

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

3

4 **Running Title:** Inflammation, coagulation and clinical outcomes

5

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49

50 **Abstract:**

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

53 **Methods:** Participants receiving standard of care in 2 HIV trials with measured biomarkers were
54 followed to ascertain all-cause death, non-AIDS death, AIDS, cardiovascular disease (CVD) and
55 non-AIDS-defining malignancies. HRs (95% CIs) of each endpoint for quartiles and log₂-
56 transformed IL-6, hsCRP and D-dimer were calculated using Cox models. Marginal models
57 modelling multiple events tested for equal effects of biomarkers on different endpoints.

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

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

68

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

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75 **Text:**

76 **Introduction:**

77 Activated inflammation and hypercoagulation have consistently been linked to many adverse
78 clinical outcomes during HIV infection, including progression to AIDS [1,2], anaemia [3], cancer
79 [4], cardiovascular disease [5,6], diabetes [7] and all-cause mortality [8-10]. This observation is
80 drawn from several studies that reported independent associations between raised plasma levels of
81 inflammatory and coagulation biomarkers and subsequent risk of clinical outcomes [11]. In the
82 Strategies for Management of Anti-Retroviral Therapy (SMART) trial, episodic antiretroviral
83 therapy (ART) use increased all-cause mortality compared with continuous ART; the majority of
84 the deaths being attributable to cardiovascular disease and cancer [12]. Out of a panel of six
85 biomarkers tested in SMART participants at study entry, elevated levels of three biomarkers were
86 independently and strongly associated with a subsequent risk of death during follow-up [8]: 1)
87 interleukin-6 (IL-6), a pro-inflammatory cytokine that is a proximal mediator or upstream
88 inflammatory marker [13]; 2) high-sensitivity C-reactive protein (hsCRP), a downstream acute
89 phase reactant whose hepatic production is stimulated, among other factors, by IL-6 [14]; and 3) D-
90 dimer, a degradation product of cross-linked intravascular fibrin that is a marker of
91 hypercoagulation [15].

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

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

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103 **Material and Methods:**

104

105 *Study population:*

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

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120 *Biomarker measurements:*

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

132

133 *Follow up and endpoints ascertainment:*

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

145

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147 *Statistical analyses:*

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

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

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

178

179 *Secondary analyses*

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

189

190 **Results:**

191

192 There were approximately 19,000 person-years of follow-up [PYFU] among 4,304 participants
193 (median age 42 years, median baseline CD4⁺ cell count 526/mm³, 77% men), including 157 all-
194 cause deaths (crude incidence rate [IR] 8.25/1,000 PYFU, 95% CI 6.96-9.53), 117 non-violent, non-
195 accidental and non-AIDS related deaths (6.14, 5.03-7.25), 101 progressions to AIDS (70
196 opportunist infections and 32 AIDS-defining cancers; one participant in ESPRIT had both types of
197 AIDS events) (5.39, 4.34-6.44), 121 cardiovascular disease events (6.47, 5.32-7.62) and 99 non-
198 AIDS-defining malignancies (5.27, 4.23-6.30).

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

205 When compared to hsCRP, IL-6 was more strongly associated with most outcomes investigated
206 both in univariable and multivariable models that considered log₂-transformed biomarkers. IL-6 was
207 a stronger predictor for cardiovascular disease events and non-AIDS-defining malignancies than D-
208 dimer (Figure 2, Table 2 and supplemental table 1) but confidence intervals overlapped.

209 Independent associations between log₂ IL-6 and clinical endpoints were strongest for fatal events
210 and similar for fatal and non-fatal cardiovascular disease and non-AIDS-defining malignancies in
211 multivariable analyses. For instance, adjusted HRs (95% CI) calculated using model 3 were
212 numerically higher for non-AIDS and non-violent/accidental death (1.71 [1.43-2.04] per 2 fold
213 higher IL-6 level) and for all-cause death (1.56; 1.33-1.84) than for fatal or non-fatal cardiovascular
214 disease events (1.35; 1.12-1.62) and non-AIDS-defining malignancies (1.30; 1.06-1.61) (Figure 2
215 and supplemental table 1). The Wei-Lin-Weissfeld test found evidence of heterogeneity in

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

218 In multivariable analyses comparing the highest to the lowest biomarker quartiles (Table 2,
219 Figure 3 and Supplemental Table 2), HRs for all-cause death were similar for IL-6, hsCRP and D-
220 dimer. However, higher quartiles of IL-6 were independently associated with steeper risk gradients
221 for all other outcomes than hsCRP. When compared to D-dimer, IL-6 appeared to be associated
222 with steeper risk gradients for cardiovascular disease and non-AIDS-defining malignancies (Table
223 2, Figure 3) but confidence intervals overlapped (Figure 3 and supplemental Table 2). The strength
224 of associations of IL-6 and D-dimer with the other endpoints was similar (Table 2).

225

226 *Secondary analyses*

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

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

237

238 **Discussion:**

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

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

262 In this study, IL-6 was more strongly associated with cardiovascular disease and non-AIDS-
263 defining malignancies than D-dimer. There is paucity of data on the relative associations of IL-6
264 and D-dimer with non-AIDS-defining outcomes during HIV infection. A previous report including
265 SMART and ESPRIT participants who were receiving ART and virologically suppressed at
266 baseline found that both IL-6 and D-dimer independently predicted the risk of a composite endpoint
267 of serious non-AIDS morbidity or all-cause death [10]. Higher D-dimer levels also predicted a
268 composite endpoint of non-AIDS-defining conditions in a case-control study involving HIV+
269 persons participating in ACTG trials [32]. In other reports involving the general population and
270 HIV+ persons, D-dimer was found to predict cardiovascular disease [33,34] and cancer mortality
271 [35].

272 We also observed that elevated levels of IL-6 and D-dimer were similarly linked to increased risk
273 of progression to AIDS. Elevated D-dimer levels had been previously associated with increased risk
274 of AIDS or death among participants in Flexible Initial Retrovirus Suppression Therapies (FIRST)
275 trial with advanced HIV infection (median CD4⁺ cell count of 163 cells/mm³) [2]. In a nested case-
276 control study involving participants from both arms in the SMART study; i.e. those randomized to
277 continuous use of ART and to CD4⁺ cell count-guided ART interruptions, D-dimer was not
278 associated with the development of opportunistic disease [1]. However, there appeared to be a
279 stronger association between D-dimer and opportunistic disease among those receiving ART
280 continually, although this is an underpowered analysis given the low number of events [1]. The
281 prognostic value of D-dimer in HIV+ persons, particularly its relationship with AIDS-defining and
282 non-AIDS-defining endpoints, deserves, therefore, further investigation.

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

286 different host factors and HIV-specific variables are associated with levels of IL-6 [38] and D-dimer
287 [19]. This is compatible with the hypothesis that circulating levels of IL-6 and D-dimer reflect two
288 distinct pathophysiological processes that may lead to different clinical endpoints. Indeed,
289 combined scores of IL-6 and D-dimer have proven more helpful to estimate future risk of non-
290 AIDS-related morbidity than each biomarker individually [10].

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

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

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

312 No study has thus far provided irrefutable evidence for a causal relationship between activated
313 inflammation/coagulation and clinical events during HIV infection. Among HIV+ persons with
314 early HIV infection enrolled in the Strategic Timing of AntiRetroviral Treatment (START) trial,
315 immediate ART reduced incidence of AIDS-defining events and cancer compared to treatment
316 deferral until CD4⁺ counts dropped below 350 cells/mm³[42]. Because ART decreases
317 inflammation and coagulation by suppressing HIV replication [16,43,44], START findings are
318 consistent with the hypothesis of activated inflammation and coagulation as a contributing cause of
319 morbidity, although the benefits of immediate ART may also have been mediated by other
320 mechanisms. The Evaluating the Use of Pitavastatin to Reduce the Risk of Cardiovascular Disease
321 in HIV-Infected Adults (REPRIEVE) trial will randomise 6,500 HIV+ individuals with low to
322 moderate cardiovascular risk receiving ART to start statin or placebo. In the Justification for the
323 Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER),
324 which recruited HIV-uninfected individuals with elevated hsCRP levels at baseline, statins were
325 found to reduce risk of cardiovascular disease by 50% [45]. However, because REPRIEVE and
326 JUPITER have cardiovascular disease as the main study endpoint, it is impossible to determine
327 whether the clinical benefit of statin therapy is mediated by a reduction cholesterol, a reduction of
328 inflammation or a combination of both [17,46]. Three additional placebo-controlled trials of anti-
329 inflammatory therapies to reduce risk of cardiovascular events started enrolling participants: the
330 Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS), which is assessing a
331 monoclonal antibody against interleukin-1 β [47]; the Cardiovascular Inflammation Reduction Trial
332 (CIRT), which is assessing low dose methotrexate among HIV-uninfected persons [48], and the
333 NCT01949116 trial, which is assessing methotrexate among HIV+ persons. By deploying

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

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

345

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

353

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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.**

Study Endpoint	IL-6		hsCRP		D-dimer	
	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)

