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Permalink

https://escholarship.org/uc/item/31v3n0gh

Journal

AIDS Research and Human Retroviruses, 30(2)

ISSN

0889-2229

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Publication Date

2014-02-01

DOI

10.1089/aid.2013.0183

Peer reviewed

Plasma Monocyte Chemoattractant Protein-1 and Tumor Necrosis Factor-α Levels Predict the Presence of Coronary Artery Calcium in HIV-Infected Individuals Independent of Traditional Cardiovascular Risk Factors

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Abstract

Coronary artery calcium (CAC) is a validated subclinical measure of atherosclerosis. Studies in the general population have linked blood inflammatory biomarkers including monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor (TNF)-α with the burden of CAC, but this relationship is often lost following correction for traditional cardiovascular risk factors. We assessed the relationship of various biomarkers to CAC, specifically in HIV-infected individuals on potent antiretroviral therapy (ART). Analyses utilized entry data from participants in the Hawaii Aging with HIV–Cardiovascular (HAHC-CVD) study. Computerized tomography examinations for CAC were obtained locally and analyzed by a central reading center in blinded fashion. Plasma biomarkers were assessed by multiplexing using Milliplex Human Cardiovascular Disease panels. Among a cohort of 130 subjects [88% male, median (IQR) age of 51 (46–57) years, CD4 count of 492 (341–635) cells/mm^3, 86.9% with HIV RNA ≤50 copies/ml], CAC was present in 46.9% of subjects. In univariate analyses higher levels of log-transformed MCP-1 and TNF-α were associated with the presence of CAC ($p < 0.05$). In multivariate logistic regression models, MCP-1 and TNF-α remained significant after adjustment for traditional cardiovascular (CVD) risk factors. Similar results were found when analyses were assessed by Framingham risk score categories or when restricted to subjects with plasma HIV RNA ≤50 copies/ml. In contrast to findings in the general population, higher MCP-1 and TNF-α predict the presence of CAC independent of traditional CVD risk factors in HIV-infected subjects fully suppressed on ART, suggesting that HIV-mediated immune activation may play a role in CVD risk.

Introduction

Coronary artery calcium (CAC) is a validated subclinical measure of atherosclerosis. Studies have linked inflammatory mediators including monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor (TNF)-α with the burden of coronary atherosclerosis as assessed by CAC.[1,2] However, in the general population, this relationship is often lost following correction for traditional cardiovascular disease (CVD) risk factors. We present data that high MCP-1 and TNF-α levels similarly predict the presence of CAC among older HIV-infected individuals on potent antiretroviral therapy

(ART), but that this association is independent of traditional CVD risk factors.

Materials and Methods

Subjects and study design

Analyses utilized baseline (entry) data from 130 participants in the Hawaii Aging with HIV-Cardiovascular (HAHC-CVD) study, a 5-year longitudinal cohort study of HIV-infected individuals on ART designed to investigate the role of immune activation and mitochondrial-specific oxidative stress on the

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pathogenesis of CVD. The study was linked to an HIV-CVD consortium funded by the National Heart, Lung and Blood Institute (NHLBI), which provided centralized services for reading of CAC at the Los Angeles Biomedical Research Institute. The HAHC-CVD study was approved by the University of Hawaii Committee on Human Subjects and informed consents were obtained from all subjects.

Entry criteria for the HAHC-CVD study required subjects to have documentation of HIV infection, be ≥40 years old, and to be on stable ART for ≥6 months. Routine HIV- and CVD-specific blood assessments were performed at baseline, including fasting (nothing by mouth for 12 h) blood tests for total, high-density lipoprotein (HDL) and directly measured low-density lipoprotein (LDL) cholesterol, triglycerides, glucose, and insulin. Blood processing with plasma separation was performed within 30 min of the draw. Plasma was separated and banked frozen at −80°C prior to shipment for plasma biomarkers.

Plasma was forwarded for biomarker multiplexing to the Blood Systems Research Institute, San Francisco, CA (S. Keating). Multiplexing was conducted using Milliplex Human Cardiovascular Disease panels (EMD Millipore, USA). Soluble biomarkers assessed included sE-selectin, sVCAM-1, sICAM-1, MMP-9, MPO, tPAI-1, CRP, SAA, SAP, IL-1β, IL-6, IL-8, IL-10, TNF-α, MCP-1, VEGF, IFN-γ, and NT-proBNP.

Computerized tomography examinations for CAC were obtained locally in Honolulu, Hawaii at Accuimaging, a free standing imaging center, using a dual source CT (DSCT) scanner (Siemens 64-slice Somatom) following previously published methods.[3] Technical quality assurance and centralized analyses were provided by the Los Angeles Biomedical Research Institute (M. Budoff). A radiologist or cardiologist blinded to clinical data quantified the CAC, using an interactive scoring system to calculate the Agatston score, with any Agatston score >0 defining the presence of CAC.[4]

Statistical analyses

Univariate and multiple logistic regressions were utilized to assess the association between plasma biomarkers and the presence of CAC, dichotomized as present (Agatston score >0) or absent (Agatston score =0). Log-transformed plasma biomarkers with a p-value less than 0.05 in univariate logistic regression were selected for examination in separate multivariate logistic regression models, adjusting for age, gender, CD4 percent, and the following traditional CVD risk factors: presence of hypertension, diabetes, smoking history, and total cholesterol/HDL ratio. Hypertension was defined as a patient-reported history of hypertension, use of antihypertensive medications, or blood pressure (BP) on entry visit of systolic BP >140 mm Hg or diastolic BP >90 mm Hg. BP measurements were performed in triplicate in a resting state. Diabetes was defined as self-reported history of diabetes, use of diabetic medications, fasting blood glucose >125 mg/dl, or a 2-h blood glucose >200 mg/dl on an oral glucose tolerance test (OGTT). A p-value less than 0.05 was considered significant.

Multivariate logistic regression models were also repeated substituting categories as defined by the Framingham risk score (FRS) for the traditional CVD risk factors. Framingham risk score was calculated online using the National Cholesterol Education Program website (http://hp2010.nhlbihin .net/atpiii/calculator.asp). Subjects were then classified into FRS risk categories. Subjects with FRS less than 10% were categorized as *low risk,* subjects with 10–20% FRS as *intermediate risk*, and subjects with >20% FRS as *high risk*. Subjects with clinical CVD (history of myocardial infarction, angina, coronary disease-related cardiac surgery, or ischemic stroke) or those with diabetes as a CVD equivalent were also automatically classified as *high risk.* Clinical CVD was adjudicated by two physician-researchers (C.M.S. and D.C.). Multiple logistic regression was also repeated utilizing only those subjects with an undetectable plasma HIV RNA (≤50 copies/ml).

Results

Cohort characteristics

The baseline demographic, HIV-specific, metabolic, and body composition characteristics of the subjects are shown in Table 1 together with a summary of CAC scores. The population was predominantly male (88%), 60% white, with a median age of 51 years. The median current CD4 count was 492, with 86.9% having undetectable plasma HIV RNA. CAC was present in approximately half of the cohort.

Univariate logistic regression (Table 2) showed significant associations between CAC and age, hypertension, and intermediate and high FRS class. Of the soluble biomarkers assessed, only log-transformed MCP-1 and tumor necrosis factor (TNF)-α were significantly associated with the presence of CAC.

MCP-1 and TNF-α were adjusted for traditional CVD risk factors using separate multivariate logistic regression models (Table 3). Both MCP-1 and TNF-α remained significant even when adjusted for age, gender, current CD4 percent, hypertension, diabetes, smoking history, and total cholesterol/HDL ratio. Similar results were found when the FRS risk category was substituted for traditional CVD risk factors with significant associations for MCP-1 (adjusted OR 10.59, $p=0.05$) and TNF-α (adjusted OR 4.29; $p=0.01$). Similar results were also obtained when both analyses were performed only among subjects with undetectable plasma HIV RNA levels [with use of individual CVD risk factors—MCP-1 (adjusted OR 21.88, $p=0.04$) and TNF-α (adjusted OR 4.81, $p=0.02$) and with the use of FRS risk categories—MCP-1 (adjusted OR 9.37, $p=0.08$) and TNF-α (adjusted OR 4.23, $p=0.02$)].

Discussion

We found an association between the presence of CAC, a validated surrogate marker of arterial injury, and increased plasma levels of MCP-1 and TNF-α. Moreover, this association was independent of traditional CVD risk factors.

There is increasing evidence that HIV-infected individuals are at higher risk of cardiovascular disease. Among patients in the Partners HealthCare System in Boston, rates of acute myocardial infarction were elevated approximately 2-fold among HIV-infected individuals compared to those not infected with HIV, and this difference was seen over multiple age ranges.[5] However, HIV-infected individuals typically have higher levels of traditional CVD risk factors, as was the case in this study, and the role of such traditional risk factors versus the role of HIV per se in CVD risk in this population is unclear. Increases in traditional risk factors may occur by lifestyle choices (i.e., smoking), may be a direct or indirect result

TABLE 1. BASELINE CHARACTERISTICS OF SUBJECTS IN THE HAWAII AGING WITH HIV–CARDIOVASCULAR STUDY

Gender (% males)	87.7%
Race/ethnicity	
% White	60%
% African-American	3.1%
% Native American/Alaskan	2.3%
% Native Hawaiian (NH)/Pacific Islanders (PI)	12.3%
% Asian	7.7%
% Mixed (other than NH/PI)	14.6%
Age [median (Q1,Q3)]	51 (46, 57)
Nadir CD4 [median (Q1,Q3)]	154 (30, 250)[a]
Current CD4 [median (Q1,Q3)]	492 (341, 635)
HIV RNA [% undetectable (≤50 copies/ml)]	86.9%
History of clinical cardiovascular disease	7.7%
% with history of smoking—past /current	63.9%/22.3%
% with hypertension	36.2%
Body mass index [median (Q1, Q3)]	25.8 (23.9, 28.3)
Waist circumference (cm) [median (Q1, Q3)]	
Males	92.5 [89, 99]
Females	91 [84, 99]
HDL-cholesterol (mg/dl) [median (Q1, Q3)]	
Males	42 [33, 53]
Females	56 [43.5, 64]
Directly measured LDL-cholesterol (mg/dl) [median (Q1, Q3)]	108 (89, 130)
Triglycerides (mg/dl) [median (Q1, Q3)]	114 (83, 164)
HOMA-IR [median (Q1, Q3)]	1.37 (0.80, 2.27)
Impaired glucose metabolism	
% with IFG	8.5%
% with IGT	6.7%
% with diabetes (by history, fasting blood sugar, or OGTT)	6.9%
FRS risk category	
Low (0–10%)	67.7%
Intermediate (11–20%)	16.9%
High (>20%)	15.4%
Coronary artery calcium [median (Q1, Q3)]	0 [0, 47.66]
% with 0 Agatston score	53.1%
1–100 Agatston score	26.9%
101–400 Agatston score	10.8%
>400 Agatston score	9.2%

[a]By self-report, data available from $n=123$ subjects.

Data from $N=130$ subjects with cytokine data and coronary artery calcium assessment.

HDL, high-density lipoprotein; LDL, low-density lipoprotein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; FRS, Framingham risk score.

TABLE 2. UNIVARIATE LOGISTIC REGRESSION ANALYSIS OF CLINICAL FACTORS AND SOLUBLE BIOMARKERS ASSOCIATED WITH THE PRESENCE OF CORONARY ARTERY CALCIUM

	Odds ratio	p-value	95% Confidence interval	
Clinical parameters				
Age	1.15	<0.001[a]	1.08	1.22
Male gender	1.55	0.42	0.53	4.56
Hypertension	2.23	0.03[a]	1.08	4.63
Diabetes	0.90	0.88	0.23	3.51
With history of smoking	1.32	0.45	0.64	2.71
Total cholesterol/ HDL ratio	0.98	0.55	0.91	1.05
FRS risk category				
Low risk (<10%)	Ref	Ref	Ref	Ref
Intermediate risk (10–20%)	3.06	0.02[a]	1.16	8.09
High risk (>20%)	5.25	0.003[a]	1.75	15.80
Current CD4%	1.00	0.78	0.97	1.03
Soluble biomarkers[b]				
sE selectin	1.08	0.90	0.29	4.12
sVCAM-1	8.97	0.13	0.52	153.78
sICAM-1	2.40	0.33	0.42	13.67
MMP-9	1.10	0.87	0.33	3.74
MPO	1.09	0.88	0.37	3.21
tPAI-1	1.73	0.54	0.30	10.14
CRP	1.28	0.30	0.80	2.04
SAA	1.14	0.54	0.75	1.75
SAP	1.01	0.98	0.44	2.34
IL-1β	0.95	0.96	0.12	7.48
IL-6	1.84	0.15	0.81	4.22
IL-8	1.44	0.68	0.25	8.10
IL-10	1.12	0.65	0.68	1.83
TNF-α	3.70	0.01[a]	1.30	10.48
MCP-1	12.2	0.03[a]	1.27	117.77
VEGF	1.87	0.12	0.84	4.16
IFN-γ	0.81	0.61	0.36	1.84
NT-proBNP	1.34	0.38	0.70	2.58

[a]Statistically significant.
[b]Odds ratios were calculated based on log-transformed values of soluble biomarkers.

rithms, such as the FRS, uniquely for this population, and may alter how preventive or therapeutic options for CVD should optimally be approached.

Both atherosclerosis and chronic HIV infection are disease states characterized by the presence of low-grade chronic inflammation.[6,7] HIV-induced inflammation and immune dysfunction have been suggested as key factors in the increase in CVD and other non-AIDS complications currently being seen among HIV-infected individuals on potent ART who are otherwise doing well with fully suppressed plasma HIV RNA levels.[6] While inflammation is reduced by ART, it is not eliminated, and the detection of inflammatory cytokines has been associated with increased morbidity in this treated population.[8] Among plasma biomarkers, both MCP-1 and TNF-α have been linked to CVD in the general population.[1,9–11] In HIV-infected individuals, higher levels of MCP-1 have been associated with higher levels of carotid intima-media thickness (cIMT)[12] and with thoracic aorta vessel wall area and vessel

of HIV or its immune dysregulation (i.e., low HDL), and/or may be mediated by the side-effects of HIV therapy (i.e., lipodystrophy). As all the factors leading to increased CVD risk in this population may not be part of traditional risk factors, the issue of whether HIV per se increases the risk of CVD above and beyond traditional risk factors is a clinically relevant issue. Determination that HIV per se adds to CVD risk would suggest a need to alter CVD risk assessment algo-

Table 3. Multivariate Logistic Regression Analysis of Tumor Necrosis Factor-α and Monocyte Chemoattractant Protein-1 and their Association with the Presence of Coronary Artery Calcium

	Adjusted odds ratio	p-value	95% Confidence interval	
Log-TNF-α	4.44	0.02[a]	1.24	15.90
Age	1.16	<0.001[a]	1.09	1.24
Male gender	0.86	0.83	0.23	3.17
Hypertension	2.43	0.049[a]	1.01	5.88
Diabetes	0.31	0.22	0.05	2.01
With history of smoking	2.39	0.06	0.97	5.91
Total cholesterol/ HDL ratio	0.98	0.79	0.88	1.10
Current CD4%	1.01	0.66	0.97	1.05
Log-MCP-1	20.36	0.03[a]	1.31	316.03
Age	1.17	<0.001[a]	1.10	1.25
Male gender	1.15	0.83	0.33	4.04
Hypertension	2.28	0.07	0.95	5.50
Diabetes	0.28	0.17	0.05	1.69
With history of smoking	2.29	0.07	0.93	5.64
Total cholesterol/ HDL ratio	0.99	0.89	0.92	1.08
Current CD4%	1.01	0.62	0.97	1.05

[a]Statistically significant.
TNF, tumor necrosis factor; MCP-1, monocyte chemoattractant protein-1.

wall thickness,[13] and higher levels of TNF-α with cIMT, specifically for the internal carotid artery.[14]

Our study assessed subclinical atherosclerosis utilizing CAC, an established surrogate measure of increased cardiovascular risk in the general population. Numerous studies have demonstrated a relationship between increasing burden of CAC and future coronary heart disease events.[15,16] Studies in the general population have also reported an association between various soluble plasma markers of inflammation and CAC.[2] One study exploring the association between MCP-1 and CAC, however, reported that this specific association disappeared following adjustment for traditional cardiovascular risk factors and age,[1] and a systematic review that more broadly assessed the relationship of various inflammatory markers to CAC in the general population subsequently concluded similarly that in most studies in which a positive relationship was found, this relationship disappeared after appropriate correction for the presence of traditional risk factors.[2] One interpretation of this loss of association upon adjustment is that inflammatory markers simply function in the causal pathway between risk factors and atherosclerosis, mediating some of the effects of these traditional risk factors.[1]

Our study, in contrast, found that elevations in MCP-1 and TNF-α were associated with the presence of CAC independent of traditional risk factors, either assessed as a collection of various traditional CVD risk factors or as a composite utilizing the FRS. It should be noted that the 95% confidence interval for the adjusted odds ratios for both MCP-1 and TNF-α is large, leading to difficulty in determining the true extent of this association. Our study cohort consisted of individuals with chronic HIV infection on potent ART, with the majority

of subjects having plasma HIV RNA levels below the level of detection (≤50 copies/ml). Restricting the analysis to subjects with undetectable plasma HIV RNA levels or excluding subjects with documented cardiovascular events made no difference in the results. Our results suggest that both MCP-1 and TNF-α may reflect the contribution of HIV per se to CVD risk in this population above and beyond traditional risk factors. Our study is in concordance with a study by Parra et al. that assessed subclinical atherosclerosis by cIMT and concluded that the FRS underestimates the presence of subclinical atherosclerosis in the HIV-infected population.[17]

Our study has several important limitations including the small sample size, which limited our ability to assess interactions between covariates, the lack of HIV seronegative controls, and the cross-sectional nature of the study.

In summary, our study found that MCP-1 and TNF-α predict the presence of CAC in a population of individuals with chronic HIV on ART, and that, unlike the situation in the general population, this is independent of traditional risk factors. This study lends support to the hypothesis that HIV-induced inflammation contributes to the development of subclinical atherosclerosis in this population.

Acknowledgments

Author Disclosure Statement

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