Full Title: IL-6 is a stronger predictor of clinical events than hsCRP or D-dimer during HIV infection.

Running Title: Inflammation, coagulation and clinical outcomes

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Abstract:

Background: IL-6, hsCRP and D-dimer levels are linked to adverse outcomes in HIV, but the strength of their associations with different clinical endpoints warrants investigation.

Methods: Participants receiving standard of care in 2 HIV trials with measured biomarkers were followed to ascertain all-cause death, non-AIDS death, AIDS, cardiovascular disease (CVD) and non-AIDS-defining malignancies. HRs (95% CIs) of each endpoint for quartiles and log_2-transformed IL-6, hsCRP and D-dimer were calculated using Cox models. Marginal models modelling multiple events tested for equal effects of biomarkers on different endpoints.

Results: Among 4304 participants, there were 157 all-cause deaths, 117 non-AIDS deaths, 101 AIDS, 121 CVD and 99 non-AIDS-defining malignancies. IL-6 was more strongly associated with most endpoints than hsCRP. IL-6 appeared to be a stronger predictor for CVD and non-AIDS-defining malignancies than D-dimer but CIs overlapped. Independent associations of IL-6 were stronger for non-AIDS death (1.71; 1.43-2.04) and all-cause death (1.56; 1.33-1.84), and similar for CVD (1.35; 1.12-1.62) and non-AIDS-defining malignancies (1.30; 1.06-1.61). There was heterogeneity of IL-6 as a predictor for different endpoints ($p<0.001$), but not hsCRP ($p=0.15$) or D-dimer ($p=0.20$).

Conclusions: IL-6 is a stronger predictor of fatal events than CVD and non-AIDS-defining malignancies. Adjuvant anti-inflammatory and anti-thrombotic therapies should be tested in HIV.

Keywords: HIV; coagulation; inflammation; IL-6; hsCRP; D-dimer; cardiovascular disease; cancer; AIDS

Text:

Introduction:

Activated inflammation and hypercoagulation have consistently been linked to many adverse clinical outcomes during HIV infection, including progression to AIDS [1,2], anaemia [3], cancer [4], cardiovascular disease [5,6], diabetes [7] and all-cause mortality [8-10]. This observation is drawn from several studies that reported independent associations between raised plasma levels of inflammatory and coagulation biomarkers and subsequent risk of clinical outcomes [11]. In the Strategies for Management of Anti-Retroviral Therapy (SMART) trial, episodic antiretroviral therapy (ART) use increased all-cause mortality compared with continuous ART; the majority of the deaths being attributable to cardiovascular disease and cancer [12]. Out of a panel of six biomarkers tested in SMART participants at study entry, elevated levels of three biomarkers were independently and strongly associated with a subsequent risk of death during follow-up [8]: 1) interleukin-6 (IL-6), a pro-inflammatory cytokine that is a proximal mediator or upstream inflammatory marker [13]; 2) high-sensitivity C-reactive protein (hsCRP), a downstream acute phase reactant whose hepatic production is stimulated, among other factors, by IL-6 [14]; and 3) D-dimer, a degradation product of cross-linked intravascular fibrin that is a marker of hypercoagulation [15].

Many previous studies on IL-6, hsCRP and D-dimer have investigated associations with all-cause death and different diseases in the setting of HIV infection [11], but the strength of associations these biomarkers have with different types of clinical endpoints is not well understood. For instance, it remains to be determined which biomarker best predicts AIDS-defining and non-AIDS-defining morbidity among HIV+ persons receiving standard-of-care [16]. This poses an important research question as to whether activated inflammation and hypercoagulation contribute

in a general way to the development of multiple diseases or are instead more strongly associated with the development of specific pathologies [17]. Here, we set out to evaluate the relative value of IL-6, hsCRP and D-dimer as predictors of different clinical endpoints in a large cohort of HIV+ persons.

Material and Methods:

Study population:

This cohort study combined the control arms of two international HIV trials conducted by the International Network for Strategic Initiatives in Global HIV Trials: the SMART study (NCT00027352) [12] and the Evaluation of Subcutaneous Proleukin® in a Randomized International Trial (ESPRIT) (NCT00004978) [18]. Participants who had consented to storing blood for future research and whose plasma levels of IL-6, hsCRP and D-dimer were measured at study entry prior to randomization were included (N= 4,304). All participants in the control arms received standard of care according to HIV guidelines and were to be continuously maintained on ART. Prior to the enrolment of participants in the SMART and ESPRIT trials, the institutional review board at each site had approved the original study protocols, and written informed consent was obtained from all participants. SMART and ESPRIT were conducted in compliance with the Declaration of Helsinki Guidelines, were registered on clinical trials databases and reviewed by independent data and safety interim monitoring boards.

Biomarker measurements:

For SMART participants, biomarkers were measured at the Laboratory for Clinical Biochemistry Research at the University of Vermont (Burlington). In the ESPRIT trial, laboratory measurements were performed by SAIC-Frederick (Frederick, MD). IL-6 was measured by the same method at each laboratory (Chemiluminescent Sandwich enzyme-linked immunosorbent assay [ELISA], R&D Sytems). hsCRP levels were determined by means of highly sensitive ELISA by both laboratories. For SMART participants, a NBTMII nephelometer, N Antiserum to Human CRP (Siemens Diagnostics) was used. For ESPRIT, an R&D Systems ELISA assay was used. D-dimer levels were determined by ELISA on the Sta-R analyzer, Liatest D-DI (Diagnostic Stago) for SMART participants and on a VIDAS instrument (BioMerieux Inc., Durham, North Carolina, USA) for ESPRIT participants. As described in detail elsewhere [6,19], the assays for hsCRP and D-dimer, while different, compared well and measurements on duplicate samples were well correlated.

Follow up and endpoints ascertainment:

Patients were followed from study entry until the first endpoint of interest, death, loss to follow-up, or the closing date of each study, whichever occurred first. The following clinical endpoints were considered: 1) all-cause mortality, 2) non-AIDS and non-violent/accidental death, 3) fatal and non-fatal progression to AIDS (CDC category C events), 4) fatal and non-fatal cardiovascular disease (defined as myocardial infarction, stroke or coronary artery disease requiring a surgical procedure) and 5) fatal and non-fatal non-AIDS-defining malignancies (excluding basal and squamous cell skin cancers). All clinical endpoints were systematically reported to and centrally adjudicated by an endpoint review committee using pre-specified criteria [20]. Causes of death were determined using CoDe [21]. Clinical assessment intervals and total follow-up time varied by study. Median follow-up time was 29 months in SMART and 81 months in ESPRIT; overall, median follow-up was 48 months for the entire cohort.

Statistical analyses:

Hazard ratios (HR) with 95% confidence interval (CI) of each study endpoint for IL-6, hsCRP and D-dimer levels, modelled as log_2-transformed values (i.e., per 2 fold higher), were calculated and used to compare the association of plasma levels of each biomarker with the risk of developing different clinical endpoints. Models for each biomarker were stratified by study to account for differences in underlying risk for the different cohorts. This stratification also grouped participants by the two laboratories that measured biomarkers. We used sequential adjustment to fit three Cox proportional hazards regression models [models (1) – (3)], for each of the five clinical endpoints, and we repeated these three models for all three biomarkers. The Cox proportional hazards models were fit as follows: model (1) univariable; model (2) adjusted for demographics (age, sex, race and body mass index), nadir and baseline CD4+ cell count, baseline ART use and baseline HIV RNA, prior AIDS and cardiovascular disease, diabetes mellitus and hepatitis B virus/hepatitis C virus (HBV/HCV) co-infection; model (3) adjusted as in model (2) with further adjustment which included fitting biomarkers of inflammation and coagulation simultaneously. However, we did not include both biomarkers of inflammation in the same model because IL-6 and hsCRP have biological redundancy. The hepatic production of hsCRP is determined by IL-6 [14]. Therefore, elevated plasma levels of both biomarkers could have indicated similar pathophysiological processes.

We computed crude incidence rates and HRs (95% CIs) of clinical endpoints for each quartile of biomarker levels. To control for any differences in biomarker distributions among studies and inter laboratory variability, quartiles were defined separately for participants in SMART and ESPRIT.

To compare the strength of associations of IL-6, hsCRP and D-dimer with each endpoint investigated, competing risk or marginal Cox model described by Wei, Lin & Weissfeld [22] were used to model multiple unordered events and to test for equal effects of biomarkers on different types of endpoints. With this approach, the fit of a model which assumed a common association for biomarkers with each endpoint was compared with the fit of a model that allowed the association to vary. The corresponding p-value is the probability that the magnitude of differences in estimated HRs for the endpoints could have arisen by chance if there were no real differences in the actual HRs. We also evaluated the strength of associations of each biomarker with specific endpoints by estimating the HRs of the highest vs. lowest biomarker quartiles and the HRs per 2-fold higher biomarker levels

Secondary analyses

To test the robustness of our main findings and adjust for other biologically plausible confounders, we carried out two pre-planned secondary analyses. First, we recalculated HRs of clinical endpoints for quartiles of biomarker levels excluding events in the first two years of follow-up. This analysis was aimed at addressing the possibility of bias by reverse causality. Because cardiovascular disease and cancer may have a long subclinical period prior to diagnosis, there is a possibility that elevated biomarkers levels were a consequence of the disease outcome instead of vice versa. Second, among SMART participants for whom information on smoking at the entry was collected, analyses further adjusting for smoking were carried out because smoking is causally related to cancer and cardiovascular disease.

Results:

There were approximately 19,000 person-years of follow-up [PYFU] among 4,304 participants (median age 42 years, median baseline $CD4^+$ cell count 526/mm^3, 77% men), including 157 all-cause deaths (crude incidence rate [IR] 8.25/1,000 PYFU, 95% CI 6.96-9.53), 117 non-violent, non-accidental and non-AIDS related deaths (6.14, 5.03-7.25), 101 progressions to AIDS (70 opportunist infections and 32 AIDS-defining cancers; one participant in ESPRIT had both types of AIDS events) (5.39, 4.34-6.44), 121 cardiovascular disease events (6.47, 5.32-7.62) and 99 non-AIDS-defining malignancies (5.27, 4.23-6.30).

Baseline characteristics are presented for both the entire cohort and separately for people who experienced the different clinical endpoints and are shown in Table 1. Levels of IL-6, hsCRP and D-dimer at study entry were higher among participants experiencing each clinical endpoint when compared to the entire cohort (Table 1). Crude incidence rates of all clinical endpoints increased across higher quartiles of all biomarkers (Figure 1). This was particularly true for IL-6 and D-dimer quartiles where a clearly graded response pattern was observed.

When compared to hsCRP, IL-6 was more strongly associated with most outcomes investigated both in univariable and multivariable models that considered log_2-transformed biomarkers. IL-6 was a stronger predictor for cardiovascular disease events and non-AIDS-defining malignancies than D-dimer (Figure 2, Table 2 and supplemental table 1) but confidence intervals overlapped.

Independent associations between log_2 IL-6 and clinical endpoints were strongest for fatal events and similar for fatal and non-fatal cardiovascular disease and non-AIDS-defining malignancies in multivariable analyses. For instance, adjusted HRs (95% CI) calculated using model 3 were numerically higher for non-AIDS and non-violent/accidental death (1.71 [1.43-2.04] per 2 fold higher IL-6 level) and for all-cause death (1.56; 1.33-1.84) than for fatal or non-fatal cardiovascular disease events (1.35; 1.12-1.62) and non-AIDS-defining malignancies (1.30; 1.06-1.61) (Figure 2 and supplemental table 1). The Wei-Lin-Weissfeld test found evidence of heterogeneity in

the association of $\log_2$ IL-6 with different endpoints ($p<0.001$), but not of $\log_2$ hsCRP ($p=0.15$) or $\log_2$ D-dimer ($p=0.20$).

In multivariable analyses comparing the highest to the lowest biomarker quartiles (Table 2, Figure 3 and Supplemental Table 2), HRs for all-cause death were similar for IL-6, hsCRP and D-dimer. However, higher quartiles of IL-6 were independently associated with steeper risk gradients for all other outcomes than hsCRP. When compared to D-dimer, IL-6 appeared to be associated with steeper risk gradients for cardiovascular disease and non-AIDS-defining malignancies (Table 2, Figure 3) but confidence intervals overlapped (Figure 3 and supplemental Table 2). The strength of associations of IL-6 and D-dimer with the other endpoints was similar (Table 2).

Secondary analyses

After censoring the events occurring in the first two years of follow-up, confidence intervals of hazard ratios became larger owing to the smaller number of clinical endpoints (supplemental Table 3). As a result, some associations between biomarker quartiles and certain types of clinical endpoints were no longer significant. This was the case, for instance, for the association between higher D-dimer quartiles and increased risk of progression to AIDS. However, most associations followed the same direction of and were broadly consistent with the main analyses including all follow-up (supplemental Table 2).

In SMART, adjustment for smoking weakened the association between higher quartiles of IL-6 and death endpoints, but strengthened associations between higher quartiles of IL-6 and increased risk of cardiovascular disease and non-AIDS-defining malignancies (supplemental Table 4).

Discussion:

In a large cohort of HIV+ persons, we confirmed that activated inflammation and coagulation, as demonstrated by elevated levels of IL-6, hsCRP and D-Dimer, respectively, are linked to a variety of clinical endpoints representative of AIDS-associated and non-AIDS-associated morbidity. These three biomarkers are of particular interest because they have similarly been linked to disease in the general population. We found that the pathophysiological pathways that involve raised plasma levels of IL-6, hsCRP and D-dimer seem to be associated with different types of clinical endpoints. Higher IL-6 levels at study entry were more strongly associated with subsequent risk of non-AIDS-defining endpoints such as cardiovascular disease and non-AIDS-defining malignancies than hsCRP or D-dimer. Furthermore, higher IL-6 was more strongly associated with fatal events than with fatal and non-fatal cardiovascular disease or non-AIDS-defining malignancies. These findings were broadly consistent after adjustment for confounders and accounting for potential reverse causality.

We demonstrated that IL-6 was superior to hsCRP to predict cardiovascular and non-AIDS-defining malignancies. Gene-association studies using principles of Mendelian randomisation support a causal role of IL-6 in cardiovascular disease [23] and cancer [24]. With respect to hsCRP, polymorphisms in genes that are associated with higher levels of hsCRP were not associated with increased risk of cardiovascular disease [25,26] or cancer [27]. hsCRP seems, therefore, to be more of a epiphenom of underlying pathophysiological processes than a biomarker causally linked to adverse endpoints. However, some studies in the general population have found hsCRP to be more strongly associated with cardiovascular disease than IL-6 after adjustment for confounders [28,29]. The reasons why hsCRP is a less informative biomarker than IL-6 in the setting of HIV infection are unclear. Since hsCRP is a terminal product of the inflammatory cascade produced by the liver, subclinical hepatic impairment determined by conditions that may affect HIV+ persons disproportionally, such as HCV/HBV co-infection or hepatic steatosis, could play a role [30,31].

In this study, IL-6 was more strongly associated with cardiovascular disease and non-AIDS-defining malignancies than D-dimer. There is paucity of data on the relative associations of IL-6 and D-dimer with non-AIDS-defining outcomes during HIV infection. A previous report including SMART and ESPRIT participants who were receiving ART and virologically suppressed at baseline found that both IL-6 and D-dimer independently predicted the risk of a composite endpoint of serious non-AIDS morbidity or all-cause death [10]. Higher D-dimer levels also predicted a composite endpoint of non-AIDS-defining conditions in a case-control study involving HIV+ persons participating in ACTG trials [32]. In other reports involving the general population and HIV+ persons, D-dimer was found to predict cardiovascular disease [33,34] and cancer mortality [35].

We also observed that elevated levels of IL-6 and D-dimer were similarly linked to increased risk of progression to AIDS. Elevated D-dimer levels had been previously associated with increased risk of AIDS or death among participants in Flexible Initial Retrovirus Suppression Therapies (FIRST) trial with advanced HIV infection (median $CD4^+$ cell count of 163 cells/mm^3) [2]. In a nested case-control study involving participants from both arms in the SMART study; i.e. those randomized to continuous use of ART and to $CD4^+$ cell count-guided ART interruptions, D-dimer was not associated with the development of opportunistic disease [1]. However, there appeared to be a stronger association between D-dimer and opportunistic disease among those receiving ART continually, although this is an underpowered analysis given the low number of events [1]. The prognostic value of D-dimer in HIV+ persons, particularly its relationship with AIDS-defining and non-AIDS-defining endpoints, deserves, therefore, further investigation.

Activated innate immunity seems to be a stronger determinant of IL-6 and D-dimer levels than adaptive immunity [32,36,37] but higher levels of each biomarker are related to different phenotypes of monocyte activation [36]. Furthermore, we had previously demonstrated that

different host factors and HIV-specific variables are associated with levels of IL-6 [38] and D-dimer [19]. This is compatible with the hypothesis that circulating levels of IL-6 and D-dimer reflect two distinct pathophysiological processes that may lead to different clinical endpoints. Indeed, combined scores of IL-6 and D-dimer have proven more helpful to estimate future risk of non-AIDS-related morbidity than each biomarker individually [10].

The Wei-Lin-Weissfeld test indicated that higher IL-6 levels were more strongly associated with death endpoints than cardiovascular disease and cancer in our study. From our studies, higher levels of IL-6 at study entry were associated with greater risk of fatal cardiovascular disease and greater risk of death after a nonfatal cardiovascular event [6]. As for non-AIDS-defining malignancies, we also found that the risk associated with elevated plasma levels of IL-6 was higher for fatal than for non-fatal events among HIV+ persons (unpublished data). Furthermore, raised inflammatory biomarkers were more strongly associated with cancer death than with non-fatal cancer in healthy elderly individuals [39] and with shorter survival following cancer diagnosis [40]. Taken together, these findings suggest that higher IL-6 is not only a marker of disease risk, but also of disease severity.

Our study had several limitations. First, we only studied three biomarkers. Other biomarkers not tested might have provided useful information. Second, we studied IL-6, hsCRP and D-dimer at study entry only because follow-up biomarkers were only available in a subset of participants; therefore, it was not possible to investigate the effects of changes in plasma levels of these biomarkers over time. The fact that biomarker levels may have changed over time during follow-up means that the association between these biomarkers and the clinical endpoints could be greater than those observed. Third, associations of each biomarker were investigated for several clinical endpoints. This means that false positive results may have arisen from multiple testing. Fourth, the strong and independent associations between raised biomarker levels and clinical endpoints

observed in this large cohort of HIV+ persons do not necessarily mean that these biomarkers are valuable for risk stratification of individual patients [41].

No study has thus far provided irrefutable evidence for a causal relationship between activated inflammation/coagulation and clinical events during HIV infection. Among HIV+ persons with early HIV infection enrolled in the Strategic Timing of AntiRetroviral Treatment (START) trial, immediate ART reduced incidence of AIDS-defining events and cancer compared to treatment deferral until $CD4^+$ counts dropped below 350 cells/mm^3[42]. Because ART decreases inflammation and coagulation by suppressing HIV replication [16,43,44], START findings are consistent with the hypothesis of activated inflammation and coagulation as a contributing cause of morbidity, although the benefits of immediate ART may also have been mediated by other mechanisms. The Evaluating the Use of Pitavastatin to Reduce the Risk of Cardiovascular Disease in HIV-Infected Adults (REPRIEVE) trial will randomise 6,500 HIV+ individuals with low to moderate cardiovascular risk receiving ART to start statin or placebo. In the Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER), which recruited HIV-uninfected individuals with elevated hsCRP levels at baseline, statins were found to reduce risk of cardiovascular disease by 50% [45]. However, because REPRIEVE and JUPITER have cardiovascular disease as the main study endpoint, it is impossible to determine whether the clinical benefit of statin therapy is mediated by a reduction cholesterol, a reduction of inflammation or a combination of both [17,46]. Three additional placebo-controlled trials of anti-inflammatory therapies to reduce risk of cardiovascular events started enrolling participants: the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS), which is assessing a monoclonal antibody against interleukin-1β [47]; the Cardiovascular Inflammation Reduction Trial (CIRT), which is assessing low dose methotrexate among HIV-uninfected persons [48], and the NCT01949116 trial, which is assessing methotrexate among HIV+ persons. By deploying

therapies that directly target inflammation, these trials will be important to test the inflammatory hypothesis of cardiovascular disease. However, their results will not address the question as to whether enhanced inflammation and coagulation is causally linked to cancer and other non-AIDS-defining morbidities.

To conclude, IL-6 is a stronger predictor of fatal events than fatal and non-fatal cardiovascular disease and non-AIDS-defining cancer. There is a need for clinical endpoint-driven trials to determine whether anti-thrombotic and anti-inflammatory therapies to lower levels of IL-6, hsCRP and D-dimer can reduce morbidity and mortality in treated HIV infection. Such trials would not only determine the potential role of adjuvant anti-inflammatory and anti-thrombotic therapies in the management of HIV infection, but also elucidate whether enhanced inflammation and coagulation are causally linked to the development of AIDS-defining and non-AIDS-defining morbidity.

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AHB, JLO, ANP, JDN, BG and JDL conceived the study. JLO and ANP performed all statistical analyses. AHB drafted the manuscript. All authors contributed to data interpretation, critically revised the manuscript and approved the final version.

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Table 1:
Baseline characteristics
Participants in the control arms of the SMART and ESPRIT trials

Baseline Characteristics N (%) or Median [IQR]	All participants N= 4,304	All-cause deaths N=157	non-AIDS and non-violent/ accidental deaths N=117	AIDS N=101	CVD N=121	non-AIDS-defining malignancies N=99
Age (years)	42 [36 , 49]	48 [40 , 54]	48 [41 , 55]	44 [38 , 50]	49 [43 , 56]	50 [44 , 57]
Female sex	1,002 (23.3)	28 (17.8)	17 (14.5)	22 (21.8)	14 (11.6)	14 (14.1)
Black Race	907 (21.1)	29 (18.5)	25 (21.4)	18 (17.8)	26 (21.5)	26 (26.3)
BMI (kg/m^2)	24.4 [22.1 , 27.1]	23.5 [21.4 , 27.3]	23.3 [21.3 , 27.1]	25.2 [22.6 , 29.0]	24.0 [22.1 , 27.4]	23.9 [21.7 , 25.8]
Prior AIDS	1,093 (25.4)	46 (29.3)	32 (27.4)	42 (41.6)	47 (38.8)	21 (21.2)
Hepatitis B/C	761 (17.7)	54 (34.4)	41 (35.0)	16 (15.8)	18 (14.9)	27 (27.3)
Prior CVD	112 (2.6)	14 (9.0)	12 (10.3)	1 (1.0)	16 (13.3)	5 (5.1)
Diabetes	217 (5.1)	14 (9.0)	10 (8.6)	7 (6.9)	14 (11.7)	9 (9.1)
PI based ART	1,478 (34.3)	53 (33.8)	45 (38.5)	31 (30.7)	52 (43.0)	41 (41.4)
NNRTI based ART	1,643 (38.2)	52 (33.1)	39 (33.3)	34 (33.7)	30 (24.8)	30 (30.3)
CD4 ($cells/mm^3$)	526 [415 , 701]	451 [370 , 594]	470 [384 , 639]	466 [376 , 599]	515 [401 , 673]	526 [404 , 679]
CD4 Nadir ($cells/mm^3$)	230 [120 , 337]	194 [93 , 297]	194 [85 , 282]	190 [90 , 298]	187 [74 , 301]	219 [97 , 311]
HIV RNA ≤500 copies/mL)	3,263 (75.8)	97 (61.8)	77 (65.8)	51 (50.5)	88 (72.7)	75 (75.8)
IL-6 (pg/mL)	1.80 [1.18 , 2.90]	3.09 [2.10 , 4.40]	3.17 [2.10 , 4.49]	2.42 [1.40 , 3.33]	2.60 [1.78 , 4.30]	2.50 [1.81 , 3.58]
hsCRP (μg/mL)	1.60 [0.69 , 3.67]	2.83 [1.53 , 6.27]	2.70 [1.57 , 6.18]	2.03 [0.83 , 4.50]	2.33 [1.02 , 5.05]	2.54 [1.13 , 4.93]
D-dimer (μg/mL)	0.24 [0.15 , 0.38]	0.33 [0.23 , 0.55]	0.35 [0.23 , 0.55]	0.31 [0.22 , 0.53]	0.31 [0.20 , 0.51]	0.28 [0.18 , 0.52]

Table 2:

Hazard ratios [a] (95% CIs) of study endpoints for plasma levels of IL-6, hsCRP and D-dimer.

	IL-6		hsCRP		D-dimer	
Study Endpoint	**2 fold higher level**	**4th/1st quartile**	**2 fold higher level**	**4th/1st quartile**	**2 fold higher level**	**4th/1st quartile**
All-cause deaths	1.64 (1.41-1.92)	3.07 (1.70-5.55)	1.29 (1.17-1.42)	2.73 (1.56-4.69)	1.32 (1.14-1.52)	3.00 (1.61-5.59)
Non-AIDS and non-violent/ accidental deaths	1.77 (1.50-2.10)	6.31 (2.68-14.83)	1.30 (1.16-1.46)	3.10 (1.59-6.05)	1.30 (1.10-1.53)	3.38 (1.62-7.08)
Progression to AIDS	1.32 (1.07-1.62)	1.63 (0.83-3.21)	1.07 (0.94-1.21)	1.35 (0.74-2.48)	1.35 (1.12-1.63)	2.20 (1.07-4.50)
Cardiovascular disease events	1.40 (1.17-1.67)	3.34 (1.66-6.70)	1.11 (0.99-1.24)	1.89 (0.99-3.63)	1.22 (1.04-1.43)	1.80 (0.98-3.29)
Non-AIDS-defining malignancies	1.31 (1.07-1.61)	3.12 (1.42-6.84)	1.16 (1.02-1.32)	1.68 (0.86-3.28)	1.09 (0.91-1.31)	1.03 (0.55-1.94)

[a] Hazards ratios adjusted for demographics (age, sex, race and BMI), nadir and baseline CD4+ cell count, ART use and baseline HIV RNA, prior AIDS and cardiovascular disease, diabetes mellitus and HBV/HCV co-infection, stratified by study (model 2). For detailed information on results using models 1 and 3, refer to supplemental Table 1 and supplemental Table 2.

Figures legends:

Figure 1: Crude incidence rates of clinical endpoints across biomarker quartiles. Quartile cut-points were defined differently for each study as described in the supplemental Table 2. NADM: non-AIDS-defining malignancies

Figure 2: HRs (95% CI) for clinical endpoints associated with baseline biomarkers. NADM: non-AIDS-defining malignancies

Model 1: unadjusted associations, stratified by study

Model 2: adjusted for demographics (age, sex, race and BMI), ART use, nadir and baseline CD4+ cell count, baseline HIV RNA, prior AIDS and cardiovascular disease, diabetes mellitus and HBV/HCV co-infection, stratified by study

Model 3: HRs for IL-6 and D-dimer using models (2) and including both biomarkers simultaneously and HRs for hsCRP using model (2) also adjusted for D-dimer, stratified by study

Figure 3: Adjusted HRs* (95% CI) for clinical endpoints associated with biomarker quartiles. NADM: non-AIDS-defining malignancies Quartile cut-points were defined differently for each study as described in the supplemental Table 2 *HRs adjusted for factors included in model 2; namely demographics (age, sex, race and BMI), ART use, nadir and baseline CD4+ cell count, baseline HIV RNA, prior AIDS and cardiovascular disease, diabetes mellitus and HBV/HCV co-infection. HRs calculated as in models (1) and (3) showed consistent results (data not shown)