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Association of Markers of Hemostasis with Death in HIV-infected Women

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Abstract

In HIV-negatives, markers of hemostasis including D-dimer, Factor VIII, plasminogen activator inhibitor-1 antigen (PAI-1) and total protein S are associated with all-cause and cardiovascular disease mortality. In HIV-positives, studies of D-dimer and Factor VIII with death were limited to short follow-up; associations of PAI-1 and total protein S with death have not been examined.

In 674 HIV-infected women from the Women's Interagency HIV Study (WIHS), markers from the first visit after enrollment were exposures of interest in multivariate analyses of death (AIDS and non-AIDS) in separate models at 5 and 16 years.

There were 87 AIDS and 44 non-AIDS deaths at 5 years, and 159 AIDS and 113 non-AIDS deaths at 16 years. An inverse association of total protein S quartiles with non-AIDS deaths was observed at 5 (p-trend=0.002) and 16 years (p-trend=0.02); there was no association with AIDS deaths. The 3rd quartile of PAI-1 was associated with AIDS deaths at 5 (hazard ratio (HR)=4.0, 95%

confidence interval (CI)=1.9–8.4) and 16 years (HR=3.4, 95% CI=1.9–5.9); and with non-AIDS deaths at 5 years (HR=4.8, 95%CI=1.6,13.9). D-dimer and Factor VIII were not associated with AIDS or non-AIDS death at 5 or 16 years.

Lower total Protein S was a consistent marker of non-AIDS death. We found no association between D-dimer with AIDS or non-AIDS death, in contrast to previous studies showing increased short term (<5 years) mortality, which may represent sex differences or population heterogeneity. Given longer survival on HAART, further studies of these markers are needed to determine their prognostic value.

INTRODUCTION

In the general HIV-uninfected population, certain plasma markers of hemostasis including D-dimer, Factor VIII, plasminogen activator inhibitor-1 antigen (PAI-1) and total protein S are associated with incident cardiovascular disease (CVD), CVD mortality, thrombosis and all-cause mortality[1–11]. Because of the clinical significance of these markers, our group and others have studied whether levels of these markers differ between HIV-infected patients and HIV-uninfected controls[12–19]. These studies found that, as compared to those who are HIV-uninfected, HIV-infected persons have higher D-dimer, Factor VIII and PAI-1 levels and lower total Protein S levels[13–19]. Further, in a prior study we found that Factor VIII and D-dimer levels are higher and total Protein S levels are lower, with higher HIV RNA viral load[12].

Beyond understanding the relationship between HIV infection and markers of hemostasis, an important priority is to understand which if any of these markers is associated with clinically meaningful events and death in HIV-infected persons. We are unaware of any studies of total Protein S or PAI-1 with mortality or clinical events in persons with HIV. However, in patients with advanced HIV disease who initiated ART, high D-dimer levels measured prior to ART were associated with increased all-cause mortality in 4 previous studies, and cardiovascular/non-AIDS deaths in 3 previous studies[20–26]. A recent case-control study in two large cohorts reported increased mortality associated with increased Factor VIII[27]. Although informative, these prior studies of D-dimer levels and Factor VIII in HIV-positives examined deaths over relatively short mean follow-up periods (12 months to 4.9 years)[20,22–26], did not all distinguish between AIDS and non-AIDS deaths[20,22–24,27,28], and included few women[20,22–26,28].

To expand upon prior marker research with these issues in mind, and to increase understanding of the relationship between plasma markers of hemostasis and long term mortality in HIV-positive persons, we examined associations between D-dimer, Factor VIII, PAI-1 and total Protein S with AIDS and non-AIDS mortality in a cohort of 674 HIV-infected women with 16 years of follow-up and independently reviewed and verified mortality data.

MATERIALS AND METHODS

Study Population

The Women's Interagency HIV Study (WIHS) is a multicenter, prospective study of women with or at risk for HIV. Participants were recruited using similar methods at six US sites during three recruitment waves: i) 1994–1995, ii) 2001–2002 and iii) 2011–2012. Detailed methods and characteristics of the WIHS population have been described previously[29,30]. At enrollment and semiannually thereafter, interviews are conducted, a physical exam performed, and blood specimens collected. The study protocol was approved by the Institutional Review Boards at each study site, and all participants provided written informed consent.

Included in the current study are WIHS participants recruited during 1994–1995 and 2001–2002. From this population, we selected from our previous parent study a subset of non-pregnant women in whom we measured D-dimer, Factor VIII, total protein S, and PAI-1 in specimens obtained at the first visit after WIHS enrollment. This subset included 900 women randomly selected on hepatitis C virus (HCV) status: 450 randomly selected women with chronic HCV infection (defined as HCV-seropositive with detectable plasma HCV RNA) from 1,023 non-pregnant women with chronic HCV, and 450 randomly selected HCV-seronegative women from 2,709 non-pregnant women without HCV antibody. This sampling strategy allowed us to characterize independent cross-sectional associations of HIV and HCV status with markers of hemostasis, as previously described, and yielded a total sample of 674 HIV-infected and 226 HIV-uninfected women[31]. For the current longitudinal study reported in this paper, we followed all 674 HIV-seropositive women derived from the parent substudy described above (Supplemental Figure 1).

Clinical Laboratory Testing

Markers of hemostasis (D-dimer, FVIII, PAI-1 and total protein S) were measured at the University of Vermont, Laboratory for Clinical Biochemistry Research, as previously described[31]. T cell subsets (cells/μL) and plasma HIV viral loads were measured in laboratories participating in the AIDS Clinical Trials Quality Assurance Program. HCV serostatus at enrollment was determined using a commercial enzyme immunoassay, and HCV viremia was determined in HCV-seropositive women using either the COBAS Amplicor Monitor 2.0 or the COBAS Taqman assay (both from Roche Diagnostics, Branchburg, New Jersey, USA).

Ascertainment of Deaths

Ascertainment and classification of deaths in WIHS have been previously described[32–34]. All death certificate data were reviewed independently by two clinicians using specific criteria which classified a death as AIDS-related if an AIDS-defining infection or malignancy was the cause of death, or if the cause of death was pneumonia or sepsis in the setting of a recent CD4+ count <200 cells/μL. Cause of death was classified as indeterminate if the cause of death was entirely nonspecific (most frequently "cardiopulmonary arrest"), if the death certificate had conflicting causes, or if HIV was the only cause of death for a woman whose CD4 count was ≥200 cells/μL at the most recent

WIHS visit. Deaths were classified as non-AIDS if a non-AIDS event was the primary cause of death.

Statistical Analysis

Associations between levels of each marker of hemostasis (measured at the first visit after enrollment) with AIDS and non-AIDS deaths during the first 5 years of follow-up were examined. These analyses characterized the relationship of these markers with deaths over a relatively short follow-up period, to ensure comparability between the current investigation and prior studies of D-dimer and all-cause mortality, where the maximum average follow-up time was 4.9 years[21,25]. Then, to use all the available data, we determined associations of hemostasis markers with AIDS and non-AIDS deaths using up to 16 years of follow-up time. Markers of hemostasis were categorized in quartiles to account for possible non-linear associations. Statistical significance of these associations between unadjusted unordered quartiles and mortality was determined using log-rank tests (with three degrees of freedom). Multivariate associations of D-dimer, FVIII, PAI-1, and total protein S with AIDS and non-AIDS deaths were determined using unordered quartiles, and using Cox models with the first quartile of the hemostasis marker as the reference category. These models included each of the four markers of hemostasis (in four separate models) along with covariates described below. We also analyzed each model using the quartile (1,2,3,4) number of each marker of hemostasis as a linear trend variable, reporting a p-value for the trend in quartiles and a hazard ratio for each quartile increase on outcome risk.

We included covariates known to be associated with AIDS or non-AIDS death, including age (in years), race/ethnicity (Black including Hispanic, White including Hispanic and other), smoking (never, former, or current in the last 6 months), recruitment wave (as more women were on HAART therapy in the 2001–2002 wave compared to the 1995–1995 wave), and site/laboratory testing date ("batch effect", as described in analyses from our previous paper[31]). In our previous study we noted significant associations of Factor VIII and total Protein S with chronic HCV infection[31] and thus adjusted for chronic HCV (HCV antibody positive with detectable RNA). We included HIV viral load (VL) and CD4+ T cell count from the first visit after enrollment. If CD4+ count or VL was missing at that visit, we instead used CD4+ and VL data from the enrollment visit. HIV treatment was self-reported at each subsequent follow up visit and was included as a time-dependent variable because of its significant effect on CD4+ count, viral load, and mortality. We categorized treatment as none, monotherapy (single agent use), or combination therapy (more than one antiretroviral agent not meeting definition of highly active antiretroviral therapy (HAART)) or HAART. The definition of HAART was guided by the DHHS/Kaiser Panel guidelines and is defined as: the reported use of three or more antiretroviral medications, one of which has to be a PI, an NNRTI, one of the NRTIs abacavir or tenofovir, an integrase inhibitor (e.g., raltegravir), or an entry inhibitor (e.g., Maraviroc or enfuvirtide)[35]. Trends in HAART and non-HAART therapy use among WIHS women have been previously reported[36,37].

RESULTS

Demographic and clinical characteristics of the study population at enrollment

Table 1 shows demographic and clinical characteristics of the 674 HIV-positive women at enrollment. Most were black (60%) and the median age was 38 years (interquartile range (IQR) 33–42 years). Most (59%) were current smokers. Median CD4+ count and $\log_{10}$ HIV viral load were 333 cells/μL (IQR 170–520 cells/μL) and 4.2 $\log_{10}$ copies/ml (IQR 3.3–4.9 $\log_{10}$ copies/mL), respectively. Only 14% of women were receiving HAART at enrollment, reflecting that 80% of the study population was recruited in 1994–1995, prior to the widespread availability of HAART. 55% of the study population was HIV/HCV co-infected.

Markers of hemostasis and deaths in the study cohort

We examined each of the four markers of hemostasis for normality. Three markers, Factor VIII, PAI-1 and total Protein S, were approximately normal, but D-dimer was skewed to the right. Median serum levels, IQR and median marker levels by demographic and clinical characteristics are presented in Supplemental Table 1. Quartiles of each marker of hemostasis were similar by age (data not shown). The level of PAI-1 and total Protein S differed by smoking status (p=0.01 and p=0.002, respectively). Significant differences in all four markers were seen by race (D-dimer, p=0.01; PAI-1 p=0.0001; total Protein S, p=0.005; Factor VIII, p<0.0001). Significant differences of D-Dimer (p=0.004), PAI-1 (p<0.0001) and total protein S (p<0.0001) were also seen between strata of antiretroviral therapy use at baseline. The distributions of Factor VIII (p<0.0001), PAI-1 (p=0.03) and total Protein S (p<0.0001) differed significantly by chronic HCV infection. After 5 years of follow-up, there were 87 AIDS deaths, 44 non-AIDS deaths, 4 indeterminate deaths and 1 death of unknown cause (Supplemental Figure 1). By 16 years, there were a total of 159 AIDS deaths, 113 non-AIDS deaths, 9 indeterminate deaths and 15 deaths of unknown causes (Supplemental Figure 1). The number of deaths by HCV status and enrollment cohort are shown in Supplemental Table 2.

Associations of markers of hemostasis with AIDS death

Initial analyses determined unadjusted unordered quartile associations between markers of hemostasis and AIDS death and are shown as Kaplan-Meier plots in Supplemental Figure 2, Panels A–D. There were strong significant associations between higher quartiles of D-dimer and Factor VIII with more rapid death from AIDS in unadjusted analyses at 5 years (D-dimer: p=0.01; Factor VIII: p=0.03), and at 16 years (D-dimer: p=0.01; Factor VIII: p=0.005). No significant associations of PAI-1 and total Protein S with AIDS death were seen in unadjusted analyses.

In multivariate analyses including adjustment for age, race, CD4+ count, HIV VL, HCV, smoking, enrollment cohort, and batch effect, and time-dependent antiretroviral therapy use, the association of higher D-dimer with AIDS death was not statistically significant at 5 years (p-trend=0.15) or 16 years of follow-up (p-trend=0.44) (Table 2). The trends observed with the unadjusted KM curve for the association of D-dimer with AIDS deaths at both 5 and 16 years were mitigated in the adjusted analyses primarily by the inclusion of CD4 count (HR 0.52, p<.0001 at 5 years and HR 0.71 per 100 cells/μl, p<.0001 at 16 years), viral load (HR

2.14, p<.001 at 5 years and HR 1.79 per $\log_{10}$, p<.0001 at 16 years), and time-dependent HAART use (compared to no treatment HR 0.20, p<.001 at 5 years and HR 0.34, p<.001 at 16 years).

In contrast to the unadjusted analyses, in multivariate analysis there was no significant association of higher Factor VIII with AIDS death at 5 years (p-trend=0.98) or 16 years (p-trend=0.09). There was also no significant association of total protein S with AIDS deaths at 5 (p-trend=0.54) or 16 years (p-trend=0.07).

After multivariate adjustment, we observed a significant association of the 3rd quartile of PAI-1 (vs. the 1st quartile) with AIDS deaths at 5 years (HR=3.98, 95% confidence interval (CI) 1.89–8.38, p<0.001); and at 16 years (HR =3.39, 95% CI=1.94–5.90, p<0.001). The 4th quartile of PAI-1 (vs. quartile 1) was also significantly associated with AIDS deaths at 16 years (HR=2.02, 95% CI 1.10–3.72, p=0.02). The trend of increasing quartiles of PAI-1 and AIDS death was not significant at 5 years (p-trend,=0.08), but was significant at 16 years (p-trend, p=0.01).

Associations of markers of hemostasis with non-AIDS death

In unadjusted unordered quartile analyses (Supplemental Figure 3, Panels A–D), no significant associations of D-dimer with non-AIDS deaths were observed (p=0.77, 5 years; p=0.15, 16 years). Factor VIII was not associated with more rapid non-AIDS deaths at 5 years (p=0.10) but the association was significant at 16 years (p=0.03). In contrast, there was an association between PAI-1 and more rapid non-AIDS deaths at 5 years (p=0.04) which was not seen at 16 years (p=0.35). Lastly, lower total Protein S was significantly associated with more rapid non-AIDS death at both 5 (p=0.002) and 16 years (p=0.004).

After multivariate adjustment, increasing quartiles of D-dimer or Factor VIII were not associated with non-AIDS deaths at 5 years (D-dimer, p-trend=0.44; Factor VIII, p-trend=0.71) or 16 years (D-dimer, p-trend=0.45; Factor VIII, p-trend=0.55). There was one significant association of the third quartile of D-dimer with non-AIDS deaths at 16 years (p=0.04) (Table 2).

In multivariate analysis, higher PAI-1 levels were not associated with increased risk of non-AIDS deaths at 5 years (p-trend=0.50) or at 16 years (p-trend=0.12). There was one significant association for the 3rd quartile compared to the 1st, (HR 4.75, 95% CI=1.63,13.9; p=0.004) which was attenuated and not statistically significant at 16 years.

There was a significant inverse association of total Protein S with non-AIDS death at 5 years (p-trend=0.002) and16 years (p-trend=0.02). Total protein S quartiles 2 through 4 (vs. the 1st quartile) were significantly associated with a reduction in risk of non-AIDS deaths after 5 years of follow up (quartile 2 HR=0.41, 95% CI 0.19–0.93, p=0.03; quartile 3 HR=0.19, 95% CI 0.07–0.51, p=0.001; quartile 4 HR=0.27, 95% CI 0.09–0.80, p=0.02). This inverse association remained significant at 16 years, however only the 3rd quartile (compared to the 1st) remained significant (quartile 3 HR=0.50, 95%CI 0.28–0.90, p=0.02).

DISCUSSION

This study investigated whether four markers of hemostasis were associated with AIDS and non-AIDS mortality after 5 and 16 years in HIV-infected women in the United States. To our knowledge, this is the first study of multiple markers to discriminate between AIDS and non-AIDS deaths in women, and the study with the longest follow-up time to date. In our study, the majority of deaths were AIDS deaths. We found no significant consistent associations of D-dimer, Factor VIII or total Protein S with AIDS death, but found a significant association of PAI-1 with AIDS deaths at 5 and 16 years. We found no significant consistent associations of D-dimer or Factor VIII with non-AIDS death. However, we found a significant positive association of PAI-1 with non-AIDS deaths at 5 years, and a strong inverse association of lower total Protein S with non-AIDS deaths at 5 years, with a continued significant trend at 16 years.

Among HIV infected patients, D-dimer has been the most-studied of the markers presented in this investigation. Four previous studies found associations between D-dimer and all-cause mortality[20,22–24]. In contrast, we did not find an association between D-dimer and AIDS deaths. Compared to these four previous studies, our study had longer follow-up time, distinguished AIDS deaths from non-AIDS deaths, and included a larger percentage of HCV/HIV co-infected participants. HCV was an independent risk factor for death for both AIDS and non-AIDS deaths at 5 and 16 years (data not shown). Further, our study included only women, of whom fewer had advanced disease: the median cell count was higher (333 cells/μL) at the first visit after enrollment, and fewer women were on antiretroviral treatment at baseline[20,22–24].

We failed to find similar associations with much longer follow up time than in these studies, even after adjustment for time-dependent ART use. Not surprisingly, HAART use in our study (compared to no treatment) conferred an independent, significant protective effect out to 16 years. It is established that early deaths may occur after HAART initiation, likely due to infectious diseases, conditions that pre-existed HAART, or diseases related to immune reconstitution inflammatory syndrome (IRIS), especially in developing countries[38,39]. Our results suggest that among those patients who survive pre-HAART comorbidities or IRIS, the long-term implications of inflammatory response and endothelial dysfunction may be mitigated by other factors, namely HAART use. In our study, HAART use remained a significant, independent predictor of decreased mortality in all four marker models at 5 and 16 years of follow up data.

We found no association between Factor VIII or total Protein S with AIDS deaths. These results contrast the findings of Baker et.al. study of two large combined cohorts (SMART and ESPRIT) which after adjustment including hepatitis infection, found a significant association of higher Factor VIII at enrollment date and all cause mortality in cases compared to nested controls[27]. Both high Factor VIII and low total Protein S are observed in HIV infected individuals and are risk factors for thrombosis [15,17–19]. While the possibility of Type II error does exist, it is also possible that the mechanisms for increased thrombosis in HIV from these abnormalities in Factor VIII and total Protein S do not translate into increased risk of AIDS-specific deaths in our population.

We found a significant association of the 3rd quartile of PAI-1 with AIDS death at 5 years, and a significant association of the 3rd and 4th quartile at 16 years. This association could be a threshold effect above which PAI-1 is associated with AIDS death, where the 3rd quartile appears larger than the 4th due to a Type II error. There was a non-significant trend test at 5 years, which became significant at 16 years, suggesting that increasing quartiles of PAI-1 could be associated with AIDS death at the long term follow up. PAI-1 in HIV is associated with hyperinsulinemia[40] and lipodystrophy syndrome[41], and in non-HIV infected patients is associated with a number of diseases, including ischemic cardiovascular disease and atherosclerosis[42,43], thrombosis[44,45], and the metabolic syndrome[46,47]. In our previous study, we found no association between PAI-1 levels with HIV or HCV, although one other study found an association between worsening HIV disease and higher PAI-1 levels[14]. PAI-1 is produced in many cells including endothelial cells and cardiac myocytes, and increased PAI-1 leads to inhibition of fibrinolysis and a hyperfibrinolytic state which could contribute to AIDS deaths[48,49].

We did not find an association between D-dimer and non-AIDS death at 5 or 16 years, after adjustment. We did find one significant independent association between the third quartile of D-dimer and non-AIDS deaths at 16 years (p=0.04), which likely represents a Type 1 error given that we did not see this association at 5 years. Our results contrast with three previous studies including Ford et. al[26], the SMART study[25], and a recent meta analysis of 3,766 patients with well controlled HIV[21]. In the meta analysis, D-dimer predicted the development of non-AIDS disease at an average follow up of 4.9 years (2.7 years in SMART, 6.7 in ESPRIT, and 7.0 in SILCAAT)[21]. D-dimer and IL-6 together increased the risk of a combined endpoint of non-AIDS diagnoses and non-AIDS deaths. Several previous studies in a non-HIV infected population have suggested that associations between D-dimer and cardiovascular disease differ qualitatively by sex[50,51], and it is possible that we did not find an association in women due to these sex differences. In non-HIV infected populations, previous studies found increases in D-dimer were associated with recurrent coronary events[52], and obstructive coronary disease in men[51], but not women, and similar hormonally driven mechanisms may also be driving a sex-difference in HIV-infected women.

We found no association of Factor VIII with non-AIDS deaths. Although this is the first study known to us conducted in HIV infected patients, one previous study of non-HIV infected persons in the Multi-Ethnic Study of Atherosclerosis (MESA) found an association of factor VIII with both total and cancer mortality[11]. It is possible that Factor VIII is not in the causal pathway to mortality as we did not find any association at either follow up time for either AIDS or non-AIDS deaths.

We found a significant association of the 3rd quartile only of PAI-1 with non-AIDS deaths at 5 years. Interestingly, the 3rd quartile was also significantly associated with AIDS deaths at 5 years and 16 years. While the possibility of a Type I error for non-AIDS deaths is possible, these associations likely warrant further investigation.

We found a strong inverse association of total Protein S with non-AIDS deaths at 5 years, which remained significant at 16 years. Protein S deficiencies predispose to thromboembolic complications[53] and we previously reported in WIHS an independent association of lower

total Protein S with HCV and HIV[31]. Protein S is synthesized by endothelium and previous studies suggest that HIV viral alteration of endothelial function may play a role in this deficiency, although the pathway remains to be fully elucidated [19,54].

Our study has several limitations. The WIHS cohort may not be representative of women living with HIV or HCV in the United States and caution must be used in applying results from this cohort study to the general population. Only 14% of women were on HAART at the first visit after enrollment, when the markers were drawn, and thus the generalizability of this study may be limited. However, we did adjust for time-dependent HAART, which has accounted for the impact of treatment on both AIDS and non-AIDS deaths.

In summary, in a cohort of 674 HIV positive women, we found that only PAI-1 was associated with AIDS death. We found no associations of Factor VIII and total Protein S with AIDS death. There was no association between D-dimer with AIDS or non-AIDS death, in contrast to previous studies of D-dimer which found increased short term (<5 years) mortality. This again may represent a sex difference, as we know that hemostasis and clotting diatheses are different in men and women [55–57]. We also found a significant association between the 3rd quartile of PAI-1 and non-AIDS deaths at 5 years and a strong inverse association of total Protein S with non-AIDS deaths at 5 years and 16 years. Given the longer survival of persons on HAART therapy and increased interest in HIV-related inflammation and aging, further studies are needed to determine the long-term prognostic value of these markers in both women and men.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Demographic and clinical characteristics at the first visit after enrollment of 674 HIV positive women*

Demographic Characteristics	Median (IQR)
Age (years)	38 (33, 42)
Race	
White	141 (21%)
Black	406 (60%)
Other (Including Hispanic)	127 (19%)
Current Smoking	396 (59%)
Enrollment Cohort	
2001–2002	138 (20%)
1994–1995	536 (80%)
Clinical Characteristics	
CD4+ T cell count (cells/μL)	333 (170,520)
HIV viral load (copies/mL)	14,934 (1,856, 89,000)
HIV $\log_{10}$ viral load (copies/mL)	4.2 (3.3, 4.9)
Antiretroviral treatment at baseline	
None	308 (45%)
Monotherapy	166 (25%)
Combotherapy	106 (16%)
HAART	94 (14%)
Chronic HCV (HCV Ab+/HCV RNA+)	370 (55%)

IQR:interquartile range; HAART: highly active anti-retroviral therapy; HCV: hepatitis C virus

*models adjusted for batch effect

Table 2

Association of markers of hemostasis with AIDS and non-AIDS deaths at 5 years and 16 years of follow up*

	AIDS deaths 5 years			AIDS deaths 6 years			Non-AIDS deaths 5 years			Non-AIDS deaths 16 years		
	Deaths	**HR (95% CI)**	**p**	**Deaths**	**HR (95% CI)**	**p**	**Deaths**	**HR (95% CI)**	**p**	**Deaths**	**HR (95% CI)**	**p**
D-dimer												
Unordered			0.31			0.41			0.79			0.18
1st quartile (ref)	14			35			13			31		
2nd quartile	20	1.51 (0.71,3.20)	0.29	31	0.73 (0.43,1.22)	0.23	10	0.68 (0.29,1.63)	0.39	31	0.93 (0.55,1.58)	0.79
3rd quartile	22	1.19 (0.57,2.49)	0.64	45	0.92 (0.57,1.48)	0.72	9	0.71 (0.28,1.79)	0.47	18	**0.52 (0.28,0.97)**	**0.04**
4th quartile	31	1.82 (0.91,3.64)	0.09	48	1.08 (0.67,1.73)	0.76	12	0.68 (0.28,1.61)	0.38	33	0.91 (0.54,1.55)	0.73
p-trend		1.17 (0.95,1.44)	0.15		1.06 (0.91,1.24)	0.44		0.89 (0.67,1.19)	0.44		0.93 (0.78,1.11)	0.45
Factor VIII												
Unordered			0.52			0.31			0.57			0.50
1st quartile (ref)	16			30			8			19		
2nd quartile	14	0.56 (0.26,1.24)	0.15	30	0.99 (0.58,1.69)	0.97	8	0.81 (0.28,2.37)	0.70	28	1.52 (0.81,2.84)	0.20
3rd quartile	29	0.74 (0.34,1.60)	0.44	51	1.41 (0.83,2.38)	0.20	9	0.66 (0.22,1.97)	0.46	28	1.13 (0.58,2.18)	0.72
4th quartile	28	0.81 (0.34,1.90)	0.62	46	1.54 (0.84,2.84)	0.17	17	1.20 (0.38,3.76)	0.76	36	1.46 (0.69,3.08)	0.32
p-trend		1.00 (0.75,1.32)	0.98		1.19 (0.97,1.44)	0.09		1.08 (0.74,1.57)	0.71		1.07 (0.85,1.35)	0.55
PAI-1												
Unordered			**0.001**			**<0.001**			**0.006**			0.33
1st quartile (ref)	17			32			6			22		
2nd quartile	19	1.53 (0.75,3.14)	0.25	33	1.63 (0.97,2.73)	0.07	13	2.42 (0.90,6.52)	0.08	30	1.53 (0.86,2.73)	0.15
3rd quartile	28	**3.98 (1.89,8.38)**	**<0.001**	46	**3.39 (1.94,5.90)**	**<0.001**	17	**4.75 (1.63,13.9)**	**0.004**	28	1.75 (0.90,3.40)	0.10
4th quartile	23	1.82 (0.79,4.20)	0.16	48	**2.02 (1.10,3.72)**	**0.02**	8	1.33 (0.38,4.72)	0.66	33	1.78 (0.90,3.51)	0.10
p-trend		1.26 (0.97,1.64)	0.08		**1.28 (1.05,1.55)**	**0.01**		1.13 (0.80,1.60)	0.50		1.19 (0.96,1.48)	0.12
total Protein S												
Unordered			0.33			0.10			**0.005**			0.07
1st quartile (ref)	17			32			22			42		
2nd quartile	20	0.81 (0.40,1.63)	0.56	39	1.12 (0.68,1.83)	0.67	10	**0.41 (0.19,0.93)**	**0.03**	32	0.93 (0.57,1.54)	0.79

	AIDS deaths 5 years			AIDS deaths 6 years			Non-AIDS deaths 5 years			Non-AIDS deaths 16 years		
	Deaths	**HR (95% CI)**	**p**	**Deaths**	**HR (95% CI)**	**p**	**Deaths**	**HR (95% CI)**	**p**	**Deaths**	**HR (95% CI)**	**p**
3rd quartile	19	0.70 (0.34,1.45)	0.34	40	1.05 (0.63,1.76)	0.85	6	**0.19 (0.07,0.51)**	**.001**	21	**0.50 (0.28,0.90)**	**0.02**
4th quartile	31	1.23 (0.60,2.52)	0.58	48	1.74 (1.01,2.98)	0.05	6	**0.27 (0.09,0.80)**	**0.02**	18	0.54 (0.27,1.09)	0.09
p-trend		1.08 (0.85,1.36)	0.54		1.18 (0.99,1.41)	0.07		**0.56 (0.39,0.81)**	**0.002**		**0.78 (0.63,0.97)**	**0.02**

HR: hazard ratio; 95% CI: 95% confidence interval

*adjusted for age, race, CD4+count, viral load, time-dependent antiretroviral therapy use, HCV status, smoking, enrollment period and batch effect