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Plasma interleukin-6 (IL-6), angiopoietin-2, and C-reactive protein levels predict subsequent type 1 myocardial infarction in persons with treated HIV infection

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Abstract

Background: HIV infection leads to endothelial activation, promoting platelet adhesion and accelerating atherosclerosis. Our goal was to determine whether biomarkers of endothelial

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Contributions: SMG, HMC, JAL, and WCL designed the study, with input from PWH, and SMG and JAL acquired funding. JNM and HMC oversaw CNICS data collection, and SRH, MJB, and HMC adjudicated cases. RNM and JMJ oversaw clinical data collection at the participating sites. RMN assisted with identification of eligible cases and controls, and SMG and RMN analyzed the data. Laboratory testing was overseen by JC, MMW and JAL. SMG wrote the initial draft of the manuscript. All authors contributed to the final draft and approved the manuscript for submission.

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activation and hemostasis/thrombosis were elevated in people with treated HIV (PWH) before myocardial infarction (MI).

Methods: In a case-control study nested within the CFAR Network of Integrated Clinical Systems (CNICS) cohort, we compared 69 adjudicated cases with type 1 MI to 138 controls matched for ART regimen. We measured angiopoietin-1, angiopoietin-2 (ANG-2), ICAM-1, VCAM-1, ADAMTS13, von Willebrand factor, C-reactive protein (CRP), interleukin-6 (IL-6), plasminogen activation inhibitor-1, P-selectin, serum amyloid-A, soluble CD14, and apolipoprotein A1 in stored plasma. Conditional logistic regression identified associations with subsequent MI, with and without adjustment for Atherosclerotic Cardiovascular Disease (ASCVD) and Veterans Aging Cohort Study (VACS) scores.

Results: Higher IL-6 was associated with MI after adjustment for ASCVD score (adjusted odds ratio [AOR] 1.51, 95% CI, 1.05–2.17 per standard-deviation-scaled $\log_2$ increment). In a separate model adjusting for VACS score, higher ANG-2 (AOR 1.49, 95% CI 1.04–2.14), higher CRP (AOR 1.45, 95% CI 1.06–2.00), and higher IL-6 (AOR 1.68, 95% CI 1.17–2.41) were associated with MI. In a sensitivity analysis excluding PWH with viral load ≥400 copies/mL, higher IL-6 remained associated with MI after adjustment for ASCVD score and after adjustment for VACS score.

Conclusions: Among PWH, higher levels of plasma IL-6, CRP, and ANG-2 predict subsequent type 1 MI, independent of conventional risk scores. IL-6 had the most consistent associations with type 1 MI, regardless of viral load suppression.

Keywords

HIV infection; angiopoietin-2; C-reactive protein; interleukin-6; myocardial infarction

INTRODUCTION

While antiretroviral therapy (ART) has increased survival and greatly reduced the risk of opportunistic infections among persons living with HIV infection (PWH), evidence has accumulated that PWH are at increased risk for cardiovascular disease (CVD), including myocardial infarction (MI).[1–3] The risk for acute MI among PWH is estimated to be 50% higher than among their uninfected counterparts, after accounting for demographic characteristics, clinical confounders, and lifestyle or behavioral factors.[1,2,4] Drivers of this increased risk appear to include residual HIV viremia, immune dysregulation, inflammation, and altered hemostatic pathways.[4–6] In order to identify PWH at greatest risk and prevent HIV-associated MI events, a better understanding of the mechanistic pathways and pathogenesis of HIV-associated MI risk is urgently needed.

Endothelial activation may be a critical link between immune activation, inflammation, hemostasis/thrombosis, and CVD in HIV infection.[7] Soon after HIV-1 acquisition, soluble forms of intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1), and E-selectin, and the ratio of ANG-2 (an endothelial activation biomarker) to ANG-1 (a biomarker of a quiescent endothelium) increases.[8] These biomarkers do not return to normal levels after effective ART.[9] Notably, sICAM-1 and sVCAM-1 have been implicated as biomarkers of symptomatic atherosclerotic plaque,[10,11] and ANG-2 has

been shown to predict incident MI independent of traditional risk factors in populations without HIV infection.[12] A persistent state of endothelial activation could be an important mechanism of increased CVD risk in PWH.

In the setting of endothelial activation, von Willebrand factor (VWF) is released and attaches to the endothelium's luminal surface, enabling platelets to adhere to the vessel wall.[13] Plasma VWF antigen levels increase in acute HIV, correlate with plasma viral load, and are associated with HIV disease progression and death.[14–17] Moreover, elevated VWF antigen levels have been associated with ischemic stroke among PWH receiving ART in a recent study.[18] It is unclear whether elevated VWF antigen levels in PWH are associated with MI.

To address gaps in the knowledge base regarding MI pathogenesis in the context of HIV infection, the objective of this study was to determine whether levels of plasma biomarkers of endothelial quiescence (ANG-1) or activation (ANG-2, sICAM-1, sVCAM-1) and of hemostasis/thrombosis (VWF antigen, ADAMTS13) predict acute MI among PWH receiving effective ART.

METHODS

Study population.

This study was conducted using data from the CFAR Network of Integrated Clinical Systems (CNICS), a national network of 8 HIV clinical sites that follows ~30,000 PWH enrolled in standard care.[19] Patients are eligible for CNICS enrollment from the date they initiate HIV care at the participating site. Because CNICS data reflect standard clinical practice, they are less subject to volunteer and non-response biases than data from interval or other types of research cohorts.[19] Standards for data terminology, format, verification, and quality assurance are established by a CNICS Data Management Core, and each site has its own human subjects approval.[19]

Study design.

We evaluated associations of the biomarkers studied with subsequent MI using a nested case-control design, with time since enrolment as the sampling frame. Cases were participants from any of the 7 CNICS sites that had the necessary data who had experienced a type 1 MI ≥6 months after ART initiation, had attained viral suppression (i.e., <400 copies/mL) on ART prior to the type 1 MI, had not experienced a type 2 MI or stroke during available follow-up, and had stored plasma available following viral suppression and within 12 months before the MI event. Controls were participants from these same 7 sites who had not experienced a type 1 MI at the time of the case's event date, had attained viral suppression on ART, had not experienced a type 2 MI or stroke during available follow-up, and had stored plasma available following viral suppression and before the case's event date. In addition, to minimize confounding by different antiretroviral medications, controls were matched to cases by ART regimen prescribed on the date of blood collection. Two controls were identified for each case; in six instances, controls were used for more than one case. Participants were followed in CNICS between 1997 and 2015.

Primary outcome.

MI events were adjudicated using a state-of-the-art protocol described previously.[20] Briefly, for all potential events, sites assembled de-identified packets with electrocardiograms, physician notes, procedure reports, and cardiac biomarker results (e.g., troponin), which were uploaded to a central web-based platform for review by two physicians experienced in cardiovascular event review, followed by a 3rd reviewer if discrepancies occurred. Reviewers categorized each MI as type 1 (due to plaque rupture) or type 2 (due to oxygen supply-demand mismatch [e.g., due to sepsis] at the time of MI);[21] other MI types are rare among PWH.[22] This rigorous adjudication procedure minimizes false-positive events and increases sensitivity over using diagnosis codes alone.[20]

Comorbidity and health-related variables.

Variables from the CNICS data repository included demographic characteristics (sex, age, and race/ethnicity) and clinical data, including MI risk factors, vital signs, and laboratory results measured at the visit before the type 1 MI event for cases and at a similar point in follow-up for controls. Hypertension was defined as a documented diagnosis of hypertension and use of antihypertensive drugs.[23] Diabetes was defined as a hemoglobin A1c level >6.5% or use of a diabetes-specific medication such as insulin or a diabetes-related medication (e.g., biguanides) in the setting of a diabetes diagnosis.[24] The atherosclerotic coronary artery disease (ASCVD) score, which predicts 10-year risk for atherosclerotic CVD,[25] was calculated based on sex, age, race, systolic blood pressure, hypertension treatment status, diabetes status, tobacco use, total cholesterol, and high-density lipoproteins (HDL). The Veterans Aging Cohort Study (VACS) index, which predicts both all-cause and cardiovascular mortality in PWH,[26] was calculated based on sex, age, race, hemoglobin, platelet count, creatinine, aspartate aminotransferase, alanine aminotransferase, hepatitis C status, CD4 count, and plasma viral load.

Biomarker predictors.

After identification of stored plasma specimens meeting inclusion criteria, a 400 μL aliquot was shipped to Seattle on dry ice and stored at −80°C until testing. The Meso Scale Discovery (Rockville, MD, USA) immunoassay platform was used to measure concentrations of ANG-1, ANG-2, C-reactive protein (CRP), interleukin 6 (IL-6), plasminogen activation inhibitor 1, P-selectin, serum amyloid A (SAA), soluble CD14 (sCD14), sICAM-1, sVCAM-1, apolipoprotein A1, ADAMTS13, and VWF. Biomarkers of HIV disease status were also evaluated as predictors, including most recent CD4 count and viral load, and peak viral load and CD4 count nadir during included follow-up time. Non-HIV biomarkers were $\log_2$-transformed to normalize skewed data and enhance interpretability, as a $\log_2$ increase corresponds to doubling of concentration (and a $\log_2$ decrease to a 50% decrease in concentration). Plasma viral load was $\log_{10}$-transformed. Each biomarker was then standard-deviation scaled to reduce measurement error.

Data analysis.

Demographic and clinical data at time of sample collection were summarized using descriptive statistics. For most variables, data were complete; however, ASCVD scores

were missing for 15.5% of participants due to missing score components (details in Table 1). To preserve statistical power and minimize bias, missing data were imputed prior to regression analysis using multiple imputation by chained equations. Imputation included all Table 1 variables as well as CNICS site, alcohol use, marijuana use, illicit drug use, coronary artery disease, heart failure, warfarin use, statin use, dyslipidemia, body mass index, glomerular filtration rate, and triglycerides. After imputation, dot plots were created to visualize differences in distributions of biomarker levels between MI cases and matched controls.

Conditional logistic regression was performed to determine if individual biomarkers were independently associated with MI in unadjusted analyses and after adjustment for age, race/ethnicity and sex (Model 1). To investigate the predictive value of each biomarker above and beyond standard prediction rules (i.e., calculated ASCVD and VACS scores), we also controlled for these scores, in separate models due to overlap in score components (Model 2 for ASCVD score; Model 3 for VACS score). Results are expressed as the odds ratio for each standard deviation increase in each $\log_2$-transformed biomarker level, $\log_{10}$ increase in viral load or 100-cell/μL increase in CD4 count.

Because some participants had viremia on the sampling date despite attaining viral suppression after CNICS enrollment, we conducted a sensitivity analysis in which the study population was restricted to individuals with plasma viral load <400 copies/mL. The impact of adjustment for years on ART was also evaluated for each regression analysis. Finally, Pearson correlations were calculated to evaluate associations between ASCVD score, VACS score, and biomarkers that were significant at $p<0.20$ in unadjusted conditional regression analysis, separately within cases and within controls. A heat map was generated to show the strength and direction of correlations for each group, highlighting correlations significant at $p\leq0.01$ after Bonferroni correction. Stata version 14.2 (StataCorp, College Station, Texas, USA) was used, and P values <0.05 were considered significant.

RESULTS

Study population.

The study included 69 cases (i.e., individuals with an MI event) and 138 matched controls (representing 132 individuals without an MI event, as 6 individuals served as controls for more than one case). Table 1 presents demographic and clinical characteristics of cases and controls, indicating which variables are included in the ASCVD and VACS scores. In terms of ART regimen, 43.5% of participants were taking a protease inhibitor (PI)-based regimen, 23.2% a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen, and 2.9% an integrase strand transfer inhibitor (INSTI)-based regimen; 11.6% had no regimen prescribed at the time of sample collection. Overall, 24.6% of participants were taking an abacavir-containing regimen at this time point. Median time on ART was 8.5 years (interquartile range [IQR], 5.7 – 11.5 years) for cases and 6.9 years (IQR, 3.6 – 10.8 years) for controls. Overall, 79.2% of participants had a viral load <400 copies/mL at the time of sampling; 12 cases and 31 controls had viral loads over this threshold, ranging from 400 to 821,000 copies/mL (median, 16,406 copies/mL). In general, cases were older, male,

Caucasian, and had been on ART longer, with lower nadir CD4 counts, higher peak viral loads, and higher ASCVD and VACS scores than controls.

Regression analysis.

Table 2 presents the results of unadjusted and adjusted conditional logistic regression. Figure 1 presents dot plots of $\log_2$-transformed biomarker levels in cases and controls that differed at p <0.20 in unadjusted analysis. In the model adjusted for age, race/ethnicity, and sex (Model 1), higher ANG-2 (adjusted odds ratio [AOR] 1.47, 95% confidence interval [CI], 1.03–2.10 per $\log_2$ increment) and higher CRP (AOR 1.49, 95% CI 1.04−2.14) were both associated with increased odds of MI. In the model adjusted for ASCVD score (Model 2), higher IL-6 (adjusted odds ratio [AOR] 1.51, 95% confidence interval [CI], 1.05–2.17 per $\log_2$ increment) was associated with MI. After adjustment for VACS score (Model 3), higher ANG-2 (AOR 1.49, 95% CI 1.04–2.14), higher CRP (AOR 1.45, 95% CI 1.06–2.00), and higher IL-6 (AOR 1.68, 95% CI 1.17–2.41) were all associated with MI. Estimated odds ratios for IL-6, ANG-2, and CRP were broadly consistent across models, and did not change after adjustment for years on ART.

Sensitivity analysis.

Table 3 presents the results of a sensitivity analysis restricted to the 55 cases and 99 matched controls with plasma viral load <400 copies/mL. In this sensitivity analysis, no biomarker was associated with MI in the model adjusted for age, race/ethnicity, and sex only (Model 1). In the model adjusted for ASCVD score (Model 2), higher IL-6 (AOR 1.73, 95% CI, 1.11–2.71 per $\log_2$ increment) was associated with MI. After adjustment for VACS score (Model 3), higher CRP (AOR 1.48, 95% CI 1.02–2.13) and higher IL-6 (AOR 1.97, 95% CI 1.25–3.10) were associated with MI. Higher peak viral load was also associated with an increased odds of MI (AOR 1.61, 95% CI 1.07–2.43) in Model 3. Results of the sensitivity analysis were also broadly consistent across models (although with lower power and large confidence intervals), and did not change after adjustment for years on ART.

Correlations.

Figure 2 presents a heat map of correlations between the standard-deviation-scaled $\log_{10}$-transformed biomarkers that were associated with MI at p<0.20 in unadjusted regression analyses and both ASCVD score and VACS score. CRP and IL-6 were positively correlated among both cases (0.598, p<0.0001) and controls (0.573, p<0.0001). In addition, ANG-2 and IL-6 had moderate positive correlations among both cases (r=0.471, p=0.0019) and controls (r=0.516, p<.0.0001). In contrast, ANG-2 and CRP were not significantly correlated among cases, and were only weakly correlated among controls (r=0.379, p=0.001). While there were no significant correlations between any of these three biomarkers and the ASCVD score, ANG-2 was positively correlated with the VACS score among cases (r=0.429, p=0.007) and controls (r=0.445, p<0.0001). CRP and IL-6 were correlated with VACS score among controls only (r=0.346, p=0.001 and r=0.428, p<0.0001, respectively).

DISCUSSION

In this case-control study of PWH who were followed at 7 CNICS sites in the United States, we found that among PWH, higher levels of plasma IL-6, CRP, and ANG-2 predict subsequent type 1 MI, independent of conventional risk scores. In the main analysis, all three biomarkers were associated with increased odds of MI after adjustment for the VACS score, a validated predictor of subsequent cardiovascular disease and mortality among PWH specifically. In sensitivity analyses, these associations were most robust for IL-6, an inflammatory biomarker that is correlated with both CRP and ANG-2. VWF levels were not associated with MI in any of the models.

High-sensitivity CRP, an acute phase protein that increases in response to inflammatory stimuli, is an established biomarker of CVD risk regardless of HIV status, with a level ≥2.0 mg/L (equivalent to 2.0 mcg/mL) indicating elevated risk.[27,28] IL-6 is a cytokine produced by activated T cells, macrophages, and adipocytes as part of the innate immune response. Engagement with receptors on monocytes, hepatocytes, and endothelial cells leads to leukocyte recruitment, the acute phase response, and endothelial activation.[29,30] The role of IL-6, CRP, and other inflammatory biomarkers, such as interleukin-1β and tumor necrosis factor (TNF), in the pathogenesis of CVD, independent of cholesterol levels and atherosclerosis, is not well understood.[30]

Angiopoietins have received less investigation in cardiovascular disease until recently, despite their important role in modulating the endothelial response to inflammatory stimuli;[30] however, new data are emerging about their role in MI in populations without HIV. For example, in a case-control study including 695 MI cases and 690 controls individually matched on age, gender and race/ethnicity, ANG-2 levels were higher in MI cases than controls, and this association remained after adjustment for sociodemographic and clinical factors, with a 1.63-fold (95% CI 1.09–2.45) increased odds of MI for those in the upper quartile relative to the lowest quartile of ANG-2 levels.[12] Moreover, in a prospective study of 138 patients hospitalized due to non-ST elevation MI (NSTEMI), ANG-2 levels were significantly higher in NSTEMI patients than in those without coronary artery disease.[31] Finally, after percutaneous coronary intervention for coronary artery disease, higher levels of ANG-2 have been associated with an increased risk of cardiovascular events (i.e., cardiac death, nonfatal MI, repeat revascularization, readmission for severe deterioration of angina or new onset heart failure) in the18 months following intervention.[32] The mechanism for these poor outcomes may relate to the role of ANG-2 in promoting abnormal vascular remodeling and polarizing macrophages towards a pro-inflammatory phenotype, exacerbating cardiac hypoxia and inflammation after MI.[33]

How does HIV-1 infection influence these biomarkers? The strong association of plasma viral load with HIV-1 RNA levels, suggests that HIV infection causes sustained innate immune activation resulting in increased levels of IL-6 and related biomarkers, despite viral suppression. IL-6 has been a consistent and strong predictor of adverse outcomes in PWH, including CVD events.[34] After an ART interruption, viral load rebounds, with an associated increase in IL-6 levels[35] and elevated MI risk.[36] Higher pre-event IL-6 levels have been associated with greater risk of fatal CVD and greater risk of death after nonfatal CVD events

among PWH.[37] In the present study, we found that IL-6 was the most consistent predictor across all models, supporting its importance as a biomarker of CVD risk among PWH. Of note, PWH have increased arterial inflammation on cardiac 18F-FDG-PET scan compared to controls with similar cardiac risk factors, and this inflammation is associated with soluble CD163, a biomarker of monocyte/macrophage activation that correlates with IL-6 levels.[38]

Relatively few studies of PWH have investigated ANG-2 as a CVD biomarker. In one cross-sectional study of children living with HIV on ART with suppressed viral loads, an "endothelial activation" index derived by factor analysis from ANG-2, soluble vascular endothelial growth factor-1, and soluble endoglin levels was associated with IL-6 levels.[39] In another cross-sectional study of adults, untreated PWH had higher ANG-2 levels than PWH on effective treatment, but ANG-2 was not related to flow-mediated vasodilation of the brachial artery as a measure of endothelial dysfunction.[40] In previous work, we have shown that the ANG-1:ANG-2 ratio increases after HIV-1 acquisition, and that ANG-1 levels decrease over time in untreated HIV,[8] while treatment with ART increases ANG-1 levels and decreases levels of soluble ICAM-1 and ANG-2.[9] In our study of participants taking ART, ANG-2 levels were also associated with higher plasma viral load,[9] which may explain our finding in the current study that ANG-2 was no longer associated with MI in our sensitivity analysis restricted to participants with viral load <400 copies/mL.

Viral suppression is clearly important to decrease the risk of type 1 MI among PWH. For example, in an analysis of data from the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) with comparison to data from the Atherosclerosis Risk in Communities (ARIC) cohort, there was a higher incidence of type 1 MI in NA-ACORD, and this risk among PWH was associated with lower CD4 count and detectable viral load.[41] This link with viral load may underlie a declining relative risk for MI among PWH compared to uninfected individuals in recent years,[42] as newer HIV medications have led to higher rates of viral suppression.[43] In the CNICS cohort in which the present study was conducted, higher viral load, renal dysfunction, and older age were predictors of mortality after a type 1 MI.[44] It is unclear from work to date how important viremia during hospitalization could be for outcomes after cardiac ischemia, but the use of recently developed long-acting injectable ART in patients unable to take their oral medications could be a potential adjunct to standard peri-MI care for PWH.

While inflammation plays a clear role in small vessel disease and drives microvascular endothelial dysfunction in both MI and ischemic stroke,[45] it is interesting that we found that ANG-2, but not VWF, was a biomarker associated with subsequent MI in this study. This is in contrast to results of our similar study in the CNICS cohort examining biomarkers of subsequent ischemic stroke, in which the converse was true: VWF, but not ANG-2 was associated with subsequent ischemic stroke.[18] While the pathogenesis of MI and that of ischemic stroke have some similarities, it is possible that endothelial activation and hemostasis/thrombosis play different roles in the two processes. Additional studies are needed to confirm our results.

While our findings indicate that higher levels of plasma IL-6, CRP, and ANG-2 may be independent predictors of subsequent type 1 MI among PWH, confirmation of these

findings in other cohorts is an important next step. In addition, further investigation into the incremental value of adding these plasma biomarkers to established prediction rules, such as the ASCVD and VACS scores, is needed. Many of the components of the ASCVD and VACS scores reflect physiologic processes (e.g., hypertension, lipid metabolism) that may be in the causal pathway from these elevated plasma biomarkers to atheroembolic MI events, complicating analysis. In addition, while it is currently unclear whether interventions to decrease the level of any of these biomarkers would effectively reduce cardiovascular risk, this is an area of active inquiry. Of note, ongoing work investigating the role of IL-6 signaling in cardiovascular disease suggests a causal role for IL-6 in systemic atherothrombosis and aneurysm formation.[46] In addition, a trial of the IL-6 inhibitor ziltivekimab demonstrated significant reductions in C-reactive protein and other biomarkers of inflammation and thrombosis (i.e., fibrinogen, serum amyloid A, haptoglobin, secretory phospholipase A2, and lipoprotein(a)), as well as the safety and tolerability of ziltivekimab.[47] While we could identify no trials of agents to reduce endothelial activation-related cardiovascular disease, a number of therapeutic agents are in development to prevent infection-related microvascular endothelial activation and dysfunction,[48] and these agents could potentially have therapeutic implications for the reduction of HIV-associated cardiovascular disease.

Our study has a number of strengths, including the use of a case-control study nested in a large cohort reflecting clinical practice, measurement of biomarkers in plasma collected prior to the MI event, matching of cases and controls by ART regimen at the time of plasma collection, and rigorous adjudication of type 1 MIs, which increases sensitivity over using diagnoses alone and allows MI types with common pathogenic features to be identified.[49,50] This is particularly important among PWH where approximately half of all MIs documented in US cohorts have been type 2 (due to causes such as sepsis or cocaine-induced vasospasm) rather than type 1 atheroembolic events.[22] However, several limitations must be acknowledged. First, although loss to follow-up is relatively uncommon (10%) in the CNICS cohort, attrition bias may be present.[19] Second, we had no data on some CVD risk factors, such as aspirin use or physical activity levels, and data for some variables collected were missing and had to be imputed. Despite these limitations, we were able to adjust for validated predictors of MI risk, including both the ASCVD score, commonly used in general population patients,[51] and the VACS score, used for PWH specifically.[26] Third, because participants in this study were followed between 1997 and 2015, before modern INSTI regimens were widely available, rates of viral suppression were relatively low by today's standards. Finally, our results may not be generalizable to PWH not meeting this study's inclusion criteria, especially PWH who are not on ART or have yet to attain viral suppression.

In conclusion, among persons living with treated HIV infection, higher levels of plasma IL-6, CRP, and ANG-2 predict subsequent type 1 MI, independent of conventional risk scores. Of these biomarkers, IL-6 had the most consistent associations with type 1 MI, regardless of viral load suppression, and ANG-2 was no longer significant when persons with viremia were excluded. Inflammation and endothelial activation may increase HIV-related cardiovascular risk, especially during transient viremia.

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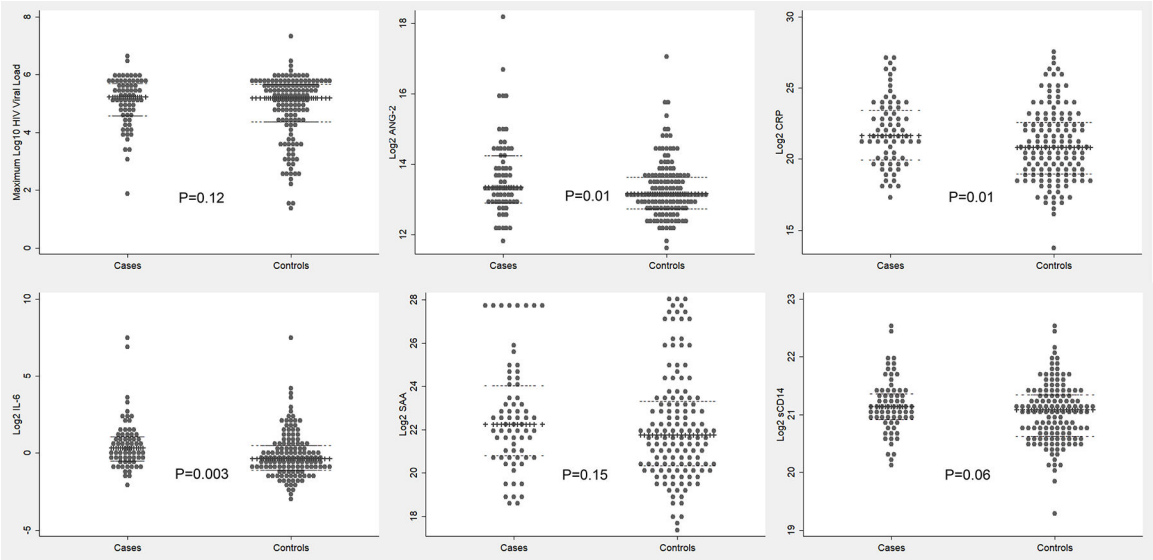

Figure 1. Dot plots of log_2-transformed biomarker levels in cases and controls.

Individual data points are graphed with grey circles, a horizontal line of black Xes indicates the median, and horizontal dashed lines indicate the upper and lower quartiles. Biomarkers presented are measures for which cases and controls differed in unadjusted conditional logistic regression analysis by $p<0.20$; p values are also presented.

ANG-2 = angiopoietin-2, CD4 = cluster of differentiation 4, CRP = C reactive protein, HIV = human immunodeficiency virus, IL-6 = interleukin-6, SAA = serum amyloid A, sCD14 = soluble CD14

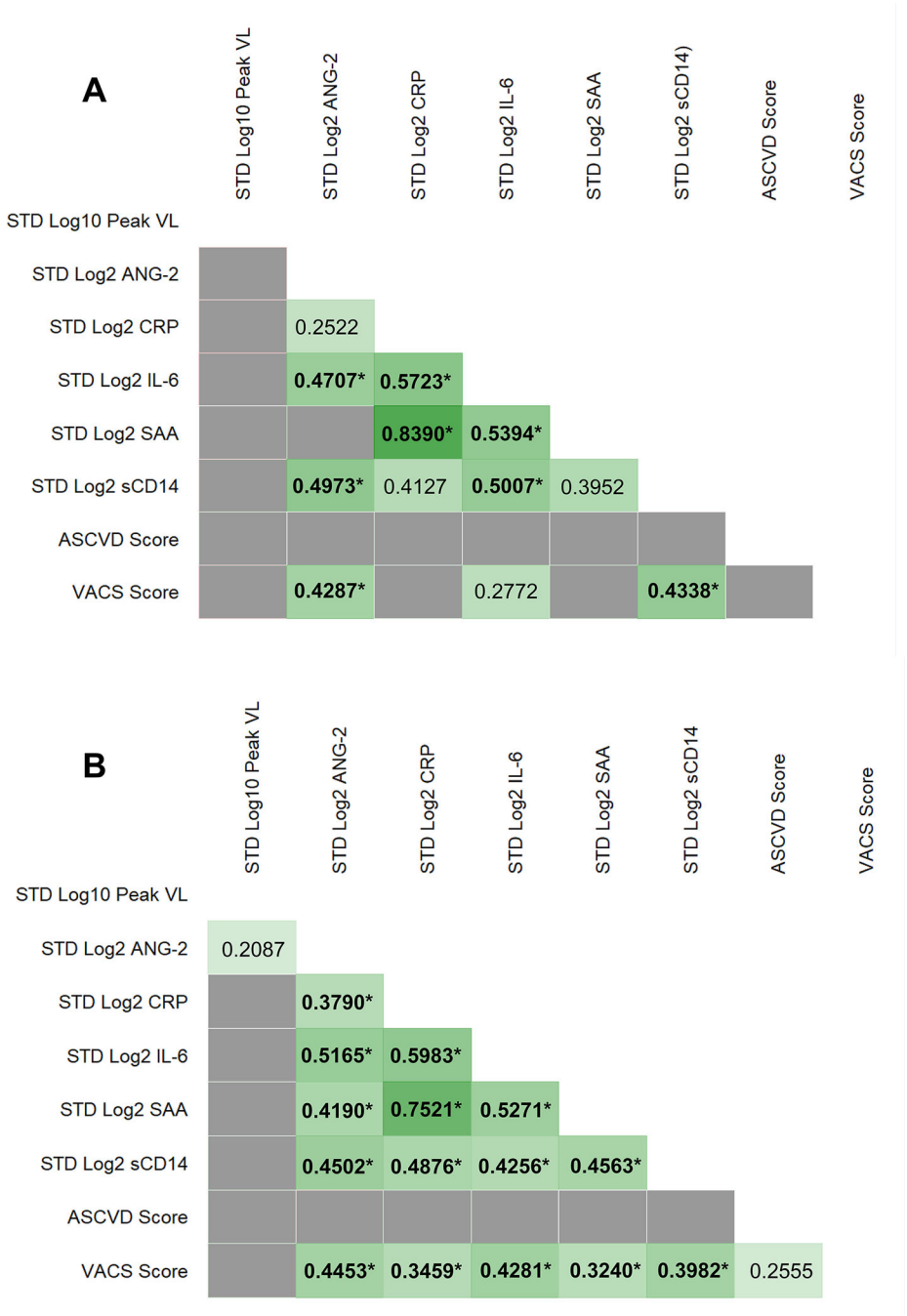

Figure 2. Heat map of correlations between standard-deviation-scaled log_{10}-transformed biomarkers, ASCVD score, and VACS score among cases (A) and controls (B).

Biomarkers included are measures for which cases and controls differed in unadjusted conditional logistic regression analysis by $p<0.20$. Positive correlations are in green, with a perfect positive correlation (i.e., a Pearson coefficient of +1) in dark green. Non-significant correlations are in grey. There were no negative correlations.

ANG-2 = angiopoietin-2, ASCVD = Atherosclerotic Cardiovascular Disease, CRP = C reactive protein, IL-6 = interleukin-6, SAA = serum amyloid A, sCD14 = soluble CD14, STD = standard-deviation scaled, VACS = Veterans Aging Cohort Study, VL = viral load

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Table 1.

Characteristics of cases and controls at the time of sample collection

Characteristic	N (%) or Median (IQR)	
	Myocardial Infarction Cases (n = 69)	Controls (n = 138)
Matching variable		
ART regimen type		
INSTI	2 (2.9)	4 (2.9)
INSTI/PI	1 (1.4)	2 (1.4)
NNRTI	16 (23.2)	32 (23.2)
NNRTI/PI	1 (1.4)	2 (1.4)
NRTI	3 (4.4)	6 (4.4)
PI	30 (43.5)	60 (43.5)
Quad (INSTI/NNRTI/NRTI/PI)	1 (1.4)	2 (1.4)
Triple (INSTI/NNRTI/PI)	7 (10.1)	14 (10.1)
None	8 (11.6)	16 (11.6)
Abacavir-containing regimen	17 (24.6)	34 (24.6)
Sociodemographic characteristics		
Male sex [a,b]	55 (79.7)	101 (73.2)
Age at specimen collection, years [a,b]	51 (46 – 57)	46 (40 – 52)
Race/ethnicity [a,b]		
White	36 (52.2)	40 (29.0)
Black	27 (39.1)	89 (64.5)
Hispanic	4 (5.8)	7 (5.1)
Other	2 (2.9)	2 (1.4)
Clinical characteristics		
Diabetes history [a]	18 (26.1)	17 (12.3)
Hypertension history [a]	37 (53.6)	35 (25.4)
Systolic blood pressure, mm Hg [a,c]	131 (116 –146)	123 (114 – 136)
Tobacco use [a]	32 (46.4)	36 (26.1)
Laboratory values		

Characteristic	N (%) or Median (IQR)	
	Myocardial Infarction Cases (n = 69)	Controls (n = 138)
Hemoglobin, g/dL [b,d]	13.7 (12.7 – 14.9)	13.8 (12.5 – 14.9)
Platelet count, /μL [b]	244,000 (189,900 – 295,000)	231,000 (187,000 – 277,000)
Creatinine, mg/dL [b]	1.0 (0.8 – 1.2)	0.9 (0.8 – 1.1)
Alanine aminotransaminase, IU/L [b,e]	23 (16 –39.5)	27 (21 – 39)
Aspartate aminotransferase, IU/L [b]	26 (20 – 38)	27.5 (22 – 39)
Total cholesterol, mg/dL [a,f]	184 (156 – 217)	180 (145 – 213)
High-density lipoprotein, mg/dL [a,g]	36.5 (31 – 45)	44.5 (35 – 54)
Hepatitis C infection status [b]	13 (18.8)	34 (24.6)
HIV-related characteristics		
Years on ART	8.5 (5.7 – 11.5)	6.9 (3.6 – 10.8)
Log_{10} HIV viral load, IU/mL [b]	1.4 (1.3 – 1.9)	1.4 (1.3 – 2.5)
Log_{10} peak HIV viral load, IU/mL [h]	5.2 (4.6 – 5.7)	5.2 (4.4 – 5.7)
CD4 count, cells/μL [b]	391 (262 – 730)	439 (254 – 719)
Nadir CD4 count, cells/μL [i]	114 (38 – 211)	118.5 (26 – 298)
Viral suppression (<400 copies/mL)	57 (82.6)	107 (77.5)
Composite risk scores		
ASCVD score [j]	0.10 (0.07 – 0.15)	0.05 (0.02 – 0.09)
VACS score [k]	28 (18 – 38)	22 (10 – 38)
Plasma biomarkers		
Angiopoietin-1, ng/mL [d]	4.6 (2.5–7.6)	4.8 (2.0–7.7)
Angiopoietin-2, ng/mL [l]	10.3 (7.8–19.7)	9.1 (6.9–13.0)
C-reactive protein, mcg/mL	3.3 (1.1–11.7)	1.8 (0.5–6.7)
Interleukin-6, pg/mL [m]	1.2 (0.7–2.2)	0.8 (0.5–1.5)
Plasma activation inhibitor-1, mcg/mL	0.6 (0.4–1.2)	0.7 (0.4–1.1)
P-selectin, ng/mL [n]	35.6 (27.7–51.0)	36.3 (24.6–53.1)
Soluble ICAM-1, mcg/mL	0.4 (0.3–0.5)	0.4 (0.3–0.5)

Characteristic	N (%) or Median (IQR)	
	Myocardial Infarction Cases (n = 69)	Controls (n = 138)
Soluble VCAM-1, mcg/mL	0.4 (0.3–0.6)	0.4 (0.3–0.5)
Serum amyloid A, mcg/mL	5.0 (1.9–17.7)	3.5 (1.4–10.8)
Soluble CD14, mcg/mL [n]	2.3 (2.0–2.7)	2.2 (1.6–2.7)
Apolipoprotein A1, mcg/mL	0.14 (0.10–0.21)	0.14 (0.10–0.18)
ADAMTS-13, ng/mL	0.15 (0.11–0.20)	0.15 (0.11–0.20)
Von Willebrand factor, ng/mL	19.5 (12.5–28.8)	19.3 (13.1–28.1)

[a] ASCVD score component

[b] VACS score component

[c] Missing for 3 cases and 10 controls

[d] Missing for 3 controls

[e] Missing for 1 case

[f] Missing for 3 cases and 15 controls

[g] Missing for 3 cases and 20 controls

[h] Peak viral load is the highest measure recorded during CNICS follow-up from enrolment through the sampling date.

[i] Nadir CD4 count is the lowest measure recorded during CNICS follow-up from enrolment through the sampling date.

[j] Missing for 6 cases and 26 controls

[k] Missing for 6 cases and 2 controls

[l] Missing for 1 control

[m] Missing for 3 cases and 6 controls

[n] Missing for 2 controls

ART = antiretroviral therapy, ASCVD = Atherosclerotic Cardiovascular Disease, CD4 = cluster of differentiation 4, HIV = human immunodeficiency virus, INSTI = integrase strand transfer inhibitor, IQR = interquartile range, IU = international units, NNRTI = non-nucleotide reverse transcriptase inhibitor, NRTI = nucleoside/nucleotide reverse transcriptase inhibitor, PI = protease inhibitor, VACS = Veterans Aging Cohort Study

Table 2.

Associations between plasma biomarkers and myocardial infarction using conditional logistic regression with the imputed dataset and all participants included (n=207)

Biomarker	OR (95% CI)	P value	Model 1: Adjusted for age, race/ethnicity, and sex AOR (95% CI)	P value	Model 2: Adjusted for ASCVD score AOR (95% CI)	P value	Model 3: Adjusted for VACS score AOR (95% CI)	P value
Log_{10} viral load	0.80 (0.52–1.25)	0.33	1.09 (0.66–1.80)	0.74	0.58 (0.32–1.05)	0.07	0.70 (0.43–1.12)	0.14
Log_{10} peak viral load	1.28 (0.94–1.76)	0.12	1.29 (0.89–1.87)	0.17	1.15 (0.81–1.63)	0.45	1.26 (0.91–1.73)	0.16
CD4 count, per 100 cells	0.98 (0.73–1.33)	0.92	0.98 (0.68–1.43)	0.92	1.11 (0.79–1.57)	0.54	1.12 (0.79–1.59)	0.54
Nadir CD4 count, per 100 cells	0.88 (0.64–1.21)	0.43	0.86 (0.59–1.24)	0.42	1.04 (0.71–1.53)	0.84	0.93 (0.66–1.29)	0.66
Angiopoietin-1 [a]	1.08 (0.80–1.46)	0.60	0.90 (0.62–1.30)	0.57	0.93 (0.65–1.34)	0.71	1.12 (0.83–1.53)	0.45
Angiopoietin-2 [a]	**1.49 (1.08–2.06)**	**0.01**	**1.47 (1.03–2.10)**	**0.04**	1.41 (0.99–2.01)	0.06	**1.49 (1.04–2.14)**	**0.03**
C-reactive protein [a]	**1.48 (1.08–2.03)**	**0.01**	**1.49 (1.04–2.14)**	**0.03**	1.29 (0.90–1.84)	0.16	**1.45 (1.06–2.00)**	**0.02**
Interleukin-6 [a]	**1.70 (1.20–2.41)**	**0.003**	1.35 (0.93–1.95)	0.11	**1.51 (1.05–2.17)**	**0.02**	**1.68 (1.17–2.41)**	**0.01**
Plasma activation inhibitor-1 [a]	1.11 (0.82–1.51)	0.50	0.91 (0.62–1.34)	0.63	1.05 (0.73–1.50)	0.81	1.18 (0.85–1.63)	0.32
P-selectin [a]	1.14 (0.83–1.56)	0.42	1.03 (0.71–1.49)	0.90	0.83 (0.56–1.22)	0.34	1.13 (0.82–1.56)	0.45
Soluble ICAM-1 [a]	1.19 (0.87–1.64)	0.28	1.16 (0.79–1.70)	0.45	1.08 (0.73–1.59)	0.70	1.13 (0.81–1.58)	0.46
Soluble VCAM-1 [a]	1.23 (0.89–1.70)	0.22	1.04 (0.71–1.52)	0.84	1.06 (0.72–1.56)	0.78	1.15 (0.78–1.69)	0.49
Serum amyloid A [a]	1.25 (0.92–1.70)	0.15	1.24 (0.89–1.75)	0.21	1.16 (0.83–1.63)	0.39	1.22 (0.89–1.67)	0.22
Soluble CD14 [a]	1.38 (0.98–1.94)	0.06	1.30 (0.89–1.90)	0.18	1.12 (0.76–1.66)	0.56	1.33 (0.92–1.92)	0.12
Apolipoprotein A1 [a]	1.11 (0.78–1.58)	0.55	1.08 (0.74–1.59)	0.69	0.87 (0.59–1.27)	0.47	1.09 (0.77–1.55)	0.62
ADAMTS-13 [a]	0.98 (0.72–1.35)	0.92	1.12 (0.76–1.65)	0.58	1.02 (0.71–1.46)	0.92	0.99 (0.72–1.35)	0.93
Von Willebrand factor [a]	1.07 (0.79–1.45)	0.67	1.21 (0.85–1.74)	0.29	0.92 (0.65–1.30)	0.64	1.03 (0.76–1.41)	0.84

ADAMTS-13 = a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, AOR = adjusted odds ratio, ASCVD = Atherosclerotic Cardiovascular Disease, CD4 = cluster of differentiation 4, CI = confidence interval, ICAM-1 = intercellular adhesion molecule 1, OR = odds ratio, VACS = Veterans Aging Cohort Study, VCAM-1 = vascular cell adhesion molecule 1

[a] All plasma biomarkers were log2-transformed and standard deviation scaled.

Table 3.

Associations between plasma biomarkers and myocardial infarction using conditional logistic regression with the imputed dataset, restricted to matched cases and controls whose viral load was <400 copies/mL (<2.60 $\log_{10}$ copies/mL) at the visit on which samples were collected (N=154)

Biomarker	OR (95% CI)	P value	Model 1: Adjusted for age, race/ethnicity, and sex AOR (95% CI)	P value	Model 2: Adjusted for ASCVD score AOR (95% CI)	P value	Model 3: Adjusted for VACS score AOR (95% CI)	P value
Log_{10} viral load [a]	0.33 (0.10–1.06)	0.06	0.47 (0.13–1.66)	0.24	0.49 (0.13–1.86)	0.29	0.31 (0.10–1.02)	0.05
Log_{10} peak viral load	**1.64 (1.10–2.46)**	**0.02**	1.57 (0.97–2.57)	0.07	1.50 (0.96–2.34)	0.07	**1.61 (1.07–2.43)**	**0.02**
CD4 count, per 100 cells	0.93 (0.65–1.32)	0.68	0.99 (0.63–1.55)	0.96	1.17 (0.75–1.81)	0.49	1.12 (0.73–1.71)	0.61
Nadir CD4 count, per 100 cells	**0.64 (0.41–1.00)**	**0.05**	0.64 (0.38–1.07)	0.09	0.79 (0.46–1.34)	0.38	0.68 (0.43–1.08)	0.10
Angiopoietin-1 [b]	1.16 (0.82–1.63)	0.40	1.04 (0.66–1.62)	0.88	1.18 (0.77–1.82)	0.45	1.22 (0.86–1.74)	0.26
Angiopoietin-2 [b]	1.42 (0.98–2.07)	0.07	1.21 (0.79–1.84)	0.38	1.46 (0.95–2.23)	0.08	1.34 (0.90–1.99)	0.16
C-reactive protein [b]	**1.51 (1.05–2.16)**	**0.03**	1.42 (0.92–2.20)	0.11	1.43 (0.93–2.21)	0.11	**1.48 (1.02–2.13)**	**0.04**
Interleukin-6 [b]	**2.04 (1.31–3.17)**	**0.002**	1.50 (0.92–2.47)	0.11	**1.73 (1.11–2.71)**	**0.02**	**1.97 (1.25–3.10)**	**0.003**
Plasma activation inhibitor-1 [b]	1.15 (0.81–1.62)	0.44	1.00 (0.62–1.62)	0.99	1.13 (0.74–1.74)	0.57	1.24 (0.86–1.79)	0.25
P-selectin [b]	1.24 (0.87–1.76)	0.24	1.06 (0.67–1.68)	0.81	0.96 (0.63–1.48)	0.86	1.22 (0.85–1.75)	0.27
Soluble ICAM-1 [b]	1.16 (0.79–1.69)	0.44	0.96 (0.59–1.57)	0.88	1.18 (0.71–1.96)	0.52	1.09 (0.74–1.61)	0.66
Soluble VCAM-1 [b]	1.27 (0.86–1.88)	0.23	0.92 (0.57–1.51)	0.75	1.23 (0.76–2.00)	0.41	1.13 (0.73–1.77)	0.58
Serum amyloid A [b]	1.36 (0.95–1.95)	0.09	1.34 (0.88–2.06)	0.17	1.35 (0.89–2.07)	0.16	1.34 (0.93–1.93)	0.12
Soluble CD14 [b]	1.34 (0.88–2.04)	0.18	1.03 (0.64–1.67)	0.89	1.12 (0.66–1.90)	0.68	1.22 (0.77–1.95)	0.40
Apolipoprotein 1 [b]	1.29 (0.87–1.93)	0.21	1.30 (0.82–2.06)	0.26	1.03 (0.65–1.62)	0.92	1.25 (0.83–1.88)	0.28
ADAMTS-13 [b]	0.88 (0.60–1.28)	0.50	1.04 (0.66–1.64)	0.87	0.90 (0.57–1.40)	0.63	0.87 (0.60–1.27)	0.47
Von Willebrand factor [b]	1.07 (0.76–1.51)	0.68	1.17 (0.77–1.76)	0.46	0.88 (0.59–1.31)	0.52	1.01 (0.71–1.44)	0.97

ADAMTS-13 = a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, AOR = adjusted odds ratio, ASCVD = Atherosclerotic Cardiovascular Disease, CD4 = cluster of differentiation 4, CI = confidence interval, ICAM-1 = intercellular adhesion molecule 1, OR = odds ratio, VACS = Veterans Aging Cohort Study, VCAM-1 = vascular cell adhesion molecule 1

[a] Plasma viral load ranged from <20 copies/mL to 387 copies/mL in this restricted sample. Of note, some assays used during the study period had a lower limit of detection of 400 copies/mL.

[b] All plasma biomarkers were log2-transformed and standard deviation scaled.