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Serum Osteoprotegerin is Increased and Independently Associated with Coronary-Artery Atherosclerosis in Patients with Rheumatoid Arthritis

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Abstract

Osteoprotegerin (OPG), a soluble decoy receptor for receptor activator of nuclear factor B ligand, is implicated in the pathogenesis of atherosclerosis. Patients with rheumatoid arthritis (RA) have inflammation and increased atherosclerosis. We examined the hypothesis that OPG concentrations are increased in patients with RA and are associated with coronary-artery atherosclerosis. Serum OPG concentrations were measured by ELISA and coronary-artery calcification by electron beam computer tomography in 157 patients with RA and 87 control subjects. OPG concentrations were higher in patients with long-standing RA (n=67) [median (interquartile range)]: [1895 (1337–2847) pg/mL, and early RA (n=90): [1340 (1021–1652) pg/mL, than controls 1068 (692–1434) pg/ml; (P<0.001)]. In patients with RA, OPG concentrations were associated with erythrocyte sedimentation rate (p<0.001), homocysteine (p=0.001), disease duration (p=0.02), coronary calcium score (p=0.03), and cumulative dose of corticosteroids (p=0.04) after adjustment for age and sex. In patients with long-standing RA, OPG was associated with coronary artery calcification independently of cardiovascular risk factors and disease activity [OR for every increase in 500 pg/mL of OPG = 2.22 (1.43–3.34), p<0.001]. In conclusion, OPG concentrations are increased in patients with RA and are associated with inflammation. In patients with long-standing disease, OPG is independently associated with coronary-artery calcification.

Keywords

rheumatoid arthritis; osteoprotegerin; atherosclerosis; coronary calcification; cardiovascular disease

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by increased mortality largely attributable to cardiovascular disease.¹ Chronic inflammation plays an important role in the pathogenesis of both atherosclerosis and RA,^{2, 3} and there is increasing evidence from controlled clinical studies that patients with RA have accelerated atherosclerosis.^{4, 5} The mechanisms for this are unclear, but mediators related to both inflammation and atherosclerosis are attractive candidates. One such candidate is osteoprotegerin (OPG).

OPG, a member of the tumor necrosis factor (TNF) receptor super-family, is a naturally occurring decoy receptor for receptor activator of nuclear factor κ B ligand (RANKL), and its expression and production are regulated by several inflammatory cytokines including interleukin-1 and TNF- α .^{6, 7} OPG binds RANKL, preventing stimulation of RANK and decreasing osteoclast differentiation, activation and survival, thus regulating bone resorption.^{6, 7} However, skeletal bone loss and vascular calcification share mechanistic pathways, and OPG plays a role in both.⁸ Targeted deletion of the OPG gene in mice results, not only in severe osteoporosis, but also in arterial calcification.⁹

Coronary artery calcification is a risk factor for plaque rupture¹⁰ and is associated with an increased risk of future cardiovascular events.¹¹ Recent clinical studies in the general population demonstrated that increased concentrations of OPG were associated with the presence and severity of coronary artery disease.^{12, 13} Serum concentrations of OPG are also elevated in patients with RA and decrease after anti-TNF therapy.¹⁴ The relationship between OPG concentrations and accelerated atherosclerosis in patients with RA has not been established, nor has this relationship been examined early in the course of RA – a time when prediction of atherosclerosis or identification of novel risk markers of atherosclerosis is particularly important. Therefore, we examined the hypothesis that patients with early and long-standing RA have increased concentrations of OPG, and that OPG is associated with coronary artery atherosclerosis.

Methods

Study Subjects

We studied 157 patients with RA and 87 control subjects. Patients were recruited from clinical RA cohorts, an early RA registry, local rheumatologists, and by advertisements. Enrolled patients were over the age of 18 years and fulfilled the classification criteria for RA. These patients are part of an ongoing study to identify cardiovascular risk factors in RA.⁴ In order to determine if any findings occurred early in the disease process, and thus might play a role in pathogenesis, we recruited two groups of patients: those with early RA (<6 years of disease) and those with long-standing RA (>10 years of disease). Control subjects were recruited from patients' acquaintances, by advertisement, and from a database of volunteers maintained by the General Clinical Research Center. Control subjects did not have RA any other inflammatory disease and were frequency matched for age, sex, and race with the entire group of RA patients. Current and cumulative use of medications was determined by combining the information provided by patients and medical records. The study was approved by the Institutional Review Board of Vanderbilt University Hospital and all subjects gave written informed consent.

Clinical assessment

Information was obtained through interview, review of medical records, self-reported questionnaires, physical examination, laboratory tests, and electron-beam CT. A structured interview was performed to obtain information about age, race, medical history, family history

of cardiovascular disease, and use of medications. A family history of coronary artery disease was defined as a first degree relative having a myocardial infarction or stroke before the age of 55 in males, and before the age of 65 in females. Height and weight were measured and body mass index, the weight in kilograms divided by the square of the height in meters, calculated. Blood pressure was determined as the average of 2 measurements obtained 5 minutes apart after subjects had rested in the supine position for at least 10 minutes. Subjects were considered to have hypertension if they were taking antihypertensive agents or if they had a systolic blood pressure of ≥ 140 mmHg and/or a diastolic blood pressure of ≥ 90 mmHg. In patients with RA, disease activity was measured using the Disease Activity Score based on the evaluation of 28 joints (DAS28).¹⁵ The DAS 28 is a validated composite index containing a 28 joint count for tenderness, a 28 joint count for swelling, the erythrocyte sedimentation rate (ESR), and the patient's overall assessment of well-being. Patients were asked to indicate on a 10 cm visual analog scale (VAS) the point that best described their fatigue and pain, with higher scores indicating more severe symptoms. The modified Health Assessment Questionnaire (MHAQ), a standard 8-question instrument, was used to assess functional capacity based on the difficulty in performing activities of daily living. This questionnaire is scored from 0 to 3, with higher scores indicating lower functional capacity.

Determination of serum OPG concentrations

Blood samples were collected from all subjects after an overnight fast. Serum was separated by centrifugation of blood samples and stored at -70°C until assayed. OPG concentrations were determined by enzyme-linked immunosorbent assay method using commercial kits according to the supplier's instructions (R&D systems, Minneapolis, MN, USA). Monoclonal mouse anti-human OPG antibody was used as a capture antibody and a biotinylated polyclonal goat anti-human OPG antibody was used for detection. The mean coefficient of variation was 4.3%.

Other laboratory tests

Fasting blood samples were collected from all subjects for a complete blood cell count and determination of creatinine, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, lipoprotein (a) [Lp (a)], homocysteine, and glucose, and low-density lipoprotein (LDL) cholesterol was calculated. In patients with RA, Westergren erythrocyte sedimentation rate (ESR) and concentrations of C-reactive protein (CRP) were measured.

Coronary-artery calcification

All subjects underwent imaging with an Imatron C-150 scanner (GE/Imatron, South San Francisco, CA). Imaging was performed with a 100-msec scanning time and a single-slice thickness of 3 mm. A total of 40 slices were obtained during a single breath-holding period. Tomographic imaging was electrocardiographically triggered at 60 percent of the RR interval. All areas of calcification within the borders of a coronary artery with a minimal attenuation of 130 Hounsfield units were computed. A calcified coronary plaque was considered present if at least three contiguous pixels were measured (voxel size: 1.03mm^3). If a patient had undergone prior coronary-artery stenting or bypass surgery, the stent and all metal clips were excluded from the computation of the coronary calcium score. The acquired images were reviewed at the imaging laboratory on a NetraMD workstation (ScImage, Los Altos, CA). Patients were included in this study only if complete data were available from their scans, without misregistration of slices due to artifacts of motion, respiration, or asynchronous electrocardiographic triggering. To ensure the continuity and consistency of the interpretation of scores, a single expert investigator (PR), blinded to the subjects' clinical status, reviewed all the scans. The extent of coronary-artery calcification was calculated as described by Agatston et al.¹⁶ by multiplying the area of each calcified lesion by a weighting factor

corresponding to the peak pixel attenuation for each lesion to yield a lesion-specific calcium score. The sum of all individual plaque scores represented a patient's total calcium score.

Statistical Analysis

It is estimated that a sample size of at least 62 in each group will have 90% power to detect a difference in mean of 200 pg/ml, assuming a common standard deviation of SD=340pg/ml (based on published data of subjects without coronary disease)¹² and using a two group t-test with a 0.05 two-sided significance level.

Statistical analyses were done in two phases. First, differences in baseline characteristics among control subjects and patients with early and long-standing RA were assessed with Kruskal-Wallis tests for continuous variables and Chi-square tests for categorical variables. A multivariable general linear model was used to examine the association between serum OPG and RA; logarithmic transformation of serum OPG was applied to achieve normality of the residuals. Covariates for adjustment chosen *a priori* included age, sex, homocysteine, HDL cholesterol, LDL cholesterol, triglycerides, Lp(a) lipoprotein, hypertension, body mass index, diabetes, pack-years of smoking, creatinine and family history of coronary disease.

The second phase of the analyses included only patients with RA and examined the association between coronary-artery calcium score and serum OPG in early and long-standing RA. Spearman rank correlation coefficients were used to assess the univariate association between serum OPG and continuous variables. For categorical variables, serum OPG concentrations were compared using Wilcoxon rank sum tests, and then multiple linear regression was used to adjust for age and sex. Because of the skewed distribution of calcium scores, non-linearity was assessed and it was included as a flexible smooth parameter (restricted cubic spline). Linear test for trend on the effect of coronary artery calcium score as an ordered continuous variable was calculated using multiple linear regression to compare log-transformed OPG in the categories of coronary-artery calcium graded as none (calcium score =0), mild (calcium score =1–125) or moderate-to-severe (calcium score >125).

A proportional odds logistic regression model for an ordinal categorical outcome variable of categories of coronary-artery calcium was used to examine the independent effect of serum OPG concentrations among RA patients stratified by early and long-standing RA. The proportional odds assumption was assessed with the score test. Covariates included strong cardiovascular risk factors: age, sex, race homocysteine, diabetes, pack-years of smoking, and hypertension. To ensure regression power, HDL and LDL cholesterol, triglycerides, Lp(a) lipoprotein were combined using principal component methods into one factor (cholesterol profile) and included in the model. Data are expressed as median and interquartile range (IQR) and a two sided 5% significance level was regarded as statistically significant. Statistical analyses were performed using R 2.1.0 (The R Project for Statistical Computing) and SAS 9.1 (SAS Institute Cary, NC).

Results

Characteristics of patients with RA and control groups

Clinical characteristics and cardiovascular risk factors for the cohort have been reported previously⁴ and are summarized in Table 1. Patients with long-standing RA were older with a median age of 59 (IQR 48–67) years compared with 51 (43–60) years in patients with early RA. The median disease duration was 2 (1–3) years in patients with early RA and 20 (14–24) years in patients with long-standing RA. In the control group, 9% were current smokers compared to 21% and 25% in patients with early and long-standing RA, respectively ($p=0.02$). Exposure to smoking in pack-years was similar among the three groups. The prevalence of

hypertension was 67.2% in patients with long-standing RA, 40.0% in patients with early RA and 39.1% in control subjects ($P<0.001$). There were no significant differences in family history of coronary artery disease, coronary artery procedure (angiography, stent, or bypass graft) and menopausal status among the three groups. Concentrations of creatinine, total cholesterol, HDL and LDL cholesterol, Lp(a), triglycerides and blood glucose were similar among patients and control groups. The median coronary-artery calcium score was 63 (0–368) Agatston units in patients with long-standing RA compared with 0 (0–47) in those with early RA and 0 (IQR 0–18) in controls ($p<0.001$).

Disease characteristics in patients with early and long-standing RA

There was no significant difference in disease activity as determined by the DAS 28 [median (IQR), 3.1 (2.0–4.0) in patients with early RA and those with long-standing RA 3.4 (2.6–4.4) ($p=0.13$)]. ESR and concentrations of CRP were similar in patients with early and long-standing RA. Sixty-eight percent of patients with early and 74% of those with long-standing RA tested positive for rheumatoid factor ($p=0.40$). As expected, cumulative exposure to medications for treatment of RA (cumulative dose of corticosteroids, hydroxychloroquine, or methotrexate) was greater in patients with long-standing RA than those with early RA.

Association between osteoprotegerin and rheumatoid arthritis

The median concentrations of OPG were 1895 pg/mL (IQR 1337–2847) in patients with long-standing RA, 1340 pg/mL (IQR 1021–1652) in those with early RA, and 1068 pg/mL (IQR 692–1434) in control subjects ($p<0.001$). The association between serum OPG concentrations and RA was significant in patients with early ($\beta=0.24$, 95% CI 0.09–0.39, $p=0.001$) and long-standing RA ($\beta=0.62$, 95% CI 0.46–0.78, $p<0.001$) compared to control subjects (Table 2). After adjustment for age, race, sex, and cardiovascular risk factors (hypertension, pack-years of smoking, body mass index, diabetes, family history of coronary artery disease, homocysteine, HDL and LDL cholesterol, triglycerides, Lp(a) and creatinine), the association remained significant in both patients with long-standing [$(\beta=0.47$, 95% CI 0.31–0.62, $p<0.001$) and those with early RA ($\beta=0.25$, 95% CI 0.11–0.39, $p<0.001$)].

Association between osteoprotegerin and clinical characteristics in patients with RA

OPG concentrations were higher in women ($p=0.001$), in patients with hypertension ($p<0.001$), and in postmenopausal patients ($p=0.007$). There were no significant differences in OPG concentrations in relation to race ($p=0.36$), smoking ($p=0.60$), history of coronary disease ($p=0.80$), history of any coronary artery procedures ($p=0.40$), and presence of diabetes ($p=0.49$). Table 3 shows the correlation between concentrations of OPG and demographic and disease characteristics in patients with RA ($n=157$). OPG concentrations were significantly correlated with age ($\rho=0.39$, $p<0.001$), systolic blood pressure ($\rho=0.28$, $p<0.001$), disease duration ($\rho=0.37$, $p<0.001$), homocysteine concentration ($\rho=0.28$, $p<0.001$), coronary artery calcium score ($\rho=0.27$, $p<0.001$), DAS28 ($\rho=0.21$, $p=0.01$) and markers of inflammation such as ESR ($\rho=0.31$, $p<0.001$), CRP ($\rho=0.16$, $p=0.04$), and cumulative exposure to corticosteroids ($\rho=0.15$, $p=0.05$) and were inversely correlated with hemoglobin ($\rho=-0.27$, $p<0.001$). After adjustment for age and sex, OPG remained significantly associated with increased concentrations of homocysteine, severe coronary artery calcification, higher levels of CRP, and ESR, increased disease activity determined by DAS28, and with lower hemoglobin concentrations. OPG concentrations were not associated with cumulative exposure to hydroxychloroquine, or methotrexate.

Association between osteoprotegerin and coronary-artery calcification in patients with RA

Figure 1 shows that serum concentrations of OPG increased significantly with higher coronary-artery calcium scores (no calcium: score =0, mild calcium score: 1–125 Agatston units,

moderate-to-severe calcium score: >125 Agatston units), among all three groups -controls, patients with early and patients with long-standing RA (p-value for trend for long-standing RA=0.003).

As shown in Figure 2, increased serum OPG concentrations were associated with severity of coronary artery calcification in patients with long-standing RA (OR 1.64, 95% CI: 1.23–2.17, $p<0.0001$). After adjustment for age, race, sex, homocysteine, cholesterol profile (HDL and LDL cholesterol, triglycerides, Lp(a)), hypertension, diabetes, and total pack-years of smoking, this association remained significant (OR 2.22, 95% CI: 1.43–3.44, $p<0.001$). When CRP was added to the model the association between OPG and coronary artery calcification remained significant (OR=1.40, $P=0.01$).

Discussion

Our results confirm increased concentrations of serum OPG in patients with both early and long-standing RA. They also show for the first time that OPG concentrations are independently associated with severity of coronary artery calcium score in patients with longstanding disease.

OPG is a member of the tumor necrosis factor receptor super-family and a natural decoy for receptor activator of nuclear factor κ B ligand. Its expression and production are regulated by several inflammatory cytokines including interleukin-1 and TNF- α .^{6, 7, 17} Among its functions, by binding RANKL and thus preventing stimulation of RANK, OPG regulates bone resorption,^{6, 7} and prevents bone loss and erosions in RA.¹⁸ In addition to its importance in bone metabolism, OPG plays a role in atherosclerotic vascular disease and vessel calcification.¹⁹

In humans, OPG is present in advanced atherosclerotic lesions,²⁰ and in clinical studies OPG concentrations have been associated not only with the presence but also with the severity of coronary artery disease and predict future cardiovascular events.¹² In the general population, patients within the highest tertile of serum OPG concentrations had higher levels of inflammation as determined by CRP, ESR and fibrinogen;²¹ OPG was also an independent predictor of more rapid progression of carotid atherosclerosis.²¹ Similarly, in populations characterized by accelerated atherosclerosis, OPG is associated with vascular disease. For example, in patients receiving dialysis, higher OPG concentrations predicted progression of calcified plaques in the aorta,²² and in patients with diabetes, OPG concentrations were independently associated with the presence of coronary artery calcification,²³ and predicted near-term cardiovascular events.²³ In patients with a previous myocardial infarction complicated by congestive heart failure, OPG concentrations within the highest quartile were associated with a four-fold increase in age-adjusted cardiovascular and all-cause mortality, and were also associated with inflammation, as evidenced by higher CRP concentrations,²⁴ reinforcing the notion that OPG links inflammation and cardiovascular disease.

In keeping with the relationship between OPG and inflammation, and consistent with previous reports,^{14, 25} we found that patients with RA had higher serum OPG concentrations than control subjects. Several mechanisms may explain this elevation in the context of RA. OPG modulates, and is modulated by the immune system, and may act as a counter-regulatory molecule^{26, 27} that compensates for increased production of RANK ligand.¹⁴ We also found positive associations between OPG concentrations and ESR, CRP and duration and activity of RA. This observation, together with those of Kubota, who found that OPG is up-regulated by tumor necrosis alpha (TNF- α),²⁸ and Ziolkowska, who found that elevated concentrations of OPG normalized after anti TNF-alpha therapy,¹⁴ suggest that OPG is a marker of inflammation in RA. In addition to its association with inflammatory markers in RA, OPG is associated with an increased burden of atherosclerosis.

Our data show that every increase in 500 pg/ml of OPG is associated with greater odds of having higher coronary calcium scores in patients with established RA. When we examined the relationship between OPG concentrations and other cardiovascular risk factors we found, as reported previously in the general population,²⁹ that OPG concentrations increased with age. In addition, OPG concentrations were also associated with homocysteine concentrations, but were not associated with any other traditional cardiovascular risk factor, such as dyslipidemia, obesity or smoking. This is important because it suggests that the mechanisms underlying the role of OPG in atherosclerosis may be independent of current targets for cardiovascular risk intervention.

The mechanistic role of OPG in atherosclerosis is not clear, but the link between atherosclerosis and osteoporosis suggests that OPG may be fundamental in explaining why these two conditions frequently occur together.¹⁹ A potential mechanism may involve the immune system since OPG ligand is produced by T cells,³⁰ and these cells are important in the development of atherosclerosis. Thus, both OPG and its ligand may be part of a cytokine system affecting bone and the vasculature.¹⁹ Initially, it appeared intuitive that a shift of calcium from the bones to the vessels explained both, with OPG as the key regulatory molecule. However, in genetically engineered OPG-deficient mice osteoporosis and vascular calcification occur simultaneously,⁹ suggesting that the absence of OPG may lead to vascular changes, including increased deposition of calcium. Thus, low rather than high, OPG concentrations would be expected to be associated with coronary artery calcification. Our findings, and those of others that have reported that higher OPG concentrations are associated with atherosclerotic burden and cardiovascular outcomes,^{12, 13, 22} suggest that OPG may be a compensatory and perhaps protective agent, with its concentration increasing in response to atherosclerosis or factors that predispose to atherosclerosis.

There are some limitations of this study. First, OPG was only measured once and longitudinal data may be more informative. However, the fact that OPG concentration is elevated in patients with both early and long-standing disease, and that OPG is independently associated with coronary-artery calcification only in patients with long-standing disease suggests that elevation of OPG concentrations may precede detectable atherosclerosis. Second, the patients studied had, on average, moderate disease activity, and the results may not be generalizable to other populations of patients. Thus, although an association between OPG and atherosclerosis in patients with severely active RA is plausible, evidence is needed to support that.

In conclusion, increased serum concentrations of OPG are present in patients with RA and are independently associated with severity of coronary artery calcification in those with long-standing disease. OPG may provide a mechanistic link between coronary artery calcification and inflammation.

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Figure 1.

Association between serum OPG and coronary artery calcification in patients with RA. OPG concentrations (means and 95% C.I.) among patients and controls with no, low or severe calcification. P-value for trend=0.05 among patients with early RA, $p=0.003$ among patients with long-standing RA, and $P=0.16$ among control subjects.

FIGURE 2.

Association between serum OPG and coronary calcification in patients with RA[†]

[†] Applying proportional odds model to calculate the odds ratio and 95% CI for every 500 pg/ml increase of osteoprotegerin.

* Adjusted for age and sex.

** Adjusted for age, race, sex, homocysteine, cholesterol profile (HDL cholesterol, LDL cholesterol, triglycerides, lipoprotein a), hypertension, diabetes, and pack-years of smoking.

TABLE 1
Clinical characteristics of patients with rheumatoid arthritis and controls subjects

	Controls (n=87)	Early RA (n=90)	Established RA (n=67)	P-value
Age (years)	53 (44–60)	51 (43–60)	59 (48–67)	<0.001
Female (%)	63%	64%	73%	0.39
Caucasians (%)	85.1%	91.1%	86.6%	0.45
Disease duration (yrs)	NA	2 (1–3)	20 (14–24)	<0.001
Smoking current (%)	9%	21%	25%	0.02
Smoking (pack-years)	0 (0–9)	0 (0–22)	0 (0–18)	0.45
Body mass index (kg/m ²)	27.0 (24.8–31.6)	28.4 (24.3–33.5)	27.4 (23.8–31.4)	0.32
Hypertension (%)	39.1%	40.0%	67.2%	<0.001
Family history of CHD (%)	30%	26%	27%	0.81
Total cholesterol (mg/dL)	195 (170–216)	181 (156–209)	190 (151–211)	0.25
HDL cholesterol (mg/dL)	45 (39–54)	41 (36–52)	46 (40–57)	0.07
LDL cholesterol (mg/dL)	121 (104–145)	110 (92–135)	115 (83–136)	0.09
Lp (a) lipoprotein (mg/dL)	10.5 (4.0–33.3)	7.8 (2.0–24.3)	7.7 (3.9–25.9)	0.17
Triglycerides (mg/dL)	103.0 (74.5–133.0)	108.5 (78.0–141.0)	110.0 (79.5–159.5)	0.62
Homocysteine (mg/dL)	8.2 (7.2–9.6)	9.5 (8.1–11.4)	11.0 (8.2–12.5)	<0.001
Diabetes	5%	11%	10%	0.25
Coronary calcium score (Agatston units)	0 (0–18)	0 (0–47)	63 (0–368)	<0.001
Serum OPG (pg/mL)	1068 (692–1434)	1340 (1021–1652)	1895 (1337–2847)	<0.001

P values are for Kruskal-Wallis test or for Chi-square test as appropriate for continuous or categorical variables.

TABLE 2

Association between serum osteoprotegerin concentrations and early and long-standing RA.

	Unadjusted coefficient (95%CI)	P-value	Adjusted coefficient [¶] (95%CI)	P-value
Controls	reference		reference	
Early RA	0.24 (0.09–0.39)	0.001	0.25 (0.11–0.39)	<0.001
Long-standing RA	0.62 (0.46–0.78)	<0.001	0.47 (0.31–0.62)	<0.001

* Applying the general linear model on the dependent variable log serum OPG

[¶] Adjusted for age, race, sex, homocysteine, HDL cholesterol, LDL cholesterol, triglycerides, Lp(a) lipoprotein, hypertension, body mass index, diabetes, pack-years of smoking, creatinine, family history of coronary disease.

TABLE 3

Factors associated with OPG concentrations in patients with RA (n=157)

Continuous variables Variable	Rho	P-value *	Age and sex adjusted **
Age	0.39	<0.001	
Systolic blood pressure	0.28	<0.001	0.06
Diastolic blood pressure	0.06	0.49	0.21
Body mass index	-0.13	0.11	0.22
Disease duration	0.37	<0.001	0.02
Hemoglobin	-0.27	<0.001	0.03
Homocysteine	0.28	<0.001	0.001
Smoking (pack-yrs)	-0.009	0.91	0.20
Coronary-calcium score	0.27	<0.001	0.03
Cholesterol	0.07	0.38	0.72
HDL cholesterol	0.15	0.07	0.82
LDL cholesterol	0.01	0.87	0.85
Triglycerides	0.08	0.30	0.52
Lp (a) lipoprotein	0.01	0.87	0.70
Duration of corticosteroid use	0.17	0.04	0.05
Corticosteroids (cumulative dose)	0.15	0.05	0.04
Hydroxychloroquine - cumulative dose	0.07	0.39	0.92
Duration of hydroxychloroquine use	0.06	0.42	0.89
Erythrocyte sedimentation rate	0.31	<0.001	<0.001
C-Reactive Protein	0.16	0.04	0.03
MHAQ	0.10	0.23	0.05
DAS28	0.21	0.01	0.02

* Spearman rank correlation coefficient statistic

** General linear model adjusted for age and sex.