

Clinical utility of biomarkers of endothelial activation and coagulation for prognosis in HIV infection

A systematic review

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Introduction: HIV infection is associated with vascular dysfunction and adverse cardiovascular outcomes. Our objective was to review the evidence regarding the clinical utility of endothelial activation and coagulation biomarkers for the prognosis of HIV-infected patients.

Results: Seventeen studies were identified that fulfilled the inclusion criteria, of which 11 investigated endothelial activation biomarkers and 12 investigated coagulation biomarkers. Biomarkers and outcomes varied widely across studies. Overall, published studies support an association between P-selectin and venous thromboembolism in HIV-infected patients, an association between tissue-type plasminogen activator and death, and associations between d-dimer and several clinical outcomes, including venous thromboembolism, cardiovascular disease, and all-cause mortality.

Methods: We searched PubMed and Embase for publications using the keywords “HIV” or “HIV infection” and “endothelium” or “coagulation”. We reviewed reference lists and hand-searched for additional relevant articles. All clinical studies that enrolled non-pregnant, HIV-infected adults, measured biomarkers reflecting endothelial activation or coagulation, and prospectively evaluated their associations with vascular dysfunction or clinical outcomes were included.

Conclusions: Several studies have demonstrated associations between biomarkers of endothelial activation and coagulation and clinically important outcomes in HIV-1 infection. Additional large-scale prospective investigations to determine the utility of endothelial activation and coagulation biomarkers for risk stratification and prediction of adverse outcomes are clearly warranted.

Introduction

Since effective antiretroviral therapy (ART) became widely available, the risks for morbidity and mortality due to opportunistic infections have greatly decreased for persons living with HIV

infection.^{1,2} Unfortunately, recent evidence shows that HIV-infected persons are at higher risk for cardiovascular, renal, and hepatic disease, despite effective ART.^{3–6} Increasing evidence points to chronic inflammation among individuals who develop HIV-related end-organ disease and other complications.^{7,8} Such inflammation may activate the coagulation cascade, leading to a pro-thrombotic tendency in HIV-infected persons that could lead to arterial or venous thromboembolism (VTE).^{9,10}

Subclinical atherosclerotic disease and vascular dysfunction have also been identified in HIV-infected individuals.¹¹ While traditional risk factors are still important, traditional risk assessments such as the Framingham Risk Score underestimate cardiovascular risk in HIV-infected persons.^{12,13} Inflammation, endothelial activation, and oxidative stress, all increased in HIV-1 infection, are known to be major driving forces for the initiation of coronary plaques, their progression to instability, and eventual plaque disruption.^{14–16} Indeed, several studies have demonstrated increased levels of biomarkers of endothelial activation (e.g., VCAM-1, ICAM-1, E-selectin) and coagulation (e.g., P-selectin, D-dimer, fibrinogen) in HIV-infected persons compared with healthy, uninfected controls.^{17–20}

There are multiple mechanisms whereby HIV-1 proteins and antiretroviral drugs may lead to endothelial damage (reviewed in ref. 21). The HIV-1 envelope protein gp120 and the regulatory protein Tat are both associated with endothelial cell apoptosis and increased cellular adhesion molecules, adhesion, permeability, and reactive oxygen species (ROS). Tat is also associated with decreased endothelial relaxation, and increased monocyte chemoattractant protein-1, matrix metalloproteinase, chemotaxis, proliferation, and angiogenesis. Other accessory proteins may augment these effects: Nef and Vpr by increasing endothelial cell apoptosis, and Vpu by increasing expression of cellular adhesion molecules (Table 1 in ref. 21). Antiretroviral drugs may exacerbate HIV-1-related endothelial effects. Nucleoside or nucleotide reverse transcriptase inhibitors decrease mitochondrial function, levels of reduced glutathione, and vasorelaxation, and increase ROS, vasoconstrictor release, endothelial proliferation, and vascular permeability. Protease inhibitors have been associated with decreased mitochondrial function, endothelial nitric oxide

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synthase, vasorelaxation, and flow-mediated dilation, as well as increased ROS, mitochondrial DNA damage, vascular permeability, and carotid intima media thickness. There are no known endothelial effects of non-nucleoside reverse transcriptase inhibitors, integrase inhibitors, or entry inhibitors (Table 2 in ref. 21).

Because endothelial activation and coagulation may each play key mechanistic roles leading to adverse outcomes in HIV-infected patients, biomarkers of these processes may prove valuable for the diagnosis, prognosis, or risk stratification of HIV-infected patients. In addition, a better understanding of the mechanisms leading to HIV-1-related vascular dysfunction may lead to specific treatments aimed at reducing endothelial activation or coagulopathy. Our objective was therefore to review the evidence regarding the clinical utility of biomarkers of endothelial activation and coagulation for prognosis in HIV-infected patients.

Results

Our search identified 1134 unique articles (see Fig. 1). Of these, 55 studies met our initial screening criteria (i.e., studies of HIV-infected adults in which biomarkers were measured). After retrieval of the full-text publication, 38 studies were excluded for the following reasons: 25 studies were cross-sectional, 11 studies did not report a relevant outcome, and 2 studies were interventional trials: one study evaluated the short-term effect of vaccination on inflammatory biomarkers,²² and the other study examined the effect of telmisartan on blood pressure and proteinuria.²³ The remaining 17 studies were included in our review. Four studies were randomized trials of interventions aimed at improving vascular function: salsalate,²⁴ pentoxifylline,²⁵ rosiglitazone,²⁶ and NRTI-sparing vs. standard triple PI-based ART.²⁷ All remaining studies were observational designs, two of which were secondary analyses of data collected during a prospective clinical trial.^{10,28}

Biomarkers of endothelial activation. We identified 11 studies investigating associations between biomarkers of endothelial activation and vascular dysfunction or clinical outcomes in HIV infection (see Table 1). Nine studies evaluated ICAM-1, 8 evaluated VCAM-1, 3 evaluated E-selectin, and one each evaluated ICAM-3, P-selectin, and VEGF, respectively. Two studies used nested case-control designs, and one used a retrospective case-control with prospective follow-up of cases. Five studies evaluated vascular function using measures including carotid intima-media thickness (c-IMT, $n = 2$), flow-mediated dilation ($n = 2$), arterial

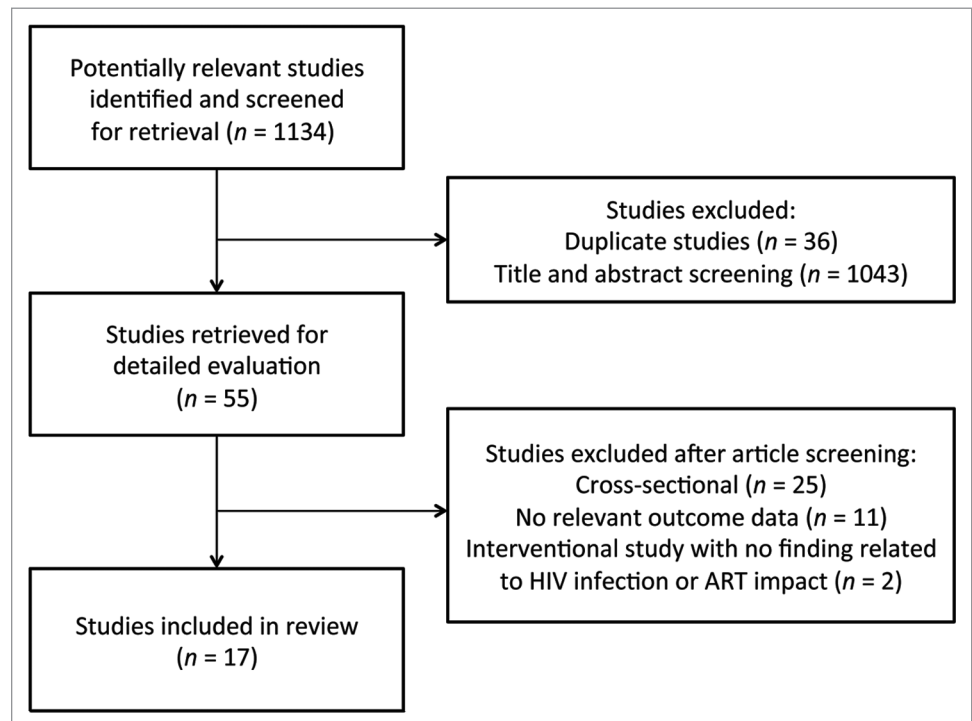


Figure 1. Study flow diagram.

stiffness ($n = 1$), circulating endothelial cells ($n = 1$), finger arterial pulse wave amplitude ($n = 1$), and nitroglycerin-mediated dilation ($n = 1$). Six studies evaluated clinical outcomes, including CD4 count decline ($n = 1$), cytomegalovirus retinitis ($n = 1$), cardiovascular disease (CVD) events ($n = 1$), death ($n = 1$), Kaposi sarcoma ($n = 1$), and VTE ($n = 1$). The study of CVD events used a composite outcome including acute myocardial infarction, silent myocardial infarction, coronary revascularization, acute coronary syndrome, cerebrovascular accident, lower extremity revascularization, and sudden cardiac death.²⁹

Only one study reported positive findings: in the study by Musselwhite et al., P-selectin levels were associated with VTE.³⁰ Two studies reported associations that were not significant in multivariable analysis. In one, higher ICAM-1 levels were associated with shorter time to death.³¹ In the other, higher VCAM-1 levels were associated with CVD events.²⁹ The results of three studies suggested an indirect association between endothelial activation biomarkers and outcomes. In the first, VCAM-1 and ICAM-1 decreased over 24 mo of ART, while c-IMT increased and arterial stiffness decreased.²⁷ In the second, pentoxifylline reduced VCAM-1 levels and improved flow mediated dilation (FMD) over 8 weeks of follow-up.²⁵ In the third, VCAM-1 levels decreased to a greater extent among participants taking tenofovir-containing regimens, in whom endothelial function also improved to a greater extent than among participants taking abacavir-containing regimens.³² In all three of these studies, no evaluation was presented to determine whether baseline biomarker levels predicted outcomes. Five studies demonstrated no difference between the outcome of interest and endothelial activation biomarkers.

Table 1. Biomarkers of endothelial activation

Author	Year	Biomarkers	Study design	Patient population and follow-up	Outcomes studied	Finding
Zangerle ⁴³	1994	ICAM-1	Prospective	47 HIV-infected adults followed for mean 12.7 mo (range, 8–16 mo)	CD4 decline	• No association between ICAM-1 levels and CD4 count decline
Greenwood ⁴⁴	1998	ICAM-1, VCAM-1	Prospective	25 HIV-infected adults with and without CMV retinitis followed for up to 19 mo	CMV retinitis	• No difference in ICAM-1 or VCAM-1 levels between patients with and without retinitis • No difference in ICAM-1 or VCAM-1 levels between active flares and quiescent periods
Renwick ⁴⁵	2002	VEGF	Prospective	40 HIV-infected men followed in the Amsterdam Cohort Studies (follow-up time not reported)	Kaposi sarcoma	• No change in VEGF levels after HIV-1 or HHV-8 seroconversion • No difference in VEGF concentration in men who did or did not develop KS
Sipsas ³¹	2003	E-selectin, ICAM-1	Prospective	64 HIV-infected adults followed for median 46 mo (range, 2–78 mo)	Death	• Baseline levels of E-selectin and ICAM-1 higher in those who died • ICAM-1 associated with time to death, but not in multivariate analysis
van Vonderen ²⁷	2009	ICAM-1, VCAM-1	Prospective	37 HIV-infected, ART-naïve men randomized to receive either AZT/3TC/LPV/r or NVP/LPV/r and followed for 24 mo	c-IMT, arterial stiffness	• Levels of VCAM-1 and ICAM-1 decreased in both groups during treatment • c-IMT increased and arterial stiffness decreased, with no difference between arms
Ford ²⁹	2010	ICAM-1, VCAM-1	Nested case-control within prospective NIH cohort	52 HIV-infected adults with a CVD event (cases) and 102 matched controls followed for mean 8.9 and 8.4 y from ART initiation, respectively	CVD event (see text for details)	• Elevated VCAM-1 associated with CVD events, but association not significant in multivariable analysis
Gupta ²⁵	2010	E-selectin, ICAM-1, VCAM-1	Prospective	9 HIV-infected adults not requiring ART, randomized to pentoxifylline or placebo, followed for 8 weeks	FMD, NTGMD	• Pentoxifylline reduced VCAM-1 levels and improved FMD over 8 weeks
Hileman ²⁴	2010	ICAM-1, VCAM-1	Prospective	40 HIV-infected adults with virologic suppression on ART, randomized to salsalate or placebo, followed for 13 weeks	FMD	• Neither ICAM-1 nor VCAM-1 levels correlated with change in FMD • Change in FMD did not differ between arms
Francisci ³²	2011	VCAM-1	Prospective, with retrospective case-control component	69 HIV-infected adults initiating ABC- or TDF-containing ART followed for 6–12 mo, 20 HIV-infected untreated controls, and 10 healthy controls	Finger arterial pulse wave amplitude, circulating endothelial cells	• HIV-infected adults had increased circulating endothelial cells and impaired tonometry • Endothelial function was worse in adults taking ABC than those taking TDF • VCAM-1 levels elevated in HIV infection, and decreased to a greater extent among adults treated with TDF

3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; AZT, zidovudine; c-IMT, carotid intima-media thickness; CMV, cytomegalovirus; CVD, cardiovascular disease; FMD, flow-mediated dilation; HIV, human immunodeficiency virus; KS, Kaposi sarcoma; LPV/r, boosted lopinavir; NIH, National Institutes of Health; NTGMD, nitroglycerin-mediated dilation; NVP, nevirapine; TDF, tenofovir; VEGF, vascular endothelial growth factor; VTE, venous thromboembolism.

Biomarkers of coagulation. We identified 12 studies investigating associations between biomarkers of coagulation and vascular dysfunction or clinical outcomes in HIV infection (see Table 2). D-dimer was evaluated in seven studies and VWF in six studies, while fibrinogen, PAI-1, prothrombin fragment

1+2, and tissue factor were evaluated in two studies each. Thrombomodulin and tPA were each evaluated in only one of these 12 studies. One study conducted an extensive coagulation work-up including activated protein C sensitivity ratio, endogenous thrombin potential, protein C, prothrombin, PT, PTT,

Table 1. Biomarkers of endothelial activation (continued)

Author	Year	Biomarkers	Study design	Patient population and follow-up	Outcomes studied	Finding
Musselwhite ³⁰	2011	E-selectin, ICAM-1, ICAM-3, P-selectin, VCAM-1	Nested case-control within prospective NIH cohort	23 HIV-infected adults with VTE (cases) and 69 matched HIV-infected controls followed for median 6.9 and 7.2 y from ART initiation, respectively	VTE	• Increased P-selectin levels associated with VTE
Tungsiripat ²⁶	2011	ICAM-1, VCAM-1	Prospective	71 HIV-infected adults with lipotrophy on thymidine-sparing ART, randomized to rosiglitazone or placebo and followed for 48 weeks	c-IMT	• VCAM-1 levels decreased and c-IMT increased, with no difference between groups • Neither ICAM-1 nor VCAM-1 levels associated with c-IMT

3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; AZT, zidovudine; c-IMT, carotid intima-media thickness; CMV, cytomegalovirus; CVD, cardiovascular disease; FMD, flow-mediated dilation; HIV, human immunodeficiency virus; KS, Kaposi sarcoma; LPV/r, boosted lopinavir; NIH, National Institutes of Health; NTGMD, nitroglycerin-mediated dilation; NVP, nevirapine; TDF, tenofovir; VEGF, vascular endothelial growth factor; VTE, venous thromboembolism.

thrombin-antithrombin complex, total and free protein S, as well as D-dimer and VWF.³³ Five studies used nested case-control designs. Four studies evaluated vascular function using measures including c-IMT ($n = 3$), arterial stiffness ($n = 1$), and FMD ($n = 1$). Eight studies evaluated clinical outcomes, including all-cause mortality ($n = 3$), CVD events ($n = 1$), deep venous thrombosis ($n = 1$), opportunistic infections ($n = 1$), and VTE ($n = 1$). One study evaluated HIV disease progression, defined as AIDS-defining illness, death, or a CD4 count < 50 cells/ μ L.³⁴

Five studies demonstrated associations between endothelial activation and outcomes of interest, four of which involved D-dimer. In these four studies, D-dimer predicted all-cause mortality ($n = 2$),^{10,35} CVD events ($n = 1$),²⁹ and VTE ($n = 1$).³⁰ In the fifth positive study, tPA and CD4 count independently predicted death.³⁶ Results from two studies suggested an indirect association between biomarkers and outcomes. In the first, a marked rise in VWF levels was associated with disease progression, with a positive correlation between VWF levels and HIV-1 RNA levels.³⁴ There was not a direct evaluation of whether baseline VWF levels predicted disease progression. In the second study, VWF levels decreased while c-IMT increased and arterial stiffness decreased during ART.²⁷ Again, there was not a direct evaluation of whether baseline VWF levels predicted vascular function. Four studies reported no association between coagulation biomarkers and outcomes of interest. One study identified no outcomes, and so had no power to determine associations.³³

Discussion

We conducted a comprehensive systematic review of the clinical utility of biomarkers reflecting endothelial activation and coagulation in HIV-1 infection. Our objective in conducting this review has been to evaluate the status of work in this area and identify productive avenues for future research. Increased coagulation and endothelial activation biomarkers have been reported in a number of studies of HIV-infected adults, with decreases in these markers after ART initiation (reviewed in refs. 7, 21, and 37). However, only a small number of studies have evaluated the

association of endothelial activation and coagulation with either vascular dysfunction (a surrogate marker of future adverse outcomes) or clinical outcomes in this patient population. Seventeen studies were identified that fulfilled the inclusion criteria, of which 11 investigated endothelial activation biomarkers and 12 investigated coagulation biomarkers. The biomarkers and outcomes studied varied widely. Sample sizes were relatively small, ranging from 9 to 499 HIV-infected participants, with the exception of one very large study of over 1300 participants.³⁵

Our review of endothelial activation biomarkers identified the platelet activation biomarker P-selectin as having clinical utility for the diagnosis of VTE.³⁰ Notably, P-selectin has been previously reported as a predictor of VTE in the general population,³⁸ and increased plasma levels of P-selectin have been reported among pregnant HIV-infected women who developed preeclampsia, compared with those who did not.³⁹ While higher ICAM-1 levels were associated with mortality in one study,³¹ and higher VCAM-1 levels with CVD events in another,²⁹ neither finding was an independent predictor of outcomes. Other studies we identified failed to evaluate the prognostic value of endothelial activation biomarkers despite longitudinal follow-up.

Are biomarkers of endothelial activation therefore not useful for the prediction of outcomes other than VTE? We have recently published a prospective study of endothelial activation biomarkers in HIV-1 seroconverters that was not included in this review. In this study, we found that levels of ICAM-1 and VCAM-1 were persistently elevated from the date of HIV-1 acquisition and that plasma VCAM-1 levels measured during chronic infection were independently associated with time to HIV progression or death.⁴⁰ Our search did not identify eligible studies of several other biomarkers in this category, including Ang-1, Ang-2, ADMA, and the soluble forms of endocan, endothelin-1, Flt-1, and Tie2 receptor. Investigation of these biomarkers is recommended as holding promise. Indeed, we have found in a small study of Kenyan women initiating ART that soluble ICAM-1 and plasma Ang-2 levels decreased after ART initiation, with concomitant increases in the beneficial protein Ang-1. Although both biomarkers predicted mortality after ART initiation in this cohort, Ang-2 had better predictive value.⁴¹

Table 2. Biomarkers of coagulation

Author	Year	Biomarkers	Study design	Patient population and follow-up	Primary outcome	Finding
Schved ³⁶	1992	PAI-1, tPA, VWF	Prospective	85 HIV-infected adults, 65 of whom were followed prospectively for a median 22 mo	Death	<ul style="list-style-type: none"> • Higher PAI-1, tPA, and VWF in advanced disease • Increased VWF and tPA in non-survivors • tPA and CD4 count independently predicted death
Aukrust ³⁴	2000	VWF	Prospective	43 HIV-infected adults followed for a median of 5 y (range, 3.8 to 6 y) and 19 healthy controls	Disease progression (see text for details)	<ul style="list-style-type: none"> • Marked rise in VWF associated with disease progression • Positive correlation between VWF levels and HIV-1 RNA
Hsue ⁴⁶	2004	Fibrinogen	Prospective	148 HIV-infected adults followed for 12 mo, and 63 healthy controls	c-IMT, c-IMT progression	<ul style="list-style-type: none"> • Higher fibrinogen levels and c-IMT in HIV-infected adults • No association between fibrinogen and c-IMT or c-IMT progression
Kuller ¹⁰	2008	d-dimer, prothrombin fragment 1+2	Nested case control within the SMART study ⁴⁷	499 HIV-infected adults randomized to drug conservation or viral suppression strategies followed for 1 mo, with nested case-control comparing 85 adults who died to 170 matched HIV-infected controls (follow-up time not reported)	All-cause mortality	<ul style="list-style-type: none"> • Higher levels of d-dimer at study entry associated with increased risk of all-cause mortality • d-dimer levels associated with HIV-1 RNA levels, with both higher in the drug conservation group
Rodger ²⁸	2009	d-dimer, prothrombin fragment 1+2	Nested case control within the SMART study ⁴⁷	91 HIV-infected adults with opportunistic infections (cases) and 182 HIV-infected controls (follow-up time not reported)	Opportunistic infection	<ul style="list-style-type: none"> • Neither baseline d-dimer nor prothrombin fragment 1+2 predicted opportunistic infections
van Vonderen ²⁷	2009	PAI-1, VWF	Prospective	37 HIV-infected, ART-naïve men randomized to receive either AZT/3TC/LPV/r or NVP/LPV/r and followed for 24 mo	c-IMT, arterial stiffness	<ul style="list-style-type: none"> • VWF levels decreased in both groups during treatment • c-IMT increased and arterial stiffness decreased, with no difference between arms
Ford ²⁹	2010	d-dimer, tissue factor	Nested case-control within prospective NIH cohort	52 HIV-infected adults with a CVD event (cases) and 102 matched controls followed for mean 8.9 and 8.4 y from ART initiation, respectively	CVD event (see reference for details)	<ul style="list-style-type: none"> • Elevated d-dimer and tissue factor associated with CVD events • Only d-dimer independently associated with CVD events in multivariable analysis
Hileman ²⁴	2010	d-dimer, fibrinogen	Prospective	40 HIV-infected adults with virologic suppression on ART, randomized to salsalate or placebo, followed for 13 weeks	FMD	<ul style="list-style-type: none"> • Neither d-dimer nor fibrinogen levels correlated with change in FMD • Change in FMD did not differ between arms
Jong ³³	2010	APCsr, d-dimer, endogenous thrombin potential, protein C, prothrombin, PT, PTT, thrombin-antithrombin complex, total and free protein S, VWF	Prospective	123 HIV-infected adults initiating ART followed for a median 7.2 mo (\pm 1.6 mo) and 71 healthy controls	DVT	<ul style="list-style-type: none"> • No asymptomatic DVT in 57 HIV-infected adults tested • All biomarkers of coagulation except APCsr improved after ART initiation • Persistent differences with uninfected controls

3TC, lamivudine; AIDS, acquired immunodeficiency syndrome; APCsr, activated protein C sensitivity ratio; ART, antiretroviral therapy; AZT, zidovudine; c-IMT, carotid intima-media thickness; DVT, deep venous thrombosis; FMD, flow-mediated dilation; HIV, human immunodeficiency virus; LPV/r, boosted lopinavir; NVP, nevirapine; PAI-1, plasminogen activator-inhibitor 1; PT, prothrombin time; PTT, partial thromboplastin time; tPA, tissue-type plasminogen activator; VACS, Veterans Aging Cohort Study; VTE, venous thromboembolism; von Willebrand Factor, VWF.

Table 2. Biomarkers of coagulation (continued)

Author	Year	Biomarkers	Study design	Patient population and follow-up	Primary outcome	Finding
Musselwhite ³⁰	2011	d-dimer, tissue factor, thrombomodulin, VWF	Nested case-control within NIH cohort	23 HIV-infected adults with VTE (cases) and 69 matched HIV-infected controls followed for median 6.9 and 7.2 y from ART initiation, respectively	VTE	<ul style="list-style-type: none"> • Increased D-dimer levels were associated with VTE
Tungsiripat ²⁶	2011	VWF	Prospective	71 HIV-infected adults with lipatrophy on thymidine-sparing regimens, randomized to rosiglitazone or placebo, followed for 48 weeks	c-IMT	<ul style="list-style-type: none"> • VWF levels decreased and c-IMT increased, with no difference between groups • VWF levels not associated with c-IMT
Justice ³⁵	2012	d-dimer	Nested case control followed in VACS	1302 HIV-infected adults (follow-up time not reported)	Death	<ul style="list-style-type: none"> • D-dimer correlated with VACS Index, which was more predictive of mortality than any biomarker. • Addition of D-dimer to the VACS Index improved classification by 7%

3TC, lamivudine; AIDS, acquired immunodeficiency syndrome; APCsr, activated protein C sensitivity ratio; ART, antiretroviral therapy; AZT, zidovudine; c-IMT, carotid intima-media thickness; DVT, deep venous thrombosis; FMD, flow-mediated dilation; HIV, human immunodeficiency virus; LPV/r, boosted lopinavir; NVP, nevirapine; PAI-1, plasminogen activator-inhibitor 1; PT, prothrombin time; PTT, partial thromboplastin time; tPA, tissue-type plasminogen activator; VACS, Veterans Aging Cohort Study; VTE, venous thromboembolism; von Willebrand Factor, VWF.

Our review of coagulation biomarkers showed that D-dimer is the most promising biomarker, given its associations with several clinical outcomes, including venous thromboembolism, cardiovascular disease, and all-cause mortality.^{10,29,30,35} Interestingly, D-dimer has been found to be a predictor of mortality in Crimean-Congo hemorrhagic fever,⁴² suggesting the possibility that this biomarker may have utility in other viral infections as well. In addition, tPA was identified as a potential biomarker for mortality in HIV-infected patients.³⁶ Future studies of biomarkers in HIV-1 infection should include D-dimer and consider inclusion of tPA, in order to help establish the clinical utility of these biomarkers for risk stratification and the prediction of clinically relevant endpoints. Our search did not identify eligible studies of several other coagulation biomarkers, including ADAMTS13, factor VIII activity, soluble fibrin, and thrombospondin.

Our study has several limitations. First, our search strategy was broad, but we may have missed some articles in which the biomarkers were not discussed in terms of their effects on endothelial activation or coagulation. We tried to address this by adding searches of biomarker names and by hand-searching the reference lists of identified studies. Second, many publications were produced by single-center teams or were retrospective analyses of previously collected specimens and data, limiting the generalizability to other populations or jurisdictions. Third, the identified studies included a wide range of biomarkers, and did not always evaluate the association of biomarkers with the later development of outcomes. In addition, the selection of outcomes varied across studies, and their definitions may have differed. Finally, because many of the biomarkers studied are not standardized, available literature can only report similarities in the direction and relative magnitude of associations across studies. We were unable to identify a sufficiently large number of adequately powered

studies with prospective designs, careful selection of biomarkers, and standardized outcomes to confirm conclusively whether any of these biomarkers have clinical utility in most HIV-infected patient populations at the present time.

Materials and Methods

Data sources. We systematically and inclusively identified all studies that reported data on biomarkers of: (1) endothelial activation (including angiotensin-1 [Ang-1], angiotensin-2 [Ang-2], asymmetric dimethylarginine [ADMA], and the soluble forms of endocan, endothelin-1, E-selectin, FMS-like tyrosine kinase-1 [sFlt-1], intercellular adhesion molecule 1 [ICAM-1], ICAM-3, P-selectin, Tie2 receptor, vascular cell adhesion molecule 1 [VCAM-1], and vascular endothelial growth factor [VEGF]), and (2) coagulation (including ADAMTS13, anti-thrombin, D-dimer, factor VIII activity, fibrinogen, plasminogen activator-inhibitor 1 [PAI-1], protein C, protein S, prothrombin, prothrombin fragment 1+2, soluble fibrin, thrombin, thrombomodulin, thrombospondin, tissue-type plasminogen activator [tPA], tissue factor, and von Willebrand factor [VWF]) in HIV-infected adult patients. We electronically searched PubMed (1950 to Week 27, 2012) and Embase (1980 to Week 27, 2012) databases for all pertinent articles published in English (see **Table S1**).

Study selection methods. Study selection was performed independently by two reviewers (RM and SMG), with disagreement resolved through arbitration by a third reviewer (WCL). A study was included if it met the following criteria:

- (1) Inclusion of non-pregnant, HIV-infected adults, aged 18 y or older;
- (2) Measurement of any known biomarker of endothelial activation and/or coagulation; and

(3) Measurement of vascular dysfunction (e.g., carotid intima-media thickness) or a clinical endpoint (e.g., all-cause mortality).

Studies that included only children (i.e., < 18 y of age), case reports, case series, and studies of interventions in which only short-term outcomes were evaluated (e.g., changes in blood pressure) were excluded. We excluded cross-sectional studies and prospective studies that only evaluated changes in biomarker levels, usually after ART initiation. Conference abstracts and publications in languages other than English were also excluded, as we were not able to fully assess these studies.

Study data extraction and analysis. For each of the selected studies, we extracted the biomarkers evaluated, study design, patient population, duration of follow-up, and details of the outcomes and findings. Results were tabulated for each type of biomarker (i.e., endothelial activation and coagulation) and compared across studies where appropriate. Due to broad study heterogeneity and disparate outcomes, we did not attempt to numerically combine or perform a metaanalysis of study results.

Conclusions

This systematic review of the published literature demonstrates that several biomarkers reflecting endothelial activation and coagulation, including D-dimer, P-selectin, and tPA, may represent potentially useful biomarkers for the prediction of clinical outcomes in HIV-infected patients. The clinical utility of these biomarkers is limited by the paucity and inconsistency of available evidence, the lack of standardized approaches to biomarker testing and assessment, and the lack of prospective validation in representative patient populations. Due to the increased risk of morbid outcomes in HIV-infected patients, additional large-scale prospective investigations to determine the utility of the most promising endothelial activation and coagulation biomarkers to stratify risk and predict adverse outcomes are clearly warranted. Such

research has the potential to elucidate mechanisms of endothelial injury and has the potential to identify specific treatments aimed at reducing endothelial activation or reducing risk of thrombosis.

Disclosure of Potential Conflicts of Interest

WCL is listed as a co-inventor on a patent applied for by the University Health Network (Toronto, ON Canada) to develop point-of-care tests for endothelial activation biomarkers in infectious diseases. All other authors report no conflict of interest.

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Supplemental Materials

Supplemental materials may be found here: www.landesbioscience.com/journals/virulence/articles/25221

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