Inflammation and Mortality in HIV-Infected Adults: Analysis of the FRAM Study Cohort

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Objective: To determine the association of inflammatory markers, fibrinogen, and C-reactive protein (CRP), with 5-year mortality risk.

Methods: Vital status was ascertained in 922 HIV-infected participants from the Study of Fat Redistribution and Metabolic Change in HIV infection. Multivariable logistic regression estimated odds ratios after adjustment for demographic, cardiovascular, and HIV-related factors.

Results: Over a 5-year period, HIV-infected participants with fibrinogen levels in the highest tertile (>406 mg/dL) had 2.6-fold higher adjusted odds of death than those with fibrinogen in the lowest tertile (<319 mg/dL). Those with high CRP (>3 mg/L) had 2.7-fold higher adjusted odds of death than those with CRP <1 mg/L. When stratified by CD4 count category, fibrinogen (as a linear variable) remained independently associated [odds ratio (95% confidence intervals)] per 100 mg/dL increase in fibrinogen: 1.93 (1.57 to 2.37); 1.43 (1.14 to 1.79); 1.43 (1.14 to 1.81); and 1.30 (1.04 to 1.63) for CD4 <200, 200–350, >350 to 500, and >500 cells per microliter, respectively. Higher CRP also remained associated with higher odds of death overall and within each CD4 subgroup.

Conclusions: Fibrinogen and CRP are strong and independent predictors of mortality in HIV-infected adults. Our findings suggest that even in those with relatively preserved CD4 counts >500 cells per microliter, inflammation remains an important risk factor for mortality. Further investigation should determine whether interventions to reduce inflammation might decrease mortality risk in HIV-infected individuals.

Key Words: HIV, inflammation, C-reactive protein, fibrinogen, mortality

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INTRODUCTION

Despite marked reductions in HIV-related mortality since the introduction of highly active antiretroviral therapy (HAART),[1] mortality in HIV-infected persons remains higher than in the general population.[2–5] We previously reported that HIV infection was associated with 3-fold higher odds of death even after controlling for demographic and traditional cardiovascular disease (CVD) risk factors.[5] Whether inflammation (which is thought to be a consequence of chronic infection and immune activation) contributes to death in HIV-infected individuals, beyond demographic and CVD risk factors, is the topic of the present investigation.

To our knowledge, only one published study has examined the association of inflammatory markers with mortality in HIV-infected individuals in the HAART era.[6] That study from the Strategies for Management of Antiretroviral Therapy trial found a strong association of interleukin (IL)-6 (an inflammatory cytokine), D-dimer (an inflammatory protein involved in the clotting cascade), and C-reactive protein (CRP) (a proinflammatory biomarker) with mortality.

We recently found that HIV infection was associated with higher levels of fibrinogen[7] (another inflammatory biomarker in the clotting cascade) and CRP[8] than controls. Both fibrinogen and CRP have been associated with increased vascular and nonvascular mortality in the general population.[9–11]

We evaluated the association of fibrinogen and CRP with mortality in a geographically and ethnically diverse cohort of HIV-infected individuals in clinical care in the United States. To assess the role of immunosuppression severity on the association of inflammation with mortality, we further stratified HIV-infected individuals into 4 CD4 count risk categories. We hypothesized that inflammation would be independently associated with mortality in HIV infection.

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METHODS

Study Population

Between June 2000 and September 2002, 1183 HIV-infected men and women from 16 geographically diverse sites were enrolled in the study of Fat Redistribution and Metabolic Change in HIV infection (FRAM), with a follow-up examination conducted approximately 5 years later (FRAM2). HIV-infected participants in FRAM were nationally representative of HIV-infected patients in care.[12] The study design, recruitment methods, and data collection procedures for the entire FRAM cohort have been described elsewhere,[12] and retention outcomes for participants enrolled in the first examination have been reported.[5] At the second examination, 922 HIV-infected participants were known to be alive or known to be dead; vital status could not be determined for the remaining 261 HIV-infected participants. Linkage to the National Death Index was not possible because of institutional review board and patient confidentiality issues. Baseline characteristics of those with unknown vital status and those who were alive at the second FRAM examination seemed more similar to each other than to those who were dead. For those with unknown vital status and those who were alive at the second examination, median age was 41.0 and 42.5 years, respectively, compared with 44.5 for those who died. Similarly, the proportion of African American was 34% and 39%, respectively, compared with 51% for those who died; the proportion reporting current smoking was 46% and 38% vs. 61%. Among the HIV-related factors, median CD4 count was 338 and 389 vs. 189 cells per milliliter for those who died; median CRP was 1.6 and 1.7 vs. 2.8 mg/L; and median fibrinogen was 356 and 355 vs. 443 mg/dL. The institutional review boards at all sites approved the protocols for both FRAM examinations.

Study Measurements

As described previously,[7,8] fibrinogen and CRP were quantitatively measured in frozen plasma and sera, respectively (ie, stored at −70°C and not previously thawed), from the first FRAM examination using the BNII nephelometer from Dade Behring (Deerfield, IL), which utilizes a particle-enhanced immunonephelometric assay. The intra- and inter-assay coefficient of variations for fibrinogen were 2.7% and 2.6%, respectively. The lower limit of detection for the ultrasensitive CRP assay used in FRAM was 0.16 mg/L. The interassay coefficient of variations for CRP ranged from 3.7% to 4.5%.

FRAM also measured cystatin C in frozen sera using the BNII nephelometer[13] and estimated glomerular filtration rate (eGFR) based on cystatin C calculated using the Chronic Kidney Disease Epidemiology Collaboration Equation: ($eGFR = 76.7 \times \text{cystatin C}^{-1.19}$).[14] Urinalysis was also performed to determine microalbuminuria [positive urine dipstick (1+ or greater) or a urine albumin to urine creatinine ratio greater than 30 mg/g] in real time.[15]

Predictors of Mortality

Fibrinogen was studied both as a continuous variable (per 100 mg/dL increase) and as a categorical variable determined by tertiles of fibrinogen levels. CRP was studied both as a continuous variable after log transformation ($\log_2$) and as a categorical variable based on the Center for Disease Control/American Heart Association guidelines[16]: low risk < 1 mg/L, average risk 1–3 mg/L, and high risk > 3 mg/L. Demographic characteristics included self-reported age at baseline, sex, and race. Additional candidate predictors included CVD risk factors at baseline: diabetes (hypoglycemic medication use or fasting glucose ≥126), smoking status (current, past, never; pack years), waist circumference, systolic blood pressure, diastolic blood pressure, high-density lipoprotein (HDL) and non–HDL cholesterol, and medication use (antihypertensives and hypolipidemics). Candidate predictors related to HIV infection included HIV RNA level at baseline, CD4 count at baseline, self-reported duration of HIV, AIDS (defined as CD4 count <200 or AIDS-defining opportunistic infection/malignancy) at baseline, active hepatitis C virus infection (defined as a detectable hepatitis C virus RNA at baseline), and lean body mass by magnetic resonance imaging. In addition, use of each antiretroviral (ARV) drug and class was evaluated both by ever use of the drug or class and by total duration of use.

Statistical Analysis

Multivariable logistic regression analysis was used to investigate whether there was an independent association of fibrinogen and CRP with 5-year mortality risk in HIV-infected participants. Because the exact dates of death were unknown, those who died provided left-censored observations, meaning that death was only known to have occurred sometime before the contact attempt at approximately 5 years of follow-up. We therefore used logistic regression rather than Cox proportional hazards regression as our primary analysis, with an offset term to account for variation in follow-up time. Follow-up time was defined as elapsed time from baseline to follow-up examination or last contact. For deceased subjects, the follow-up time was defined as 4.6 years (the median follow-up time for those with known vital status).

Covariates included in the multivariable analysis are the baseline demographic CVD risk factors and HIV-related factors listed above. To ensure that models were not overfit, we also built more parsimonious models using a backward stepwise procedure. Similar analyses were conducted separately within HIV-infected participants who had different baseline CD4 counts (<200, 200–350, >350 to 500, and >500) using the same models. We stratified by CD4 count and not by presence or absence of detectable HIV RNA because we previously demonstrated that after adjustment for demographic and traditional cardiovascular risk factors, detectable HIV RNA was no longer associated with mortality risk.[5] Age-standardized mortality rates were calculated within strata of CD4, fibrinogen, and CRP using fitted values from the logistic regression models, with estimates standardized to the approximate mean age of the overall cohort at enrollment (40 years).

Multiple imputation using the Markov chain Monte Carlo method for arbitrary missing data was used to impute missing covariates.[17] To account for those with unknown vital status, we performed analyses using an inverse

probability weighting approach[18,19] by modeling each participant's probability of having a known death status. The inverse of this probability was then used as a weight (applied to persons with known vital status) in the logistic regression analysis of death. We conducted additional analyses to assess the potential bias introduced to our results by limited vital status data. First, we compared main model results without the application of inverse probability weights for vital status. Second, we repeated analyses assuming that all participants who were lost to follow-up were alive. Finally, we also performed analyses excluding those with unknown vital status. All analyses were conducted using the SAS system, version 9.2 (SAS Institute, Inc., Cary, NC).

RESULTS

Demographic and baseline clinical characteristics of the 922 HIV-infected participants stratified by tertiles of fibrinogen levels are shown in Table 1. HIV-infected participants with fibrinogen levels in the highest tertile at the baseline examination were older (median: 43.3 vs. 41.1 years), more often African American (50% vs. 29%), had lower HDL (median: 38.9 vs. 42.1 mg/dL), and higher total cholesterol (median: 196.1 vs. 187.2 mg/dL) and CRP levels (median: 3.30 vs. 1.12 mg/L) than those with fibrinogen levels in the lower tertiles. Among the HIV-related factors, HIV RNA levels were higher (median: 600 vs. 400 copies/mL) and CD4 counts were lower (median: 329 vs. 388 cells/mL) in those with

TABLE 1. Baseline Characteristics of HIV-Infected Participants by Fibrinogen Tertile*

	Fibrinogen			
	<319 mg/dL (n = 313)	319–406 mg/dL (n = 306)	>406 mg/dL (n = 303)	*P*
Age (y)	41.1 (35.8–45.9)	41.4 (36.4–47.6)	43.3 (37.1–49.7)	0.008
Gender (%)				
Female	26	30	34	0.29
Male	74	70	66	
Race (%)				
African American	29	39	50	0.018
White	57	50	36	
Other	14	11	14	
Diabetes	6.7	8.4	10.9	0.35
Smoking status (%)				
Current	40	41	44	0.89
Past	24	23	24	—
Never	37	36	32	—
Antihypertensive use	16	22	28	0.029
ACE-I use	6.1	7.9	13	0.33
Hyperlipidemia treatment (%)	12	19	16	0.012
Systolic BP (mm Hg)	114.8 (106.3–122.2)	116.3 (106.9–124.5)	114.5 (105.0–124.0)	0.36
Diastolic BP (mm Hg)	77.6 (70.2–83.8)	78.7 (71.3–84.6)	76.9 (69.4–83.0)	0.16
Waist circumference (cm)	86.7 (81.1–95.1)	89.8 (81.4–96.6)	89.3 (79.9–96.8)	0.13
Total cholesterol (mg/dL)	187.2 (156.9–215.3)	189.5 (154.7–227.8)	196.1 (159.7–240.2)	0.041
HDL (mg/dL)	42.1 (34.5–53.6)	39.5 (32.9–51.8)	38.9 (31.4–50.5)	0.014
CRP	1.12 (0.51–2.29)	1.66 (0.74–3.53)	3.30 (1.50–8.34)	<0.0001
<1 mg/L	45%	34%	16%	<0.0001
1–3 mg/L	38%	34%	31%	
3–10 mg/L	15%	28%	33%	
>10 mg/L	1.8%	3.2%	21%	
HIV-related factors				
HIV RNA (/1000 copies/mL)	0.4 (0.4–4.2)	0.4 (0.3–11.7)	0.6 (0.4–29.9)	0.008
Detectable HIV RNA (%)	45	51	54	0.12
Current CD4 count (cells/μL)	388 (244–575)	354 (225–558)	329 (156–514)	0.008
Detectable HCV RNA (%)	23	20	20	0.29
Injection drug use (%)	23	19	21	0.29
History of AIDS (%)†	66	70	78	0.18
HAART use (ever)	86	88	91	0.50

*Statistics estimated using IPCW weights. Continuous data are represented as median (interquartile ranges).
†AIDS defined by CD4 <200 or history of opportunistic infection or malignancy.
ACE, angiotensin-converting enzyme; HCV, hepatitis C virus.

fibrinogen levels in the highest tertile. Fibrinogen and CRP were moderately correlated (Spearman rank correlation coefficient: 0.43).

Association of Fibrinogen and CRP With Mortality in HIV Infection

Over the 5-year period, HIV-infected participants with fibrinogen levels in the highest tertile had an unadjusted mortality rate of 24.7% compared with 9.7% and 7.4% in those with fibrinogen in the middle and low tertiles, respectively. HIV-infected participants with high CRP (>3 mg/L) also had a higher unadjusted mortality rate of 19.3% compared with 14.4% in those with CRP 1–3 mg/L and 7.3% in those with CRP <1 mg/dL (Table 2). Age-standardized mortality rates showed a similar increase across these fibrinogen and CRP categories (Fig. 1). Mortality rates were highest in those for whom both fibrinogen and CRP were high.

After adjustment for demographic, CVD and HIV-related factors (Table 2), those with fibrinogen in the highest tertile had a 3.4-fold higher odds of death compared with those in the lowest tertile. Similarly, high CRP (>3 mg/L) was associated with a 3.7-fold higher odds of death compared to those with low CRP (<1 mg/L) (Table 2). When fibrinogen and CRP were simultaneously included in the multivariable model, both high fibrinogen and high CRP remained independently associated with a 2.6- and 2.7-fold higher odds of death compared with those in the lowest fibrinogen and CRP categories, respectively.

We observed similar results when fibrinogen and CRP levels were assessed as continuous measures. Fibrinogen and CRP were individually associated with higher odds of death [odds ratio (OR) = 1.70 per 100 mg/dL increase in fibrinogen, 95% confidence interval (CI): 1.43 to 2.02; and OR = 1.36 per doubling of CRP, 95% CI: 1.22 to 1.52, respectively]. When fibrinogen and CRP were included jointly in the multivariable model, both remained independently associated with higher odds of death (OR = 1.48 per 100 mg/dL increase in fibrinogen, 95% CI: 1.21 to 1.81; and OR = 1.20 per doubling of CRP, 95% CI: 1.06 to 1.37, respectively), although associations were attenuated. There was some evidence for a fibrinogen and CRP interaction, although it did not reach statistical significance ($P = 0.071$). As illustrated in Figure 1, effects of higher fibrinogen and CRP did not seem to be additive when both were elevated. Interactions of fibrinogen and CRP with CD4 and HIV RNA level seemed to be much weaker (all $P > 0.37$).

In all sensitivity analyses, point estimates for markers of inflammation were very similar to main model results (data not shown).

Association of Fibrinogen and CRP With Mortality Stratified by CD4 Category

We next examined the role of CD4 risk category (<200, 200–350, >350 to 500, and >500) on the association of fibrinogen and CRP with mortality by analyzing fibrinogen and CRP as continuous measures. Fibrinogen and CRP were independently associated with higher odds of death in every CD4 category, after adjustment for demographic, CVD, and HIV-related factors (Table 3). When fibrinogen and CRP were simultaneously included in the multivariable model, each remained associated with higher odds of death. The OR for the associations of fibrinogen and CRP with mortality were largest in those with CD4 <200 and smallest in those with CD4 >500, although the tests for trend were not statistically significant (fibrinogen: $P = 0.38$, CRP: $P = 0.90$). Even in those with CD4 >500, a substantial proportion had inflammation (26% with fibrinogen levels in the highest tertile and 36% with high CRP >3 mg/L). There was little difference in CRP levels by CD4 count category. The median and interquartile ranges by CD4 category <200, 200–350, 350–500, and >500 were 1.80 (0.82–4.14), 1.88 (0.83–3.99), 1.72 (0.79–3.65), and 1.82 (0.69–4.31), respectively, $P = 0.88$. The median and interquartile ranges for fibrinogen were higher in those with CD4 <200 compared with those with CD4 200–350, >350–500, and >500 [379 (316–471), 355 (299–422), 353 (283–439), and 355 (286–416), respectively; $P = 0.005$].

TABLE 2. Association of Fibrinogen and CRP With 5-Year Mortality in HIV-Infected Participants*

	n	Unadjusted Death Rate (%)	Multivariable-Adjusted OR (95% CI)*		
			Fibrinogen*	CRP*	Fibrinogen + CRP†
Fibrinogen					
<319 mg/dL	313	7.4	Reference	—	Reference
319–406 mg/dL	306	9.7	1.27 (0.72 to 2.22) $P = 0.41$	—	1.12 (0.63 to 1.98) $P = 0.70$
>406 mg/dL	303	24.7	3.35 (1.99 to 5.65) $P < 0.0001$	—	2.57 (1.46 to 4.52) $P = 0.001$
CRP					
<1 mg/L	293	7.3	—	Reference	Reference
1–3 mg/L	317	14.4	—	2.34 (1.33 to 4.11) $P = 0.003$	2.08 (1.17 to 3.70) $P = 0.013$
>3 mg/L	312	19.3	—	3.72 (2.09 to 6.63) $P < 0.0001$	2.68 (1.46 to 4.93) $P = 0.002$

Multivariable-adjusted model controls for demographics, smoking, non–HDL cholesterol, hepatitis C virus infection, waist circumference, lean mass, CD4 count, and total duration of efavirenz, indinavir, and amprenavir.

*Fibrinogen and CRP are shown entering the model individually.

†Fibrinogen and CRP are shown entering the model jointly.

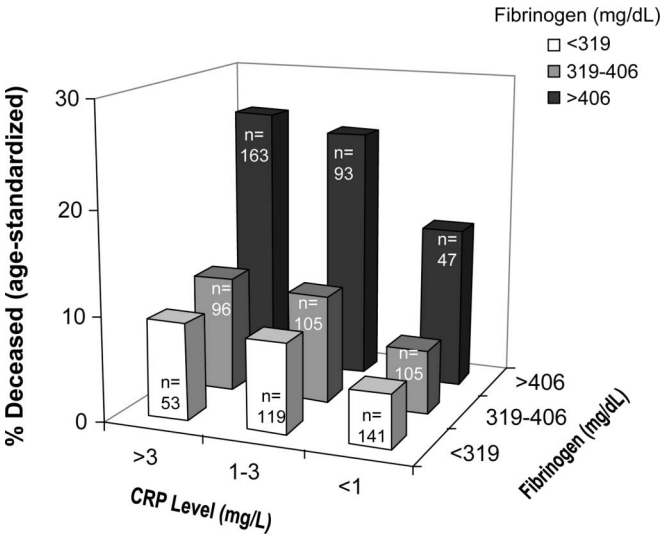

FIGURE 1. Age-standardized mortality rates* in HIV-infected patients, stratified by fibrinogen and CRP levels. *Estimates standardized to 40 years (the approximate mean age of the overall cohort at enrollment).

After further adjustment for markers of renal disease (ie, microalbuminuria and eGFR based on cystatin C), fibrinogen and CRP remained associated with higher odds of overall death and death in every CD4 category (data not shown).

DISCUSSION

In our nationally representative cohort of HIV-infected individuals in the recent HAART era, we found that elevated levels of fibrinogen and CRP were strong and independent predictors of 5-year mortality risk. Our findings suggest an important role for inflammation in mortality risk beyond demographic, cardiovascular, and HIV-related factors. Furthermore, when HIV-infected participants were stratified by degree of immunosuppression, fibrinogen and CRP were independently associated with higher odds of death in every CD4 category. Although fibrinogen and CRP seemed to have stronger associations in those with low CD4 count, the associations remained even in the highest CD4 category.

Our findings support the observations from the Strategies for Management of Antiretroviral Therapy trial,[6] which reported an association of IL-6, D-dimer, and CRP with mortality in HIV-infected participants from the recent HAART era. Their case-control analysis, however, included only 255 HIV-infected participants; the majority of whom had relatively preserved CD4 counts (median baseline CD4 count $>$ 500). Although our study did not test the association of IL-6 and D-dimer with mortality, we found that fibrinogen (also an inflammatory marker in the clotting cascade) was strongly associated. Our results are also consistent with a study from the pre-HAART era that found an association between CRP and all-cause mortality.[20] That study was limited to HIV-infected women from Brooklyn, New York, with a median CD4 count of 290. Taken together, these observations suggest that inflammation is an important risk factor for mortality in HIV-infected persons.

The strength of our study was the wide spectrum of CD4 levels in our participants, which allowed us to examine the effect of immunosuppression severity on the association of inflammation with mortality. As expected, we found that the OR for mortality associated with fibrinogen and CRP was greatest in magnitude for those with CD4 $<$200. However, more important is our finding that higher fibrinogen and CRP levels remained associated with increased mortality risk in

TABLE 3. Association of Fibrinogen and CRP With 5-Year Mortality in HIV-Infected Participants Stratified by CD4 Count

			Multivariable Adjusted, OR (95% CI)		
CD4 Category	**n**		**Fibrinogen***	**CRP***	**Fibrinogen + CRP†**
CD4 $<$200	213	Fibrinogen (per 100 mg/dL increase)	2.19 (1.83 to 2.62), $P < 0.0001$	—	1.93 (1.57 to 2.37), $P < 0.0001$
		CRP (per doubling)‡	—	1.44 (1.29 to 1.61), $P < 0.0001$	1.25 (1.10 to 1.42), $P = 0.001$
CD4 200–350	231	Fibrinogen (per 100 mg/dL increase)	1.64 (1.35 to 2.00) $P < 0.0001$	—	1.43 (1.14 to 1.79), $P = 0.002$
		CRP (per doubling)	—	1.33 (1.19 to 1.49), $P < 0.0001$	1.16 (1.02 to 1.32), $P = 0.022$
CD4 $>$350–500	189	Fibrinogen (per 100 mg/dL increase)	1.65 (1.34 to 2.02), $P < 0.0001$	—	1.43 (1.14 to 1.81), $P = 0.002$
		CRP (per doubling)	—	1.34 (1.20 to 1.50), $P < 0.0001$	1.17 (1.03 to 1.33), $P = 0.017$
CD4 $>$500	289	Fibrinogen (per 100 mg/dL increase)	1.49 (1.22 to 1.82), $P < 0.0001$	—	1.30 (1.04 to 1.63), $P = 0.022$
		CRP (per doubling)	—	1.30 (1.16 to 1.45), $P < 0.0001$	1.13 (1.00 to 1.29), $P = 0.055$

Multivariate-adjusted model controls for demographics, smoking, non–HDL cholesterol, hepatitis C virus infection, waist circumference, lean mass, CD4 count, and total duration of efavirenz, indinavir, and amprenavir.

*Fibrinogen and CRP are shown entering the model individually.

†Fibrinogen and CRP are shown entering the model jointly.

‡CRP is $\log_2$ transformed. Estimates are odds of death per doubling of CRP.

participants with CD4 >500. The lack of a substantial interaction of fibrinogen and CRP with CD4 also strengthened our hypothesis that the association of inflammation with mortality is independent of the absolute CD4 count. These findings could suggest that the CD4 cells remain immunologically activated despite CD4 cell restoration. The subsequent persistent inflammatory state could contribute to non–HIV-related comorbidities such as liver and CVD, which have been reported as the leading causes of non–HIV-related death in the HAART era.[4,21–24] Although we did observe that the OR was greater for those with CD4 count <200, this could be due to other factors such as infections or malignancies that are a consequence of a low CD4 count that may increase inflammation, for which we were not able to adequately adjust. Interestingly, a recent study found that early initiation of ARV therapy (before the CD4 count fell below 500) improved survival in HIV-infected individuals.[25] It is not yet known whether reduction of inflammation was a mechanism for the beneficial effect of ARV therapy. Whether or not levels of fibrinogen and CRP might be an additional prognostic marker warrants investigation.

The novel association of fibrinogen with all-cause mortality in HIV-infected individuals is also noteworthy. Fibrinogen is a coagulation protein that is thought to play a major role in platelet aggregation and thus vascular-related morbidity and mortality. However, a large meta-analysis of HIV-uninfected individuals found moderately strong associations between plasma fibrinogen levels and nonvascular mortality (mainly cancer), in addition to coronary heart disease, stroke, and other vascular mortality.[10] Interestingly, fibrinogen is increased in smokers (who are at risk for vascular and nonvascular morbidities) and has been shown to decrease with cessation of smoking.[26] Smoking is highly prevalent in HIV-infected individuals and is a key predictor of mortality risk in HIV infection.[5] Nevertheless, after controlling for cardiovascular risk factors including smoking, fibrinogen remained independently associated with mortality in HIV-infected individuals. The relationship of fibrinogen levels to D-dimer levels must also be explored; unfortunately, we were unable to assay D-dimer and IL-6 levels on our participants.

There are limitations to our study. First, vital status could not be determined in 23% of the HIV-infected participants who could not be contacted, which may have led to an underestimation of the mortality rate. We therefore used a multiple imputation and inverse probability weighting approach to model the participant's probability of having a known death status. Additional sensitivity analyses produced results that were similar to our primary modeling approach. Second, we did not have information regarding the cause of death and were therefore unable to discern whether the independent association of fibrinogen and CRP with mortality in HIV-infected individuals was due to cardiovascular or noncardiovascular deaths. Finally, as with all observational studies, our findings are subject to possible unmeasured confounding.

We conclude that elevated levels of fibrinogen and CRP are strong and independent predictors of all-cause mortality in HIV-infected adults. Our findings that fibrinogen and CRP remained associated with higher odds of death regardless of the degree of immunosuppression suggests that inflammation remains an important factor even in those with relatively preserved CD4 cells. Investigation is needed to determine whether interventions to reduce fibrinogen and CRP levels might decrease mortality risk in HIV-infected individuals.

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