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Coagulation and morbidity in treated HIV infection

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Abstract

HIV infected patients are at increased risk for venous and arterial thromboembolic events. Multiple markers related to inflammation (IL-6, TNF α , C-reactive protein) and coagulation (tissue factor expression, FVIII, thrombin, fibrinogen and D-dimer levels) are increased in HIV infection, and several are predictive of thrombotic risk and mortality in HIV disease. The mechanisms behind the risk for abnormal coagulation in HIV infection have not been fully elucidated, but may be related to a chronic immune activation and inflammatory state in both untreated and treated HIV infection. The contribution of traditional risk factors, including smoking and dyslipidemia, overly represented in HIV infected patients, must also be considered when assessing thrombotic risk in this setting. Currently, several interventional studies are aimed at reducing inflammation and cardiovascular risk in HIV disease and may provide insights into the determinants of clotting events in HIV infected patients.

Introduction

Activation of the intrinsic and extrinsic coagulation cascades can limit bleeding through fibrin clot formation. Inappropriate clot formation, however, may block blood flow within vessels, resulting in ischemia and tissue necrosis. The intrinsic coagulation pathway is often initiated at the surface of damaged blood vessels; tissue factor (TF), a glycoprotein expressed on cell surfaces or circulating as microparticles, is the initiator of the extrinsic pathway (1). As inappropriate clot formation, or its failure, can be life-threatening, the coagulation cascade is tightly regulated.

There is a growing recognition that the relationship between coagulation and inflammation is bidirectional, with clotting a recognized consequence of inflammation, and coagulation directly amplifying the inflammatory response (2-5). While combination antiretroviral

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therapy (ART) has improved dramatically the disease-free survival in human immunodeficiency virus (HIV) infection, recent reports indicate that infected patients are at a 2-fold to 10-fold greater risk for a venous thromboembolic event (VTE) (6-8) and an approximately 2-fold greater risk for myocardial infarction (9) than are uninfected controls. The mechanism(s) that contribute to this increased thrombotic risk in HIV disease have not been fully elucidated, but may be related to the chronic immune activation and inflammation demonstrable in these patients; indices that often do not normalize, even after years of suppressive antiretroviral therapy (10-13). Understanding the drivers of inflammation and coagulation in HIV-infected patients is a research priority, and by elucidating the mechanisms that contribute to thrombotic risk, potential therapeutic strategies may be revealed.

Mechanisms related to thrombotic risk

Several potential drivers of immune activation in treated HIV infection have been identified, including: low level viral replication in tissues (14), replication of prevalent copathogens such as cytomegalovirus (CMV) (15), exposure to bacterial products released into the circulation from a damaged GI-tract (16), exposure to pro-inflammatory lipids (17) or a homeostatic response to lymphocytopenia(18). This “residual immune dysregulation syndrome” (RIDS) is characterized by elevated expression of proinflammatory cytokines, evidence of activation of both innate and adaptive immune cells, and evidence of increased activation of the coagulation cascade(19). How are these phenomena linked?

Chronic inflammation can lead to alterations in vascular function and damage to activated endothelial cells, and this has been reported in HIV-infection (20-24). Damaged endothelial cells can act as a substrate for the initiation of coagulation. Elevated levels of von Willebrand factor (vWf), a marker of endothelial cell damage, have been reported HIV-infected patients (10, 25), and vWf expression by endothelial cells may enhance platelet adhesion and clot formation. Plasma levels of vWf remain elevated even in patients receiving suppressive ART (10).

Coagulation can also be initiated by the binding of tissue factor (TF) to factor VIIa, which eventually leads to the generation of thrombin and fibrin clots. Tissue factor can be expressed on multiple cell types (1); we, and others, have reported increased expression of TF on monocytes (26, 27), platelets (28), and within the plasma as circulating microparticles (26, 29, 30) in samples from HIV-infected patients.

Tissue factor expression can be induced by exposure of cells to pro-inflammatory cytokines and through activation of pattern recognition receptors, including Toll-like receptors (TLRs) (1, 31). Numerous TLR ligands can be found in HIV-infection and these include viral elements of co-pathogens like CMV, or HIV-1 that can be recognized by TLRs7 and 8 (32) and can induce TF expression on patrolling (CD14^{Dim}CD16⁺) monocytes(27). Early damage to the gastrointestinal tract of HIV-infected patients(33) can result in systemic translocation of microbial products, including bacterial DNAs and LPS, from the gut lumen into the circulation (16, 33). Recognition of bacterial products by TLRs can result in increased proinflammatory cytokine production (34) and increased monocyte expression of TF (27).

The proportion of monocytes that express TF correlates with levels of HIV-1 viremia, with plasma levels of soluble CD14, an LPS coreceptor and marker of monocyte activation that independently predicts mortality in HIV-infected patients (13), and with plasma levels of D-dimer products of fibrinolysis (26), linking monocyte TF expression to coagulation *in vivo*. Bioactive TF+ microparticles also provide a link between inflammation and coagulation in HIV disease, as their levels correlate with plasma levels of D-dimer, IL-6, and vWf (30).

Markers of coagulation and inflammation are related to morbidity in HIV infection

Several biomarkers, including markers related to coagulation (35, 36), inflammation (35) and monocyte activation(13) are found to be predictive of mortality and thrombotic risk in HIV infection. The Strategies for the Management of Antiretroviral Therapy (SMART) study reported that elevated D-dimer levels were predictive of all-cause mortality (35), and cardiovascular events (37) in HIV-infected patients. There was a 2.14 fold greater risk for cardiovascular events in SMART patients within the highest quartile of D-dimer levels compared to the risk in patients within the lowest quartile (37). When D-dimer levels in HIV-uninfected donors in the Multi-Ethnic Study of Atherosclerosis (MESA) and levels from participants in SMART were compared, patients from SMART had D-dimer levels that were nearly twice as high as the levels found in patients from MESA, compatible with increased ongoing coagulation in the HIV-infected population. Levels of D-dimer among SMART participants with suppressed viremia (<400copies/mL), were still half again higher than levels measured in the general population (12). The Veterans Aging Cohort study (VACS), confirmed the predictive value of D-dimer levels on mortality in HIV-infected patients (38). Also, plasma D-dimer levels have been independently associated with venous thromboembolic events (29) and cardiovascular events (39) in two retrospective case control studies among HIV-infected patients. Overall, these findings provide a strong link between D-dimer levels and both venous and arterial thrombosis in HIV-infection.

While D-dimer levels may be predictive of overall morbidity in HIV-infected patients, this is an incomplete measure of the coagulation profile. More detailed analyses of coagulation have been reported recently, with some conflicting results. Using samples from a subset of patients in the SMART study, Baker et al measured pro- and anti- coagulant factors involved in the clotting cascade in order to model thrombin generation and coagulation potential (40). The authors report that HIV replication causes increases in many procoagulant factors (including FVIII) and decreases in multiple anticoagulant factors (including antithrombin). Modeling of these multiple inputs suggests that HIV-infection results in a procoagulant state (40). These findings have not been uniformly reproduced in other studies. Though Haugaard et al confirm the finding of increased D-dimer levels in untreated HIV-infected patients compared to levels in controls, they also found reduced platelet aggregation and clot initiation in both treated and untreated HIV-infected patients (41). Hsue et al have recently reported that there was little difference between plasma levels of D-dimers, prothrombin fragment 1+2 (F1+2), and TF measured in ART treated HIV-infected patients and levels of these indices measured in controls (42). In fact, they also find that levels of antithrombin are increased in HIV-infected patients compared to levels in controls, and suggest that plasmas

of treated HIV+ patients have decreased thrombin generation *in vitro*, suggesting an overall anticoagulant phenotype (42). Differences in sample preparation and other methodologic issues may contribute to the differences found in these studies, but additional studies are needed to clarify these points.

Conclusions

Antiretroviral therapy is changing the course of HIV disease in the modern era; and now, HIV-infected patients appear to be at greater risk for major thrombotic events than for opportunistic infections. Plasma markers of inflammation and coagulation are predictive of all-cause mortality in HIV-infected patients (35, 37, 43) and for thrombotic and cardiovascular events (29, 39). The interplay between inflammation and coagulation is bi-directional, each likely contributing to the other; and while ART reduces the levels of several inflammatory and coagulation biomarkers, they often do not reach levels found in HIV-uninfected donors (10-13, 30). The mechanisms underlying the residual immune dysregulation, increased inflammation, increased T cell activation, and altered coagulation that we observe in treated HIV infection are complex and not yet adequately defined, but may include: persistent low level viral replication in tissues (14), infection due to copathogens such as cytomegalovirus (15), sustained microbial translocation (16), and a pro-inflammatory lipid environment (17) (Figure 1). These potential “drivers” may alter endothelial cell function and integrity (10, 25), activating the clotting cascade through multiple mechanisms including the induction of tissue factor expression on platelets (28) and monocytes (26, 27). More traditional risk factors for coagulation, including smoking, dyslipidemia, hypertension, and diabetes, are found with increased prevalence in HIV-infected persons and are likely to contribute to increased risks for morbidity and mortality in HIV disease (9, 44). Several strategies targeting inflammation in treated HIV infection are under study, and some have had apparent modest beneficial effects on indices of inflammation and/or coagulation (45, 46). Targeted interventional studies may help to identify the determinants of coagulation risks in treated HIV infection. And while a number of interventions, such as administration of aspirin and statins, have proven value in reducing cardiovascular risks among HIV-uninfected populations (47, 48), their clinical utility in treated HIV infection has not yet been demonstrated and merits evaluation.

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Figure 1. There are complex relationships between coagulation and inflammation in HIV disease.

Many HIV infected patients have increased levels of plasma markers related to inflammation and coagulation. How chronic HIV disease produces a procoagulant state may include mechanisms related to activation of the innate and adaptive immune system by low level HIV replication, copathogens, and microbial products translocated from the gastrointestinal tract.