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Markers of Endothelial Dysfunction, Coagulation and Tissue Fibrosis Independently Predict Venous Thromboembolism in HIV

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Abstract

Objective—HIV infection is associated with coagulation abnormalities and significantly increased risk of venous thrombosis. It has been shown that higher plasma levels of coagulation and inflammatory biomarkers predicted mortality in HIV. We investigated the relationship between venous thrombosis and HIV-related characteristics, traditional risk factors of hypercoagulability and pre-event levels of biomarkers.

Design—A retrospective case-control study of 23 HIV-infected individuals who experienced an incident venous thromboembolic (VTE) event while enrolled in National Institutes of Health studies from 1995–2010 and 69 age and sex-matched HIV-infected individuals without known VTE.

Methods—Biomarkers of inflammation, endothelial dysfunction, coagulation, tissue fibrosis, and cytomegalovirus (CMV) reactivation were assessed by ELISA-based assays and PCR using plasma obtained prior to the event.

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I.S. and C.H. designed the study, contributed to the analysis and interpretation of the data and in writing the manuscript. L.W.M. contributed to the study design, clinical and laboratory data acquisition, data analysis and interpretation, and in writing the manuscript. V.S. and T.D.N. contributed to the acquisition of clinical data. A.R. performed laboratory studies. B.O.P. contributed to study conception and revision of the manuscript. S.R.P. provided plasma samples from HIV-negative volunteers. J.S. assisted with the study design and interpretation of statistical analyses. J.M.M. provided clinical management of patients enrolled in NIH protocols that were included in the study. All authors have reviewed and approved the final version of the manuscript for publication.

Results—VTE events were related to nadir CD4 count, lifetime history of multiple opportunistic infections, CMV disease, CMV viremia, immunological AIDS, active infection and provocation (i.e. recent hospitalization, surgery or trauma). VTE events were independently associated with increased plasma levels of P-selectin, $P=0.002$; D-dimer, $P=0.01$; and hyaluronic acid, $P=0.009$ in a multivariate analysis. No significant differences in antiretroviral or interleukin 2 exposures, plasma HIV viremia, or other traditional risk factors were observed.

Conclusion—Severe immunodeficiency, active infection and provocation are associated with venous thromboembolic disease in HIV. Biomarkers of endothelial dysfunction, coagulation and tissue fibrosis may help identify HIV-infected patients at elevated risk of VTE.

Keywords

HIV; venous thrombosis; blood coagulation factors; fibrosis; P-selectin; hyaluronic acid

Introduction

The advent of highly active antiretroviral therapy (HAART) has dramatically increased life spans of HIV-infected individuals [1, 2]. Concurrently, the incidence of AIDS-defining illnesses and opportunistic infections has declined while non-infectious etiologies of morbidity and mortality have emerged including an increased risk of thrombotic disease [3–6].

HIV-infected patients are 2–10 fold more likely to have a venous thromboembolism (VTE) compared to the general population [7–9]. Epidemiologic studies have linked traditional risk factors including ongoing non-HIV infections and hospitalizations [7, 10] and, to a lesser extent, protease inhibitors (PIs) to thrombotic events [11, 12]. Mounting evidence, however, suggests that HIV-specific factors are more heavily implicated in the pathogenesis of venous thrombotic disease [5, 7, 13].

HIV-related chronic immune activation and inflammation may contribute to vascular dysfunction and VTE risk [14–18]. A wide spectrum of procoagulant abnormalities including increased levels of D-dimer, soluble thrombomodulin (sTM), P-selectin, von Willebrand factor (vWF), monocyte tissue factor expression and anticardiolipin antibodies [19] have been observed in HIV-infected compared to uninfected individuals [15, 20–23]. Many of these abnormalities correlate with the degree of immunodeficiency and concomitant presence of opportunistic infection [11, 24]. Case reports implicating co-infection with cytomegalovirus (CMV) in clot formation date back to the beginning of the HIV epidemic [24]. This may be due to impaired endothelial function and inflammation [25] of the venous vasculature [26].

Results of the SMART study showed that higher levels of pro-inflammatory cytokines and acute phase reactants (IL-6 and CRP, and D-dimer products of fibrinolysis), which are known to be associated with the coagulation pathway, were associated with increased all-cause mortality, cardiovascular disease, and opportunistic disease [27]. It is unclear whether these markers will be helpful in predicting specifically venous thrombotic disease.

In this retrospective, case-control study of HIV-infected participants with incident VTE, we hypothesized that chronic inflammation, induced by HIV infection and coexisting opportunistic disease, may contribute to the elevated risk of VTE.

Methods

Case Identification and Definitions

A retrospective review of all HIV-infected subjects (N=2072) enrolled in National Institute of Allergy and Infectious Diseases (NIAID) intramural research protocols between January 1995 and April 2010 was conducted. Subjects who experienced an incident venous thromboembolism (VTE) while participating in a research study were identified from the NIAID electronic research database. Thromboembolic events were identified and validated by detailed chart review and imaging. Events were defined by the JUPITER Trial as described elsewhere [28]. Deep venous thromboses (DVTs) were confirmed at the time of the documented event by venous ultrasonogram or venogram; pulmonary embolism (PE) by angiogram, computed tomographic scan, or ventilation-perfusion scan; and portal vein thrombosis (PVT) by abdominal ultrasonogram. Validation methods used to identify cases were the use of anticoagulation therapy and a determination of sudden death attributed to pulmonary embolism by autopsy.

Exclusion criteria included experiencing a VTE prior to enrollment in NIH research protocols or prior to documented HIV seroconversion.

Matching Design

Cases with a first occurrence of VTE and cryopreserved plasma available at a pre-VTE event time point were matched 1:3 to HIV-infected controls by sex, age (within five years), and date of NIAID study enrollment (within three years). Controls were eligible for inclusion if they had not experienced a VTE and had cryopreserved plasma available at the time of the matching event.

Clinical Data

Active infection was classified as the treatment and/or diagnosis of any non-HIV infection within three months of the matching event. Provocation was defined as any trauma, surgery, central line placement, or hospitalization within three months of the event date. Hypertension was identified by the use of any antihypertensive agent, or documented blood pressure reading >140 mmHg systolic or >90 mmHg diastolic on two or more occasions prior to the event date. Body mass index was computed using the most recent weight and height measurements preceding the matching event. Smoking history was assessed positive if patient reported smoking >100 lifetime cigarettes and classified as current if patient reported cessation <1 month prior to the event date. Diabetes was identified by use of an anti-diabetic agent, random glucose level >200mg/dL or a fasting glucose level >126mg/dL on two separate occasions. Family history of a coagulopathy was classified as any history of VTE or coagulation disorder in a first-degree relative documented prior to the matching event. History of malignancy was defined as any cancer diagnosis excluding squamous cell and basal cell skin carcinomas and was classified as current if the patient was diagnosed

and/or treated within 5 years of the VTE event date. History of cardiovascular disease was defined as any history of myocardial infarction, arterial revascularization, stroke or coronary heart disease risk equivalent using the 2004 National Cholesterol Education Program guidelines. Intravenous drug use was considered positive if patient reported any lifetime use of injection drugs. Cumulative antiretroviral exposures were determined by prescribing information and detailed chart review. Antiretroviral use was classified as current if the patient was documented as actively taking an agent within one month of the matching event. Immunological AIDS and opportunistic infections were defined according to current Department of Health and Human Services (DHHS) guidelines. The most recent clinical and laboratory values preceding the matching event were used in comparative tests. Deaths were verified using the social security death index database.

Measurement of biomarkers

The most recent cryopreserved plasma specimens available prior to the matching event were used to assess biomarkers and CMV viremia. All plasma samples were collected in EDTA at the time of an NIH study visit, shipped frozen to a central repository and cryopreserved at -70°C . CMV DNA was quantified by real-time PCR using EZ1 Virus Mini kits v2.0 (Qiagen, Germantown, Maryland, USA). Anticardiolipin antibody levels were measured using an enzyme-linked immunosorbent assay (ELISA) (Inova Quanta Lite TM, San Diego, California, USA). D-dimer was measured using an enzyme-linked fluorescent assay on a VIDAS instrument (bioMerieux Inc., Durham, North Carolina, USA). Hyaluronic acid (HA) was measured using HA test kits (Corgenix, Inc, Westminster, Colorado, USA). Von Willebrand Factor (vWF) activity was assessed using ELISA Zymutest kits (Aniara, Mason, Ohio, USA). Soluble CD14 and soluble tissue factor were measured by ELISA (R&D Systems, Minneapolis, Minnesota, USA). Interferon- γ (IFN γ), interleukin (IL)-1b, IL-6, IL-8, IL-10, IL-12p70, tumor necrosis factor- α (TNF α), eotaxin, eotaxin-3, macrophage inflammatory protein (MIP)-1b, chemokine (C-C motif) ligand (CCL)-17, interferon-inducible protein (IP)-10, monocyte chemotactic protein (MCP)-1, MCP-4, macrophage-derived chemokine (MDC), thrombomodulin (TNB), intracellular adhesion molecule (ICAM)-1, ICAM-3, vascular cellular adhesion molecule (VCAM)-1, E-selectin, P-selectin, serum amyloid A [15], high sensitivity C-Reactive Protein (CRP), and tissue inhibitor of metalloproteinase-1 [29], were measured by electrochemiluminescence using Human Vascular Injury I kits, Human Vascular Injury II kits, Human ProInflammatory-7 Ultra-Sensitive kits, Human Chemokine-9 Ultra-Sensitive kits, and Human TIMP-1 kits (Meso Scale Discovery, Gaithersburg, Maryland, USA).

Statistical methods

Non-parametric Mann Whitney U tests and Fisher's Exact test were used for comparisons between cases of VTE and HIV-infected controls using values obtained at the time of the matching event. Continuous variables were compared using Mann-Whitney U tests. Categorical variables were compared with Fisher's Exact test. To evaluate whether relevant biomarker values were sensitive to time elapsed between measurement and time of the impending event, the relationship between biomarkers determined to be significantly associated with VTE by univariate analyses and the number of days from specimen collection to the event were evaluated by Spearman rank order analysis. To assess the

independent association of risk factors with VTE, a logistic regression analysis was performed and included the variables which were identified on univariate analyses: active non-HIV infection, provocation, nadir CD4 count, one or more lifetime opportunistic infections, albumin, sCD14, D-dimer, P-selectin, thrombomodulin, vWF, SAA, CRP, IL-6, IL-8, TARC, TIMP-1, and HA. Alternative regression models, which included immunological AIDS, HIV viral load, and/or history of CMV disease, were also tested. Odds ratios for VTE were calculated for subjects with median biomarker values above the 50th percentile with logistic regression. All p-values reported are two-sided. Statistical analyses were performed using JMP software (Version 8.0 SAS Institute, Cary, North Carolina, USA).

Results

Clinical measures of traditional risk factors and HIV-specific characteristics

Twenty-three subjects experienced a first incident VTE from a cohort of 2072 HIV-infected subjects followed at the National Institutes of Health between 1995 and April of 2010. Sixty-nine HIV-infected subjects with no history of VTE were matched to cases as described. Fifty-six percent of cases had a DVT (N=13); 35% had a PE (N=8); one had a biventricular cardiac thrombus (N=1); and one had a PVT.

Subjects who had a VTE were predominantly male and the median age at which an event occurred was forty-seven (Table 1). Thirty percent of participants with a VTE and 25% of controls were black.

Participants with a VTE were four times more likely to have a history of provocation within three months of the matching event and a higher percentage had ongoing, non-HIV infections than controls (Table 1). There were no significant differences in the prevalence of intravenous drug use, hypertension, smoking, diabetes, cardiovascular disease, or malignancy between VTE cases and controls. The majority of cases (91%) and controls (85%) were ARV-experienced, and the median duration of HIV infection was 9 and 10 years, respectively. There were no differences in ARV regimens, cumulative ARV exposure, or co-infection with hepatitis C between groups. Current or previous use of IL-2 and cumulative IL-2 exposure did not differ between the groups (data not shown).

The median CD4 nadir was 40 for cases and 250 for controls (p=0.0003). Most participants in both groups had a current CD4 count >500 cells/ μ L. VTE cases though were more likely to meet diagnostic criteria for immunological AIDS, as determined by the most recent CD4 count prior to the matching event; 5 by both CD4 absolute count and percentage and 3 by percentage of total lymphocyte count. Prevalence of HIV viremia did not differ between the groups (39% cases vs. 35% controls; p=0.80). The majority of VTE cases had more than one lifetime opportunistic infection (52% vs. 22% in controls; p=0.008). A significantly higher proportion of cases had a history of CMV disease. CMV viremia was more prevalent in VTE cases (13.0% vs. 0%; p=0.02). A history of *Mycobacterium avium* complex (MAC) or tuberculosis infection was also more common in cases (Table 2) [30]. Lower albumin was observed in cases and there was a non-significant trend for higher platelet levels in cases. Other laboratory variables did not differ significantly between the groups (Table 3).

Pre-event levels of circulating biomarkers differed between VTE cases and HIV-infected controls

Plasma available prior to the matching event was obtained for VTE cases and controls at a median of 34 days (IQR, 12–44) vs. 23 days (IQR, 10–59), respectively; $p=0.26$. Cases had higher levels of markers of monocyte activation, sCD14; endothelial dysfunction and coagulation including P-selectin, thrombomodulin, and vWF; inflammation, SAA, CRP, IL-6, IL-8, TARC, TIMP-1, and D-dimer; and tissue fibrosis, HA (Fig. 1). There was a trend toward higher MCP-4 in cases than controls. Plasma levels of the remaining tested markers did not differ between the groups (Table, Supplemental Digital Content 1). There were no differences in biomarkers for patients on PI- or specifically, IDV-containing regimens. Thrombomodulin levels were higher in patients co-infected with HCV (3.71 vs. 2.61; $p=0.05$), while other biomarkers measurements were similar. Median biomarker levels in patients co-infected with CMV were not different from those without CMV. Reference plasma biomarker levels from healthy, HIV-negative subjects are provided in Supplemental Digital Content 2.

The time interval from specimen collection to VTE event was significantly correlated with D-dimer ($r=-0.44$, $p=0.03$) and vWF ($r=-0.48$, $p=0.02$) levels. There was no correlation with other biomarkers determined to be significant by univariate analyses.

The frequency of detectable anticardiolipin antibodies [19] IgG or IgM was similar in cases and controls (8.7% vs. 9.8%, $p=1.0$).

Multivariate Analysis

A multivariate regression analysis was performed that included active non-HIV infection, provocation, nadir CD4 count, more than one lifetime opportunistic infection, albumin, and all biomarkers determined by univariate analyses to be associated with VTE. P-selectin ($p=0.002$), D-dimer ($p=0.01$), and HA ($p=0.009$) were found to be independently associated with VTE risk. Alternative models including immunologic AIDS, HIV viral load and CMV disease did not alter these findings.

The odds ratios of incident VTE were calculated for subjects with baseline levels of P-selectin, D-dimer, or HA, above the median. The odds ratios of VTE for subjects with biomarker values exceeding the median value for all subjects were 11.4 (95% CI, 3.1–42.3) for D-dimer, 7.6 (95% CI, 2.3–24.9) for P-selectin, 1.8 (95% CI, 0.7–3.8) for HA, and 11.2 (95% CI, 3.7–33.6) when both D-dimer and P-selectin were above the median (Table 4).

Discussion

In this case-control study of HIV-infected patients, elevated plasma levels of P-selectin, D-dimer and hyaluronic acid were strongly and independently linked to risk of subsequent venous thromboembolism when measured prior to the event occurrence. HIV-specific risk factors including low nadir CD4, immunological AIDS, history of multiple opportunistic infections and CMV viremia were associated with thromboembolic events as well as ongoing, non-HIV infection and the presence of a provocation (recent hospitalization, surgery, or trauma).

In HIV-uninfected persons with a first venous thromboembolism, up to half of all events are unprovoked [31]. In comparison, roughly two thirds of first-time events in our HIV-infected population occurred without an identifiable provocation. The prevalence of non-HIV infection was high in cases and controls, although substantially higher in cases, consistent with its known relationship to VTE in the general population. Contrary to other traditional clinical risk factors for VTE in patients without HIV, current malignancy, family history of hypercoagulability, and cardiovascular disease were not related to thrombosis in this study. The presence of a provocation or active infection in HIV-infected individuals, even in the absence of other traditional risk factors, has clinical implications for inpatient and outpatient management; extended-distance travel, surgery, hospitalization, and ongoing non-HIV infections necessitate consideration of venous thromboprophylaxis [9].

At the time of the matching event, the vast majority of cases and controls were receiving ART with a median duration of therapy since HIV diagnosis of 6.9 years and 7.3 years, respectively. Most cases had normalized CD4 counts (>500 cells/ μ L) at the time of VTE diagnosis, which is above the threshold at which current DHHS guidelines recommend starting antiretroviral treatment [32]. On the other hand, nadir CD4 was significantly lower and multiple lifetime opportunistic infections were more prevalent among VTE cases than controls despite a similar duration of HIV infection (9.3 years vs. 10.5 years). Combined, these results support the hypothesis that earlier initiation of ART in individuals with advanced infection may modify the risk of venous thrombotic disease [3].

HIV viremia and plasma HIV-RNA levels were not associated with VTE risk in this cohort although other studies have reported otherwise [7, 10]. Persistent low level viremia has been observed in a majority of individuals with <50 copies/ μ L [33] and chronic immune activation is evident even in patients with suppressed HIV viremia below detectable levels [34]. The contribution of viral replication to observed biomarker levels and related coagulopathies cannot thus be precluded in this study [27].

Associations between D-dimer products of fibrinolysis and all-cause mortality as well as cardiovascular disease have been well established in HIV-infected and non-infected populations [27, 35–38]. The relationship between latest pre-event levels of D-dimer and venous thrombotic disease is likely attributable, in part, to subclinical clot formation. Measurement of D-dimer has significant long-term predictive value in high-risk individuals and is a strategy already used to stratify risk of VTE recurrence. Furthermore, a recent meta-analysis found that timing of testing post-VTE did not affect this association [31]. In a previous report, we linked risk of CVD in HIV to elevated D-dimer levels up to 2 years prior to the index event [35]. Combined with our current findings, these data suggest that there may be clinical utility in D-dimer measurement among HIV-infected patients at elevated risk for venous and arterial thrombosis.

In addition to elevated D-dimer levels observed in HIV-infected subjects with an impending VTE, several markers of inflammation (IL-6, CRP, and SAA) were also higher in cases compared to controls.

Increases in IL-6, CRP, and SAA are also related to levels of HIV RNA [27], risk of opportunistic infection [39], and death [27, 40] supporting our hypothesis that underlying inflammation, caused by both HIV [20] and non-HIV infections [41], contributes to venous thrombotic disease. However, due to the retrospective nature of this study, temporality between biomarkers of inflammation and activation of coagulation and fibrinolytic pathways could not be determined.

A hypercoagulable state often exists among patients with HIV including a high prevalence of detectable anticardiolipin antibodies [7]. In this study, we evaluated their role in VTE and determined that although anticardiolipin antibodies were detected more commonly in HIV-infected patients than in healthy, uninfected individuals, there was no association with VTE.

The relationship of immunodeficiency with increased coagulation factors may be due to the increased risk of opportunistic infections [7]. In support of this explanation, we found that a history of multiple lifetime opportunistic infections and advanced immunodeficiency were associated with VTE risk in the studied population. Further, CMV viremia was more prevalent among cases than controls. Active CMV infection was recently associated with hypercoagulability independent of stage of HIV disease [42]. These findings suggest that CMV disease, rather than immunodeficiency alone, may result in a heightened inflammatory state leading to activation of the coagulation cascade in co-infected patients.

Another explanation for chronic inflammation in HIV may be related to the ability of HIV to cross the endothelial cell (EC) membrane, causing endothelial disruption via induction of intracellular biochemical changes and subsequent activation of inflammatory cascades and expression of EC adhesion molecules [43]. To investigate the role of endothelial dysregulation in venous clot formation, we quantified circulating levels of P-selectin, vWF, TARC, and TIMP-1, and observed each to be significantly associated with VTE risk. A higher degree of immunodeficiency among cases may in part explain these findings due to its association with impaired vascular function and endothelial inflammation in HIV disease [44, 45].

P-selectin was found to be independently and most strongly associated with thrombosis in this cohort. Stored in endothelial cells and platelet granules, P-selectin interacts with its receptor to promote a hypercoagulable environment by inducing the generation of prothrombotic microparticles from leukocytes and upregulation of tissue factor expression on monocytes [46–49]. Prospective studies in HIV-uninfected subjects with malignancies have demonstrated that P-selectin is significantly elevated in patients with an impending or acute VTE. Furthermore, P-selectin has been shown to have comparable diagnostic value to D-dimer in patients with confirmed DVTs [50].

Plasma levels of the soluble lipopolysaccharide (LPS) receptor sCD14 can be utilized as a marker of monocyte activation [51]. In our cohort, we observed a significantly higher level of sCD14 in cases compared to controls suggesting that overstimulation of monocytes from increased peripheral exposure to microbial products may contribute to a prothrombotic state in chronic HIV infection. These findings are consistent with a recent study that showed an increased proportion of monocytes expressing surface procoagulants in HIV-infected

patients following LPS stimulation [15]. Contrary to expectations, we did not observe any appreciable differences in soluble TF plasma levels between groups. Lipopolysaccharide-mediated activation of TF-dependent coagulation pathways may be confined specifically to monocyte surface TF expression.

Monocyte production of inflammatory cytokines may also be induced by hyaluronic acid (HA) degradation products through stimulation of the Toll-like receptors (TLR)-2 and TLR4 [52]. A strong indicator of liver fibrosis in hepatitis C virus (HCV) co-infected [53] and mono-infected [54] individuals, increased HA levels are also predictive of AIDS or death [55]. Although compared to controls, fewer VTE cases in our study were co-infected with HCV, overall HA levels were higher in VTE cases, and in a multivariate analysis, independently linked to VTE disease. These results suggest that HA-mediated tissue injury and inflammation may contribute to VTE risk in subjects without other evidence of hepatic injury.

The limitations of our study include a relatively small number of events evaluated and the risk of increased type I error, given the considerable number of variables compared. However, in the multivariate analyses, three biomarkers retained significance: the associations of P-selectin, D-dimer, and HA with VTE remained strong, are biologically plausible, and relevant to other HIV-related adverse outcomes associated with their increased levels.

In summary, indices of endothelial dysfunction, coagulation, and tissue fibrosis were most strongly and independently associated with risk of venous thrombotic disease while a history of multiple lifetime opportunistic infections, clinical CMV disease and CMV viremia, immunodeficiency, and the presence of an ongoing non-HIV infection or provocation were also related. Our findings suggest that unique risk factors in HIV-infected individuals exist that may contribute significantly to augmented risk of VTE disease. Furthermore, indices of endothelial dysfunction, coagulation and tissue fibrosis that have been linked to morbidity and mortality in HIV infection were more strongly associated with VTE risk than other covariates examined in this study. Our results present a clinical impetus to further explore both their contributions to immunopathogenesis and the therapeutic role of modulating these biomarkers and associated inflammatory and coagulation pathways to potentially impact non-infectious complications of HIV infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Differences between VTE cases (n=23) and HIV-infected controls (n=69) in the latest pre event plasma levels of A) sCD14, B) D-dimer, C) P-selectin, D) Thrombomodulin, E) vWF F) SAA, G) CRP, H) IL-6, I) IL-8, J) TARC, K) TIMP-1, and L) HA were examined with the Mann-Whitney test. Data shown are medians and interquartile ranges.

Table 1

Baseline characteristics and prevalence of traditional risk factors at the time of the matching event

Characteristic	VTE cases (N=23)	HIV+ controls (N=69)	p-value
Age	47 (38–56)	47 (38–54)	.92
Male, % of subjects	91.3	91.3	1.0
Black, % of subjects	30.4	24.6	.59
Years of follow up	9.2 (2.7–13.3)	8.4 (4.8–12.1)	1.0
Active non-HIV infection, % of subjects	56.5	14.5	.0002
Provocation, % of subjects	34.8	8.7	.006
History of IDU, % of subjects	4.6	6.1	1.0
BMI, kg/m ²	25.4 (21.9–29.5)	25.5 (23.9–27.9)	.79
Hypertension, % of subjects	13.6	22.7	.54
Current smoker, % of subjects	13.0	26.1	.26
Current malignancy, % of subjects	4.4	11.6	.44
Diabetes, % of subjects	4.4	8.7	.68
History of CVD, % of subjects	9.1	7.6	1.0

Data shown are the median values and interquartile ranges, unless otherwise indicated. IDU, intravenous drug use; BMI, body mass index; CVD, cardiovascular disease

Table 2

HIV-associated variables of cases and controls

	VTE cases (N=23)	HIV+ controls (N=69)	p-value
Years of HIV infection	9.3 (4.1–12.5)	10.5 (6.1–14.7)	.32
Years of ARV exposure	6.9 (2.7–9.2)	7.2 (2.3–10.8)	.63
ARV use, % of subjects	91.3	85.5	.72
PI-containing regimen, % of subjects	43.4	60.9	.16
IDV-containing regimen, % of subjects	21.7	23.2	1.0
Hepatitis C, % of subjects	4.4	13.0	.44
Nadir CD4 T cells/ μ L	40 (5–179)	250 (91–341)	.0003
Immunological AIDS, % of subjects	34.8	11.6	.02
History of CMV disease, % of subjects	21.7	1.5	.003
History of mycobacterial infection, % of subjects	26.1	8.7	.07
One lifetime OI, % of subjects	26.1	17.4	.37
More than one lifetime OI, % of subjects	52.3	21.7	.008

HIV, human immunodeficiency syndrome; ARV, antiretroviral; PI, protease inhibitor; IDV, indinavir; OI, opportunistic infection.

Table 3

Laboratory variables at the time of the matching event

	VTE cases (n = 23)	HIV+ controls (n = 69)	p-value
HIV-RNA _{log} 10 (copies/mL)	2.70 (1.70–3.55)	1.70 (1.70–3.05)	.36
Detectable HIV RNA, % of subjects	39.1	35.3	.80
CD4 T cells/ μ L	507 (215–653)	517 (344–736)	.30
CD4% T cells	27 (9–34)	27 (21–36)	.14
CD8 T cells/ μ L	760 (39–1526)	830 (646–1164)	.98
CD8% T cells	50 (37–67)	47 (39–58)	.56
CD14 cells/ μ L	520 (372–713)	470 (402–600)	.33
White blood cells/ μ L	6260 (3970–7510)	5400 (4618–6325)	.30
Platelets ($\times 10^3$ / μ L)	255 (208–294)	226 (181–267)	<i>0.07</i>
Hemoglobin (g/dL)	14.0 (10.7–15.5)	14.6 (13.5–15.1)	.19
PT (s)	13.3 (12.0–16.2)	12.8 (12.3–13.3)	.11
PTT (s)	29.9 (26.2–32.1)	29.6 (26.9–31.6)	.95
Albumin (g/dL)	3.8 (2.9–4.2)	4.0 (3.7–4.2)	.02

Data shown are median values and interquartile ranges, unless otherwise indicated. AIDS, acquired immunodeficiency syndrome; PT, prothrombin time; PTT, partial thromboplastin time.

Table 4

Odds ratios of VTE for subjects with biomarker values above the median value.

Biomarker	Group	VTE cases No. (%)	HIV+ Controls No. (%)	Odds Ratios (95% CI)	p-value
D-dimer	>50 th % ile	20 (87%)	24 (37%)	11.4 (3.1 – 42.3)	<.0001
P-selectin	>50 th % ile	19 (83%)	25 (38%)	7.6 (2.3 – 24.9)	.0002
HA	>50 th % ile	14 (61%)	30 (46%)	1.8 (0.7 – 3.8)	0.22
D-dimer & P-selectin	Both >50 th % ile	16 (70%)	11 (17%)	11.2 (3.7 – 33.6)	<.0001

CI, confidence interval.