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Associations of Osteoprotegerin with Coronary Artery Calcification Among Women with Systemic Lupus Erythematosus and Healthy Controls

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Abstract

Objective—We tested the hypothesis that higher circulating levels of osteoprotegerin (OPG) are related to higher levels of coronary artery calcification (CAC) among women with systemic lupus erythematosus (SLE) compared with healthy controls (HC).

Methods—Among 611 women in 2 age- and race-matched SLE case-control studies, OPG was assayed in stored blood samples (HEARTS: plasma, n cases/controls = 122/124, and SOLVABLE: serum, n cases/controls = 185/180) and CAC was measured by EBCT.

Results—In both studies, SLE had higher OPG and CAC levels than HC. Higher OPG was associated with high CAC (>100 vs. 100) among SLE, and with any CAC (>0 vs. 0) among HC. Multivariable-adjusted OR (95% CI) for OPG tertile 3 vs. 1 was 3.58 (1.19, 10.76), p trend = 0.01 for SLE, and 2.28 (1.06, 4.89), p trend = 0.04 for HC. Associations were attenuated when age-adjusted, but remained significant for HC women aged 40 and SLE women aged 50. ROC analyses identified 4.60 pmol/l as the optimal OPG cutpoint for predicting high CAC (>100) among SLE with sensitivity = 0.74 and specificity = 0.61, overall, but 0.92 and 0.52, respectively, for SLE aged 50.

Conclusion—Our cross-sectional results suggest that higher OPG levels are related to higher CAC levels among women with SLE versus healthy controls.

Keywords

Systemic Lupus Erythematosus; Coronary Calcification; Osteoprotegerin

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory condition with a predisposition for premature cardiovascular disease¹, carotid atherosclerosis² and increased coronary artery calcification (CAC),³ a marker of coronary atherosclerosis that predicts cardiovascular events and mortality in women and men.^{4, 5} The bone-regulating protein osteoprotegerin (OPG), a decoy receptor (and inhibitor) of RANKL, is found in calcified

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atherosclerotic plaque,^{6, 7} is implicated in the pathogenesis of arterial calcification,⁸ and is associated with arterial calcification and cardiovascular events in population-based studies.^{9–12} OPG has been reported to be higher in premenopausal SLE women compared with controls.¹³ Higher circulating OPG is associated with presence of CAC among healthy postmenopausal women,¹⁴ and among SLE patients.¹⁵ Therefore, we hypothesized that higher OPG levels may partially explain higher levels of CAC in women with SLE compared with healthy controls (HC), and tested this hypothesis in women enrolled in 2 SLE case-control studies: HEARTS and SOLVABLE.

Patients and Methods

Study participants

Participants were from 2 case-control studies of SLE, Heart Effects on Atherosclerosis and Risk of Thrombosis in Systemic Lupus (HEARTS, n cases/controls = 125/124, age and race-matched) and Study of Long-term Vascular and Bone Outcomes in Lupus Erythematosus (SOLVABLE, n cases/controls = 185/184, age and race-matched). Prior reports have combined results from HEARTS and SOLVABLE,¹⁶ since, by design, the two studies used similar inclusion and exclusion criteria, similar data collection protocols including Electron Beam Computed Tomography (EBCT) scans, and used the same core laboratories at the University of Pittsburgh for laboratory assays, and for imaging training and interpretation. Details regarding each study sample are reported below.

In HEARTS, women (>18 years old) who fulfilled the revised 1982 American College of Rheumatology criteria for SLE but with no history of cardiovascular events were non-selectively recruited from the Pittsburgh Lupus Registry to participate in the HEARTS study, which was designed to compare the prevalence and risk factors of coronary artery calcification in women with SLE and healthy controls. All women with SLE had >2 years of disease duration. The Lupus registry includes women diagnosed with SLE and seen either at the University of Pittsburgh Medical Center or by rheumatologists in the Pittsburgh metropolitan area. In this study, the healthy women (controls) were matched to women with SLE by age (± 5 years) and race. The recruitment methods for healthy controls were based on (1) voters registration lists or motor vehicle license lists, depending on where the women with SLE were found in these lists, or (2) direct sample neighborhood control if the patients were not found in the previous lists.

For SOLVABLE, inclusion and exclusion criteria were similar to HEARTS and the participants in this study were recruited from the Chicago Lupus Database, administered by Northwestern University. Eligible women, aged ≥ 18 years were invited to participate, with the first 180 women to respond being enrolled in SOLVABLE. The recruitment methods for controls were based on direct sample of neighborhood lists and otherwise similar to HEARTS, although it included women with prior cardiovascular disease (CVD) events.

Study Protocol

This research was carried out according to the principles of the Declaration of Helsinki and the institutional review boards of Northwestern University, University of Illinois at Chicago,

and University of Pittsburgh approved the protocols. All study participants provided informed consent prior to enrollment. Data were collected using identical protocols at both sites for SOLVABLE and HEARTS.¹⁶ The initial study visit included anthropomorphic measurements (height, weight, waist and hip circumferences), 2 consecutive blood pressure readings with patients seated and at rest for 5 minutes, and a blood draw after a required overnight fast. Total cholesterol, triglycerides, and high-density lipoprotein cholesterol were assayed in stored blood samples from both studies at the Lipid Laboratory in the University of Pittsburgh Graduate School of Public Health, which has been Clinical Laboratory Improvement Amendments certified by the Centers for Disease Control and Prevention. C-reactive protein (CRP) was measured using immunonephelometric assay at the Laboratory for Clinical Biochemistry Research at the University of Vermont. Hypertension was defined by physician diagnosis as measured systolic blood pressure (SBP)/diastolic blood pressure (DBP) $\geq 140/90$ mm Hg, or antihypertensive medication use. Information was also collected on family history of CVD (first-degree relative having a minor stroke or myocardial infarction before 60 years of age), cigarette smoking (current, former, or never), childbearing history, and menopausal status. The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was completed by trained physicians as previously described.¹⁶

CAC Measurement

Both studies used the same protocol to measure CAC by Electron Beam Computed Tomography (EBCT) using an Imatron C-150 scanner (Imatron, San Francisco, California) and all EBCT scans were read by the same cardiologist (DE) at the University of Pittsburgh.¹⁴ As previously described,¹⁴ 3-mm slices were scanned at the same point in diastole (at 80% of the patient's RR interval on electrocardiography) during a single breath hold, starting at the aortic root to the apex of the heart. The individual region-of-interest scores were then summed for a total Agatston calcification score.

OPG Assays

OPG was assayed in stored (-80°C) EDTA-plasma in HEARTS and in stored (-80°C) serum in SOLVABLE. For HEARTS, circulating OPG was assayed in the Heinz lab at the University of Pittsburgh on stored EDTA plasma (-80°C) by ELISA (ALPCO Immunoassays, Salem, NH), as described in our previous studies.¹⁴ In brief, samples were incubated in wells coated with monoclonal anti-OPG antibody in the presence of biotinylated anti-OPG antibody for 18–24h at 4°C . The plates were washed, conjugate (streptavidin-horseradish peroxidase) added to each well, incubated for 1 hour at room temperature and then washed. Substrate (tetramethylbenzidine) was added to each well and the plates incubated for 20 min at room temperature in the dark. Stop solution was added and the absorbance read immediately at 450 nm against 620 nm. Blanks, controls and standards (0.37 to 30 pmol/L) were run with each set of samples. The intra- and inter-assay coefficients of variation were 7.6% and 7.6%, respectively.

For SOLVABLE, circulating OPG was assayed at Northwestern University on stored (-80°C) serum samples using a highly sensitive sandwich ELISA (OPG; Biomedica Gruppe). Plates were read using a Sunrise ELISA plate reader (Phoenix Pharmaceuticals, Belmont, CA). The detection limit is 0.14 pmol/l, with an intra-assay ($n = 16$) CV of $<10\%$

and an inter-assay ($n = 16$) CV of $<10\%$. OPG assays were performed in duplicate, with results reported as the mean $\pm$ SD. To assess comparability between the OPG assays used in SOLVABLE and HEARTS, 6 serum samples from SOLVABLE were sent to the Heinz Laboratory, and assayed for OPG using the ALPCO assay. Mean OPG results obtained from the 2 assays were within 1–2% of each other.

Statistical Analyses

All analyses were performed using SAS 9.3, with 2-sided tests and $\alpha = 0.05$. OPG was normally distributed, and was analyzed as continuous and categorized as tertiles, defined by the distribution among all study participants in both studies combined. This was done, to enable comparison between SLE and HC subjects at a given level of OPG as well as to evaluate associations across the range of OPG. The distribution of CAC scores was highly skewed, with a high prevalence of scores of 0 (56% of HEARTS and 71% of SOLVABLE). Therefore, CAC was evaluated as continuous (natural log $\ln(\text{CAC}+1)$), as well as categories: 0 (no calcification), 1 to less than 10 (minimal calcification), 10 to less than 100 (mild calcification) and 100 or more (moderate to extensive calcification). Key comparisons were any CAC ($\text{CAC} > 0$ vs. 0) and high CAC ($\text{CAC} \geq 100$ vs. <100).

Differences in participant characteristics by SLE vs. HC and study were assessed using *t*-tests or ANOVA for normally distributed continuous variables, Wilcoxon rank tests for non-normally distributed continuous variables, and chi-square tests for categorical variables. Associations of OPG with categorical risk factors, and of tertiles of OPG with $\ln(\text{CAC}+1)$ were assessed graphically and with analysis of covariance (ANCOVA), adjusted for Study (HEARTS vs. SOLVABLE) and SLE vs. HC. If statistically significant interactions terms indicated that the main association differed by subgroup, i.e., for SLE vs. HC, or by study, or both), associations were reevaluated in separate models, e.g. for SLE and for HC only. Associations of OPG with continuous risk factors were assessed using Spearman correlations, stratified by study and SLE vs. HC.

Associations of OPG tertiles with CAC categories (any CAC (> 0 vs. 0), and high CAC (≥ 100 vs. <100)) were assessed first using chi-squared analyses, and using logistic regression to estimate multivariable-adjusted associations of OPG tertiles (reference = lowest) with any CAC and high CAC. Covariates were selected from risk factors associated with OPG or CAC, in the literature or in the current study. Preliminary models evaluated unadjusted models separately by study. If ORs were similar for the 2 studies, they were pooled for primary analyses, adjusted for Study (Model 1). Models were sequentially adjusted for study, then race, smoking status (ever vs never), LDL-C and SBP (Model 2), and then additionally for age (Model 3.) Since younger women have a low prevalence of CAC, secondary analyses included restricting age to ≥ 40 or ≥ 50 years. Among SLE, sensitivity analyses included adjusting for SLE-related variables that correlated with OPG.

Within each study, we evaluated the extent to which higher OPG explained higher CAC among SLE compared with HC in formal mediation analysis, using the difference method.¹⁷ The proportion explained by OPG was calculated as the difference between parameter estimates for SLE vs. HC predicting $\ln(\text{CAC}+1)$, before and after adjusting for OPG, divided by the parameter estimate not adjusted for OPG $(\beta_1 - \beta_2)/\beta_1 * 100$). Mediation was

assessed for the entire study and among women aged ≥ 50 , adjusted for: a) no additional covariates, b) age, or c) age, race, smoking and LDL-C. Finally, among SLE patients, receiver operating curve (ROC) analysis was used to determine the area under the curve (AUC), the optimal cut-point to predict high CAC (by maximizing Youden's J, an index that maximizes sensitivity and specificity), and its sensitivity and specificity.¹⁸

Results

Participant Characteristics

After excluding 7 women with missing CAC scores (HEARTS: 1 SLE, 2 HC, SOLVABLE: 4 SLE), 611 women remained: HEARTS (n cases/controls = 124/122, frequency matched for age and race), SOLVABLE (n cases/controls = 180/185, frequency matched for age and race). By design, most risk factors were similar for SOLVABLE compared with HEARTS, but SOLVABLE had a younger mean age, higher prevalence of black women, and among SLE only, SOLVABLE had a higher disease activity (SLEDAI) and shorter duration of disease ($p < 0.05$ for all, Table 1). Comparing SLE with healthy controls (HC), SLE cases were younger, had a higher prevalence of diabetes, higher triglycerides, and lower total, LDL and HDL cholesterol levels, although not all of these differences reached statistical significance in both studies (Table 1.) Although mean OPG was higher in HEARTS than SOLVABLE (Table 1), in both studies, mean OPG was higher for SLE than HC ($p = 0.01$ in each study.)

Associations of CAC and OPG with demographics and risk factors

In both studies, CAC was strongly related to age, and was substantially higher for SLE than HC in age groups >40 years (Figure 1.). Among women aged <40 (32.5% of SOLVABLE and 20.9% of HEARTS) few women had any CAC (>0) and none had high CAC (≥ 100) (Figure 1). In logistic regression models adjusted for study (not significant) and age ($p < 0.001$), SLE cases were 2.34 (95%CI: 1.61, 3.41) times more likely than HC to have any CAC, and 7.37 (95%CI: 3.17, 17.17) times more likely to have high CAC (CAC ≥ 100) (not shown.)

For SLE and HC in both studies, black women had higher mean OPG levels than white women (Appendix Figure 1, $p = 0.01$ for race, adjusted for study, SLE vs. HC, and age.) OPG was correlated with older age, which reached statistical significance in SOLVABLE SLE and HC only. OPG was also significantly correlated with higher SLEDAI and lower LDL-C among HEARTS SLE, with higher disease duration and HDL-C among SOLVABLE SLE, and with higher HDL-C and total cholesterol among SOLVABLE HC.

Associations of OPG with Extent of CAC

The prevalence of higher CAC increased across OPG tertiles for HC and for SLE in each study (Figure 2), and with both studies combined (Table 2.) Among HC in both studies, higher OPG tertiles were significantly associated with any CAC (CAC > 0 vs. 0) ($p = 0.001$) and with high CAC (CAC ≥ 100 vs. <100) ($p = 0.04$), although only 9 HC women had high CAC (Table 2). Among SLE in both studies, OPG tertiles were strongly associated with presence of high CAC (≥ 100 vs. <100) ($p = 0.001$), but not significantly associated with

presence of any CAC (>0 vs. 0) ($p = 0.28$) (Table 2.) Similarly, $\ln(CAC+1)$ was higher with higher OPG tertiles for SLE and HC in both studies, ($p = 0.004$, adjusted for study and SLE vs. HC, not shown). However, at a given level of OPG, $\ln(CAC+1)$ was higher for SLE than HC ($p = 0.0006$, for SLE vs. HC, adjusted for study and OPG tertiles).

Multivariable-adjusted risk of any CAC or high CAC (OR (95%CI)) for higher tertiles of OPG were evaluated for HC and SLE in separate logistic regression models sequentially adjusted for potential confounders (Table 3). Among HC, associations with any CAC ($CAC > 0$ vs. 0) were similar in HEARTS and SOLVABLE (Table 3), so the studies were combined, adjusted for study. The OR (95%CI) for any CAC was 2.24 (1.10, 4.54), for the highest (vs. lowest) tertile of OPG, p for trend = 0.002 (Model 1). Results were similar when additionally adjusted for race, smoking, SBP and LDL-C (Model 2.) Further adjusting for age (Model 3) modestly attenuated associations, but higher OPG tertile remained significantly associated with any CAC among women aged ≥ 40 (p for trend = 0.04.) Results were similar if restricted to age ≥ 50 , (Table 3), to white women only, or if adjusted for hypertension instead of SBP (not shown.)

Among SLE, associations of OPG tertiles with high CAC ($CAC \geq 100$) were similar by study (Table 3), so the studies were combined and adjusted for study. The significant association of higher OPG tertiles with high CAC (Model 1, p for trend = 0.002) persisted when further adjusted for race, smoking, SBP and LDL-C, (Model 2, OR (95%CI) for OPG T3 vs. T1 = 3.58 (1.19, 10.76; p for trend = 0.01). Adjusting for age (Model 3) attenuated the OR (95%CI) to 2.25 (0.70, 7.26). Results were similar when restricted to age ≥ 40 only, but were much stronger and statistically significant when restricted to age ≥ 50 (OR (95% CI) = 14.44 (1.50, 139.50), p for trend = 0.005) (Table 3.) In sensitivity analyses, results were similar if further adjusted for disease duration or current disease activity (SLEDAI), or for hypertension instead of SBP, or if restricted to white women only (not shown.)

We also performed sensitivity analyses to evaluate whether the inclusion of women with prior CVD in SOLVABLE (Table 1, 10 HC and 45 SLE) affected our results. For SOLVABLE, multivariable-adjusted associations were modestly attenuated if adjusted for prior CVD for HC and SLE, but for SLE, OPG remained significantly associated with higher CAC (p for trend = 0.045), adjusted for study, race, LDL-C, smoking, SBP and prior CVD.

OPG Mediation of Higher CAC among SLE compared with HC

Next, we evaluated the extent to which higher OPG explained the higher CAC among SLE compared with HC. In logistic regression models predicting high CAC, the OR (95%CI) for SLE vs. HC was 7.37 (95%CI: 3.17, 17.17) adjusted for age and study, but decreased $\sim 15\%$, to 6.33 (2.68, 14.95), when further adjusted for OPG. In formal mediation analyses OPG explained 13.2% of higher $\ln(CAC+1)$ for SLE vs. HC in HEARTS, but only 6.3% in SOLVABLE (Supplemental Table 1.) However, restricted to women aged ≥ 50 , OPG explained $\sim 15\%$ of higher $\ln(CAC+1)$ for SLE vs. HC in both studies. In both studies, adjusting for age (Supplemental Table 1) substantially reduced the %CAC explained by OPG. However, among those aged ≥ 50 years, after adjusting for age, OPG explained 13.5% (HEARTS) and 10.9% (SOLVABLE) of the higher CAC for SLE compared with HC.

Similar results were seen with additional adjustment for race, smoking, and LDL-C (not shown).

Optimal OPG Cutpoint to Detect High CAC in SLE

Finally, we evaluated the optimal OPG cutpoint to identify high CAC among SLE, by maximizing Youden's J in ROC curve analyses¹⁸ (Supplemental Table 2). With both studies combined, the optimal OPG cutpoint to predict high CAC was 4.60, AUC = 0.67, sensitivity (Se) = 0.74 and specificity (Sp) = 0.61. Restricted to age ≥ 50 , the optimal cutoff remained 4.60 pmol/l, with slightly higher AUC (0.72) and Se (0.92), but lower Sp (0.52).

Specifically, among women with SLE aged ≥ 50 , 57/100 had OPG ≥ 4.60 pmol/l, of whom 21 (37%) had high CAC, vs. 3/43 (7%) of those with OPG < 4.60 pmol/l. Calculated separately by study, for SOLVABLE SLE age ≥ 50 , the optimal cutoff was 4.60 pmol/l, AUC = 0.85 with Se = 0.92 and Sp = 0.75, whereas for HEARTS SLE age ≥ 50 , the optimal cutpoint was higher, 5.85 pmol/l, with AUC = 0.68, Se = 0.83 and Sp = 0.62.

Discussion

The pathogenesis of premature coronary calcification and atherosclerotic cardiovascular events in SLE patients¹ is not well understood. This is the first report to demonstrate that higher OPG levels may partially explain the higher CAC among women with SLE in comparison to healthy controls, and to identify an optimal OPG cutpoint to identify high CAC (>100) among SLE women aged ≥ 50 . These results suggest that OPG may be clinically useful to identify increased risk for high CAC among women with SLE. Furthermore, these results suggest a link between higher OPG levels and higher CAC levels among women with SLE compared with healthy control women. Additional studies are needed to test whether cross-sectional associations of circulating levels of OPG with CAC levels reflect causal effects of OPG on the endothelium or arterial wall. If OPG and CAC are causally linked, interventions that affect the OPG-RANKL axis may prevent or slow development of CAC among high risk women with SLE as well as adults without SLE.

The novel findings we report, in two established SLE case control studies, is that OPG and CAC were higher among women with SLE than HC, and higher OPG was significantly associated with any CAC (CAC > 0 vs. 0) among HC, and with high CAC (CAC ≥ 100 vs. < 100) among SLE, independent of race, smoking and LDL-C. Furthermore, adjusting for OPG modestly attenuated the association of SLE versus HC with high CAC, and explained 10–15% of the association of SLE with higher Ln (CAC+1). Finally, this is the first report to identify an optimal OPG cutpoint for CAC among SLE, 4.60 pmol/l, which identified high CAC (>100) among women with SLE aged ≥ 50 with high sensitivity = 0.92, but low specificity = 0.52. Replication in other studies is needed. Furthermore, comparisons with prior publications suggest that standardization of OPG assays may be needed for OPG to be clinically useful as a risk-stratifying tool.

OPG was higher with older age among SLE and HC in our studies, as in other studies,^{10, 15} and associations were attenuated when adjusted for age but remained significant for women aged ≥ 40 for HC (any CAC), or aged ≥ 50 for SLE (high CAC). We have previously shown that higher serum OPG was related to the presence of CAC independent of age in 2 studies

of healthy, primarily white postmenopausal women (aged >50).¹⁴ The higher age cutoff for SLE than HC appears to be related to the greater extent of CAC among SLE compared to HC in a given age-group. Our results build on the few prior studies that measured OPG in SLE. An SLE case-control study among younger premenopausal women found higher serum OPG levels for SLE than controls.¹⁹ OPG was associated with older age and SLEDAI, as in our study, but also with higher LDL-C, carotid intima-media thickness (cIMT) and antiphospholipid antibodies.¹³ In another SLE case-control study, serum OPG was positively associated with age and disease duration, but OPG was lower among SLE compared with controls.²⁰ Another study of 66 SLE patients did not find an association of OPG with cIMT, but did find higher OPG and cIMT for postmenopausal vs. premenopausal women.²¹ Most recently, among 201 SLE patients (mean age = 45), OPG was positively associated with CAC, carotid IMT and carotid plaque, but these associations were not statistically significant after adjusting for age.¹⁵ That study did not assess whether associations of OPG with subclinical atherosclerosis were stronger if restricted to women aged ≥ 50 years, as seen among women with SLE in our 2 studies.

Associations of higher OPG with higher levels of CAC and/or aortic calcification, atherosclerosis and events have also been reported in studies of adults without SLE, including the Dallas Heart Study,²² Tromso Study²³, Framingham Heart Study,¹⁰ and others.^{9, 11, 24, 25} Furthermore, several studies have found that higher OPG is associated with incident cardiovascular events^{9, 10, 24} and mortality.^{10, 26} Higher OPG levels have also been shown to be associated with progression of CAC, among patients on dialysis,^{27, 28} and adults with type 2 diabetes,²⁴ but have not yet been evaluated among SLE patients. In SOLVABLE, OPG was associated with longer disease duration as well as with age, suggesting that longitudinal studies are needed to understand the long-term effects of elevated OPG in the blood.

Finally, among SLE and HC in both studies, OPG was higher for black women compared with white women. This agrees with a prior report showing higher OPG for black women and men compared with their white counterparts.²⁹ Race did not explain current study results, which were similar if restricted to white women only. However, a prior combined analysis of HEARTS and SOLVABLE SLE found that CAC was not different by race, despite many studies showing lower CAC among black compared with white adult, non-SLE populations.¹⁶ Higher OPG levels in black versus white women deserves further assessment in a larger, racially diverse study population, especially because disparities in lupus outcomes between blacks and whites have been described.³⁰

Our results should be interpreted carefully considering the strengths and limitations of our study. Strengths include the fact that the 2 studies were designed to use similar enrollment criteria and study protocols, including the measurement and interpretation of CAC, which was standardized, and read at the single center thereby, reducing variability in measurement. Limitations include the use of 2 case-control studies that differed in age and racial composition, the use of different biospecimens (serum vs. plasma), laboratories, and assays for OPG, as well as the cross-sectional nature of the data. However, despite these differences between studies, OPG associations with age, race and CAC were similar in both studies. Furthermore, as noted, our results agree with prior studies among SLE and the general

population. The high prevalence of CAC = 0 among these relatively young women also presented analytic challenges. Hence we performed analyses restricted to women >40 and >50 years, since CAC prevalence increases with age thereby increasing the ability to show associations between OPG and CAC, as shown here. The optimal cutoff for identifying risk of high CAC among women with SLE aged ≥ 50 was 4.60 pmol/l in SOLVABLE and in HEARTS and SOLVABLE combined, but 5.85 pmol/l in HEARTs, which had older participants. Given variation in reported OPG levels by assay, age, sex and race, the clinical utility and optimal cutpoint for OPG among women with SLE requires validation in additional studies as well as improved standardization across assays. Finally, clarification of the relationship of blood levels of OPG with OPG expression or activity in the vessel wall is required to further our understanding of the OPG-RANKL axis in vascular calcification.

In conclusion, in 2 SLE case-control studies, higher OPG was associated with SLE, age, smoking and black race, but also with higher CAC among SLE cases and controls. Higher OPG explained an estimated 10–15% of higher CAC for SLE compared with HC, even after adjusting for age. Replication is needed to validate the utility of OPG ≥ 4.60 pmol/l to identify high CAC (≥ 100) among SLE women aged ≥ 50 . Longitudinal and intervention studies are needed to determine whether OPG is causally related to the increased CAC among SLE patients, to evaluate possible interventions, and to evaluate potential differences by race.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

Appendix Figure 1. Distribution of OPG by Study, SLE vs. HC and Race

Mean OPG was higher for black than white women ($p = 0.01$) adjusted for study, SLE vs. HC, and age.

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Figure 1. Distribution of CAC Categories by Study (HEARTS and SOLVABLE), SLE vs. HC and by Age group

Bars show % CAC categories within each category defined by combinations of study, SLE vs. HC and Age group. CAC categories defined as 0 (no calcification), 1 to less than 10 (minimal calcification), 10 to less than 100 (mild calcification) and 100 or more (moderate to extensive calcification). Age groups are defined as <40, 40–49, 50–59 and ≥60. In logistic regression models adjusted for study and age, the OR (95%CI) for SLE vs. HC is 2.34 (1.61, 3.41) for any CAC and 7.37 (3.17, 17.17) for high CAC (≥100 vs. <100.) For both outcomes, age was significantly associated with CAC, and study was not.

Figure 2. Distribution of CAC Categories by Study (HEARTS and SOLVABLE), SLE vs. HC, and OPG Tertiles

Tertiles of OPG are defined by both studies combined, as T1 = <3.44, T2 = 3.44–4.80, and T3 >4.80. Bars present % of each CAC category (defined as for Figure 1) within each tertile of OPG, by SLE vs. HC, and study.

Table 1

Participant Characteristics by Study and SLE versus Healthy Controls

Characteristic	HEARTS n = 246			SOLVABLE n = 365		
	HC (N = 124)	SLE (N = 122)	p [*]	HC (N = 185)	SLE (N = 180)	p [*]
Age, years ^{a,b}	51.5 ± 9.5	49.4 ± 10.0	0.09	45.8 ± 10.3	43.0 ± 10.6	0.01
Postmenopausal, n (%) ^{a,b}	61 (50.0)	54, 56.5	0.31	61 (33.0)	67 (37.2)	0.40
Black women, n (%) ^{a,b,c}	11 (9.0)	15 (12.2)	0.42	52 (31.7)	49 (30.6)	0.83
Type 2 Diabetes, n (%) ^a	0.0	7 (5.6)	0.008	6 (3.2)	14 (7.8)	0.06
Smoking %						
Never	68 (55.7)	84 (67.7)	0.13	109 (58.9)	106 (58.9)	0.79
Former	41 (33.6)	28 (22.6)		57 (30.8)	54 (30.0)	
Current	13 (10.7)	12 (9.7)		19 (10.3)	20 (11.1)	
BMI, kg/m ²	28.7 ± 7.0	27.7 ± 6.2	0.22	28.8 ± 7.3	27.8 ± 7.4	0.19
Triglycerides, mg/dl	114 ± 54	132 ± 82	0.13	113 ± 80	127 ± 81	0.02
HDL-C, mg/dl	58 ± 16	54 ± 16	0.08	60 ± 17	55 ± 15	0.002
LDL-C, mg/dl	122 ± 35	109 ± 35	0.005	117 ± 36	106 ± 32	0.003
Total Chol, mg/dl	202 ± 38	189 ± 40	0.009	200 ± 38	187 ± 38	0.001
SBP, mmHg ^a	123 ± 18	119 ± 19	0.09	119 ± 15	118 ± 15	0.53
Hypertension, n (%)	27 (22)	57 (46)	<.0001	45 (24)	91 (51)	<.0001
CRP, mg/dl	3.7 ± 5.4	4.6 ± 6.0	0.24	3.0 ± 4.5	4.2 ± 10.4	0.13
History of CVD, n (%) ^{a,b,d}	n/a	n/a		10 (5.4)	45 (25.0)	<.0001
SLEDAI ^b	n/a	1.9 ± 2.3		n/a	4.1 ± 3.7	
Disease Duration, yrs ^b	n/a	16.2 ± 7.4		n/a	12.0 ± 8.7	
OPG ^{a,b} pmol/L	4.9 ± 1.7	5.8 ± 2.8	0.01	3.6 ± 1.3	4.2 ± 2.2	0.01

Variables are reported as mean, standard deviation, or if indicated, as n (%).

^{*} p for difference between HC and SLE.^a indicates variables that differ significantly (p<0.05) between HEARTS and SOLVABLE among HC and^b indicates variables that differ significantly (p<0.05) between HEARTS and SOLVABLE among SLE.^c Race was missing for 1 SLE in HEARTS and for 21 HC and 20 SLE in SOLVABLE.

$p_{\text{Prior CVD}}$ was an exclusion for HEARTS, but not SOLVABLE.

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Table 2
Distribution of CAC categories by OPG tertiles among Healthy Controls and SLE cases, both studies combined

OPG Tertiles (T)					
		T1	T2	T3	
OPG range (pmol/L)		<3.44	3.44–4.80	>4.80	p
<hr/>					
HC (Both Studies)		n	109	117	81
CAC categories		n (%)	n (%)	n (%)	
0		88 (80.7)	82 (70.1)	48 (59.3)	0.001 *
1–9		18 (16.5)	18 (15.4)	16 (19.8)	
10–99		3 (2.8)	12 (10.3)	13 (16.1)	
100		0 (0)	5 (4.2)	4 (4.9)	0.04 **
<hr/>					
SLE (Both Studies)		n	96	85	123
CAC categories		n (%)	n (%)	n (%)	
0		60 (62.5)	51 (60)	68 (55.3)	0.28 *
1–9		20 (20.8)	16 (18.8)	20 (16.3)	
10–99		11 (11.5)	12 (14.1)	12 (9.8)	
100		5 (5.2)	6 (6.8)	23 (18.4)	0.001 **

P values from χ^2 analyses for

* CAC >0 vs. CAC = 0 and for

** High CAC (100 vs. <100).

Table 3

Multivariable-adjusted OR (95%CI) for CAC by OPG Tertiles for HC and SLE

OPG Tertiles (range, pmol/l)		T1 (<3.44)	T2 (3.44-4.80)	T3 (>4.80)	
n		109	117	81	
Healthy Controls, Outcome: Any CAC (CAC>0 vs. 0)					
Model	Sample	OR	OR (95% CI)	OR (95% CI)	p trend
1	HEARTS only	1.0 (ref)	1.25 (0.35, 4.51)	2.22 (0.62, 8.02)	0.11
1	SOLVABLE only	1.0 (ref)	1.67 (0.77, 3.63)	1.97 (0.77, 5.04)	0.11
1	Both studies	1.0 (ref)	1.48 (0.77, 2.86)	2.24 (1.10, 4.54)	0.03
2	Both studies	1.0 (ref)	1.63 (0.80, 3.31)	2.28 (1.06, 4.89)	0.04
3	Both studies	1.0 (ref)	1.42 (0.68, 2.94)	1.95 (0.89, 4.28)	0.09
3	Both studies, age 40+	1.0 (ref)	1.71 (0.75, 3.87)	2.43 (1.01, 5.84)	0.049
3	Both studies, age 50+	1.0 (ref)	0.99 (0.36, 2.75)	1.79 (0.60, 5.32)	0.23
SLE Cases Outcome: High CAC (100 vs. <100)					
Model	Sample	OR	OR (95% CI)	OR (95% CI)	p trend
1	HEARTS only	1.0 (ref)	1.10 (0.06, 18.64)	4.53 (0.56, 36.58)	0.08
1	SOLVABLE only	1.0 (ref)	1.39 (0.36, 5.43)	4.72 (1.38, 16.12)	0.01
1	Both studies	1.0 (ref)	1.33 (0.39, 4.53)	4.72 (1.65, 13.54)	0.002
2	Both studies	1.0 (ref)	1.04 (0.30, 3.67)	3.58 (1.19, 10.76)	0.01
3	Both studies	1.0 (ref)	0.77 (0.20, 3.00)	2.25 (0.70, 7.26)	0.10
3	Both studies, age 40+	1.0 (ref)	0.71 (0.18, 2.77)	2.10 (0.65, 6.77)	0.12
3	Both studies, age 50+	1.0 (ref)	2.19 (0.17, 28.81)	14.44 (1.50, 139.50)	0.005

Model 1: adjusted for study. Model 2: adjusted for study, race, ever smoking, LDL-C and SBP. Model 3: Adjusted for Model 2 covariates and age.