Extrinsic Pathway Coagulation Factors Are Associated with Mortality During Treated HIV Disease

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Introduction

- D-dimer predicts mortality in patients with HIV infection, a finding reproduced in multiple studies.[1-4]
- These data support that activation of the coagulation system may contribute to long-term complications among patients with treated HIV disease.
- Blood thrombosis primarily involves local activation of the extrinsic coagulation pathway, and can be broken down into the following steps (**Figure 1**):
  1. Expression of tissue factor (e.g., on monocytes) combining with factor (F) VIIa to initiate a cascade of procoagulant activation (e.g., FX, FIX, and FV).
  2. The process is facilitated and amplified by cofactors (e.g., FVIII).
  3. The resulting thrombin generation facilitates cleavage of fibrinogen.
  4. Vigorous thrombin generation is then attenuated by inhibitors (e.g., antithrombin (AT), tissue factor pathway inhibitor (TFPI) and protein C).
- We previously studied the influence of HIV replication on extrinsic pathway coagulation factors, and reported that HIV replication led to:[5]
  1. Decreases hepatocyte-dependent procoagulant (FII, or prothrombin) and anticoagulant factors (AT and Protein C)
  2. Increases in non-hepatocyte dependent procoagulant factors (e.g., FVIII).
  3. The net-effect of these changes in the profile of extrinsic pathway factors was procoagulant.

Study Goal

To explore mortality risk associated with individual extrinsic pathway coagulation factors, including patterns across hepatocyte and non-hepatocyte dependent factors, among patients with treated HIV disease.

Study Design

In a nested case-control study of the SMART and ESPRIT control arms (randomized to continuous antiretroviral therapy), we evaluated all-cause mortality associations with 9 extrinsic pathway factors; 6 are hepatocyte products (FII, FVII, FIX, FX, antithrombin, and protein C) and 3 are not (FV, FVIII, and TFPI). Three controls were matched per case on enrollment date, age (+/- 5yrs), study, and country. A total of 134 deaths had specimens available for analyses in this study; 60 deaths from the viral suppression (VS) group in SMART (among 64 total deaths) and 74 deaths from the control arm in ESPRIT (among 116 total deaths).

Statistical Methods

Odds ratios (OR) with 95% confidence intervals (CI) for 4th vs. 1st quartile of each marker were estimated using conditional logistic regression accounting for matching factors and adjusting for gender and race. To study the combined effect of the hepatocyte-dependent factors, individual biomarkers for each case/control set were ranked and combined over the 6 biomarkers to obtain a "Hepatocyte Score". Additional covariates studied included: hepatitis co-infection, diabetes, history of cardiovascular disease, and baseline CD4, HIV RNA, and IL-6.

Laboratory Methods

We measured 9 extrinsic pathway factors (Table 2) along with D-dimer levels from baseline specimens. Plasma was collected using EDTA tubes. Biomarker levels were measured at the Laboratory for Clinical Biochemistry Research at the University of Vermont. D-dimer levels with immunoturbidometric methods on the Sta-R analyzer, Liatest D-DI (Diagnostica Stago). Factor (F) levels were measured using ELISA (Enzyme Research Lab, South Bend, IN), including: FII (prothrombin), FV, FVII/FVIIa, FVIII, FIX, FX and tissue factor pathway inhibitor (TFPI; R&D Systems, Minneapolis, MN). Protein C and protein S levels (free and total) were measured using ELISA (Stago Diagnostics, Parsipanny, NJ). Antithrombin (AT) and Fibrinogen levels were measured on Stago STA-R analyzer (Stago Diagnostics, Parsipanny NJ). Factors expressed as % represent the relative activity compared to standard laboratory controls. All samples were analyzed blinded to clinical data and mortality status.

Figure 1: Coagulation with Thrombin Generation and inhibition

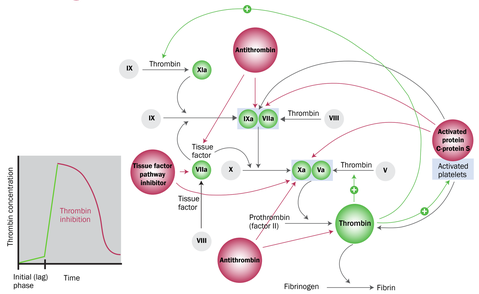


Figure Legend: Presented is Figure 1B from Tripodi A and Mannucci PM (NEJM 2011;365:147-156).6 Procoagulant factors are represented by Roman numerals, and the key procoagulant steps are highlighted in green. Key steps in thrombin inhibition are then shown in red. Examples of the in vitro curves for thrombin generation and inhibition are also shown.

Results

Table 1: Baseline Clinical and Demographic Characteristics of Death Cases (n=134) and Controls (n=388)

Baseline Characteristics of Death Cases and Controls	Cases	Controls	P-value*
N	134	388	
Age (mean, SD)	47 (39 - 54)	46 (39 - 52)	<.001
Female (%)	19.4	20.9	0.766
Black race (%)	17.9	16.0	0.438
CD4+ (median, IQR)	454 (367 - 604)	495 (405 - 660)	0.058
Nadir CD4+ (median, IQR)	194 (86 - 297)	210 (104 - 322)	0.096
HIV RNA (% ≤500)	64.9	76.8	0.005
Hepatitis co-infected (%)	36.4	18.2	<.001
BP lowering treatment (%)	22.4	13.7	0.010
Lipid lowering treatment (%)	17.9	14.7	0.411
Diabetes (%)	9.0	6.7	0.446
Prior CVD (%)	9.0	2.6	0.002
Current smoker** (%)	60.0	39.0	0.005
BMI (median, IQR)	23.3 (21.2 - 27.1)	24.4 (22.4 - 26.8)	0.288
hsCRP (median, IQR)	2.94 (1.57 - 6.73)	1.75 (0.76 - 3.84)	0.004
IL-6 (median, IQR)	3.10 (2.06 - 4.40)	1.90 (1.30 - 3.10)	<.001
D-dimer (median, IQR)	0.33 (0.23 - 0.55)	0.26 (0.15 - 0.39)	0.004

*P-value from univariate conditional logistic regression model
**Only available for SMART participants

Results Continued...

Table 2: Mortality Associations with Coagulation Markers

Coagulation Marker	Cases (n=134) Median (IQR)	Controls (n=388) Median (IQR)	Mortality OR* 4th vs.1st QRT (95%CI)	*p*-value
Non-Hepatocyte Dependent Factors				
D-dimer (µg/mL)	0.33 (0.23, 0.55)	0.26 (0.15, 0.39)	**6.61 (3.09, 14.16)**	**<0.001**
F V (%)	109 (87-135)	98 (85-120)	**2.13 (1.23-3.69)**	**0.007**
F VIII (%)	148 (110-189)	128 (100-160)	**2.68 (1.45-4.95)**	**0.002**
TFPI ng/mL	27 (21-35)	26 (21-32)	1.27 (0.71-2.27)	0.42
Hepatocyte Dependent Factors				
F II, prothrombin (%)	100 (85-116)	105 (92-121)	0.63 (0.35-1.13)	0.12
F VII (%)	94 (77-112)	102 (82-123)	**0.49 (0.26-0.93)**	**0.03**
F IX (%)	96 (82-113)	97 (84-111)	0.95 (0.53-1.71)	0.87
F X (%)	92 (78-104)	92 (80-107)	0.68 (0.38-1.22)	0.19
Antithrombin (%)	126 (109-141)	129 (114-143)	0.80 (0.45-1.42)	0.44
Protein C (%)	109 (91-126)	117 (100-134)	**0.54 (0.30, 0.98)**	**0.04**
Hepatocyte Score	2.3 (1.7, 2.8)	2.5 (2.3, 2.7)	**0.39 (0.20, 0.77)**	**0.007**

F = factor; TFPI = tissue factor pathway inhibitor
**adjusted for age, gender, race, study (SMART or ESPRIT) and date and country of enrollment*

When restricted to participants with viral suppression at entry, D-dimer (OR 7.58; p<0.001), FVIII (OR 2.28, p=0.03), FVII (OR 0.37, p=0.03) and protein C (OR 0.38, p=0.02) remained associated with mortality. When restricted to participants free of prior CVD, findings were similar for D-dimer (OR 6.55; p<0.001), FV (OR 2.17; p=0.01), and FVIII (OR 2.17, p=0.02), but slightly attenuated for FVII (OR 0.52, p=0.06) and protein C (OR 0.55, p=0.06).

Figure 2: Mortality Associations After Adjustment for Inflammation (IL-6) and Hepatitis Co-infection

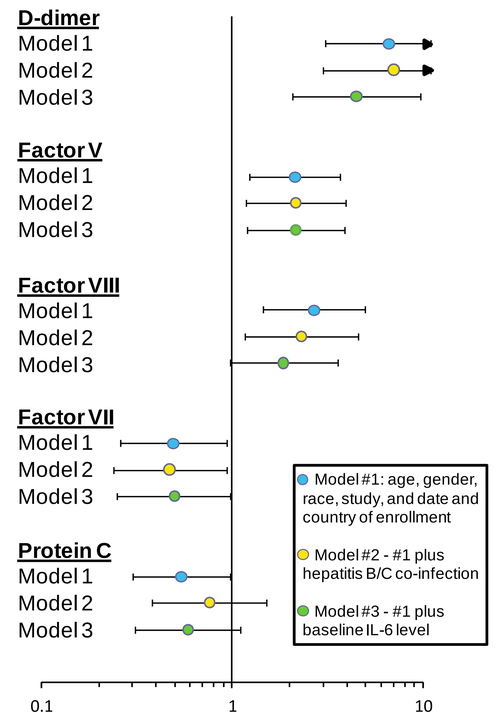


Figure above plots mortality OR for the individual coagulation factors that were significantly associated with all-cause mortality from table 2 (model #1, adjusted for age, gender, race, study, and date and country of enrollment). We then show that OR for D-dimer and factor VIII are attenuated after adjustment for IL-6 levels, and OR for Protein C is attenuated after adjustment for hepatitis B/C co-infection. In fully adjusted models including all cofactors (for age, gender, race, study [SMART or ESPRIT] and date and country of enrollment hepatitis co-infection, diabetes, cardiovascular disease, and baseline CD4, HIV RNA, and IL-6), only D-dimer and the Hepatocyte Score remained independently associated with all-cause mortality.

Table 3: Spearman Rank Correlations Between D-dimer Levels and Extrinsic Pathway Coagulation Factors (n=522)

Marker	Correlation Coefficient	*p*-value
F V (%)	**0.19**	**<0.001**
F VIII (%)	**0.18**	**<0.001**
TFPI ng/mL	**0.10**	**0.03**
F II, prothrombin (%)	0.05	0.25
F VII (%)	**-0.10**	**0.03**
F IX (%)	**0.09**	**0.04**
F X (%)	-0.03	0.49
Antithrombin (%)	-0.01	0.75
Protein C (%)	-0.07	0.10
Hepatocyte Score	**-0.10**	**0.02**

Summary and Implications

- Mortality risk increased with higher FV and FVIII levels, non-hepatocyte procoagulant cofactors reflective of systemic inflammation; the FVIII association was modestly attenuated by adjusting for IL-6 levels.
- Mortality was inversely related to the levels of hepatocyte dependent FVII and protein C (anticoagulant), as well as the composite hepatocyte score; the protein C association was attenuated by adjusting for hepatitis B/C co-infection.
- D-dimer levels demonstrated strong positive correlations with FV and FVIII levels, and inverse correlations with FVII and the Hepatocyte Score.

Findings support that hypercoagulation associated with mortality risk via alterations in the profile of extrinsic pathway coagulation factors, mediated in part by inflammation (e.g., elevated FVIII levels) and hepatocyte dysfunction (e.g., lower protein C levels).

Studies are needed to further clarify mechanisms by which these, and other, alterations in coagulation biology leads to clinical disease among treated HIV patients.

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