Title

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Osteoprotegerin (OPG), but not Receptor Activator for Nuclear Factor Kappa B Ligand (RANKL), is Associated with Subclinical Coronary Atherosclerosis in HIV-infected Men

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Abstract

Context—Abnormalities in the osteoprotegerin (OPG)/receptor activator of nuclear factor-κB ligand (RANKL) axis have been observed in HIV-infected persons and have been implicated in cardiovascular disease pathogenesis in the general population.

Objective—To determine associations of serum OPG and RANKL concentrations with HIV infection and subclinical atherosclerosis.

Design—Cross-sectional study nested within the Multicenter AIDS Cohort Study

Setting—Four US academic medical centers

Participants—There were 578 HIV-infected and 344 HIV-uninfected men.

Main Outcome Measures—Coronary artery calcium (CAC) was measured by non-contrast cardiac computed tomography (CT), and coronary stenosis and plaque characteristics (composition, presence and extent) were measured by coronary CT angiography. All statistical models were adjusted for traditional cardiovascular risk factors.

Results—OPG concentrations were higher and RANKL concentrations were lower among HIV-infected men compared to –uninfected men ($p<0.0001$ each). Among the HIV-infected men, higher OPG concentrations were associated with the presence of CAC, mixed plaque, and coronary stenosis > 50%, but not with plaque extent. In contrast, among HIV-uninfected men, higher OPG concentrations were associated with extent of both CAC and calcified plaque, but not

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their presence. RANKL concentrations were not associated with plaque presence or extent among HIV-infected men, but among HIV-uninfected men, lower RANKL concentrations were associated with greater extent of CAC and total plaque.

Conclusions—OPG and RANKL are dysregulated in HIV-infected men and their relationship to the presence and extent of subclinical atherosclerosis varies by HIV-status. The role of these biomarkers in CVD pathogenesis and risk prediction may be different in HIV-infected men.

Introduction

Among HIV-infected persons, increased risk of cardiovascular disease (CVD) compared to the general population has been reported, which results from the interplay of traditional CVD risk factors, highly active antiretroviral therapy (HAART) use, and HIV-related chronic immune activation[1–4]. Other novel pathways may include the tumor necrosis factor (TNF) cytokine superfamily, including the osteoprotegerin (OPG)/receptor activator for nuclear factor kappa B ligand (RANKL) cytokine axis[5–7]. OPG is a soluble glycoprotein expressed in most tissues, including by osteocytes, osteoblasts, endothelial cells, and vascular smooth muscle cells. RANKL is expressed by osteoblasts, activated T-cells and endothelial cells[8]. While the interaction of OPG and RANKL is best described in the regulation of bone turnover, the OPG/ RANKL axis has also been implicated in the pathogenesis of atherosclerosis[9].

In the general population, elevated OPG concentrations have been associated with coronary artery calcification, coronary angiographic abnormalities and cardiovascular mortality[10;11]. The relationship between circulating RANKL concentrations and coronary artery disease is less clear[711].

The relationships between OPG/RANKL, HIV infection and atherosclerosis are poorly understood. Higher OPG and lower RANKL concentrations are observed in antiretroviral (ART) treatment-naïve and ART-treated HIV-infected individuals compared to HIV-uninfected persons[12–14]. To date, only three studies have examined the relationship between OPG/RANKL and CVD among HIV-infected persons, with inconsistent findings[5;6;15].

We have previously reported that coronary artery plaque, especially non-calcified plaque, was more prevalent and extensive among HIV-infected men compared to HIV-uninfected men, independent of traditional CVD risk factors[16]. We hypothesized that perturbations in the OPG and RANKL axis would be observed the HIV-infected men in our cohort and that these biomarkers would be related to the prevalence and extent of coronary plaque.

Methods

Study Population

The Multicenter AIDS Cohort Study (MACS) in an ongoing prospective observational study of HIV-infected and HIV-uninfected men who have sex with men (MSM) in four cities in the United States (Baltimore, MD/Washington DC; Chicago, IL; Los Angeles, CA, and Pittsburgh, PA)[17]. MACS participants undergo semi-annual evaluation with standardized interviews and collection of blood for laboratory testing and for storage. In the

cardiovascular disease substudy, active MACS participants aged 40–70 years, without prior cardiac surgery or percutaneous coronary intervention and who weighed less than 300 pounds completed a non-contrast cardiac CT scan to measure coronary artery calcium (CAC). Coronary CT angiography (CTA) was also performed in men without a contrast allergy, atrial fibrillation, or impaired renal function (defined as an estimated glomerular filtration rate less than 60 ml/min/1.73 m^2 within 30 days of scanning or during any previous MACS examination), and who gave consent to contrast administration. Eligibility criteria were identical for HIV-infected and HIV-uninfected men. All participants gave informed consent to participate. The Institutional Review Board of each institution approved the study.

Clinical Parameters

Age, race/ethnicity, tobacco use and use of antihypertensive, glucose-lowering and lipid-lowering medication were obtained from the previous MACS study visit closest to the coronary scan date, generally within 6 months. Height and weight were measured and body mass index (BMI), the weight in kilograms divided by the square of the height in meters, was calculated. Blood pressure was measured as the average of two measurements obtained 5 minutes apart with the patient in the supine position. Subjects were considered hypertensive if they had a systolic blood pressure ≥140 mmHg and/or a diastolic pressure ≥90 mmHg, or self-reported use of antihypertensive medications. Lipid-lowering medication use included statin, niacin, fibrate or bile acid sequestrant drugs. Diabetes mellitus was defined as a fasting serum glucose ≥ 126 mg/dL or use of glucose lowering medications at the previous visit closest to the CT scan. Among HIV-infected men, measures related to HIV-disease and its treatment including the most recent CD4+ T lymphocyte cell count (CD4), nadir CD4, plasma HIV RNA level, history of an AIDS illness, and duration of HAART use.

Laboratory Measurements

OPG and RANKL were measured at the University of Vermont Laboratory for Clinical Biochemistry Research Laboratory (Burlington, VT) using serum collected at the substudy visit and stored at −70 degrees Celsius until analysis. OPG concentrations were determined by a sandwich enzyme-linked immunosorbent assay method (Biomedica, Salem NH). The lower limit of detection was 0.05 pmol/L and the interassay coefficient of variation ranged from 8.7–14.7%. Soluble RANKL concentrations were measured using a multiplex panel (Millipore, Billerica, MA). The assay range was from 2–40,000 pg/mL and the interassay coefficient of variation ranged from 10.1–12.5%. Additional biomarkers used in exploratory analyses described below, including interleukin-6 (IL-6), high sensitivity C-reactive protein (hsCRP), soluble(s) CD163, and sCD14, soluble tumor necrosis factor receptor (sTNFR)1 and sTNFR 2, and monocyte chemoattractant protein-1 (MCP-1) were also measured at the University of Vermont from samples obtained at the substudy visit. hsCRP was measured using the BNII Nephelometer (Siemens Healthcare Diagnostics, Deerfield, IL). IL-6 and MCP-1 were measured by chemiluminescent ELISA (R&D Systems, Minneapolis, MN and Quansys Biosciences, Logan, UT respectively). sCD163 and sCD14 were measured by ELISA (R&D Systems, Minneapolis, MN). sTNFR1 and sTNFR2 were measured with multiplexing using a Milliplex soluble cytokine receptor panel (Millipore, Billerica, MA).

Interassay coefficients of variation ranged from 4.5–5.2% for hsCRP, 7–12% for IL-6, 3.2–4.1% for MCP1, 4.6–10.8% for sTNFR1 and 4.2–7.9% for sTNFR2.

Fasting blood samples were used to measure serum levels of glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation or was measured directly in men with triglyceride levels greater than 400 mg/dL or with only a non-fasting sample available[18].

Imaging Parameters

A non-contrast cardiac CT scan was performed to measure CAC and CAC scores were computed using the Agatston method[19]. Eligible participants underwent coronary CT angiography using radiation dose reduction techniques, as previously described[20]. Plaque grading was performed according to the American Heart Association's 15-segment coronary artery classification grading system[21]. Within each segment, plaques with calcification comprising ≥ 50% of the plaque area were classified as calcified plaques, plaques with >0 but < 50% calcification were considered mixed plaques, and lesions without any calcium were classified as noncalcified plaques. Plaque size was scored as none (0), mild (1), moderate (2) or severe (3) in each coronary segment. Semiquantitative measures of overall coronary artery plaque burden were calculated. The total calcified plaque (CP) score, non-calcified plaque (NCP) score, and mixed plaque (MP) scores were the sum of the scores of identified calcified, noncalcified and mixed plaques, respectively, in the coronary segments. The total plaque score (TPS) was the sum of the CP, MP and NCP scores and thus represents the summary measure of overall plaque burden[22]. Segment stenosis was defined as 0= no plaque, 1= 1–29% (minimal) stenosis, 2= 30–49% (mild) stenosis, 3=50–69% (moderate) stenosis, or 4= ≥ 70% (severe) stenosis.

Statistical Analysis

Continuous variables are presented as means and standard deviations or medians and IQR (25%–75%). The distributions of demographic and clinical variables across subject groups were compared with the Wilcoxon rank-sum test or chi-square test, as appropriate. The distributions of OPG, RANKL, other biomarkers, and measures of plaque burden were non-normally distributed and therefore were natural log-transformed. Two individuals had OPG below the lower limit of detection; thus regression analyses were performed with left censoring. Linear regression was used to assess the relationship between OPG and RANKL with HIV-associated factors, first adjusting for age and race, then additionally for CVD risk factors (BMI, use of antihypertensive, diabetes, and lipid-lowering medications, systolic blood pressure among men not receiving antihypertensive medications, fasting glucose among those men not receiving diabetic medications, total and HDL cholesterol among men not receiving cholesterol-lowering medications, and cumulative pack years of tobacco use). Logistic regression modeling was used to investigate associations between serum OPG and RANKL concentrations and the presence of CAC, coronary stenosis > 50% and the presence of any, non-calcified, calcified, and mixed plaque. Multivariate linear regression was used to assess associations of individual biomarkers with the extent of CAC and plaque among men with plaque present, using the same adjustments as above. Stratified analyses by HIV-

serostatus were performed. Tests for differential associations between OPG, RANKL and plaque outcomes by HIV-serostatus were performed using interaction terms in the models of the full population. Associations between the OPG/RANKL ratio and plaque outcomes yielded results similar to associations observed between RANKL and plaque outcomes and are therefore not presented in this report. Multiple imputation was used for missing cardiovascular covariate data for the multivariable models, with missing values imputed five times based on the distribution of the covariates (age, race/ethnicity, HIV serostatus, BMI (missing n=18), systolic blood pressure (n=34), antihypertensive medications (n=6), diabetes medications (n=6), fasting glucose (n=24), fasting HDL(n=19), fasting total cholesterol (n=19), lipid lowering medications (n=15) and cumulative pack years tobacco use (n=4) using a Markov chain Monte Carlo method, assuming multivariate normality[23]. Analyses were performed using SAS 9.2 (SAS Institute, Cary, NC). Statistical significance was taken as $P < 0.05$.

We undertook a series of exploratory analyses among the HIV-infected men to determine associations between markers of inflammation (IL-6, hsCRP, sTNFR1, sTNFR2, MCP-1) and monocyte activation (sCD14 and sCD163), OPG and RANKL, and subclinical atherosclerosis. We first used linear regression to determine associations between each inflammatory/monocyte activation marker, OPG, and RANKL after adjustment for demographic and CVD risk factors. In the multivariate models in which there was a statistically significant relationship between OPG and measures of subclinical atherosclerosis, we sequentially added each of the inflammatory/monocyte activation markers to determine whether the associations between OPG and subclinical atherosclerosis were independent of inflammation/immune activation.

Results

There were 578 HIV-infected and 344 HIV-uninfected men who underwent a cardiac CT and OPG and RANKL measurements; 711 of these men (77%; 429 HIV-infected, 282 HIV-uninfected) additionally underwent coronary CTA. HIV-infected men were younger, more likely to be non-White, had a lower mean BMI, and were more likely to be current smokers (Table 1). HIV-infected men had lower median LDL- and HDL-cholesterol concentrations and higher triglycerides, and were more likely to be using lipid-lowering medication.

OPG and RANKL Concentrations in HIV-infected and HIV-infected Men

HIV-infected men had higher unadjusted OPG concentrations than HIV-uninfected men, and lower RANKL levels ($p<0.0001$) (Table 1). After adjusting further for age, race and CV risk factors in linear regression models, serum OPG concentrations remained significantly higher in HIV-infected men than in HIV-uninfected men ($p<0.001$), and serum RANKL concentrations remained lower ($p<0.001$).

Associations of OPG and RANKL Concentrations with HIV Clinical Characteristics

Among HIV-infected men, in models adjusted for age, race and CVD risk factors, a history of AIDS and a lower current CD4 count were associated with higher serum OPG concentrations ($p=0.002$, $p=0.051$), although the latter association was of borderline

statistical significance. Men with undetectable plasma HIV RNA (< 50 copies/ml) had significantly lower RANKL concentrations than men with detectable HIV RNA levels (p=0.001) (Table 2).

Associations of OPG and RANKL Concentrations with Coronary Plaque Among HIV-Infected Men

Associations of OPG Concentrations with Coronary Plaque—Among HIV-infected men, higher OPG levels were associated with the presence of CAC, mixed plaque, and coronary stenosis >50%, but not calcified plaque, non-calcified plaque, or any plaque (Total Plaque Score) (Table 2). Further adjustment for CD4 T-cell count and history of AIDS yielded similar results (data not shown). Given our findings regarding OPG and the presence of coronary plaque, we conducted a series of stratified analyses to determine whether the effect was more or less prominent in certain subgroups. In those with current PI use (n=205), the association between OPG and the presence of mixed plaque and stenosis >50% appeared to be greater than those not on PIs (n=224)(Mixed Plaque: PI: adjOR 3.8 (95% CI:1.3,10.7) vs no PI adjOR 1.3 (95%CI: 0.5, 3.8) and Stenosis > 50%: adjOR 5.5 (95%CI:1.2, 25.5) vs no PI adjOR 2.08 (95%CI: 0.4, 10.9)). The associations between OPG and the presence of CAC did not appear to differ by PI use (data not shown). The associations between OPG and CAC, mixed plaque, and stenosis >50% did not appear to differ depending on the use of lipid lowering therapy (data not shown). In contrast to the statistically significant findings regarding the associations and the presence of coronary plaque, there were no significant associations between OPG concentrations and extent of coronary plaque among HIV-infected men with plaque present (Table 3).

Associations of RANKL Concentrations with Coronary Plaque—RANKL concentrations were not associated with the presence or extent of any type of coronary plaque among HIV-infected men (Tables 2 and 3).

Exploratory Analyses in HIV-infected Men

OPG secretion by endothelial cells is induced by pro-inflammatory cytokines[24]. Given the observed associations between OPG concentrations and the presence of coronary plaque and stenosis > 50% among HIV-infected men, we explored whether OPG and RANKL concentrations were associated with biomarkers of inflammation and monocyte activation, and if adjustment for concentrations of these biomarkers in the statistical models altered the observed associations between OPG and coronary plaque. In models adjusted for age, race, and CVD risk factors, OPG was positively associated with IL-6, sCD163, sCD14, MCP-1, sTNFR1, sTNFR2, with the strongest correlations with sCD163 and sTNFR2 (r=0.26 for both) (Table 4). RANKL was associated, albeit modestly, IL-6, sCD163, sTNFR1, and sTNFR2. We then added each of these biomarkers individually as covariates in multivariate models that had demonstrated statistically significant associations between OPG and measures of subclinical atherosclerosis. We did not find any change in the magnitude of the associations between OPG and CAC, mixed plaque, or stenosis >50% with the addition of biomarkers in the model (data not shown).

Associations of OPG and RANKL Concentrations with Coronary Plaque Among HIV-Uninfected Men

In contrast to our findings among HIV-infected men, there were no significant associations between OPG concentrations and the presence of CAC, any plaque subtype, or coronary stenosis >50% among HIV-uninfected men (Table 2). However, higher OPG concentrations were associated with greater extent of CAC and calcified plaque) among the HIV-uninfected men with coronary plaque present (Table 3), an association which was not seen in HIV-infected men. Despite these apparent differences in the relationship between OPG and coronary plaque presence and extent by HIV serostatus, formal statistical testing of interaction did not reach statistical significance, except for the relationship between OPG and the extent of CAC (p=0.04).

Associations of RANKL Concentrations with Coronary Plaque—Similar to the findings among HIV-infected men, RANKL concentrations were not associated with the presence of any type of coronary plaque among HIV-uninfected men (Table 2). However, among HIV-uninfected men with plaque present, lower RANKL concentrations were associated with greater extent of CAC (p= 0.04) and total plaque (p=0.02) (Table 3). Formal testing of an interaction between HIV-serostatus and RANKL was statistically significant ($p < 0.05$).

Discussion

In this large cross-sectional analysis of men with and at risk for HIV infection, we found that HIV-infected men had significantly higher serum OPG concentrations and lower RANKL concentrations than HIV-uninfected men. Among HIV-infected men, higher OPG concentrations were independently associated with the presence of CAC, mixed plaque, and coronary stenosis > 50%. In contrast, RANKL was not associated with subclinical CVD among HIV-infected men. Among HIV-uninfected men, the patterns of associations between OPG, RANKL, and subclinical CVD were different; higher OPG concentrations were associated with the extent of CAC and calcified plaque, but not their presence; lower RANKL was associated with the extent of CAC and total plaque, but not their presence. Our findings suggest that the OPG/RANKL axis is dysregulated in HIV-infected persons and that the role that these biomarkers play in the pathogenesis of CVD may differ by HIV-serostatus.

In the general population, higher OPG levels have been positively associated with the presence and severity of coronary artery disease in persons with stable or suspected CVD who are undergoing coronary catheterization[10;25;26] and the progression of calcification and endothelial dysfunction in patients with diabetes[27;28]. The present study is consistent with these findings: among HIV-uninfected men, higher OPG was independently associated with the severity of coronary calcification among men with plaque present.

To our knowledge, this is the first report linking serum OPG concentrations with subclinical coronary atherosclerosis in HIV-infected persons. Several small studies have investigated the relationship between OPG and CVD in HIV-infected populations and have shown mixed results[5;6;15;29]. Similar to our study, these studies used CT angiography, but did not find a

positive association between OPG concentrations and coronary plaque. In fact, in univariate analyses, Hwang et al (n=78) found lower OPG concentrations in persons with plaque compared to those without plaque[5]. Similarly, another small study by D'Abramo et al (n=35) showed lower OPG levels in HIV-infected persons with higher-grade coronary stenosis[15]. In contrast to these findings, higher OPG concentrations have been positively associated with peripheral arterial disease in HIV-infected persons as measured by ankle-brachial indices[29]. Reasons for these disparate findings are unclear. While different sample sizes and varying degrees of adjustment for traditional CVD risk factors may have contributed, different assay kits using varied antibodies for OPG measurement as well as different biological material (plasma vs serum) make the studies difficult to compare[30].

In our study, the relationship between OPG and coronary atherosclerosis appeared to differ by HIV serostatus, with OPG associated with atherosclerosis presence in the HIV-infected men and atherosclerosis severity in the HIV-uninfected men. There is conflicting evidence as to whether OPG is a better marker of plaque initiation or progression. In a large population-based study, OPG was associated with carotid artery plaque growth but not new plaque formation[31]. Longitudinal studies using sensitive measures such as CT angiography are needed to clarify the associations between OPG and CVD development and progression and whether these relationships differ by HIV serostatus.

There is debate whether the relationship between higher OPG concentrations and atherosclerosis is causal, compensatory, or is only indirect (i.e., an epiphenomenon)[24]. In animal studies, the targeted knock-out of the OPG gene in mice was associated with aortic calcification in mice with mixed genetic background and increased atherosclerosis lesion size in atherosclerosis-prone ApoE−/− mice[32;33], suggesting that OPG plays a protective role against vascular calcification[34]. Alternatively, it has been suggested that higher OPG levels may be a marker of other biological processes that lead to atherosclerosis and vascular calcification, such as chronic inflammation. IL-1β and TNF-alpha stimulate OPG expression in endothelial and vascular smooth muscle cells, and serum levels of OPG in humans have been associated with increased markers of inflammation and chronic infection[8].

HIV infection is a chronic inflammatory condition which may be reflected in our finding that OPG concentrations were higher among HIV-infected than HIV-uninfected men. Previous studies comparing serum concentrations of OPG by HIV serostatus have been inconsistent, with some studies showing higher concentrations among HIV-infected populations[13;35] and others showing similar levels by HIV serostatus[5;6]. While multiple cell types secrete these cytokines, an immune source is plausible among HIV-infected persons since OPG concentrations decrease with ART initiation[36] and increase with interruption[37] and, as our study showed, are associated with clinical parameters of HIV infection, such as CD4 count and a history of AIDS. We also found that OPG was positively correlated with markers of inflammation and monocyte activation. However, the relationship between OPG and subclinical atherosclerosis among HIV-infected men was not altered after adjustment for these biomarkers, suggesting that other pathways may be involved.

In the present study, serum RANKL concentrations were not associated with the presence or extent of subclinical CVD in HIV-infected men, consistent with a previous study[6]. However,

our findings differ from those of Hwang et al. who found that among HIV-infected participants, lower RANKL levels were independently associated with increased CAC and calcified and non-calcified coronary plaque[5]. Nevertheless, a consistent finding across all these studies is that RANKL concentrations were lower among HIV-infected men compared to HIV-uninfected men. The mechanisms underlying and clinical significance of this finding are unclear. Among HIV-uninfected men in our study, however, lower concentrations of RANKL were associated with greater extent of CAC and total plaque among those with plaque present. We speculate that RANKL may be differentially regulated in HIV-infected persons and that this may distort the relationship between RANKL and CVD that was observed in the HIV-uninfected men. However, further work needs to be done to test this hypothesis.

Our study has several limitations. Because it was limited to men, the results may not be generalizable to women, in whom the OPG/RANKL may be regulated differently[38]. In addition, the measured serum OPG and RANKL concentrations may not be reflective of levels expressed locally in the vasculature. Finally, despite apparent differences in the magnitude of the point estimates between OPG and subclinical CVD among HIV-infected and HIV–uninfected men, few tests of interaction by HIV-serostatus were statistically significant. A larger sample size may be needed to evaluate these interactions with adequate statistical power.

In conclusion, we found that concentrations of OPG and RANKL differed by HIV serostatus among men, and that OPG concentrations was associated with coronary atherosclerosis among HIV-infected men that persisted after adjustment for CVD risk factors, measures of HIV disease severity, and markers of inflammation/monocyte activation. Further studies are needed to determine the utility of these markers to predict clinical cardiovascular events and to predict whether interventions aimed at reducing inflammation and OPG concentrations alter the development and progression of coronary atherosclerosis among HIV-infected patients.

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Table 1

Characteristics of study population

	HIV-infected N= 578	HIV-uninfected N= 344	p value
Age mean (SD), year	53.2(6.6)	55.7(7.3)	<0.0001
Race, n (%)			
Black	196(33.9)	84(24.4)	<0.0001
Body Mass Index mean (SD), kg/m^2	26.0 (4.5)	27.2(4.8)	<0.0001
Current smoker n (%)	181(31.7)	75(22.1)	0.003
Diabetes [1] n (%)	74(13.2)	32(9.5)	0.092
Fasting glucose median (IQR) mg/dL	98(90–107)	96(88–106)	0.010
Hypertension [2] n (%)	271(48.9)	150(44.9)	0.247
Lipid lowering medication use n (%)	201(35.4)	96(28.3)	0.028
Cholesterol median (IQR) mg/dl			
Total	186(159–212)	190(166–216)	0.053
HDL	46(39–55)	51(43–61)	<0.0001
LDL	103(81–127)	112(91–137)	<0.0001
Triglycerides	134(95–205)	103(75–156)	<0.0001
OPG median (IQR) pmol/L	4.8(3.9–6.0)	4.3(3.6–5.2)	<0.0001
RANKL median (IQR) pg/mL	8.3(2.6–21.2)	14.0(7.9–29.1)	<0.0001
Current CD4+ T cell count median (IQR) cells/mm^3	600(426–760)	N/A	-
Nadir CD4+ T cell count median (IQR) cells/mm^3	249(143–333)	N/A	-
Time on HAART years median (IQR)	12.4(8.8–14.1)	N/A	-
Undetectable HIV RNA level (<50 copies/ml) (%)	81%	N/A	-
Prior AIDS diagnosis(%)	14.2%	N/A	-

HDL= high density lipoprotein; LDL= low density lipoprotein

[1] fasting glucose >126 mg/dl or use of glucose lowering medications.

[2] systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 or use of antihypertensive medications.

HAART = highly active antiretroviral therapy

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Table 2

Associations of OPG and RANKL concentrations with presence of subclinical coronary atherosclerosis by HIV serostatus

	OPG				RANKL			
	HIV+		HIV−		HIV+		HIV−	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
	N=578		N=344		N=578		N=344	
CAC Model 1	**1.89(1.15,3.09)**	**0.012**	1.51(0.77,2.98)	0.23	1.09(0.95,1.26)	0.20	0.92(0.75,1.14)	0.45
Model 2	**2.0(1.17,3.39)**[a]	**0.011**	1.31(0.61,2.83)	0.49	1.12(0.97,1.3)	0.13	0.93(0.74,1.17)	0.55
	N=429		N=282		N=429		N=282	
Noncalcified Plaque Model 1	1.29(0.74,2.26)	0.37	1.25(0.67,2.33)	0.49	0.93(0.79,1.09)	0.37	0.97(0.79,1.2)	0.77
Model 2	1.26(0.71,2.24)	0.44	1.26(0.65,2.44)	0.49	0.96(0.81,1.14)	0.64	0.97(0.78,1.2)	0.75
Calcified Plaque Model 1	0.99(0.56,1.75)	0.97	1.12(0.56,2.27)	0.744	1.12(0.95,1.31)	0.17	0.83(0.66,1.04)	0.10
Model 2	1.04(0.56,1.93)	0.90	1.03(0.47,2.24)	0.94	1.16(0.97,1.38)	0.10	0.82(0.64,1.05)	0.11
Mixed Plaque Model 1	**2.19(1.15,4.2)**	**0.018**	1.33(0.61,2.92)	0.47	1.13(0.96,1.33)	0.16	1.05(0.84,1.33)	0.66
Model 2	**2.28(1.15,4.5)**[b]	**0.018**	1.28(0.52,3.13)	0.59	1.14(0.96,1.35)	0.14	1.08(0.84,1.38)	0.564
Any Plaque Model 1	1.7(0.9,3.2)	0.10	1.66(0.8,3.46)	0.18	1(0.82,1.22)	0.995	0.96(0.73,1.27)	0.7
Model 2	1.59(0.81,3.15)	0.18	1.55(0.71,3.39)	0.27	1.06(0.86,1.3)	0.59	0.97(0.73,1.31)	0.86
Stenosis>50% Model 1	**4.02(1.56,10.37)**	**0.004**	1.41(0.46,4.29)	0.55	1.12(0.91,1.37)	0.28	0.82(0.61,1.11)	0.21
Model 2	**4.31(1.52,12.18)**[c]	**0.006**	1.02(0.31,3.42)	0.97	1.16(0.93,1.43)	0.19	0.87(0.63,1.2)	0.39

Data represent odds ratio (OR) and 95% confidence interval (CI) of associations between OPG and RANKL concentrations with coronary plaque and stenosis. OPG and RANKL concentrations were natural log transformed. Model 1 adjusted for age and race. Model 2 additionally adjusted for CVD risk factors. CVD risk factors include body mass index, cumulative pack years of tobacco use, antihypertensive medication use, systolic blood pressure, diabetes medication use, fasting glucose, lipid medication use, total cholesterol, HDL cholesterol.

CAC: Coronary Artery Calcium;.

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Table 3

Association between OPG, RANKL, OPG/RANKL concentrations and extent of plaque among those men with plaque present, by HIV serostatus

	OPG				RANKL			
	HIV+		**HIV−**		**HIV+**		**HIV−**	
	β (SE)	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**
Coronary Artery Calcium	N=305		N=176		N=305		N=176	
Model 1	0.088(0.256)	0.73	**0.837(0.399)**	**0.037**	0.038(0.074)	0.61	**−0.233(0.104)**	**0.027**
Model 2	0.114(0.257)	0.66	**0.867(0.431)**	**0.044**	0.082(0.075)	0.28	**−0.22(0.107)**	**0.040**
Noncalcified Plaque	N=270		N=149		N=270		N=149	
Model 1	0.037(0.123)	0.76	0.022(0.168)	0.90	0.043(0.033)	0.20	−0.079(0.045)	0.08
Model 2	0.053(0.129)	0.68	−0.08(0.17)	0.64	0.039(0.034)	0.26	−0.072(0.044)	0.10
Calcified Plaque	N=146		N=110		N=146		N=110	
Model 1	0.175(0.169)	0.30	**0.449(0.22)**	**0.044**	0.01(0.049)	0.83	−0.116(0.064)	0.07
Model 2	0.12(0.177)	0.50	**0.507(0.226)**	**0.025**	0.025(0.05)	0.61	−0.099(0.065)	0.13
Mixed Plaque	N=148		N=91		N=148		N=91	
Model 1	0.16(0.173)	0.36	0.062(0.261)	0.81	−0.068(0.044)	0.13	−0.07(0.063)	0.27
Model 2	0.252(0.183)	0.17	0.003(0.272)	0.99	−0.082(0.046)	0.07	−0.066(0.064)	0.31
Total Plaque	N=332		N=210		N=332		N=210	
Model 1	0.109(0.128)	0.40	0.181(0.187)	0.33	0.032(0.033)	0.34	**−0.129(0.047)**	**0.007**
Model 2	0.138(0.131)	0.29	0.133(0.181)	0.46	0.04(0.034)	0.24	**−0.11(0.045)**	**0.015**

Data represent estimates and standard error (SE) for the associations between OPG and RANKL concentrations with extent of coronary plaque. Plaque outcomes and OPG and RANKL concentrations were natural log transformed. Model 1: adjusted for age and race. Model 2 additionally adjusted for CVD risk factors (body mass index, cumulative pack years of tobacco use, antihypertensive medication use, systolic blood pressure, diabetes medication use, fasting glucose, lipid medication use, total cholesterol, HDL cholesterol).

Table 4

Associations between OPG and RANKL Concentrations and Markers of Inflammation and Monocyte Activation among HIV-infected Men

	OPG		RANKL	
	r	**p**	**r**	**p**
IL-6	**0.19**	**<0.001**	**0.09**	**0.02**
hsCRP	0.07	0.09	−0.2	0.71
sCD163	**0.26**	**<0.001**	**0.09**	**0.04**
sCD14	**0.10**	**0.02**	0.01	0.77
MCP-1	**0.15**	**<0.001**	−0.01	0.79
sTNFR1	**0.19**	**<0.001**	**0.10**	**0.02**
sTNFR2	**0.26**	**<0.001**	**0.10**	**0.02**

All inflammatory and monocyte activation markers are natural log transformed. Models adjusted for age, race, and CVD risk factors (see Table 3).

IL-6: Interleukin-6;hsCRP: high sensitivity C-Reactive Protein; MCP-1:Monocyte chemoattractant protein-1; sTNFR1: soluble tumor necrosis factor alpha receptor 1; sTNFR2: soluble tumor necrosis factor alpha receptor 2