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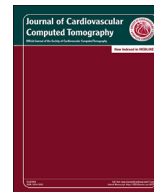
Research paper

Extra-coronary calcification (aortic valve calcification, mitral annular calcification, aortic valve ring calcification and thoracic aortic calcification) in HIV seropositive and seronegative men: Multicenter AIDS Cohort Study

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ABSTRACT

Introduction: Previous studies have demonstrated an association between HIV infection and coronary artery disease (CAD); little is known about potential associations between HIV infection and extra-coronary calcification (ECC).

Methods: We analyzed 621 HIV infected (HIV+) and 384 HIV uninfected (HIV−) men from the Multicenter AIDS Cohort Study who underwent non-contrast computed tomography (CT) from 2010-2013. Agatston scores were calculated for mitral annular calcification (MAC), aortic valve calcification (AVC), aortic valve ring calcification (AVRC), and thoracic aortic calcification (TAC). The associations between HIV infection and the presence of each type of ECC (score > 0) were evaluated by multivariable logistic regression. We also evaluated the association of ECC with inflammatory biomarker levels and coronary plaque morphology.

Results: Among HIV+ and HIV− men, the age-standardized prevalences were 15% for TAC (HIV+ 14%/HIV− 16%), 10% for AVC (HIV+ 11%/HIV− 8%), 24% for AVRC (HIV+ 23% HIV− 24%), and 5% for MAC (HIV+ 7%/HIV− 3%). After adjustment, HIV+ men had 3-fold greater odds of MAC compared to HIV− men (OR = 3.2, 95% CI: 1.5−6.7), and almost twice the odds of AVC (1.8, 1.1−2.9). HIV serostatus was not associated with TAC or AVRC. AVRC was associated with higher Il-6 and sCD163 levels. TAC was associated with higher ICAM-1, TNF-α RII, and Il-6 levels. AVC and AVRC calcification were associated with presence of non-calcified plaque in HIV+ but not HIV− men.

Conclusion: HIV infection is an independent predictor of MAC and AVC. Whether these calcifications predict mortality in HIV+ patients deserves further investigation.

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1. Introduction

Cardiovascular disease (CVD) is a significant cause of morbidity

and mortality among the approximately 1.1 million people infected with the human immunodeficiency virus (HIV) in the United States [1]. Due to highly active antiretroviral therapy (HAART), fewer individuals with HIV are dying of opportunistic infections[2,3]. However, more are dying from age-related illnesses such as CVD[4]. Previous studies have shown that HIV−infected (HIV+) individuals have higher rates of myocardial infarction, CVD risk factors, sudden cardiac death, and non-calcified plaque when compared to HIV-uninfected (HIV−) individuals[4–6]. The reason for and etiology of these differences is not clear, but may include several factors including metabolic side effects of HAART, chronic increased systemic inflammation from HIV itself, or a higher prevalence of CVD risk factors, such as smoking[7–9].

In the general population, extra-coronary calcification (ECC) including mitral annular calcification (MAC), aortic valve calcification (AVC), thoracic aortic calcification (TAC), and aortic valve-ring calcification (Fig. 1) have been well described and are associated with systemic atherosclerosis[10–12]. In the Multi-Ethnic Study of Atherosclerosis (MESA), Owens et al found AVC to be predictive of future CVD events independent of inflammatory biomarkers and traditional risk factors[13]. A subsequent study also found an association between AVC and CVD events, which was independent of Framingham risk factors[14]. However, ECC has not been well studied in HIV+ populations. To address this issue, we compared the amount and type of ECC between HIV+ and HIV− men in the Multicenter AIDS Cohort Study (MACS).

2. Methods

2.1. Patient population

The MACS is a prospective cohort study, conducted in four U.S. cities, of HIV+ and HIV− men who have sex with men. The MACS has been well characterized previously[15]. We performed cardiac CT scans to characterize the prevalence and predictors of subclinical atherosclerosis. Participants completed non-contrast cardiac computed tomography (CT) scanning for coronary artery calcium (CAC) scoring between January 2010 and August 2013. Eligibility criteria for this MACS CVD ancillary study included being an active MACS participant, age 40 to 70 years, weighing less than 136 kg (300 pounds), and without a history of cardiac surgery or percutaneous coronary intervention as these procedures could interfere with the measurement of coronary atherosclerosis.

2.2. Demographic and CVD risk factors

Participants were seen during routine MACS semi-annual research visits for standardized interviews, physical examination, and blood and urine collection for concurrent laboratory analyses and storage. Data from the MACS visit prior to the CT measurements were used and included demographic, HIV clinical parameters, and CVD risk factors, including age, race, measured blood pressure, fasting glucose, fasting lipid panel, body mass index (weight/height^2), and self-reported medication use. Cumulative pack-years of smoking were calculated from the longitudinal self-report of smoking status and cigarette quantity.

2.3. Non-contrast CT acquisition

Our analytic sample included 1005 men, 384 were HIV−, and 621 were HIV+. Details of the CT scanning procedures have been described[16]. Briefly, computed tomography scanning equipment included 64-slice multidetector CT at 3 centers and 320-row multidetector CT at 1 center. Prospective electrocardiography-
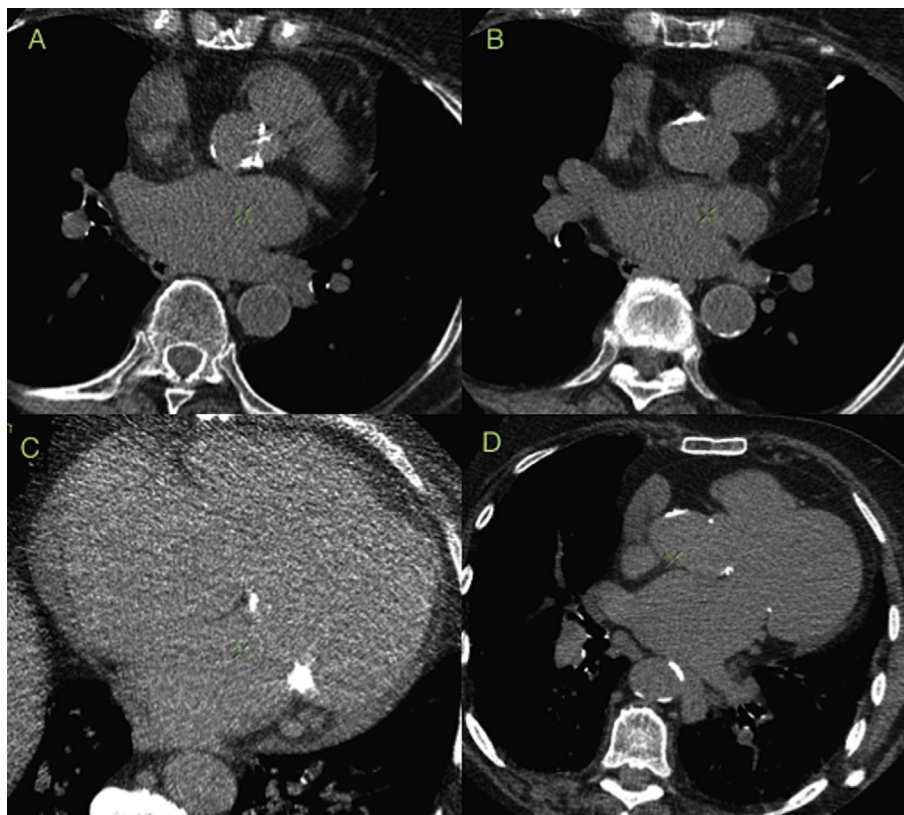

Figure 1. CT Imaging Showing A) Aortic Valve, B) Aortic-valve ring, C) Mitral Annular, and D) Thoracic Aortic Calcifications.

triggering non-contrast cardiac protocols were used to obtain the CAC scan. The CT images were transferred to the core CT reading center (Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center) and were analyzed by trained, experienced readers who were blinded to participant characteristics and HIV serostatus. The main outcome was the calcification score, calculated as the Agatston Score[17].

2.4. Coronary CTA analysis

Participants also underwent coronary CT angiography (CTA) to assess plaque morphology unless contraindicated by chronic kidney disease (estimated glomerular filtration rate <60 ml/min/m^2 using the MDRD equation within 30 days), atrial fibrillation, or IV contrast allergy. Coronary CTA images were analyzed using the modified 15-segment model of the American Heart Association for plaque presence and extent, coronary artery stenosis, and plaque composition[18]. Partially calcified plaque was defined as lesions with less than 50% of plaque area occupied by calcium. Calcified plaque included any structure with attenuation >130 Hounsfield units (HU) visualized separately from the intravascular lumen. Non-calcified atherosclerotic plaque was defined as any structure clearly assignable to the vessel wall, with a CT density less than the contrast-enhanced coronary lumen but greater than the surrounding connective tissue. Plaque types were identified in at least two independent planes. Coronary stenosis was defined as greater than 50% in any coronary segment. The segment involvement score was defined as the number of coronary segments with plaque present. The total plaque score was calculated by adding the plaque size score for each coronary segment that had any plaque (maximum score of 45).

2.5. Extra-coronary calcification assessment

MAC, AVC, AV-ring calcification and TAC scores were computed using the Agatston method from non-contrast cardiac CT scans[17]. According to this method, three contiguous pixels with a density over 130 HUs qualified as an atherosclerotic plaque. The absence of calcium was deemed a score of 0. AVC was defined as any calcified lesion within the aortic valve leaflets, excluding the aortic annulus, proximal aorta, and coronary arteries. AV-ring (root or annulus) calcium was measured at the level of the aortic-ring, and excluded calcium of the valve leaflets as well as the ascending aorta wall. MAC included calcification along the mitral annulus, excluding the mitral leaflets and differentiated from the left circumflex artery. TAC was defined as either ascending or descending thoracic aortic calcium measured from the pulmonary artery bifurcation superiorly to the cardiac apex inferiorly.

2.6. HIV clinical factors

HIV− men were tested for HIV at each MACS visit, and HIV serostatus was established by a positive ELISA test confirmed by Western blot. Measures of HIV disease activity, including CD4$^+$ T-cell counts and plasma HIV RNA levels (Roche ultrasensitive assay with limit of detection of 50 copies/mL; Roche Diagnostics, Nutley, NJ), were measured at each visit for HIV+ men. Duration of highly active antiretroviral therapy (HAART), protease inhibitor (PI), non-nucleoside reverse transcriptase inhibitor (NNRTI), and nucleoside reverse transcriptase inhibitor (NRTI) use was calculated based on the longitudinal participant self-report of antiretroviral medication use at each MACS visit. A history of clinically defined AIDS was determined by medical record confirmation of self-reported outcomes.

2.7. Laboratory measurement

A panel of biomarkers was measured from blood samples drawn on the day of CT scanning and stored at −70 °C until analysis. Biomarkers included high sensitivity C-reactive protein (hsCRP), fibrinogen, monocyte chemotactic protein 1 (MCP-1), interleukin 6 (IL-6), D-dimer, intercellular adhesion molecule 1 (ICAM-1), soluble tumor necrosis factor alpha receptor type I (sTNFαRI), type 2 (sTNFαRII), sCD14, and sCD163. High-sensitivity CRP and fibrinogen were measured using the BNII Nephelometer (Siemens Healthcare Diagnostics, Deerfield, IL). Samples of IL-6, ICAM-1, sCD14, and sCD163 were tested using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). D-dimer was measured using a Stago STA-R analyzer (Parsippany, NJ). A Milliplex (Millipore, Billerica, MA) measured sTNFαRI and sTNFαRII via multiplexing.

2.8. Statistical analyses

The distributions of demographic, behavioral, and clinical characteristics between HIV+ and HIV− men were compared using the chi-square or Kruskal-Wallis tests for categorical and continuous variables, respectively. The prevalence of each extra-coronary plaque type was described by HIV serostatus, both crude and age-adjusted, assuming a common age distribution for the HIV+ and HIV− men (we used the age distribution of the total sample) using direct standardization. We used multiple imputation to estimate values for nine CVD risk factor covariates with a small percentage of missing values (<4%). The predictors for the multivariate imputation model included all covariates and outcome variables from the multivariable analyses models. Missing values were imputed five times based on the distribution of covariates using the data augmentation Markov chain Monte Carlo (MCMC) method assuming a multivariate normal distribution[19]. We used logistic regression models, pooled across the multiple imputation datasets, to assess the association between HIV infection and extra-coronary plaque. Multivariable models included age, race, education, study site, enrollment period into the MACS (enrolled pre-vs. post-2001), body mass index (BMI), current smoking status, pack-years of tobacco smoking, use of anti-hypertensive, diabetes or lipid-lowering medications, and systolic blood pressure (SBP), fasting glucose, total and HDL cholesterol for those not on anti-hypertensive, diabetes or lipid-lowering medications. After adjustment for demographic, HIV clinical factors, and CVD risk factors, levels of each of the nine biomarkers were log transformed (base-*e*) and analyzed for their associations with ECC outcomes in separate multivariable models. Further analyses were conducted among the HIV+ men to evaluate associations between measures of HIV disease and treatment exposures with ECC outcomes. We assessed nadir and current CD4 cell count, HIV viral suppression, history of AIDS, and use of protease inhibitors sequentially, and retained them in the multivariable model if their association with an ECC outcome had a p-value <0.05. Among HIV+ men on HAART, we determined the effects of years of PI, NNRTI, and NRTI use on ECC presence. In both our HIV+ and HIV− groups, we assessed the association between ECC and the presence of coronary plaque morphology after adjusting for age and race. All analyses used Stata 13.1 (StataCorp, College Station, TX).

3. Results

Our analytic sample included 1005 men who underwent CT scanning [n = 621 (61.8%) HIV+/n = 384 (38.2%) HIV−]. TAC was evaluated among 988 men since 17 CT scan images did not include the thoracic aorta. Demographic and clinical characteristics stratified by HIV serostatus are described in Table 1. Compared to the

Table 1
Descriptive characteristics of 1005 men in the MACS cardiovascular study, by HIV serostatus.

	HIV+ N = 621		HIV− N = 384		P-value
Age (years)	52	(48, 58)	54	(50, 61)	<0.001
Race					<0.001
Caucasian	327	(52.7%)	257	(66.9%)	
Hispanic/Other	83	(13.4%)	32	(8.3%)	
African-American	211	(34.0%)	95	(24.7%)	
Systolic Blood Pressure (mmHg)	126	(115, 137)	128	(118, 137)	0.25
On Hypertensive Medication	221	(35.7%)	121	(31.8%)	0.20
Fasting Glucose (mg/dL)	98	(90, 108)	96	(89, 106)	0.01
On Diabetes Medication	53	(8.6%)	29	(7.6%)	0.57
Total Cholesterol (mg/dL)	185	(157, 212)	190	(166, 216)	0.03
HDL-Cholesterol (mg/dL)	46	(38, 55)	52	(43, 61)	<0.001
LDL-Cholesterol (mg/dL)	104	(81, 128)	112	(91, 137)	<0.001
Triglycerides (mg/dL)	132	(95, 201)	105	(74, 147)	<0.001
On Lipid Lowering Medication	211	(35.0%)	113	(30.1%)	0.11
Body Mass Index (kg/m^2)	26	(23, 29)	27	(24, 30)	<0.001
Smoking Status					<0.01
Current	192	(31.0%)	83	(21.8%)	
Former	271	(43.8%)	201	(53.9%)	
Never	156	(25.2%)	96	(25.3%)	
Cumulative pack years	6	(0, 24)	2	(0, 22)	0.02
Injection drug use (ever)	116	(18.7%)	28	(7.3%)	<0.001
HIV-specific factors					
Undetectable Viral Load (<50 copies)	503	(82.1%)			
HIV RNA (copies/mL) (1)	695	(139, 16400)			
On HAART	540	(88.4%)			
Time on HAART (years)	9.5	(6.4, 12.4)			
CD4+ T-cell count (cells/mm^3)	599	(421, 766)			
CD4+ T-cell nadir (cells/mm^3)	284	(172, 400)			
History of AIDS	91	(14.7%)			

HDL = high-density lipoprotein, LDL = low-density lipoprotein, HAART = highly active antiretroviral therapy. Prevalence (%) or median and interquartile range. P-values determined using the Kruskal-Wallis or chi-square test as appropriate. (1) HIV viral load among the 109 HIV+ men with detectable current HIV RNA (>50 copies/mL) levels.

HIV− men, HIV+ men were younger, African-American, had higher fasting glucose and triglyceride levels, were current smokers, and previously injected drugs. In addition, HIV+ men had lower total, HDL and LDL cholesterol levels, and lower BMIs compared to HIV− men.

Thirty-five percent of our sample had extra-coronary calcification in one or more areas (Table 2). Prevalence was highest for AV-ring (24%), followed by TAC (15%), AVC (10%), and MAC (5%). HIV+ men had higher age-standardized prevalences of AVC (HIV+ 11%/HIV− 8%) and MAC (HIV+ 7%/HIV− 3%) when compared to HIV− men. Of note, even among the 495 men with no coronary artery calcium (CAC = 0), 19% had extra-coronary calcification, with no difference by HIV serostatus (data not shown).

After adjustment for demographic and CVD risk factors, HIV infection was associated with increased odds of AVC (odds ratio, 95% CI: 1.7, 1.1−2.8) and MAC (3.0, 1.4−6.2) (Table 3), but was not associated with AV-ring calcification or TAC. Each decade increase in age was associated with greater odds of all four ECC types. African American men had 40 percent lower odds than white men of AV-ring calcification (0.6, 0.3−0.9). Men on lipid lowering medication had significantly greater odds of TAC (1.6, 1.0−2.4) and AV-ring calcification (1.6, 1.1−2.3). Each additional pack-year of smoking increased the odds of AVC and TAC by 10% (both 1.1, 1.0−1.1). We did not find any statistically significant associations between other demographic or CVD risk factors and any of the four ECC types.

We evaluated the association between each measure of ECC and HIV-specific factors after adjustment for demographics and CVD risk factors. A previous history of AIDS was associated with two-fold higher odds of AV-ring calcification (2.33, 1.26−4.29, p < 0.01), but not with AVC, MAC, or TAC. We did not find any statistically significant associations between other ECC measures and detectable HIV RNA levels, current CD4, nadir CD4, years of HAART, years of NNRTI duration, or NRTI duration. For each year of

PI use, however, we found a trend towards increased MAC and AVC (p = 0.08 and 0.06, respectively).

To assess inflammation as a potential mechanism for the greater ECC observed in HIV+ men, we evaluated the association between ECC and levels of 9 biomarkers in separate models (Table 4). After full adjustment, TAC was positively associated with levels of ICAM-1 (1.82, 1.07−3.11), TNF-α RII (2.10, 1.18−3.72), and Il-6 (1.32, 1.01−1.72). AV-ring calcifications were positively associated with levels of Il-6 (1.30, 1.04−1.62) and sCD163 (1.77, 1.09−2.88). Only sCD163 was marginally associated (p < 0.10) with AVC. However, its inclusion in multivariate models attenuated the association between HIV infection and AVC [from 1.84 (1.10−3.09) to 1.62 (0.95−2.77)], suggesting it may be a mediating factor. We did not find an association between MAC and any of the biomarkers we tested.

Since ECC has been associated with CVD events[13,14], we used coronary CTA to investigate the associations between the presence of each ECC and coronary artery plaque presence, extent and composition, stratified by HIV serostatus (Table 5A and B). The presence of AVC was associated with the presence of CAC, calcified plaque, partially calcified plaque, coronary stenosis >50%, a greater segment involvement score, and total plaque score among both HIV+ and HIV− participants. Among men with AVC, there was a greater prevalence of non-calcified plaque in HIV+ men compared with HIV− men (81.3% vs. 52.9%), p-value for HIV serstatus interaction = 0.001. After adjustment for age and race, HIV+ participants with AVC were 2.2 times more likely to have non-calcified plaque than men without AVC. Similar associations were seen with AV-ring calcification. TAC presence was associated with all coronary plaque types except non-calcified plaque among both HIV+ and HIV− men. After adjustment for age and race, there were no statistically significant associations between MAC and plaque morphologies.

Table 2
Crude and age-adjusted prevalence of extra-coronary calcification, by HIV status among HIV+ and HIV− men in the MACS.

	Total (N = 1005)		HIV− (N = 384)			HIV+ (N = 621)		
	Crude		Crude	Adjusted	Adj 95% CI	Crude	Adjusted	Adj 95% CI
TAC[a]	0.15		0.18	0.16	[0.13,0.20]	0.12	0.14	[0.11,0.16]
MAC	0.05		0.03	0.03	[0.01,0.05]	0.06	0.07	[0.05,0.09]
AVC	0.10		0.10	0.08	[0.06,0.11]	0.10	0.11	[0.09,0.14]
AV-ring	0.24		0.28	0.24	[0.20,0.28]	0.21	0.23	[0.20,0.26]
Any ECC	0.35		0.40	0.35	[0.31,0.39]	0.33	0.35	[0.32,0.39]

MACS-Multicenter AIDS Cohort Study, TAC- thoracic aortic calcification, MAC- mitral annular calcification, AVC- aortic valve calcification, AV-ring- aortic valve-ring, ECC-extra-coronary calcification. HIV-specific rates were adjusted to a common age distribution using direct standardization.

[a] TAC was evaluated among 988 men (n = 381 HIV−/n = 607 HIV+) since 17 CT scan images did not cover the thoracic aorta.

4. Discussion

In the MACS, we found that HIV+ men were 70% more likely than HIV− men to have AVC and 3 times more likely to have MAC. Using coronary CTA, the presence of ECC was strongly correlated with the presence and extent of coronary artery plaque and stenosis. Furthermore, the presence of AVC and AV-ring calcification in HIV+ but not HIV− men was associated with the presence non-calcified plaque, a plaque type previously demonstrated to exist in excess among HIV+ compared to HIV− men in this cohort[6]. In contrast, the presence of MAC was not independently associated with coronary plaque type or extent overall or by HIV serostatus.

Although the presence and extent of CAC has a well-established link with future CVD events[20,21] in the general population, growing evidence suggests that further prognostic information can be gleaned from calcifications in other-than-coronary vascular beds[22–24]. ECC has the added benefit of high reproducibility and visualization with several imaging modalities, such as CT and echocardiography[25–27]. Additionally, ECC has been associated with angiographic coronary artery disease (CAD) extent and severity. Yamamoto et al found that AVC was the strongest predictor of obstructive coronary disease. Further, descending aorta calcifications were associated with multivessel coronary disease[12]. Our findings build upon these earlier reports and go one step further by comparing ECC types and their associations with coronary plaque morphology. These findings could potentially offer clinicians valuable information regarding the likelihood of coronary disease if ECC is found. Moreover, they could trigger further testing and potentially early intervention of CAD.

In our cohort, HIV+ men with AVC were more likely to have non-calcified coronary plaque, which is associated with a higher risk of coronary events[28,29] than calcified plaque. If AVC is found on imaging with CT or other modalities, it could alert clinicians to the increased likelihood of high-risk plaque, which may not be represented by CAC scoring. Our group has previously described the increased prevalence of non-calcified coronary plaque in HIV+ men[6]. These analyses suggest that there may be a shared mechanism whereby HIV infection predisposes to a greater prevalence of non-calcified plaque as well as to AVC.

Two studies from different cohorts (MESA and The Heinz Nixdorf Recall Study) have previously described the predictive ability of AVC for future CVD events[13,14]. A subsequent study, also from MESA, included all four ECC types and showed their predictive value for CVD and all-cause mortality risk beyond traditional CVD risk factors[22]. Furthermore, they found that the odds of CVD events, CVD mortality, and all-cause mortality increased with incremental sites of ECC involvement. Risk prediction was further improved with the addition of traditional CVD risk factors and CAC. Their study built upon findings from both the Rotterdam Study and Cardiovascular Health Study, each of which reported upon the prognostic ability of ECC as assessed by echocardiography[27,30]. However, the applicability and use of ECC in CVD risk prediction for other populations such as persons with HIV infection is unclear.

The development of ECC appears to share similar cardiovascular risk factors and mechanisms with atherosclerosis[31–33]. All four ECC types included in these analyses are areas of altered shear stress and turbulent blood flow, both of which predispose to endothelial dysfunction, lipid accumulation, and calcium deposition[33–36].

Table 3
Predictors of extra-coronary calcification among 1,005 men in the MACS from multivariable logistic regression model.

	AVC OR	95% CI	MAC OR	95% CI	TAC[a] OR	95% CI	AV-ring OR	95% CI
HIV Infection	**1.7***[*]	**[1.1,2.8]**	**3.0**[**]	**[1.4,6.2]**	0.8	[0.5,1.2]	0.9	[0.7,1.3]
Age (per 10 year increase)	**3.4**[***]	**[2.3,5.2]**	**3.7**[***]	**[2.1,6.8]**	**4.4**[***]	**[3.0,6.4]**	**4.0**[***]	**[2.9,5.5]**
White (Ref)	1		1		1		1	
African American	0.7	[0.3,1.4]	0.3	[0.1,1.0]	1.5	[0.8,2.8]	**0.6**[*]	**[0.3,0.9]**
Hispanic/Other	0.4	[0.1,1.5]	0.4	[0.1,2.1]	0.9	[0.3,2.2]	0.7	[0.3,1.3]
Systolic Blood Pressure (per 10 mm Hg)	1.2	[1.0,1.4]	1.3	[1.0,1.8]	1.0	[0.8,1.1]	0.9	[0.8,1.1]
On Hypertension Medication	1.0	[0.6,1.7]	1.7	[0.9,3.4]	1.5	[0.9,2.2]	1.1	[0.7,1.5]
On Diabetes Medication	1.2	[0.5,2.6]	2.3	[1.0,5.4]	0.9	[0.5,1.9]	0.9	[0.5,1.6]
Fasting glucose per 10 mg/dL	0.9	[0.7,1.1]	1.0	[0.8,1.3]	1.1	[0.9,1.2]	1.0	[0.9,1.1]
Total cholesterol per 5 mg/dL	1.0	[1.0,1.1]	1.0	[0.9,1.1]	1.0	[1.0,1.1]	1.0	[1.0,1.1]
HDL cholesterol (mg/dL)	1.0	[1.0,1.1]	1.0	[0.9,1.2]	0.9	[0.9,1.0]	1.0	[0.9,1.1]
On lipid lowering medication	1.6	[1.0,2.6]	1.5	[0.8,3.0]	**1.6**[*]	**[1.0,2.4]**	**1.6**[*]	**[1.1,2.3]**
BMI	1.0	[1.0,1.1]	1.0	[1.0,1.1]	1.0	[1.0,1.1]	1.0	[1.0,1.1]
Cumulative smoking (pack-year)	**1.1**[*]	**[1.0,1.1]**	1.0	[0.9,1.1]	**1.1**[***]	**[1.0,1.1]**	1.0	[1.0,1.1]

Models are adjusted for all shown covariates as well as study site, recruitment cohort and current smoking status. TAC- thoracic aortic calcification, MAC- mitral annular calcification, AVC- aortic valve calcification, AV-ring- aortic valve-ring.

[*] $p < 0.05$, [**] $p < 0.01$, [***] $p < 0.001$.
Bolded results are statistically significant.

[a] TAC was evaluated among 988 men (n = 381 HIV−/n = 607 HIV+) since 17 CT scan images did not cover the thoracic aorta.

Table 4
Adjusted odds ratio of extra-coronary calcification among MACS participants, sequentially adjusting for biomarkers.

	MAC		AVC		AV-ring		TAC	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
ICAM-1	0.75	0.37–1.50	0.71	0.44–1.14	1.13	0.76–1.67	1.82	**1.07–3.11**
TNF-α RI	0.55	0.24–1.27	1.23	0.68–2.22	1.03	0.66–1.60	1.42	0.86–2.35
TNF-α RII	0.59	0.23–1.50	1.01	0.51–1.98	1.33	0.82–2.17	2.10	**1.18–3.72**
Il-6	0.77	0.48–1.24	1.04	0.76–1.42	1.30	**1.04–1.62**	1.32	**1.01–1.72**
CD14	2.01	0.60–6.79	1.40	0.76–2.56	1.07	0.75–1.52	1.00	0.67–1.47
CD163	1.93	0.73–5.11	1.78*	0.89–3.57	1.77	**1.09–2.88**	1.74*	0.96–3.18
MCP-1	0.60	0.29–1.26	0.94	0.54–3.57	1.51	1.00–2.28	1.44	0.87–2.37
D-dimer	0.92	0.65–1.29	1.13	0.87–1.46	1.11	0.91–1.35	0.94	0.75–1.19
Fibrinogen	0.94	0.49–1.81	0.74	0.41–1.33	1.81*	0.99–3.31	1.69	0.81–3.50

TAC- thoracic aortic calcification, MAC- mitral annular calcification, AVC- aortic valve calcification, AV-ring- aortic valve-ring.
Exponentiated coefficients. Bolded values are statistically significant. * = marginally significant ($p < 0.10$).
Odds ratios are per natural log increase in the biomarker. Models controlled for demographic, HIV clinical factors, and CVD risk factors. SBP values are limited to those not on hypertension medication, fasting glucose limited to those not on diabetes medication, and cholesterol values among those not on lipid-lowering medication.

Furthermore, inflammation and inflammatory cells, including monocyte activation, play a large role in vascular calcifications[37–39]. We also know that inflammation plays a significant role in HIV disease and related complications[9,40], and that HIV leads to advanced vascular aging[41].

In the Strategies for Management of Antiretroviral Therapy (SMART) study, investigators interrupted HAART therapy, which resulted in higher levels of inflammatory markers, such as IL-6, and greater CVD events as well as mortality compared to persons in whom HAART was not interrupted[9]. We found that both AV-ring calcifications and TAC were associated with elevated levels of IL-6. Furthermore, higher levels of sCD163, a marker of monocyte activation[42], were positively associated with AV-ring calcification and borderline associated with TAC. Elevation of these and other inflammatory markers could partially explain the higher prevalence of ECC in HIV+ men in our cohort. Also supporting inflammation as a potential mechanism included our findings that a history of AIDS, which has been associated with higher levels of HIV-related inflammation, was associated with a two times greater risk of AV-ring calcifications.

Another potential mechanism for the increased CVD morbidity and mortality of HIV + individuals are ART medications, especially protease inhibitors, and resulting metabolic disorders[7,8]. However, we did not find that overall HAART duration was associated with any of the ECC types. Of the specific HAART classes, years of NNRTI and NRTI were not associated with ECC. However, the presence of MAC and AVC was more likely with increasing years of protease inhibitor use, but these relationships did not meet statistical significance. It may be that cumulative use of specific protease

inhibitors increases the risk of ECC, but this sample is too small to parse out these associations.

Racial differences in ECC have also been well described[10]. Similar to previous studies from MESA on CAC[43], they found that whites had the highest prevalence of all four ECC types compared to African-Americans, Hispanics, and Chinese participants. In our cohort, we did not find an association between race and AVC, MAC, or TAC prevalence. However, African-American men had lower odds of AV-ring calcification compared to whites, which persisted after adjustment for other demographic and CVD risk factors.

Our study has several important strengths, including a large sample size with an HIV- group from a similar, ethnically diverse population. More importantly, to our knowledge, this is the first study addressing the association of ECC with HIV infection. It also includes novel results comparing the presence of ECC with coronary plaque composition. However, our study needs to be interpreted in light of its limitations. Although we statistically adjusted for demographic and CVD risk factors differences between the two populations (HIV+ and HIV−), it is possible there may be residual or unmeasured confounding. Furthermore, we measured prevalent extracoronary calcification at a single time point, and therefore the associations that we found may differ from the factors associated with its development or progression. Finally, given sex-based differences in the manifestations of subclinical cardiovascular disease, our findings may have limited generalizability to HIV+ women.

5. Conclusions

We found that HIV+ men were more likely than HIV− men to

Table 5A
Association between presence of extra-coronary calcification and presence and extent of coronary atherosclerosis in HIV+ men (N = 453[a]).

	AVC		MAC		TAC		AV-ring	
	Prevalence	OR	Prevalence	OR	Prevalence	OR	Prevalence	OR
Coronary artery calcium	75.6%***	2.2*** (1.4–3.5)	70.3%*	0.8(0.4–1.8)	82.7%***	2.8** (1.4–5.4)	78.6%***	2.5*** (1.6–4.1)
Non-calcified plaque	81.3%***	2.2**(1.2–3.8)	76%	0.9(0.5–2.1)	74.4%	1.0(0.5–2.1)	79.8%***	1.8(1.0–3.2)
Calcified plaque	55.1%***	2.3*** (1.4–3.8)	52.0%	1.1(0.5–2.6)	60.5%***	2.4*(1.2–4.7)	59.5%***	2.7*** (1.6–4.5)
Partially calcified plaque	53.3%***	1.9*(1.2–3.1)	40.0%	0.5(0.2–1.2)	62.8%***	2.5*(1.2–5.0)	53.6%***	1.7*(1.0–2.9)
Coronary Stenosis >50%	29.0%***	2.0*(1.1–3.6)	40.0%***	2.1(0.8–5.3)	32.6%**	2.0(0.9–4.3)	29.8%***	1.9*(1.0–3.4)
	Mean difference		Mean difference		Mean difference		Mean difference	
Segment Involvement Score	1.8*** (1.4–2.4)		0.9 (0.6–1.5)		1.9** (1.3–2.7)		1.8*** (1.4–2.4)	
Total Plaque Score	2.0*** (1.5–2.7)		1.0 (0.6–1.7)		2.1*** (1.4–3.1)		1.9 (1.4–2.7)	

TAC- thoracic aortic calcification, MAC- mitral annular calcification, AVC- aortic valve calcification, AV-ring- aortic valve-ring, ECC- extra-coronary calcification. Prevalence—Proportion of individuals with each plaque type among participants with each type of ECC. OR—odds ratio (adjusted for age and race). Mean diff—adjusted mean difference (adjusted for age and race). CI—95% confidence interval. For percentage or medians and interquartile ranges, the differences by presence of ECC type are evaluated by χ^2 test or Wilcoxon rank-sum test, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
 [a] n = 453 is the sample size for plaque outcomes measured by coronary CTA; n = 621 for CAC measured by CT. Due to CT scan images that did not cover the thoracic aorta, the sample size for the associations with TAC are n = 440 for plaque outcomes measured by coronary CTA and n = 607 for CAC measured by CT.

Table 5B
Association between presence of extra-coronary calcification and presence and extent of coronary atherosclerosis in HIV− men (N = 311[a]).

	AVC		MAC		TAC		AV-ring	
	Presence	OR	Presence	OR	Presence	OR	Presence	OR
Coronary artery calcium	79.0%***	3.3***(1.9—5.6)	61.5%	0.7(0.2—2.5)	87.1%***	5.6***(2.6—12.2)	81.5%***	3.8*** (2.1—6.8)
Non-calcified plaque	52.9%	0.9(0.5—1.5)	36.4%	0.4(0.1—1.5)	46.2%	0.6(0.3—1.2)	50.5%	0.8(0.5—1.4)
Calcified plaque	61.5%***	2.5***(1.5—4.4)	27.3%	0.4(0.1—1.5)	69.2%***	3.3***(1.7—6.7)	63.7%***	2.7*** (1.6—4.7)
Partially calcified plaque	54.8%***	3.4***(2.0—5.9)	54.5%	1.9(0.5—6.5)	57.7%***	2.6**(1.4—5.0)	57.1%***	3.6*** (2.1—6.4)
Stenosis >50%	24.0%***	1.9(0.9—3.9)	27.3%	1.7(0.4—6.8)	30.8%***	2.5*(1.2—5.4)	24.2%**	1.8(0.9—3.6)
	Mean difference		Mean difference		Mean difference		Mean difference	
Segment Involvement Score	1.8*** (1.3—2.4)		0.9 (0.4—1.8)		1.7** (1.2—2.5)		1.8*** (1.3—2.4)	
Total Plaque Score	2.0*** (1.4—2.8)		1.0 (0.4—2.2)		2.1*** (1.4—3.1)		2.0*** (1.4—2.8)	

TAC- thoracic tic calcification, MAC- mitral annular calcification, AVC- aortic valve calcification, AV-ring- aortic valve-ring, ECC- extra-coronary calcification. Prevalence—Proportion of individuals with each plaque type among participants with each type of ECC. OR—odds ratio (adjusted for age and race). Mean diff—adjusted mean difference (adjusted for age and race). CI—95% confidence interval. For percentage or medians and interquartile ranges, the differences by presence of ECC type are evaluated by χ^2 test or Wilcoxon rank-sum test, as appropriate. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.
  [a] n = 311 is the sample size for plaque outcomes measured by coronary CTA, n = 384 for CAC measured by CT. Due to CT scan images that did not cover the thoracic aorta, the sample size for the associations with TAC are n = 308 for plaque outcomes measured by coronary CTA and n = 381 for CAC measured by CT.

have AVC and MAC. Furthermore, AVC was associated with the presence of non-calcified plaque in HIV+ but not HIV− men. Levels of several inflammatory and immune activation biomarkers were positively associated with AV-ring calcification and TAC. Given the higher rates of CVD morbidity among HIV+ compared HIV− persons, further studies are needed to evaluate whether the increased prevalence of AVC and MAC is predictive of CVD clinical events among HIV+ persons.

Disclosures

Dr. Jacobson is a consultant for Bristol-Myers Squibb. Dr. Palella is on the speaker's bureau for Gilead Sciences, Janssen Pharmaceuticals, Merck & Co., Inc., and Bristol- Myers Squibb. Dr. Matthew Budoff has received grants from General Electric Company and National Institute of Health (NIH).

Conflicts of interest

The other authors have no conflicts of interest to disclose.

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References

1. Centers for Disease Control (U.S.). *Centers for Disease Control (U.S.). Office of the Deputy Director (HIV), and Centers for Disease Control and Prevention (U.S.), HIV/AIDS prevention newsletter.* Atlanta, GA: U.S. Dept. of Health and Human Services, Public Health Service, Centers for Disease Control, Office of the Deputy Director (HIV); 1990.
2. May MT, Sterne JA, Costagliola D, et al. Antiretroviral Therapy (ART) Cohort Collaboration. HIV treatment response and prognosis in Europe and North America in the first decade of highly active antiretroviral therapy: a collaborative analysis. *Lancet*. 2006;368:451—458.
3. Wada N, Jacobson LP, Cohen M, et al. Cause-specific life expectancies after 35 years of age for human immunodeficiency syndrome-infected and human immunodeficiency syndrome-negative individuals followed simultaneously in long-term cohort studies, 1984-2008. *Am J Epidemiol*. 2013;177:116—125.
4. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab*. 2007;92:2506—2512.
5. Tseng ZH, Secemsky EA, Dowdy D, et al. Sudden cardiac death in patients with human immunodeficiency virus infection. *J Am Coll Cardiol*. 2012;59:1891—1896.
6. Post WS, Budoff M, Kingsley L, et al. Associations between HIV infection and subclinical coronary atherosclerosis. *Ann Intern Med*. 2014;160:458—467.
7. Holmberg SD, Moorman AC, Williamson JM, et al. Protease inhibitors and cardiovascular outcomes in patients with HIV-1. *Lancet*. 2002;360:1747—1748.
8. Rossi R, Nuzzo A, Guaraldi G, et al. Metabolic disorders induced by highly active antiretroviral therapy and their relationship with vascular remodeling of the brachial artery in a population of HIV-infected patients. *Metabolism*. 2009;58:927—933.
9. Kuller LH, Tracy R, Belloso W, , et alINSIGHT SMART Study Group. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med*. 2008;5:e203.
10. Nasir K, Katz R, Takasu J, et al. Ethnic differences between extra-coronary measures on cardiac computed tomography: multi-ethnic study of atherosclerosis (MESA). *Atherosclerosis*. 2008;198:104—114.
11. Allison MA, Cheung P, Criqui MH, Langer RD, Wright CM. Mitral and aortic annular calcification are highly associated with systemic calcified atherosclerosis. *Circulation*. 2006;113:861—866.
12. Yamamoto H, Shavelle D, Takasu J, et al. Valvular and thoracic aortic calcium as a marker of extent and severity of angiographic coronary artery disease. *Am Heart J*. 2003;146:153—159.
13. Owens DS, Budoff MJ, Katz R, et al. Aortic valve calcium independently predicts coronary and cardiovascular events in a primary prevention population. *JACC Cardiovasc Imaging*. 2012;5:619—625.
14. Kalsch H, Lehmann N, Mahabadi AA, et al. Beyond Framingham risk factors and coronary calcification: does aortic valve calcification improve risk prediciton? The Heniz Nixdorf Recall Study. *Heart*. 2014;100:930—937.
15. Kaslow RA, Ostrow DG, Detels R, et al. The multicenter AIDS cohort study: rationale, organization, and selected characteristics of the participants. *Am J Epidemiol*. 1987;126:310—318.
16. Hacioglu Y, Gupta M, Choi T-Y, et al. Use of cardiac CT angiography imaging in an epidemiology study — the methodology of the multicenter aids cohort study cardiovascular disease substudy. *Anadolu Kardiyol Derg*. 2013 May;13:207—214.
17. Agatston AS, Janowitz WR, Hildner FJ, et al. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15:827—832.
18. Austen WG, Edwards JE, Frye RL, et al. A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc committee for grading of coronary artery disease, council on cardiovascular surgery, American Heart

Association. *Circulation*. 1975;51:5e40.

19. Schafer JL. *Analysis of Incomplete Multivariate Data*. New York: Chapman & Hill; 1997.

20. Greenland P, LaBree L, Azen SP, Doherty TM, Detrano RC. Coronary artery calcium score combined with Framingham score for risk prediction in asymptomatic individuals. *JAMA*. 2004;291:210–215.

21. Detrano R, Guerci AD, Carr JJ, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. *N Engl J Med*. 2008;358:1336–1345.

22. Tison GH, Guo M, Blaha M, et al. Multisite extracoronary calfication indicates increased risk of coronary heart disease and all-cause mortality: the multi-ethnic study of atherosclerosis. *J Cardiovasc Comput Tomogr*. 2015;9:406–416.

23. Fox CS, Vasan RS, Parise H, et al. Mitral annular calcification predicts cardiovascular morbidity and mortality: the Framingham Heart Study. *Circulation*. 2003;107:1492–1496.

24. Craiem D, Chironi DG, Casciaro ME, et al. Association of thoracic aorta calcium and non cardiac vascular events in cardiac disease-free individuals. *Atherosclerosis*. 2015 [Epub ahead of print].

25. Madaj P, Budoff MJ. Risk stratification of non-contrast CT beyond the coronary calcium scan. *J Cardiovasc Comput Tomogr*. 2012;6:301–307.

26. Budoff MJ, Takasu J, Katz R, et al. Reproducibility of CT measurements of aortic valve calcification, mitral annulus calcification, and aortic wall calcification in the multi-ethnic study of atherosclerosis. *Acad Radiol*. 2006;13:166–172.

27. Van der Meer IM, Bots ML, Hofman A, et al. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarctino: the Rotterdam Study. *Circulation*. 2004;109:1089–1094.

28. Ahmadi N, Nabavi V, Hajsadeghi F, et al. Mortality incidence of patients with non-obstructive coronary artery disease diagnosed by computed tomography angiography. *Am J Cardiol*. 2011;107:10–16.

29. Motoyama S, Kondo T, Sarai M, et al. Multislice computed tomographic characteristics of coronary lesions in acute coronary syndromes. *J Am Coll Cardiol*. 2007;50:319–326.

30. Barasch E, Gottdiener JS, Marino Larsen EK, Chaves PH, Newman AB. Cardiovasuclar morbidity and mortality in community-dwelling elderly individuals with calcification of the fibrous skeleton of the base of the heart and aortosclerosis (The cardiovascular health study). *Am J Cardiol*. 2006;97:1281–1286.

31. Stewart BF, Siscovick D, Lind BK, et al. Clinical factors associated with calcific aortic valve disease. cardiovascular health study. *J Am Coll Cardiol*. 1997;29:630–634.

32. Ferreira-Gonazalez I, Pinar-Sopena J, Ribera A, et al. Prevalence of calcific aortic valve disease in the elderly and associated risk factors: a population-based study in a mediterranean area. *Eur J Prev Cardiol*. 2013;20:1022–1030.

33. Allison MA, Cheung P, Criqui MH, Langer RD, Wright CM. Mitral and aortic annular calcification are highly associated with systemic calcified atherosclerosis. *Circulation*. 2006;113:861–866.

34. Dobrin PB. Mechanical factors associated with the development of intimal and medial thickening in vein grafts subjected to arterial pressure. A model of arteries exposed to hypertension. *Hypertension*. 1995;26:38–43.

35. Stary HC, Blankenhorn DH, Chandler AB, et al. A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the committee on vascular lesions of the council of arteriosclerosis, American Heart Association. *Arterioscler Thromb*. 1992;12:120–134.

36. Olsson M, Thyberg J, Nilsson J. Presence of oxidized low density lipoprotein in nonrheumatic stenotic aortic valves. *Arter Thromb Vasc Biol*. 1999;19:1218–1222.

37. Tintut Y, Patel J, Territo M, et al. Monocyte/macrophage regulation of vascular calcification in vitro. *Circulation*. 2002;104:650–655.

38. Molad Y, Levin-laina N, Vaturi M, Sulkes J, Sagie A. Heart valve calcification in young patients with systemic lupus erythematosus: a window to premature atherosclerosis vascular morbidity and a risk factor for all-cause mortality. *Atherosclerosis*. 2006;185:406–412.

39. Fox CS, Guo CY, Larson MG, et al. Relations of inflammation and novel risk factors to valvular calcification. *Am J Cardiol*. 2006;97:1502–1505.

40. Baker JV, Hullseik KH, Singh A, et al. Immunologic predictors of coronary artery calcium progression in a contemporary HIV cohort. *AIDS*. 2014;28:831–840.

41. Guaraldi G, Zona S, Alexopoulos N, et al. Coronary aging in HIV-infected patients. *Clin Infect Dis*. 2009;49:1756–1762.

42. McKibben RA, Margolick JB, Grinspoon S, et al. Elevated levels of monocyte activation markers are associated with subclinical atherosclerosis in men with and those without HIV infection. *J Infect Dis*. 2015;211:1219–1228.

43. Bild DE, Detrano R, Peterson D, et al. Ethnic differences in coronary calcification: the multi-ethnic Study of atherosclerosis. *Circulation*. 2005;111:1313–1320.