Original article

Elevated D-dimer is independently associated with endothelial dysfunction: a cross-sectional study in HIV-infected adults on antiretroviral therapy

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Background: D-Dimer elevations have been associated with a striking increase in mortality in HIV-infected patients. However, D-Dimer has not been directly linked to endothelial dysfunction in HIV.

Methods: In this cross-sectional study, we used flow-mediated dilation (FMD) of the brachial artery to measure endothelial function and several biomarkers to measure systemic inflammation and coagulation activation in HIV-infected adults on stable antiretroviral therapy with HIV-1 RNA levels <400 copies/ml. Multivariable linear regression was used to model FMD by these markers, traditional cardiovascular risk factors and HIV-related characteristics.

Results: Analysis included 98 subjects (88\% male, median age 47.5 years, CD4+ T-cells 578.5 cells/mm\(^3\)); all on ART (52\% on protease inhibitors). The only factors independently associated with FMD were D-Dimer and body mass index.

Conclusions: We show for the first time an independent association between D-Dimer and endothelial dysfunction in virologically suppressed, HIV-infected adults on stable antiretroviral therapy, potentially explaining the link between D-Dimer and mortality in HIV.

Introduction

There is great interest in identifying biomarkers that are predictive of cardiovascular risk in patients whose HIV infection is well-controlled on antiretroviral therapy (ART). Two of the more promising candidates, fibrin D-Dimer (a fibrin degradation product that broadly reflects activation of the coagulation system) and interleukin-6 (IL-6; an inflammatory cytokine and acute phase reactant) were strongly associated with mortality in the SMART study \cite{1}. Cardiovascular disease (CVD) was an important cause of mortality in this study \cite{2,3}. High sensitivity C-reactive protein (hs-CRP) has also been linked with CVD in HIV \cite{4,5}, although not consistently \cite{6}. Little is known about the mechanisms that relate biomarkers to cardiovascular risk in HIV. As such, we sought to evaluate the relationship between markers of oxidative stress, inflammation and coagulation, and endothelial function in virologically suppressed, HIV-1-infected adults on stable ART.

Methods

In this cross-sectional study, we included HIV-1-infected adults on stable ART for at least 12 weeks with HIV-1 RNA<400 copies/ml who had endothelial function measured by flow-mediated dilation (FMD) of the brachial artery performed as part of entry into a study through the HIV Metabolic Research Center at Case Western Reserve University. Major exclusion criteria were active infection, inflammatory condition or malignancy, uncontrolled diabetes mellitus, creatinine clearance <50 ml/min, alanine aminotransferase or aspartate aminotransferase >2× the upper limit of normal, pregnancy, lactation, regular use of anti-inflammatory or anti-oxidant medication, intravenous drug use or daily alcohol use. The use of anti-hypertensive and lipid-lowering medications was allowed, but had to be stable for at least 12 weeks prior to entry. All individuals signed written informed consent for participation in the study.

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HIV Metabolic Research Center trials for which FMD was performed and also to have their blood stored for use in future HIV-related metabolic research.

Demographics, HIV and cardiovascular characteristics, laboratory values and plasma samples to store were obtained from participants in fasting state (≥8 h fast) on the date FMD was performed. Stored plasma was kept in a -80°C freezer and never thawed until biomarker measurement. The following markers were measured: IL-6, soluble tumour necrosis factor alpha receptors-I and -II (sTNFR-I and -II), hs-CRP, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), fibrinogen, D-Dimer and F2-isoprostanes. IL-6, sTNFR-I and -II, sICAM-1 and sVCAM-1 were determined by quantitative sandwich ELISAs (R&D Systems, Minneapolis, MN, USA). Inter-assay variability ranged from 2.02–15.36%, 3.66–5.77%, 2.13–3.79%, 3.43–7.37% and 4.76–8.77%, respectively. High sensitivity C-reactive protein and fibrinogen were determined by particle enhanced immunonephelometric assays on a BNII nephelometer (Siemens, Indianapolis, IN, USA). Inter-assay variability ranged from 3.01–6.46% and 3.42–7.59%, respectively.

D-Dimer was determined by immuno-turbidometric assay on a STA-R Coagulation Analyzer (Diagnostica Stago, Parsippany, NJ, USA). Inter-assay variability ranged from 2.02–15.36%, 3.66–5.77%, 2.13–3.79%, 3.43–7.37% and 4.76–8.77%, respectively. High sensitivity C-reactive protein and fibrinogen were determined by particle enhanced immunonephelometric assays on a BNII nephelometer (Siemens, Indianapolis, IN, USA). Inter-assay variability ranged from 3.01–6.46% and 3.42–7.59%, respectively.

All studies were performed by a single technologist (CAW) using a Phillips iU22 Ultrasound and a L10-7 MHz linear array transducer (Phillips Healthcare, Bothell, WA, USA) and a 5 min occlusion time. Images were read using Brachial Artery Analyzer software (Medical Imaging Applications LLC, Coralville, IA, USA), a semi-automated, border interfacing programme. For FMD determination, brachial artery diameters were measured in triplicate and averaged from a 1 cm segment of the artery. Using this technique, the coefficient of variation for FMD measurement at our site is 1.9%, which compares favourably with others [9]. FMD is expressed as percentage change from baseline artery diameter to diameter post reactive hyperaemia.

Demographic, HIV and cardiovascular factors are described for the group using median and interquartile range (IQR) for continuous variables and frequency and percent for categorical variables. Univariable followed by multivariable linear regression was performed with FMD as the dependent variable. In the first multivariable model, variables with P<0.25 in univariable analysis were included and backwards elimination was used for model selection. A cutoff value of P<0.25 was selected so that we would not miss associations that may be present if negative confounding was present. In a second model, clinically relevant variables, that is, age, sex, race, body mass index (BMI), CD4+ T-cell count, whether on a protease inhibitor and smoking status, were added to the first multivariable model regardless of statistical significance. For multivariable models, variables with P<0.05 were considered statistically significant. Final models were checked to be sure that the assumptions of linear regression were met.

**Results**

In total, 98 individuals met eligibility criteria. The median (IQR) age was 47.5 (43–52) years. Most participants were men (88%) and were African American (52%) or Caucasian (42%). Median (IQR) known duration of HIV was 11.3 (7.2–16.2) years, current CD4+ T-cell count was 578.5 (431–789) and nadir CD4+ T-cell count was 130 (32–238) cells/mm³. All participants were on ART (52% on protease inhibitors and 48% on non-nucleoside reverse transcriptase inhibitors) and had HIV-1 RNA<400 copies/ml. In total, 47% were current smokers and 22% past smokers. Thirty percent were on an anti-hypertensive medication and 27% were on lipid-lowering therapy (17% on a statin). The median (IQR) total, high-density lipoprotein (HDL) and non-HDL cholesterol and triglyceride levels were 178 (151–209), 40.5 (36–51), 131.5 (110–166) and 124.5 (84–198) mg/dl, respectively. Median (IQR) BMI was 26 (23.3–30) kg/m².

Median (IQR) IL-6, sTNFR-I, sTNFR-II, hs-CRP, sICAM-1, sVCAM-1, D-Dimer, fibrinogen and F2-isoprostanes were 2.43 (1.52–3.99) pg/ml, 1,286 (1,074–1,581) pg/ml, 2,695 (2,270–3,292) pg/ml, 1.33 (0.8–3.8) µg/ml, 227 (150–311) ng/ml, 687 (567–884) ng/ml, 0.17 (0.11–0.28) µg/ml, 373 (322–439) mg/dl and 0.05 (0.03–0.21) ng/ml, respectively. Additionally, the median (IQR) FMD for the group was 3.29% (1.58–6.17%). Table 1 shows results of univariable and multivariable regression. In univariable analysis, age, BMI, total bilirubin, D-Dimer and brachial artery diameter had P<0.25 and were entered into the first multivariable model. BMI, D-dimer and brachial artery diameter remained independently associated with FMD with this approach. Adjusting for clinically relevant variables in the model with BMI, D-Dimer and brachial artery diameter led to an attenuation of the statistical significance of D-Dimer with FMD.
Variable | Parameter estimate | Standard error | P-value
--- | --- | --- | ---
Age, years | -0.0556 | 0.0413 | 0.18
Sex (male =1) | -0.6056 | 0.985 | 0.54
Race (Caucasian/African American/other) | | | |
Caucasian | 1.5221 | 1.4914 | 0.31
African American | 0.1993 | 1.4741 | 0.89
Smoker (yes =1) | -0.1164 | 0.6481 | 0.86
Systolic BP, mmHg | -0.0244 | 0.0235 | 0.3
Diastolic BP, mmHg | 0.0077 | 0.0367 | 0.83
On anti-HTN drug (yes =1) | 0.1753 | 0.7085 | 0.81
Total cholesterol, mg/dl | -0.0008 | 0.0074 | 0.92
HDL, mg/dl | -0.0268 | 0.0235 | 0.26
Non–HDL, mg/dl | 0.0021 | 0.0078 | 0.79
Triglycerides, mg/dl | -0.001 | 0.0027 | 0.71
On lipid-lowering drug (yes =1) | -0.1384 | 0.7326 | 0.85
HOMA-IR | 0.1577 | 0.1713 | 0.36
Body mass index, kg/m² | 0.0956 | 0.0591 | 0.11
Total bilirubin, mg/dl | -0.4366 | 0.3426 | 0.21
CD4+ T-cells, cells/mm³ | -0.0002 | 0.0009 | 0.86
Nadir CD4+ T-cells, cells/mm³ | -0.0014 | 0.0021 | 0.51
HIV duration, years | 0.0024 | 0.0551 | 0.96
On PI | -0.4686 | 0.6458 | 0.47
On NNRTI | 0.1466 | 0.6474 | 0.82
IL-6, pg/ml | 0.1403 | 0.829 | 0.87
sTNFR-I, pg/ml | 1.358 | 2.8326 | 0.63
sTNFR-II, pg/ml | 1.4 | 2.804 | 0.62
hs-CRP, µg/ml | 0.077 | 0.6292 | 0.9
sICAM-1, ng/ml | 0.057 | 0.0807 | 0.48
sVCAM-1, ng/ml | 0.6908 | 2.3707 | 0.77
D-Dimer, µg/ml | -3.8247 | 2.1219 | 0.07
Fibrinogen, mg/dl | -0.0737 | 3.0505 | 0.98
F₂-isoprostanes, ng/ml | 0.355 | 0.7422 | 0.63
Brachial artery diameter, mm | -1.364 | 0.5022 | 0.008

**Multivariable linear regression**

Model 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter estimate</th>
<th>Standard error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index, kg/m²</td>
<td>0.1579</td>
<td>0.058</td>
<td>0.008</td>
</tr>
<tr>
<td>D-Dimer, µg/ml</td>
<td>-5.2156</td>
<td>2.0735</td>
<td>0.01</td>
</tr>
<tr>
<td>Brachial artery diameter, mm</td>
<td>-1.4959</td>
<td>0.4846</td>
<td>0.003</td>
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</tbody>
</table>

Model 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter estimate</th>
<th>Standard error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>-0.0172</td>
<td>0.0443</td>
<td>0.7</td>
</tr>
<tr>
<td>Sex (male =1)</td>
<td>0.9654</td>
<td>1.2617</td>
<td>0.45</td>
</tr>
<tr>
<td>Caucasian</td>
<td>0.8842</td>
<td>1.4689</td>
<td>0.55</td>
</tr>
<tr>
<td>African American</td>
<td>-0.1738</td>
<td>1.4863</td>
<td>0.91</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>0.1873</td>
<td>0.0644</td>
<td>0.005</td>
</tr>
<tr>
<td>CD4+ T-cells, cells/mm³</td>
<td>-0.0007</td>
<td>0.0009</td>
<td>0.44</td>
</tr>
<tr>
<td>Smoking status (current smoker =1)</td>
<td>-0.1484</td>
<td>0.6445</td>
<td>0.82</td>
</tr>
<tr>
<td>On PI (yes =1)</td>
<td>-0.5119</td>
<td>0.6228</td>
<td>0.41</td>
</tr>
<tr>
<td>Brachial artery diameter, mm</td>
<td>-1.7246</td>
<td>0.6172</td>
<td>0.006</td>
</tr>
<tr>
<td>D-Dimer, µg/ml</td>
<td>-3.9044</td>
<td>2.2627</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*Variables tested in univariable linear regression, but not shown include: glucose, insulin, waist-to-hip ratio, CD4+ T-cell percentage. *Homeostasis model of insulin resistance (HOMA-IR) is fasting glucose (mg/dl) × fasting insulin (µU/ml)/405. *Log-transformed. *Transformed by taking the square root. *All variables with P<0.15 included in this model and backward elimination used for model selection. Interpreted as higher D-Dimer is associated with lower flow-mediated dilation (FMD). *Clinically relevant variables added to Model 1 regardless of statistical association. BP, blood pressure; F₂-isopxs, F₂-isoprostanes; HDL, high-density lipoprotein; hs-CRP, high sensitivity C-reactive protein; HTN, hypertension; IL-6, interleukin-6; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; sICAM-1, soluble intercellular adhesion molecule-1; sTNFR-I, soluble tumour necrosis factor receptor-I; sTNFR-II, soluble tumour necrosis factor receptor-II; sVCAM-1, soluble vascular cell adhesion molecule-1.
Discussion

To date, many studies have evaluated the contribution of viral replication, ART and traditional cardiovascular risk factors in HIV-related endothelial dysfunction [10–12]. In this study, we have evaluated the relationship between several important markers of systemic inflammation, coagulation and oxidative stress, in addition to the above factors in viremically suppressed, HIV-1-infected adults on stable ART. To our knowledge, this is the first study to demonstrate an association between elevated D-Dimer and endothelial dysfunction measured by FMD in this patient group. In our study, the median (IQR) D-Dimer levels by lowest to highest FMD quartiles were 0.18 (0.125–0.355), 0.17 (0.09–0.24), 0.17 (0.008–0.25) and 0.145 (0.11–0.205) µg/ml showing the inverse relationship.

Importantly, epidemiologic studies in the general population and in HIV have shown a relationship between D-Dimer and cardiovascular risk [6,13,14]. In a case-control study, 52 HIV-infected patients experiencing a cardiovascular event were compared with 104 age- and sex-matched controls. In this study, elevated D-Dimer was independently associated with being a case, that is, experiencing a cardiovascular event [6].

Our findings suggest that impaired endothelial function is one possible mechanism by which D-Dimer may contribute to cardiovascular risk and mortality in HIV as observed by the SMART Study Group and others [1,6]. It is intuitive that increased activation of the coagulation system may lead to acute atherothrombosis and subsequent unstable coronary syndromes or stroke; however, pro-coagulant activity may be a cause or consequence of the underlying endothelial dysfunction that facilitates the chronic progression of atherosclerosis [15]. Endothelial dysfunction has been proposed as a mechanism of the increased cardiovascular risk observed in patients with unprovoked venous thromboembolism [16,17]; however, data on the association of D-Dimer and FMD in large population-based studies are limited. In a study from the Multi-Ethnic Study of Atherosclerosis (MESA), both fibrinogen and D-Dimer were associated with impaired FMD; though after adjustment for traditional risk factors, only the association with fibrinogen remained significant [18].

The major limitation of this study is the cross-sectional design. Because both D-Dimer and FMD were determined at that same time, we cannot be certain that low-levels of activated coagulation cause endothelial dysfunction in this population. This hypothesis should be further explored in a longitudinal study. Furthermore, although we cannot with certainty say that D-Dimer is associated with coronary endothelial function, brachial artery FMD is strongly correlated with coronary artery FMD (r=0.79; P<0.001) [19].

In conclusion, elevated D-Dimer was independently associated with endothelial dysfunction in viremically suppressed, HIV-1-infected adults in our study. This biomarker may be useful in identifying HIV-1-infected patients at increased cardiovascular risk.

Acknowledgements

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Disclosure statement

COH and CTL have received research grant support from Bristol–Myers Squibb through the Virology Fellows Research Program. TLC serves on a DSMB for Prairie Education and Research Cooperative. GLM serves as a consultant to Roche. GAM has received research grant support and serves as a consultant for GlaxoSmithKline, Bristol–Myers Squibb, Gilead Sciences and Tibotec, and currently serves as the DMC Chair for a Pfizer-sponsored clinical trial.

References


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