Title

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ORIGINAL RESEARCH

Effect of HIV-1 Infection on Angiopoietin 1 and 2 Levels and Measures of Microvascular and Macrovascular Endothelial Dysfunction

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BACKGROUND: Individuals infected with HIV have an increased risk of developing cardiovascular disease; yet, the underlying mechanisms remain unknown. Recent evidence has implicated the Tie-2 tyrosine kinase receptor system and its associated ligands ANG1 (angiopoietin 1) and ANG2 (angiopoietin 2) in maintaining vascular homeostasis. In the general population, lower ANG1 levels and higher ANG2 levels are strongly correlated with the development of cardiovascular disease. In this study, we aim to investigate the associations of HIV infection with angiopoietin levels and endothelial dysfunction.

METHODS AND RESULTS: In this cross-sectional study, we compared measures of ANG1, ANG2, and endothelial dysfunction using flow-mediated vasodilation of the brachial artery in 39 untreated subjects infected with HIV, 47 treated subjects infected with HIV, and 46 uninfected subjects from the SCOPE (Observational Study of the Consequences of the Protease Inhibitor Era) cohort. Compared with uninfected controls, treated individuals infected with HIV had 53.1% lower mean ANG1 levels ($P<0.01$) and similar ANG2 levels. On the other hand, untreated individuals infected with HIV had similar ANG1 levels, and 29.2% had higher ANG2 levels ($P<0.01$) compared with uninfected controls. When compared with individuals with untreated HIV infection, those with treated HIV infection had 56% lower ANG1 levels ($P<0.01$) and 22% lower ANG2 levels ($P<0.01$).Both treated and untreated HIV infection were associated with significant impairment in hyperemic velocity, a key measure of microvascular dysfunction (median 61 versus 72 cm/s, $P<0.01$), compared with uninfected controls (median 73 cm/s). This difference persisted after adjustment for ANG1 and ANG2 levels. Interestingly, when compared with untreated individuals infected with HIV, treated individuals infected with HIV had worse hyperemic velocity (–12.35 cm/s, $P=0.05$). In contrast, HIV status, ANG1 levels, and ANG2 levels were not associated with macrovascular dysfunction as measured by flow-mediated dilatation and brachial artery diameter, 2 other measures of vascular homeostasis.

CONCLUSIONS: HIV infection affects the balance between levels of ANG1 and ANG2 and may disturb endothelial homeostasis through disruption of vascular homeostasis. Individuals with treated HIV had decreased ANG1 levels and similar ANG2 levels, whereas individuals with untreated HIV had similar ANG1 levels and increased ANG2 levels, suggesting that treatment status may alter the balance between ANG1 and ANG2. HIV also promotes endothelial dysfunction via impairment of microvascular dysfunction, independent of the Tie-2 receptor system; the finding of worse microvascular dysfunction in the setting of treated HIV infection may reflect the impact of viral persistence on the microvasculature or toxicities of specific antiretroviral regimens. Further research to clarify the mechanism of HIV-mediated endothelial dysfunction is necessary to advance treatment of cardiovascular complications of HIV infection.

Key Words: angiopoietin 1 ■ angiopoietin 2 ■ endothelial dysfunction ■ endothelial homeostasis ■ HIV

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CLINICAL PERSPECTIVE

What Is New?

- Individuals infected with HIV have an increased risk of developing cardiovascular disease; yet, the underlying mechanisms remain unknown.
- In this study, we demonstrate that HIV infection affects the balance between levels of ANG1 (angiopoietin 1) and ANG2 (angiopoietin 2) and may disturb endothelial homeostasis through disruption of vascular homeostasis. Furthermore, individuals with treated HIV had decreased ANG1 levels and similar ANG2 levels, whereas individuals with untreated HIV had similar ANG1 levels and increased ANG2 levels, suggesting that treatment status may alter the balance between ANG1 and ANG2.
- We also demonstrate that HIV promotes endothelial dysfunction via impairment of microvascular dysfunction; this finding in the setting of treated HIV infection may reflect the impact of viral persistence on the microvasculature and toxicities of specific antiretroviral regimens.

What Are the Clinical Implications?

- Further research to clarify the mechanism of HIV-mediated endothelial dysfunction is necessary to advance treatment of cardiovascular complications of HIV infection.

Nonstandard Abbreviations and Acronyms

BA	brachial artery
FMD	flow-mediated dilation
HV	hyperemic velocity

Despite the advent of antiretroviral therapy, individuals infected with HIV remain at higher risk for multiple forms of cardiovascular disease (CVD) including heart failure,[1] myocardial infarction,[2,3] arrhythmias,[4] and sudden cardiac death.[5] Moreover, CVD has become a leading cause of morbidity and mortality among persons living with HIV. Although the exact mechanisms underlying this excess cardiovascular risk are unclear, mounting evidence suggests that chronic activation of inflammatory pathways as a result of HIV infection may lead to endothelial dysfunction, atherosclerosis, and subsequent clinical events. HIV infection has been associated with increased levels of macrovascular and microvascular endothelial dysfunction.[6,7] In the general population, impairments in flow-mediated dilation (FMD) of the brachial artery (BA)[8,9] and microvascular endothelial function indices[10] are predictive of cardiovascular events. Inflammation and immune activation are key mediators in endothelial dysfunction[11] and as such, they likely play a role in HIV-associated endothelial dysfunction. Namely, chronic upregulation of inflammation and immune activation in the setting of HIV infection are driven by several factors including low-level viral replication and inability to restore levels of circulating CD4+ T cells.[12] Inflammatory biomarkers including interleukin-6 (IL-6) and CRP (c-reactive protein), which rise correlating with viremia and remain elevated in chronic infection, are strongly predictive of CVD and mortality.[13–15] However, the mechanisms by which HIV leads to endothelial dysfunction, and subsequently progressive atherosclerosis and cardiovascular events, remain unclear.

The Tyrosine Kinase Receptor System and Endothelial Dysfunction

The Tie-2 tyrosine kinase receptor system and its ligands, ANG1 (angiopoietin 1) and ANG2 (angiopoietin 2), maintain vascular homeostasis. In this study, we investigated the effects of HIV infection on ANG1 and ANG2 levels. Furthermore, we correlated these levels to macrovascular endothelial function, as measured by BA diameter and FMD, as well as microvascular function, as assessed by hyperemic velocity (HV), to investigate the relationship between ANG1, ANG2, and endothelial dysfunction.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design and Population

We compared measures of ANG1, ANG2, and endothelial function in subjects infected and subjects uninfected with HIV from the SCOPE (Observational Study of the Consequences of the Protease Inhibitor Era) cohort based in San Francisco, California. SCOPE is an observational, prospective cohort of volunteers infected with HIV-1 who receive treatment at HIV/AIDS clinics in the San Francisco area. Participants undergo a baseline evaluation and sample collection and are subsequently followed approximately every 4 months. We studied (1) antiretrovirally treated and suppressed individuals who were on a stable HIV regimen and had an undetectable HIV RNA level for at least 6 months before study entry, and (2) untreated, unsuppressed individuals infected with HIV. Individuals with previous CVD as well as traditional risk factors were allowed to participate in the study as long as they had stable

medications for the previous 6 months. Control participants were individuals who answered study advertisements, received medical care in San Francisco, or were acquaintances of study participants; all underwent HIV antibody testing before study entry and were documented to be HIV negative. Individuals performing the laboratory testing as well as endothelial function tests were blinded with regard to HIV status. This study was approved by the University of California, San Francisco Institutional Review Board, and all individuals signed written witnessed informed consent.

Measurement of Angiopoietin and Inflammatory Biomarker Levels

Serum samples were processed into ethylenediaminetetraacetic acid and frozen to –38 °C. Concentrations of all samples were measured by solid phase sandwich enzyme-linked immunosorbent assays using commercially available kits (R&D Systems, Minneapolis, MN). The coefficients of variation were below 10% for both intra-assay and interassay precision. For the inflammatory biomarkers, CRP and IL-6, 1% and 22% of the cohort were missing values for each biomarker, respectively.

Measurement of Endothelial Dysfunction

Endothelial dysfunction was estimated by measuring the baseline BA diameter, BA FMD, and HV as previously described by our group.[16–18] All indices were measured on the same day as serum collection, and participants were requested to avoid food, alcohol, caffeine, illicit drugs, exercise, and nicotine products for 12 hours before testing. A 10 MHz linear array probe and the GE VividSeven Imaging System were used to make all measurements.

BA diameter was measured under basal conditions after a 10-minute rest period. B-mode ultrasound was used to identify the BA during its superficial course 5 to 9 cm proximal to the antecubital fossa. Arterial location was confirmed using Doppler. Images were acquired for 5 cardiac cycles, and BA diameter was determined to be the average end-diastolic diameter.

To assess FMD, an endothelial-dependent process, basal BA diameter was compared with BA diameter following an ischemic stimulus, which has been shown to significantly predict cardiovascular events.[19] With participants lying supine in a dark room, a blood pressure cuff was placed on the forearm and inflated to suprasystolic pressures for 5 minutes to induce forearm ischemia. The cuff was then deflated, and between 30 and 120 seconds following cuff deflation, B-mode ultrasound was used to measure the BA diameter at 15 second intervals. FMD was characterized as the percent change between the maximum BA diameter post cuff deflation and the resting BA diameter.

To assess HV within the BA, the forearm cuff was inflated to either 200 mm Hg or 50 mm Hg above baseline systolic blood pressure, whichever was higher. After 4 minutes and 30 seconds, pulse wave Doppler recording was initiated. At 5 minutes, the forearm cuff was deflated. Between 30 and 120 seconds following cuff deflation, 5 beat loop B-mode images were obtained every 15 seconds. Maximal HV was characterized as the Doppler mean velocity-time integral of the first 3 complete beats after cuff release.

Acquisition and analysis of the digitized images were performed using dedicated software (Medical Imaging Applications, LLC, Coralville, IA). Images were analyzed by a technician who was blinded to the subjects' HIV disease and treatment status. The protocols in our laboratory for these endothelial dysfunction measures are highly reproducible, as described previously.[16]

Covariates

Covariates included demographic characteristics (age, sex, and race), traditional risk factors for CVD, and HIV-related risk factors. Traditional risk factors included coronary artery disease, hypertension, hyperlipidemia, diabetes, chronic kidney disease, tobacco dependence, cardiovascular medication use (aspirin, antihypertensive medications, and statins), body mass index, Framingham Risk Score, and low-density lipoprotein levels. HIV-related risk factors included median viral load, duration of infection, nadir CD4 cell count, current CD4 and CD8 counts, CD4/CD8 ratio, and hepatitis C virus coinfection.

Statistical Analysis

We compared demographic and clinical characteristics by disease category (HIV-uninfected versus untreated HIV versus treated HIV) with chi-square test for categorical variables; and ANOVA (all 3-group comparisons), *t* test (untreated HIV versus treated HIV), or Kruskal-Wallis tests for continuous variables, respectively. We used Spearman coefficients to summarize correlations between markers of inflammation and angiopoietin levels due to right-skewed distributions of these variables. We estimated cross-sectional associations of disease category with measures of angiopoietin and endothelial dysfunction using linear regression models that were sequentially adjusted for the following: (1) demographic characteristics only; and (2) demographics and traditional CVD risk factors. Models of endothelial dysfunction were further adjusted for ANG1, ANG2, and the ANG2/ANG1 ratio. We used natural logarithm to transform angiopoietin, FMD, and BA to approximate normal distribution before fitting the regression models. Regression coefficients were back-transformed to produce relative (%)

differences. HV was used in its original form as it is normally distributed and the regression coefficients were presented as absolute differences. All analyses were performed using SAS 9.4 (SAS Institute, Cary, NC).

RESULTS

Demographic and Clinical Characteristics

Table 1 summarizes the demographic and clinical characteristics of the 132 participants (46 controls who were uninfected; 39 individuals who were HIV infected and untreated; and 47 individuals who were HIV infected and treated) in our cohort. Participants who were HIV uninfected were on average older and more often male compared with HIV-infected participants. There was no significant difference in racial distribution between the groups.

The most prevalent comorbid conditions were hypertension (32%), hyperlipidemia (50%), and tobacco dependence (36%). There were no significant differences in the prevalence of comorbid medical conditions between the groups, with the exception of tobacco dependence, which was lower in the treated group infected with HIV compared with the untreated groups who were HIV uninfected and HIV infected (21% versus 46% versus 41%, P=0.04). Body mass index, Framingham risk score, and use of aspirin and antihypertensives were similar between the groups. Use of statins was lower in the untreated cohorts uninfected with HIV and infected with HIV compared with the treated cohort infected with HIV (13% versus 5% versus 26%, P=0.03).

There were 86 participants who were HIV infected including 39 who were untreated with detectable viral loads and 47 who were virally suppressed (defined as having a viral load <75 copies/mL) on antiretroviral therapy. The rate of hepatitis C virus coinfection was similar between the 2 groups.

Association Between Levels of Inflammatory Markers and ANG1 and ANG2

As summarized in Table 1, levels of CRP and IL-6 were similar across all groups, regardless of HIV status. We studied the association between levels of these inflammatory biomarkers and ANG1 and ANG2. In general, CRP and IL-6 showed only weak correlations with levels of ANG1 and ANG2, regardless of HIV status. The strongest correlations were seen in treated individuals infected with HIV, where ANG2 showed modest, positive correlations for CRP (r=0.41, P=0.004) and IL-6 (r=0.51, P<0.001). There was a modest but significant correlation in individuals uninfected with HIV between ANG2 and IL-6 (r=0.48, P=0.004).

Association Between HIV Infection Status and ANG1 and ANG2 Levels

Table 2 summarizes levels of ANG1 and ANG2 stratified by HIV status, and Table 3 summarizes differences in ANG1 and ANG2 levels between study groups. After adjusting for demographic and traditional CVD risk factors, individuals with treated HIV had 53% lower ANG1 levels compared with uninfected controls (P=0.007). ANG2 levels were similar between groups. In contrast, untreated individuals infected with HIV had similar ANG1 levels and 29% higher ANG2 levels compared with uninfected controls (P=0.01). Compared with untreated individuals infected with HIV, those with treated HIV infection had 56% lower ANG1 levels (P=0.007) and 22% lower ANG2 levels (P=0.02). The associations between HIV treatment status and ANG1 and ANG2 levels remained after adjustment for duration on antiretroviral therapy.

After adjustment for demographic factors, ANG2/ANG1 ratio was significantly higher in treated individuals infected with HIV compared with uninfected controls (mean demographic adjusted difference of 162%, P<0.01); this difference was attenuated to 85% after further adjustment for traditional CVD risk factors (P=0.08). ANG2/ANG1 ratio was similar between the untreated groups infected with HIV and uninfected with HIV, as well as between treated and untreated groups infected with HIV.

Associations of Demographic and Clinical Factors With ANG1 and ANG2 Levels

Associations between demographic and clinical risk factors and ANG1 and ANG2 levels among our cohort are summarized in Tables S1 through S3. These risk factors were modeled individually (not jointly). After multivariable adjustment for demographic factors and HIV treatment status, several significant associations emerged.

Among uninfected controls, hypertension was associated with 56% lower ANG1 levels (P=0.03) and 176% higher ANG2/ANG1 ratio (P=0.01). Antihypertensive use was associated with 50% higher ANG2 levels (P=0.03); for each 10 mg/dL rise in low-density lipoprotein cholesterol, ANG2 levels were 7% lower (P=0.001).

Among treated individuals infected with HIV, doubling of CD4/CD8 ratio was associated with 39% decrease in ANG1 levels (P=0.002); increase by 100 cells/mm^3 of nadir CD4 count and current CD8 count were associated with 17% decrease (P=0.05) and 11% increase (P=0.003) in ANG1 levels, respectively. Concurrent hepatitis C virus infection was associated with 39% increase in ANG2 levels (P=0.04).

Table 1. Demographic and Clinical Characteristics, Stratified by HIV-Infection Status

	All (n=132)	HIV uninfected (n=46)	HIV infected, untreated (n=39)	HIV infected, treated (n=47)	*P* value
Age, y	49 (41–55)	53 (45–55)	43 (32–54)	48 (43–57)	0.01
Sex (male)	87.9%	97.8%	74.4%	89.4%	0.01
Race and ethnicity					0.31
White	56.8%	43.5%	59.0%	68.1%	
Black	29.6%	41.3%	25.6%	21.3%	
Hispanic	9.1%	8.7%	10.3%	8.5%	
Other races/ethnicities*	4.6%	6.5%	5.1%	2.1%	
Comorbidities					
Coronary artery disease	2.3%	2.2%	0.0%	4.3%	0.42
Hypertension	31.8%	28.3%	38.5%	29.8%	0.56
Hyperlipidemia	50.0%	52.2%	41.0%	55.3%	0.39
Diabetes type 2	4.6%	2.2%	5.1%	6.4%	0.61
Chronic kidney disease	9.9%	2.2%	15.4%	12.8%	0.09
Tobacco dependence	35.6%	45.7%	41.0%	21.3%	0.04*
Hepatitis C virus	9.9%	2.2%	15.4%	12.8%	0.09*
Medication use					
Aspirin	15.9%	10.9%	10.3%	25.5%	0.08*
Antihypertensive	26.5%	21.7%	25.6%	31.9%	0.53
Statin	15.2%	13.0%	5.2%	25.5%	0.03
Body mass index, kg/m^2	24.9 (22.8–28.7)	26.4 (23.1–28.3)	24.1 (22.1–29.7)	24.6 (22.6–29.1)	0.91
Framingham Risk Score	5 (2–9)	9 (6–11)	4 (2–6)	4 (2–6)	0.11
Framingham Risk Score (using lipids)	0.08 (0.04–0.14)	0.10 (0.05–0.16)	0.06 (0.03–0.13)	0.08 (0.04–0.11)	0.10
Low-density lipoprotein, mg/dL	108 (88–128)	114.5 (96–132)	97.5 (71–129)	102 (88–125)	0.31
HIV-related factors					
Viral load, copies/mL	40 (40–3380)	…	5233 (573–28 400)	40 (40–40)	<0.0001
Duration of infection, y	9 (4–19)	…	6 (2–20)	10 (6–19)	0.10
CD4 nadir, cells/mm^3	305.5 (164–28)	…	350 (248–458)	253 (100–390)	0.02
Current CD4, cells/mm^3	597 (380–707)	…	517 (357–691)	632 (476–786)	0.14
Current CD8, cells/mm^3	869 (602–1234)	…	1128 (779–1431)	1028 (728–1028)	0.00
CD4/CD8 ratio	67.6 (39.4–96.4)	…	0.45 (0.32–0.73)	0.88 (0.57–1.38)	<0.0001
HCV coinfection	14.0%	…	15.4%	12.8%	0.76
Protease inhibitor, y	…	…	…	2.7 (0–8.9)	…
Nucleoside/nucleotide reverse transcriptase inhibitor, y	…	…	…	6.8 (3.1–10.7)	…
Nonnucleoside reverse transcriptase inhibitor, y	…	…	…	1.9 (0–4.7)	…
Antiretroviral duration, y	…	…	…	6.6 (2.8–10.1)	…
C-reactive protein, µg/mL	1.6 (0.7–3.3)	1.4 (0.7–3.3)	1.6 (0.8– 3.5)	1.4 (0.7–3.3)	0.94
Interleukin-6, pg/mL	2.2 (1.2–3.9)	1.9 (1.1–3.1)	2.4 (1.3–4.6)	2.6 (1.2–3.9)	0.44

Data are presented as median (IQR) or numbers (percent).
* Other races refers to individuals from all races/ethnicities that are not White, Black, or Hispanic.

Among untreated individuals infected with HIV, concurrent chronic kidney disease was associated with 82% lower ANG1 levels (P=0.02) and 625% higher ANG2/ANG1 ratio (P=0.02). For each 10 mg/dL rise in low-density lipoprotein cholesterol, ANG2/ANG1 ratio decreased by 14% (P=0.04). Finally, doubling of CD4/CD8 ratio was associated with 23% decrease in ANG2 levels (P<0.001).

Association Between HIV Infection and Markers Endothelial Dysfunction

The relationships between HIV status and markers of macrovascular dysfunction (FMD, BA diameter)

Table 2. Association Between HIV Infection Status and Levels of ANG1 and ANG2

	HIV negative	HIV infected, treated	HIV infected, untreated
	n=46	n=47	n=39
ANG1, pg/mL			
Median (IQR)	3755.9 (1566.7–9740.7)	1668.2 (799.3–3392.1)	2812.6 (1181.2– 8254.1)
Mean (SD)	6565.8 (6662.2)	3095.6 (3974.4)	5405.7 (6715.6)
ANG2, pg/mL			
Median (IQR)	1838.8 (1452.2–2349.7)	1853.5 (1318.3–2895.3)	2502.2 (2001.3–3200.2)
Mean (SD)	2433.2 (2410.1)	2692.8 (2757.4)	2955.2 (1802.8)
ANG2/ANG1			
Median (IQR)	0.52 (0.20–1.09)	0.97 (0.40–4.20)	0.99 (0.31–2.61)
Mean (SD)	1.50 (3.71)	2.89 (4.23)	1.89 (2.24)

ANG1 indicates angiopoietin 1; ANG2, angiopoietin 2; and IQR, interquartile range.

and microvascular dysfunction (HV) are summarized in Table 4. A comparison of differences in endothelial dysfunction measures by HIV status is summarized in Table 5. Measures of macrovascular dysfunction showed little difference between treated and untreated individuals infected with HIV and uninfected controls. These differences remained small after multivariable adjustment for traditional risk factors and levels of ANG1 and ANG2.

In contrast, measures of microvascular dysfunction were significantly different between the groups. When compared with uninfected controls, treated individuals infected with HIV had significantly impaired HV (demographic adjusted mean difference of –0.26 cm/s, P=0.006) despite adjustment for demographic and cardiovascular risk factors as well as levels of ANG1 and ANG2. This association was amplified in untreated individuals infected with HIV when compared with uninfected controls (–10.72 cm/s, P=0.05); however, after adjustment for ANG2, this difference was attenuated (–9.25 cm/s, P=0.10). Interestingly, when compared with untreated individuals infected with HIV, treated individuals infected with HIV had worse HV (–12.35 cm/s, P=0.05), but this significant difference was attenuated after adjustment for ANG1 (–10.42 cm/s, P=0.114). This association remained after adjustment for duration on antiretroviral treatment. We also performed an analysis examining the impact of duration of treatment with the following antiretroviral and antiretroviral classes: protease inhibitor, nonnucleoside reverse transcriptase inhibitor, nucleoside/nucleotide reverse transcriptase inhibitor, abacavir, tenofovir, and didanosine. Overall, we found modest positive correlation between duration on each antiretroviral and HV, with the exception of nonnucleoside reverse transcriptase inhibitors, which had a weakly negative correlation with HV. This suggests that longer duration of treatment with common antiretroviral regimens is associated with improved HV.

Associations Between ANG1 and ANG2 Levels With Markers of Endothelial Dysfunction

Finally, we studied the association of angiopoietin levels with macrovascular and microvascular endothelial dysfunction. In general, correlations were weak and patterns were inconsistent. For example, the correlation of ANG1 with BA was –0.13 (P=0.38) in treated HIV, –0.20 (P=0.23) in untreated HIV, and 0.15 (P=0.31) in the group uninfected with HIV. An exception was seen for ANG2 where higher levels were correlated with lower (worsened) HV regardless of HIV status (HIV uninfected: r=–0.20, P=0.18; treated HIV infected: r=–0.16, P=0.32; untreated HIV infected: r=–0.32, P=0.04), shown in Figure 1.

DISCUSSION

In this cross-sectional study of individuals who were HIV -infected and uninfected, we examined the association between HIV infection, angiopoietin levels, and measures of endothelial dysfunction.

Two key findings emerged from our study: first, individuals with treated HIV had significantly lower levels of ANG1 and similar levels of ANG2 compared with uninfected controls, whereas individuals with untreated HIV had similar levels of ANG1 and significantly higher levels of ANG2 compared with uninfected controls. The untreated group infected with HIV had significantly higher levels of both ANG1 and ANG2 compared with the treated group infected with HIV.

Second, although HIV status did not have any significant association with macrovascular endothelial dysfunction (measured by BA and FMD), both groups of individuals infected with HIV had significantly impaired microvascular endothelial function (measured by HV) compared with uninfected controls, with a more pronounced effect seen in the untreated group infected

Table 3. Differences in ANG1 and ANG2 Levels Among Groups Based on HIV and Treatment Status

	ANG1 (% difference)		ANG2 (% difference)		ANG2/ANG1 (% difference)	
	Demographic adjusted*	Multivariable adjusted†	Demographic adjusted	Multivariable adjusted	Demographic adjusted	Multivariable adjusted
HIV+ treated vs HIV−	−57.3%	−53.1%	9.9%	−8.9%	162.2%	84.8%
95% CI	−73.9% to −29.9%	−73.1% to −18.5%	−7.6% to 30.7%	−24.2% to 9.7%	46.2% to 370.3%	−6% to 263.2%
P value	0.0007	0.007	0.287	0.329	0.001	0.075
HIV+, untreated vs HIV−	−19.2%	−18.8%	44.7%	29.2%	71.2%	45.0%
95% CI	−56.3% to 49.2%	−56.1% to 50.1%	17.4% to 78.2%	5.3% to 58.5%	−12.5% to 234.7%	−25.1% to 180.6%
P value	0.495	0.506	0.0005	0.014	0.116	0.270
HIV+ treated vs untreated	−43.3%	−55.5%	−24.6%	−22.2%	45.9%	93.6%
95% CI	−67.5% to −1.1%	−75.3% to −20.0%	−37.5% to −9.2%	−37.5%, to −3.3%	−25.6% to 185.9%	−4.5% to 292.6%
P value	0.045	0.007	0.003	0.024	0.271	0.067
HIV+ treated vs untreated, adjusted for antiretroviral duration	−58.82%	−66.57%	−30.65	−26.19%	98.18%	185.89%
95% CI	−79.93% to −15.52%	−83.77% to −31.15%	−45.23% to −12.19%	−44.65% to −1.57%	−15.17% to 362.98%	17.53% to 595.45%
P value	0.016	0.003	0.002	0.039	0.114	0.021

ANG1 indicates angiopoietin 1; and ANG2, angiopoietin 2.

*Demographic adjusted model includes age, sex, and race.

†Multivariable adjusted model includes age, sex, and race, traditional risk factors (body mass index, current smoking, coronary artery disease, hypertension, systolic blood pressure, diastolic blood pressure, current smoker, chronic kidney disease, hyperlipidemia, hepatitis C virus, diabetes, low-density lipoprotein, and use of aspirin, anti-hypertensives, or statins).

Table 4. Association of HIV Status With Endothelial Dysfunction Measures (FMD, BA Diameter, HV)

	HIV-negative	HIV-infected, treated	HIV-infected, untreated
	n=46	n=47	n=39
FMD (%)			
Median (IQR)	4.35 (2.71–5.51)	3.6 (2.65–.09)	4.58 (2.53– 6.98)
Mean (SD)	4.34 (1.92)	4.28 (2.18)	4.77 (2.56)
BA diameter, unit			
Median (IQR)	4.38 (3.89–4.79)	4.34 (4.11–4.69)	4.34 (3.89–4.73)
Mean (SD)	4.36 (0.55)	4.44 (0.59)	4.30 (0.61)
HV, unit			
Median (IQR)	73.4 (64.8–87.8)	61.1 (48–72)	71.9 (51.3–83.9)
Mean (SD)	75.2 (23.7)	60.75 (23.27)	73.43 (64.75–87.75)

BA indicates brachial artery; FMD, flow-mediated dilation; HV, hyperemic velocity; and IQR, interquartile range.

with HIV. Compared with treated individuals infected with HIV, untreated individuals infected with HIV had improved microvascular endothelial dysfunction even after adjustment for demographic factors and duration on antiretroviral treatment, suggesting that additional factors in the setting of treated HIV may contribute to cardiovascular risk including chronic inflammation/immune activation, viral persistence, or side effects from specific antiretroviral therapy. Taken together, our findings point to a link between HIV infection and the development of endothelial dysfunction specifically through microvascular dysfunction, independent of the link between HIV infection and the Tie-2 tyrosine kinase system, as demonstrated by the schematic in Figure 2.

Both ANG1 and ANG2 have been implicated in the development of atherosclerosis. ANG1 binds to the Tie2 receptor and signals through the protein kinase B-Akt pathway to inhibit cell apoptosis, thus promoting endothelial survival.[20] In turn, Akt inactivates forkhead transcription factor FKHR-1, a potent inducer of ANG2.[20] ANG1-induced activation of Tie-2 also results in inhibition of the nuclear factor-kB pathway, thus inhibiting inflammatory responses.[20] Evidence from mouse models has shown that ANG1 induces arteriolar vasodilation by the release of nitric oxide, an important regulator of endothelial function.[21]

On the other hand, ANG2 antagonizes the effects of ANG1. ANG2 is released in response to cytokines including TNF-alpha (tumor necrosis factor-alpha) and VEGF (vascular endothelial growth factor), and environmental stressors. ANG2 binds to the same site as ANG1 triggering endothelial activation and increased permeability.[22] ANG2 antagonism at the Tie2 receptor also results in increased sensitization of the endothelium to inflammatory cytokines and modulates expression of endothelial cell adhesion molecules.[22] Overall, these studies suggest a proinflammatory and proatherosclerotic role for ANG2, although research in animal models has yielded conflicting results.

The strong association between HIV infection and angiopoietin disequilibrium is striking. Studies in other disease states with a predisposition for CVD display an imbalance between ANG1 and ANG2. For example, in subjects with chronic kidney disease, ANG1 levels were decreased and ANG2 increased when compared with controls, with ANG2 levels in particular correlating to CVD.[23,24] ANG2 levels have been found to be elevated in disease states such as hypertension,[25] systemic lupus erythematosus,[26] diabetes,[27] and sepsis.[28] Subjects with coronary artery disease, acute myocardial infarction, and unstable angina pectoris have been found to have elevated ANG2 levels compared with subjects with stable angina or to healthy controls.[29,30] Increases in ANG2 have also been shown to correlate with increasing severity of congestive heart failure.[31] These data are consistent with our study findings showing significantly higher ANG2 levels in individuals with untreated HIV as compared with both uninfected controls and individuals with treated HIV. Furthermore, higher levels of ANG2 were correlated with worse measures of microvascular dysfunction as demonstrated by HV in Figure 1. It is notable that these studies showed no difference in ANG1 levels between their subjects unlike what we have shown between subjects infected and uninfected with HIV.

On the other hand, few small studies targeting specific patient populations have demonstrated changes in ANG1 levels in the presence of HIV infection. Previously, Graham et al reported that chronic HIV infection results in decreased levels of ANG1 in a prospective cohort study of 102 antiretroviral therapy-naïve Kenyan women who were subsequently initiated on antiretroviral therapy.[32] However, this study was performed in a specific demographic with advanced HIV and prior opportunistic infections, with no uninfected control group, limiting the generalizability of its findings. Furthermore, the mechanism for this reduction in ANG1 expression was unclear. In a study of brain vasculature, direct measures of pericyte expression,

Table 5. Comparison of Differences in Endothelial Dysfunction Measures (FMD, BA Diameter, HV) by HIV Status

	HIV infected, treated vs HIV negative	HIV infected, untreated vs HIV negative	HIV infected, treated vs HIV infected, untreated	HIV infected treated vs untreated, adjusted for antiretroviral duration
	% difference	% difference	% difference	% difference
FMD (%)				
Demographic adjusted*	−0.54 (−1.6 to 0.51), *P*=0.313	0.03 (−1.14 to 1.2), *P*=0.959	−0.44 (−1.54 to 0.66), *P*=0.432	−0.45 (−1.95 to 1.05), *P*=0.558
Adjusted for ANG1	−0.34 (−1.41 to 0.74), *P*=0.537	0.09 (−0.99 to 1.16), *P*=0.875	−0.48 (−1.64 to 0.68), *P*=0.421	−0.50 (−2.09 to 1.10), *P*=0.541
Adjusted for ANG2	−0.51 (−1.55 to 0.53), *P*=0.337	0.18 (−0.98 to 1.34), *P*=0.762	−0.64 (−1.83 to 0.56), *P*=0.297	−0.57 (−2.19 to 1.05), *P*=0.489
Adjusted for ANG2/ANG1	−0.42 (−1.5 to 0.65), *P*=0.439	0.23 (−0.9 to 1.35), *P*=0.69	−0.39 (−1.60 to 0.81), *P*=0.522	−0.37 (−2.03 to 1.29), *P*=0.665
BA diameter, unit				
Demographic adjusted	0.17 (−0.11 to 0.45), *P*=0.231	0.05 (−0.26 to 0.37), *P*=0.737	0.13 (−0.14 to 0.39), *P*=0.363	0.41 (0.09 to 0.73), *P*=0.013
Adjusted for ANG1	0.22 (−0.04 to 0.49), *P*=0.098	0.06 (−0.27 to 0.38), *P*=0.728	0.11 (−0.17 to 0.40), *P*=0.432	0.39 (0.05 to 0.73), *P*=0.025
Adjusted for ANG2	0.15 (−0.14 to 0.44), *P*=0.321	0.08 (−0.24 to 0.4), *P*=0.637	0.10 (−0.18 to 0.39), *P*=0.463	0.40 (0.06 to 0.74), *P*=0.020
Adjusted for ANG2/ANG1	0.21 (−0.07 to 0.48), *P*=0.138	0.06 (−0.27 to 0.4), *P*=0.712	0.13 (−0.15 to 0.40), *P*=0.375	0.42 (0.08 to 0.75), *P*=0.016
HV, unit				
Demographic adjusted	−0.26 (−0.45 to −0.08), *P*=0.006	−10.72 (−21.59 to 0.15), *P*=0.053	−12.35 (−24.43 to −0.28), *P*=0.045	−19.91 (−35.69 to −4.13), *P*=0.013
Adjusted for ANG1	−0.26 (−0.45 to −0.07), *P*=0.009	−10.63 (−21.91 to 0.65), *P*=0.065	−10.42 (−23.33 to 2.50), *P*=0.114	−18.12 (−34.05 to −2.2), *P*=0.026
Adjusted for ANG2	−0.27 (−0.46 to −0.07), *P*=0.007	−9.25 (−20.29 to 1.8), *P*=0.101	−13.26 (−25.33 to −1.18), *P*=0.031	−21.07 (−36.09 to −6.04), *P*=0.006
Adjusted for ANG2/ANG1	−0.25 (−0.44 to −0.06), *P*=0.009	−10.79 (−21.73 to 0.15), *P*=0.053	−10.48 (−22.3 to 1.34), *P*=0.082	−17.96 (−33.4 to −2.51), *P*=0.023

ANG1 indicates angiopoietin 1; ANG2, angiopoietin 2; BA, brachial artery; FMD, flow-mediated dilation; HV, hyperemic velocity; and IQR, interquartile range.
*Demographic adjusted model includes age, sex, and race.

including ANG1, were found to be lower in the setting of HIV infection. Most recently, a retrospective study of 61 children on antiretroviral therapy showed that early and effective antiretroviral therapy was associated with higher levels of ANG1.[33] The discrepant findings regarding ANG1 in these prior studies and the current study may be because of differences in the clinical populations studied (ie, sex, age, ethnicity, traditional risk factors, and duration of HIV/antiretroviral therapy).

We found that HIV infection was associated with significant microvascular endothelial dysfunction compared with uninfected subjects; moreover, we found that individuals with treated HIV had significantly worse microvascular endothelial dysfunction compared with individuals with untreated HIV. Prior studies have found that both HIV infection and treatment with antiretrovirals are associated with endothelial dysfunction. HIV infection has been shown to be associated with elevated levels of inflammatory and coagulation markers, which may be due to increased monocyte activation and foam cell formation which in turn promote endothelial dysfunction.[34] A study by Leucker et al showed that PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibition improved endothelial dysfunction in individuals with HIV, potentially by reversing nitric oxide-mediated effects.[35] Similarly, antiretroviral regimens have been linked with dyslipidemia and increased endothelial adhesion molecule expression, promoting accelerated development of atherosclerosis. A study by Khawaja et al demonstrated increased

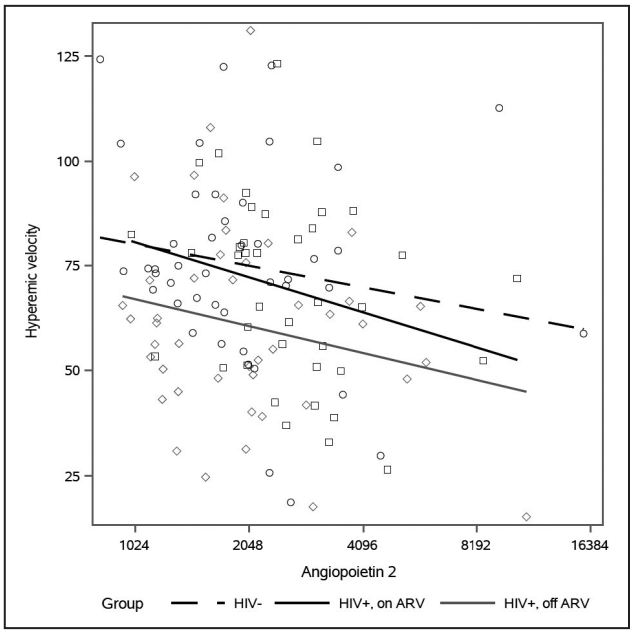

Figure 1. Association between angiopoietin 2 levels with hyperemic velocity.
Scatter plot of data from which the line of best fit is created. Circles are HIV negative; diamonds are HIV positive, off ARV; squares are HIV positive, on ARV. antiretroviral indicates antiretroval.

ICAM-1 (intercellular adhesion molecule-1) expression in individuals treated with abacavir was associated with increased cellular interaction and resulting endothelial dysfunction.[36]

However, there is conflicting evidence around the association between HIV infection and measures of macrovascular and microvascular dysfunction. A study of 38 antiretroviral-naïve individuals infected with HIV by Oliviero et al found that worsened viral control was associated with impaired FMD, with a dose-response relationship between FMD and HIV RNA copies.[37] On the other hand, a study by Stein et al with 331 antiretroviral-naïve individuals infected with HIV found that HIV infection did not have a significant impact on FMD impairment. Rather, FMD was more closely associated with traditional cardiovascular risk factors.[38] A study by our research group showed that among individuals infected with HIV treated with antiretrovirals, nadir CD4 count <350 cells/μL, which is a marker of advanced immunodeficiency, was significantly associated with impaired FMD.[39] Finally, a study by Dube et al found that HIV infection did not correlate significantly with FMD levels.[40] A more recent study by Dysangco et al examined the association of HIV infection on both macrovascular and microvascular parameters in individuals infected with HIV on antiretrovirals and found no significant association between HIV status and FMD or HV.[41]

Although we observed that HIV-mediated endothelial dysfunction is independent of the angiopoietin pathway, this disequilibrium may still play a significant role in atherogenesis, as measured by other markers of endothelial activity. Notably, an increase in CD4/CD8 ratio was associated with decreasing ANG1 levels; a prior study similarly showed that lower CD4/CD8 ratio is associated with increased CD8 T-cell activation and increased risk of non-AIDS morbidity and mortality in individuals infected with HIV, suggesting that this ratio may be an important predictor of outcomes in the HIV population.[42] Further studies should focus on elucidating the mechanism underlying angiopoietin disequilibrium and CVD. Understanding the mechanism underlying endothelial dysfunction in the setting of HIV infection can also guide novel therapeutic approaches to mitigate cardiovascular complications such as through anti-inflammatory agents and modulation of the Tie-2 tyrosine kinase receptor system.

Among HIV-infected subjects, initiation of antiretrovirals has been shown to result in increased levels of ANG1 and decreased levels of ANG2. In our study, despite effective viral suppression on antiretroviral, ANG1 levels remained depressed compared with uninfected controls; duration of antiretroviral therapy did not have an effect on this association. This suggests that HIV

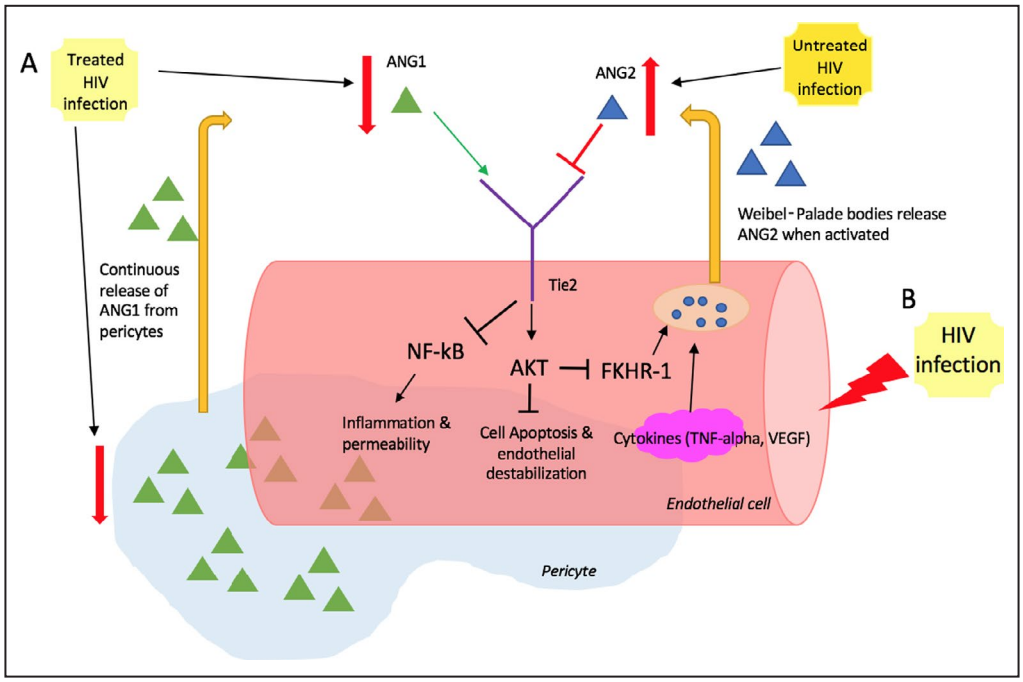

Figure 2. HIV infection affects production of angiopoietin 1 and 2, altering endothelial stability, and also directly impairs microvascular function independent of the Tie2 pathway.
A, Treated HIV infection decreases production of angiopoietin1, whereas untreated HIV infection increases production of angiopoietin2. Angiopoietin1 binds to and activates the Tie2 receptor, signaling to inhibit cell apoptosis via protein kinase B-AKT pathway. AKT inactivates forkhead transcription factor FKHR-1, which induces angiopoietin 2. Angiopoietin 1 activation of Tie2 also inhibits the nuclear factor-kB pathway, thus inhibiting inflammation and endothelial permeability. Overall, angiopoietin1 stabilizes the endothelium. On the other hand, angiopoietin2 antagonizes the effects of angiopoietin1. It is released from the endothelial cell Weibel-Palade bodies in response to cytokines and environmental stressors. Overall, angiopoietin2 destabilizes the endothelium triggering endothelial activation and permeability. **B**, Our study results suggest that HIV infection—particularly treated HIV infection—also directly impairs microvascular function independent of the Tie2 pathway. ANG indicates angiopoietin; NF-kB, nuclear factor kappa B; TNF, tumor necrosis factor; and VEGF, vascular endothelial growth factor.

infection itself may result in an altered state of vascular homeostasis mediated by the Tie2 receptor system and may play a key role in mediating the increased atherosclerosis observed among subjects infected with HIV regardless of treatment status.

Limitations

We acknowledge certain key limitations of our study. The first set of limitations relates to the study design. As a cross-sectional study, our study is limited in its ability to establish a temporal relationship between HIV infection, angiopoietin levels, and measures of endothelial dysfunction. As measurements were taken at a single time point, changes in levels over time as a result of HIV infection cannot be observed. Second, the observational nature of the study limits its ability to directly show a causal relationship between HIV infection and measures of endothelial dysfunction. Third, the sample size of this study is relatively small, therefore limiting the generalizability of results. Additionally, FMD, BA diameter, and HV are surrogate measures of macrovascular and microvascular function, respectively, and therefore may be less informative than direct measures of vascular function; however, direct measurement of peripheral or coronary vascular function is likely not feasible given increased risk from unnecessary invasive procedures. Finally, this is a single-center study, which may limit its generalizability to other settings.

CONCLUSIONS

Our findings suggest that HIV infection alters the balance between ANG1 and ANG2, disturbing endothelial homeostasis through disruption of vascular homeostasis; moreover, treatment of HIV appears to alter the balance between ANG1 and ANG2 levels, with individuals with treated HIV having decreased ANG1 and similar ANG2 levels, and individuals with untreated HIV having similar ANG1 levels and increased ANG2 levels.

HIV infection also promotes endothelial dysfunction specifically via impairing microvascular function, independent of the Tie-2 receptor system. The finding of worse microvascular dysfunction in individuals with treated HIV infection as compared with untreated HIV infection may reflect prolonged viral persistence and accelerated pathogenesis of atherosclerosis from certain antiretroviral regimens.

Future studies should further investigate the downstream implications of HIV-mediated angiopoietin disequilibrium. It is well known that individuals infected with HIV have higher risk of CVD—particularly, atherosclerosis and myocardial infarction. The imbalance between ANG1 and ANG2 may result in derangements in endothelial quiescence and stability, which are essential to the prevention of atherosclerosis. Gaining further understanding of the relationship between HIV infection, HIV treatment, and the mechanisms behind endothelial stability and quiescence will be essential in mitigating CVD risk in the population infected with HIV.

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Supplementary Material

Tables S1–S3

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SUPPLEMENTAL MATERIAL

Table S1. Estimated Association between clinical risk factors and levels of ANG1 and ANG2 among HIV-negative individuals in the cohort

(coefficient: percentage difference)

	HIV-negative (n=46)		
	Ang1	***Ang2***	***Ang2/Ang1***
BMI	*-0.2% (-8.4%, 8.9%), p= 0.971*	*0.5% (-3.1%, 4.2%), p= 0.79*	*1.6% (-6.9%, 10.9%), p= 0.718*
Current smoker	*87.8% (-7%, 279%), p= 0.079*	*25.6% (-1.7%, 60.5%), p= 0.069*	*-35% (-70.5%, 43.1%), p= 0.285*
Coronary Artery Disease	*-0.7% (-89.1%, 808.1%), p= 0.995*	*95.9% (-22.7%, 396.6%), p= 0.157*	*70.9% (-84.9%, 1838.8%), p= 0.665*
Hypertension	*-55.8% (-79.3%, -5.9%), p= 0.034*	*28% (-7.1%, 76.2%), p= 0.131*	*176.2% (23.6%, 516.9%), p= 0.013*
SBP	*1.7% (-1.5%, 5.1%), p= 0.302*	*0.4% (-1%, 1.9%), p= 0.539*	*-1.1% (-4.4%, 2.3%), p= 0.512*
DBP	*-0.9% (-6%, 4.4%), p= 0.725*	*-1.1% (-3.4%, 1.2%), p= 0.358*	*-0.5% (-5.8%, 5.2%), p= 0.866*
Hyperlipidemia	*-4% (-52.1%, 92.3%), p= 0.909*	*-21.3% (-40.1%, 3.4%), p= 0.085*	*-7.2% (-56.4%, 97.4%), p= 0.846*
Aspirin Use	*26.9% (-57.6%, 279.7%), p= 0.67*	*53.8% (-2.3%, 142.2%), p= 0.063*	*34.8% (-59.7%, 350.7%), p= 0.628*
Anti-hypertensive Use	*-34% (-71.2%, 51.1%), p= 0.325*	*49.6% (3.1%, 117.2%), p= 0.034*	*106.4% (-17.6%, 416.7%), p= 0.122*
Statin	*53.4% (-53%, 400.7%), p= 0.479*	*-5.8% (-40.9%, 50.1%), p= 0.8*	*-37.3% (-79.4%, 91%), p= 0.411*
HCV infection status	*-0.7% (-89.1%, 808.1%), p= 0.995*	*95.9% (-22.7%, 396.6%), p= 0.157*	*70.9% (-84.9%, 1838.8%), p= 0.665*
CKD	*-1.8% (-93%, 1280.8%), p= 0.989*	*-30.9% (-78.5%, 122.1%), p= 0.535*	*-29.6% (-96.2%, 1202.3%), p= 0.814*
DM	*27.1% (-86.3%, 1074.9%), p= 0.833*	*12.3% (-58.6%, 204.4%), p= 0.82*	*-10.4% (-92.3%, 945.6%), p= 0.93*
LDL (per 10)	*1% (-10.1%, 13.4%), p= 0.87*	*-6.9% (-10.8%, -2.8%), p= 0.001*	*-6.4% (-17.5%, 6.1%), p= 0.302*
Viral Load (per doubling)	*NA*	*NA*	*NA*
Duration of infection	*NA*	*NA*	*NA*
CD4 Nadir (per 100)	*NA*	*NA*	*NA*
Current CD4 (per 100)	*NA*	*NA*	*NA*
Current CD8 (per 100)	*NA*	*NA*	*NA*
CD4/CD8 Ratio (per doubling)	*NA*	*NA*	*NA*

Table S2. Estimated Association between clinical risk factors and levels of ANG1 and ANG2 among treated HIV-infected individuals

(coefficient: percentage difference)

	HIV-infected, treated (n=47)		
	Ang1	***Ang2***	***Ang2/Ang1***
BMI	*-2.9% (-10%, 4.6%), p= 0.437*	*-0.6% (-3.2%, 2.1%), p= 0.661*	*1.4% (-7%, 10.7%), p= 0.746*
Current smoker	*-37.4% (-72.2%, 41.1%), p= 0.259*	*13.6% (-15.8%, 53.3%), p= 0.403*	*100.9% (-22%, 417.3%), p= 0.148*
Coronary Artery Disease	*147.4% (-46.2%, 1036.8%), p= 0.244*	*63.4% (-6.5%, 185.6%), p= 0.085*	*-38.2% (-90.4%, 298.2%), p= 0.613*
Hypertension	*-13.1% (-58.9%, 83.6%), p= 0.713*	*8.1% (-18.8%, 43.8%), p= 0.595*	*35.4% (-45.3%, 235.2%), p= 0.512*
SBP	*-1.9% (-4.9%, 1.2%), p= 0.22*	*-0.9% (-2%, 0.3%), p= 0.133*	*0.2% (-3.5%, 4.1%), p= 0.903*
DBP	*2.5% (-1.7%, 6.8%), p= 0.252*	*1% (-0.5%, 2.6%), p= 0.183*	*-0.3% (-5.2%, 4.8%), p= 0.904*
Hyperlipidemia	*26.9% (-38.2%, 160.3%), p= 0.516*	*-13.8% (-32.5%, 10.1%), p= 0.234*	*-32.4% (-70.8%, 56.1%), p= 0.359*
Aspirin Use	*-47.8% (-77.4%, 20.6%), p= 0.128*	*-2.9% (-27.4%, 29.9%), p= 0.845*	*78.3% (-33.2%, 375.9%), p= 0.248*
Anti-hypertensive Use	*-9.6% (-56.5%, 87.9%), p= 0.786*	*18.5% (-11.1%, 57.9%), p= 0.247*	*73.1% (-27.6%, 313.9%), p= 0.217*
Statin	*98% (-1.9%, 299.8%), p= 0.057*	*-5.2% (-28.8%, 26.1%), p= 0.713*	*-56.8% (-81.4%, 0.2%), p= 0.051*
HCV infection status	*16.8% (-55.1%, 203.8%), p= 0.749*	*38.5% (1.2%, 89.7%), p= 0.042*	*38.5% (-53.4%, 311.9%), p= 0.558*
CKD	*33.8% (-54.8%, 296.3%), p= 0.599*	*7.7% (-26.6%, 57.9%), p= 0.706*	*6.1% (-69.1%, 264.3%), p= 0.926*
DM	*-40.6% (-84%, 120.3%), p= 0.436*	*-25.2% (-52.7%, 18.4%), p= 0.216*	*12.2% (-75.7%, 417.7%), p= 0.882*
LDL (per 10)	*1.9% (-8%, 12.9%), p= 0.72*	*-2.5% (-5.9%, 1.1%), p= 0.172*	*-4.5% (-16%, 8.5%), p= 0.477*
Viral Load (per doubling)	*33.1% (-75.7%, 628.8%), p= 0.742*	*22.1% (-34.2%, 126.6%), p= 0.527*	*-17.5% (-89.4%, 544.6%), p= 0.854*
Duration of infection	*4.6% (-0.2%, 9.7%), p= 0.059*	*1.2% (-0.6%, 3%), p= 0.179*	*-2.3% (-7.9%, 3.6%), p= 0.438*
CD4 Nadir (per 100)	*-16.7% (-30.9%, 0.3%), p= 0.053*	*1.8% (-5%, 9%), p= 0.621*	*24.6% (0.4%, 54.6%), p= 0.046*
Current CD4 (per 100)	*-6.5% (-19.2%, 8.3%), p= 0.372*	*-0.2% (-5.4%, 5.2%), p= 0.936*	*10.1% (-7.9%, 31.6%), p= 0.292*
Current CD8 (per 100)	*11.1% (3.7%, 19%), p= 0.003*	*2.5% (-0.2%, 5.3%), p= 0.065*	*-6.9% (-14.4%, 1.2%), p= 0.094*
CD4/CD8 Ratio (per doubling)	*-39.1% (-55.8%, -16%), p= 0.002*	*-7.5% (-18.8%, 5.5%), p= 0.248*	*48.1% (-6.5%, 134.6%), p= 0.094*

Table S3. Estimated Association between clinical risk factors and levels of ANG1 and ANG2 among untreated HIV-infected individuals.

(coefficient: percentage difference)

	HIV-infected, untreated (n=39)		
	Ang1	***Ang2***	***Ang2/Ang1***
BMI	*5% (-3.4%, 14.2%), p= 0.253*	*-0.4% (-3.2%, 2.5%), p= 0.785*	*-5.8% (-14.5%, 3.7%), p= 0.224*
Current smoker	*43.9% (-37.2%, 230.1%), p= 0.39*	*20.1% (-8.3%, 57.3%), p= 0.183*	*-20% (-68.5%, 103.4%), p= 0.64*
Coronary Artery Disease	*N/A*	*N/A*	*N/A*
Hypertension	*-34.2% (-75.1%, 73.5%), p= 0.397*	*0.8% (-26.7%, 38.5%), p= 0.962*	*60.7% (-45.9%, 377.1%), p= 0.393*
SBP	*-1.2% (-4%, 1.7%), p= 0.413*	*0% (-1%, 1.1%), p= 0.952*	*1.3% (-2%, 4.7%), p= 0.434*
DBP	*0.6% (-4.4%, 5.8%), p= 0.83*	*0.3% (-1.6%, 2.2%), p= 0.768*	*-0.6% (-6.2%, 5.4%), p= 0.845*
Hyperlipidemia	*119% (-3.3%, 395.9%), p= 0.06*	*-7.2% (-30.4%, 23.9%), p= 0.614*	*-62.8% (-85.7%, -3.3%), p= 0.042*
Aspirin Use	*-40.6% (-84.6%, 129.8%), p= 0.451*	*-33.8% (-58.5%, 5.5%), p= 0.083*	*5.6% (-77.3%, 392.3%), p= 0.945*
Anti-hypertensive Use	*-9.9% (-70.1%, 171.5%), p= 0.854*	*-3.6% (-32.5%, 37.7%), p= 0.84*	*-14.2% (-74.6%, 190%), p= 0.805*
Statin	*318.1% (-37.9%, 2715.3%), p= 0.141*	*-34% (-65.9%, 27.7%), p= 0.217*	*-84.7% (-98.2%, 27.3%), p= 0.082*
HCV infection status	*83.1% (-36.8%, 429.9%), p= 0.265*	*19.6% (-18.6%, 75.6%), p= 0.363*	*-29.6% (-79.3%, 140.1%), p= 0.575*
CKD	*-81.6% (-95.5%, -23.9%), p= 0.019*	*4% (-37.7%, 73.6%), p= 0.88*	*625.1% (37.3%, 3729.5%), p= 0.02*
DM	*254% (-48.3%, 2326.4%), p= 0.198*	*3.8% (-47.3%, 104.2%), p= 0.915*	*-70.9% (-96.8%, 167.7%), p= 0.276*
LDL (per 10)	*11.1% (-1.2%, 24.8%), p= 0.078*	*-3.9% (-7.9%, 0.3%), p= 0.069*	*-13.7% (-24.8%, -0.9%), p= 0.036*
Viral Load (per doubling)	*-0.3% (-13.7%, 15.3%), p= 0.972*	*4.3% (-1.1%, 10%), p= 0.121*	*9.9% (-5.8%, 28.1%), p= 0.229*
Duration of infection	*1.4% (-5.8%, 9.1%), p= 0.715*	*1.4% (-0.7%, 3.6%), p= 0.192*	*-0.5% (-7.8%, 7.4%), p= 0.902*
CD4 Nadir (per 100)	*5.9% (-18.6%, 37.9%), p= 0.668*	*-7% (-14.7%, 1.3%), p= 0.096*	*-14% (-35%, 13.8%), p= 0.291*
Current CD4 (per 100)	*-2.2% (-17.7%, 16.2%), p= 0.800*	*-5.3% (-10.7%, 0.4%), p= 0.067*	*-3.7% (-19.4%, 15.1%), p= 0.68*
Current CD8 (per 100)	*-2.5% (-9%, 4.4%), p= 0.469*	*1.1% (-1.2%, 3.6%), p= 0.349*	*3.8% (-3.9%, 12.1%), p= 0.347*
CD4/CD8 Ratio (per doubling)	*8.8% (-35.7%, 84.2%), p= 0.754*	*-22.7% (-32.1%, -12%), p<.001*	*-29.3% (-59.5%, 23.5%), p= 0.223*