the biomarkers on mortality risk. Third, while all three of our selected biomarkers are associated with HIV infection and increased risk of mortality among those with and without HIV, there are other potentially important biomarkers (e.g., CD163, TNF alpha) and immunologic processes that were not included in our analysis. Finally, like all observational studies, we cannot eliminate the possibility of unmeasured or residual confounding.

In conclusion, increased HIV viral loads are associated with higher levels of biomarkers of inflammation (IL6), monocyte activation (sCD14), and altered coagulation (D-dimer). Elevated levels of these biomarkers are associated with mortality among HIV infected and uninfected people and in combination, these biomarkers partially explain the excess risk of mortality among viremic HIV infected compared to uninfected people.

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Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs.

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Tables & Figures

Table 1: Characteristics of study population at baseline

Table 2: Association between HIV infection and IL-6, sCD14, and D-dimer adjusted for a) age and race-ethnicity b) all covariates.

Table 3: Mortality rates, rate ratios and risks by IL-6, sCD14 and D-dimer quartile mutually adjusted for each other and adjusted for HIV status and co-morbid conditions

Table 4: Assessing whether addition of inflammatory biomarkers to Cox regression models attenuates the association between HIV infection (stratified by HIV-1 RNA at baseline) and mortality.

Figure 1(a-d): Kaplan-Meier survival curves describing mortality by HIV status and a) IL-6, b) sCD14 and c) D-dimer and d) inflammatory burden (number of inflammatory biomarkers (0, 1 or 2+) elevated >75th percentile). IL-6, sCD14 and D-dimer elevation thresholds were defined as ≥75th percentile among those who died. For, IL-6 this threshold was 5·043 pg/mL, for sCD14 it was 2356·12 ug/mL, and for D-dimer, it was 0·88 ug/mL

Supplemental digital content Table 1: Mortality risk by a) IL-6, b) sCD14 and c) D-dimer quartile (IL-6, sCD14 and D-dimer in separate models)

Supplemental digital content Table 2: Mortality risk by IL-6, sCD14 and D-dimer quartile excluding HIV infected people with unsuppressed viremia (IL-6, sCD14 and D-dimer in same model)

Table 1: Characteristics of study population at baseline

Data are column percentages (N) unless otherwise specified	Total		Died	
	HIV-	**HIV+**	**HIV-**	**HIV+**
N	829	1521	125	289
Demographics				
Age	54·1 (9·4)	52·3 (8·2)	59·0 (10·5)	54·6 (7·6)
Female	10 (79)	3 (42)	2 (2)	1 (4)
Race				
White	21 (174)	19 (287)	30 (37)	16 (47)
Black	67 (555)	69 (1050)	57 (71)	74 (206)
Hispanic	8 (66)	8 (126)	10 (13)	6 (18)
Other	4 (34)	4 (58)	3 (4)	4 (11)
Comorbid diseases				
Prevalent CVD	25 (209)	14 (215)	44 (55)	22 (63)
Prevalent cancer	7 (57)	6 (97)	10 (13)	12 (36)
Controlled hypertension	57 (473)	50 (758)	58 (72)	50 (144)
Uncontrolled hypertension	27 (224)	24 (360)	37 (46)	29 (85)
Diabetes	30 (248)	20 (302)	40 (50)	24 (68)
COPD	18 (149)	15 (225)	28 (36)	22 (66)
Never smoker	23 (194)	24 (366)	15 (19)	16 (45)
Current smoker	47 (391)	50 (758)	49 (61)	61 (176)
Past smoker	29 (242)	26 (396)	36 (45)	24 (68)
BMI>30 kg/m^2	46 (385)	16 (244)	42 (53)	16 (45)
Total cholesterol/(mg/dL)				
<200, no cholesterol-lowering drugs	37 (303)	42 (633)	38 (48)	40 (117)
<200, on cholesterol-lowering drugs	36 (300)	31 (473)	45 (56)	37 (108)
>=200	26 (213)	27 (404)	16 (20)	21 (62)
Substance use				

Data are column percentages (N) unless otherwise specified		Total		Died	
		HIV-	HIV+	HIV-	HIV+
Cocaine use in past year		38 (312)	36 (549)	37 (46)	46 (132)
Alcohol					
Not hazardous drinking		17 (143)	23 (355)	17 (21)	13 (38)
Not current drinking		40 (330)	36 (551)	43 (54)	43 (123)
At-risk or heavy episodic drinking		12 (97)	12 (181)	6 (8)	8 (24)
Alcohol abuse/dependence diagnosis		28 (230)	24 (372)	28 (35)	32 (93)
VACS index components					
Median (IQR) VACS Index score (includes HIV-1 RNA and CD4 count)			29 (18, 45)		46 (33, 62)
Median (IQR) modified VACS Index score (excludes HIV-1 RNA and CD4 count)		18 (11, 27)	21 (11, 33)	27 (20, 42)	32 (21, 43)
Hepatitis C		31 (257)	47 (714)	45 (56)	62 (180)
FIB-4					
>3·25		4 (33)	9 (134)	13 (16)	20 (59)
1·45-3·25		25 (206)	36 (549)	35 (44)	44 (127)
<1·45		69 (575)	55 (831)	51 (64)	35 (102)
Hemoglobin <12 g/dL		7 (59)	12 (178)	11 (14)	22 (65)
eGFR<60 mL/min/1·73 m^2		9 (76)	8 (117)	22 (27)	13 (38)
HIV specific factors					
CD4/ cells/mm^3	Median (IQR)		392 (232, 583)		292 (123, 453)
	>=500		35 (532)		22 (65)
	200-<500		46 (704)		42 (122)
	<200		19 (285)		35 (102)

Data are column percentages (N) unless otherwise specified		Total		Died	
		HIV-	HIV+	HIV-	HIV+
HIV-1 RNA/ copies/mL	Median (IQR)		75 (75, 3339)		400 (75, 20321)
	<500		66 (1006)		55 (158)
	500-9999		15 (226)		15 (44)
	≥10000		19 (289)		30 (87)
HAART (baseline)			76 (1156)		75 (217)
Inflammatory biomarkers					
IL-6/ (pg/mL)	Median	1·8	2·1	2·6	3·1
	IQR	(1·2, 3·2)	(1·4, 3·4)	(1·5, 4·6)	(1·8, 5·4)
	% elevated (N)	11 (95)	12 (177)	21 (26)	27 (77)
sCD14/ (ug/mL)	Median	1731	1719	1888	1879
	IQR	(1478, 2043)	(1448, 2085)	(1668, 2339)	(1585, 2333)
	% elevated (N)	15 (124)	16 (237)	26 (32)	25 (71)
D-dimer/ (ug/mL)	Median	0·30	0·26	0·47	0·37
	IQR	(0·21, 0·53)	(0·15, 0·49)	(0·25, 0·88)	(0·22, 0·86)
	% elevated (N)	14 (114)	13 (192)	26 (32)	25 (73)
Inflammatory burden score					

Data are column percentages (N) unless otherwise specified	Total		Died	
	HIV-	**HIV+**	**HIV-**	**HIV+**
0 biomarkers elevated	67 (556)	71 (1080)	42 (53)	52 (151)
1 biomarker elevated	24 (203)	20 (302)	42 (52)	25 (73)
Any 2 biomarkers elevated	6 (50)	6 (96)	14 (17)	15 (44)
IL-6 & sCD14 elevated	2 (18)	3 (48)	4 (5)	7 (20)
IL-6 & D-dimer elevated	2 (19)	2 (27)	6 (7)	5 (14)
D-dimer & sCD14 elevated	2 (13)	1 (21)	4 (5)	3 (10)
3 biomarkers elevated	1 (9)	2 (37)	1 (1)	7 (20)

All covariates had complete data during the analysis period except the following: blood pressure was available for 828 HIV uninfected, smoking data were available for 1520 HIV+ and 827 uninfected, BMI was available for 1517 HIV+ and 827 uninfected, cholesterol was available for 1510 HIV+ and 816 uninfected, alcohol use was available for 1459 HIV+ and 800 uninfected, FIB-4 was available for 1512 HIV+ and 804 uninfected, hemoglobin was available for 828 uninfected, eGFR data were available for 823 uninfected, IL-6 was available for 1517 HIV+ and 821 uninfected, and D-dimer was available for 1519 HIV+ and 826 uninfected..

IL-6, sCD14 and D-dimer elevation thresholds were defined as ≥75th percentile among those who died. For, IL-6 this threshold was 5·043 pg/mL, for sCD14 it was 2334·18 ug/mL, and for D-dimer, it was 0·86 ug/mL.

Table 2: Association between HIV infection and IL-6, sCD14, and D-dimer adjusted for a) age and race-ethnicity b) all covariates.

		Proportional odds ratio (95% confidence interval)*			
a) Model (age, race-ethnicity adjusted)	**Outcomes**	**HIV uninfected**	**HIV+ HIV-1 RNA<500 copies/mL**	**HIV+ HIV-1 RNA 500-9999 copies/mL**	**HIV+ HIV-1 RNA ≥10000 copies/mL**
1	IL-6 quartiles	1 (Ref)	1·14 (0·96-1·35)	1·32 (1·01-1·73)	2·99 (2·32-3·84)
2	sCD14 quartiles	1 (Ref)	0·93 (0·78-1·10)	0·85 (0·65-1·12)	2·05 (1·61-2·62)
3	D-dimer quartiles	1 (Ref)	0·50 (0·42-0·59)	0.88 (0·67-1·16)	1·91 (1·49-2·45)
b) Model (Fully adjusted)	**Outcomes**	**HIV uninfected**	**HIV+ HIV-1 RNA<500 copies/mL**	**HIV+ HIV-1 RNA 500-9999 copies/mL**	**HIV+ HIV-1 RNA ≥10000 copies/mL**
1	IL-6 quartiles	1 (Ref)	1·35 (1·11-1·64)	1·46 (1·10-1·95)	2·78 (2·11-3·65)
2	sCD14 quartiles	1 (Ref)	0·77 (0·64-0·93)	0·71 (0·54-0·95)	1·49 (1·14-1·94)
3	D-dimer quartiles	1 (Ref)	0·51 (0·43-0·62)	0·95 (0·71-1·26)	1·73 (1·32-2·26)

** The proportional odds model estimates the proportional odds of being above the Nth quartile of the biomarker distribution versus being in the Nth quartile or lower.*

Fully adjusted model adjusted for age, race-ethnicity, prevalent cardiovascular disease, cancer, diabetes, chronic obstructive pulmonary disease, hypertension, smoking, hepatitis C, obesity, total cholesterol and cholesterol lowering medication, alcohol use, cocaine use, hemoglobin, FIB-4, estimated glomerular filtration rate. IL-6, sCD14 and D-dimer quartile thresholds were defined using quartile levels (25 , 50 , and 75 percentiles) among those who died. For, IL-6 these thresholds were 1·727, 2·91, and 5·043 pg/mL. For sCD14: 1592·84, 1883·51, and 2334·18 ug/mL. For D-dimer: 0·23, 0·39, and 0·86 ug/mL

Table 1: Mortality rates, rate ratios and risks by IL-6, sCD14 and D-dimer quartile mutually adjusted for each other and adjusted for HIV status and co-morbid conditions

		Number of deaths/ Number of people	Death rate/100py (95% CI)	Mortality IRR for HIV+ vs. uninfected (95% CI)	Hazard Ratio (95% CI)	
					Model 1	Model 2
IL-6 quartile	1	103/981	1·56 (1·29-1·89)	1·05 (0·70-1·61)	**1 (ref)**	**1 (ref)**
	2	102/651	2·44 (2·01-2·96)	1.00 (0·65-1·58)	1·25 (0·94-1·65)	1·11 (0·83-1·47)
	3	103/434	3·79 (3·12-4·60)	1·41 (0·89-2·31)	1·64 (1·23-2·20)	1·25 (0·92-1·69)
	4	103/272	6·91 (5·69-8·38)	1·90 (1·20-3·08)	2·67 (1·95-3·64)	1.98 (1·43-2·74)
sCD14 quartile	1	104/896	1·73 (1·43-2·10)	1·46 (0·94-2·34)	**1 (ref)**	**1 (ref)**
	2	103/599	2·67 (2·20-3·23)	1·27 (0·83-1·98)	1·24 (0·94-1·65)	1·18 (0·89-1·56)
	3	104/494	3·37 (2·78-4·09)	1·26 (0·82-1·99)	1·38 (1·04-1·83)	1·18 (0·88-1·58)
	4	103/361	4·87 (4·01-5·90)	1·27 (0·82-1·99)	1·57 (1·16-2·12)	1·27 (0·92-1·73)
D-dimer quartile	1	102/884	1·73 (1·43-2·10)	1·16 (0·73-1·88)	**1 (ref)**	**1 (ref)**
	2	113/679	2·58 (2·15-3·10)	2·33 (1·48-3·79)	1·16 (0·88-1·53)	1·16 (0·88-1·53)
	3	94/476	3·09 (2·53-3·79)	0·93 (0·61-1·44)	1·17 (0·87-1·58)	1·09 (0·81-1·48)
	4	105/306	6.07 (5·02-7·35)	1·56 (1·01-2·44)	1·84 (1·36-2·50)	1·65 (1·20-2·25)

All models were adjusted for IL-6, sCD14, D-dimer and HIV status categorized as uninfected, HIV infected (HIV-1 RNA<500 copies/mL), HIV infected (HIV-1 RNA 500-9999 copies/mL), and HIV infected (HIV-1 RNA ≥10000 copies/mL).

Model 1 additionally adjusted for age and race-ethnicity.

Model 2 additionally adjusted for age, race-ethnicity, prevalent cardiovascular disease, diabetes, cancer, chronic obstructive pulmonary disease, hypertension, smoking, hepatitis C, BMI, total cholesterol and cholesterol lowering medication, alcohol use, cocaine use, hemoglobin, FIB-4, and estimated glomerular filtration rate.

IL-6, sCD14 and D-dimer quartile thresholds were defined using quartile levels (25th, 50th, and 75th percentiles) among those who died.

For, IL-6 these thresholds were 1·727, 2·91, and 5·043 pg/mL. For sCD14: 1592·84, 1883·51, and 2334·18 ug/mL. For D-dimer: 0·23, 0·39, and 0·86 ug/mL

CI: confidence interval; py: person years; IRR: incidence rate ratio

Table 4: Assessing whether addition of inflammatory biomarkers to Cox regression models attenuates the association between HIV infection (stratified by HIV-1 RNA at baseline) and mortality.

	Hazard Ratio (95% Confidence Interval)			
	Unadjusted for IL-6, sCD14 or D-dimer	**Adjusted for IL-6, sCD14 and D-dimer**	**Unadjusted for IL-6, sCD14 or D-dimer**	**Adjusted for IL-6, sCD14 and D-dimer**
	Model 1	**Model 1**	**Model 2**	**Model 2**
N (# deaths)	2324 (411)	2324 (411)	2324 (411)	2324 (411)
HIV uninfected	1 (ref)	1 (ref)	1 (ref)	1 (ref)
HIV+ HIV-1 RNA<500 copies/mL	1·12 (0·89-1·42)	1·20 (0·94-1·53)	1·04 (0·80-1·35)	1·09 (0·84-1·42)
HIV+ HIV-1 RNA 500-9999 copies/mL	1·55 (1·09-2·19)	1·58 (1·11-2·24)	1·34 (0·93-1·92)	1·42 (0·99-2·05)
HIV+ HIV-1 RNA ≥10000 copies/mL	2·94 (2.22-3·91)	2·29 (1·72-3·07)	2·18 (1·60-2·99)	2·00 (1·45-2·76)

Model 1 additionally adjusted for age and race-ethnicity.

Model 2 additionally adjusted for age, race-ethnicity, prevalent cardiovascular disease, diabetes, cancer, chronic obstructive pulmonary disease, hypertension, smoking, hepatitis C, BMI, total cholesterol and cholesterol lowering medication, alcohol use, cocaine use, hemoglobin, FIB-4, estimated glomerular filtration rate.

IL-6, sCD14 and D-dimer quartile thresholds were defined using quartile levels (25th, 50th, and 75th percentiles) among those who died. For, IL-6 these thresholds were 1·727, 2·91, and 5·043 pg/mL. For sCD14: 1592·84, 1883·51, and 2334·18 ug/mL. For D-dimer: 0·23, 0·39, and 0·86 ug/mL