Osteoprotegerin Concentrations Relate Independently to Established Cardiovascular Disease in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** We determined whether osteoprotegerin (OPG) concentrations are associated with established cardiovascular disease (CVD) among patients with rheumatoid arthritis (RA).

Methods. OPG concentrations were measured by ELISA in 151 patients with RA (54 with CVD) and 62 age-matched control subjects without CVD. Established CVD was composed of documented ischemic heart disease, cerebrovascular disease, and peripheral artery disease.

Results. In patients with RA, age, body mass index (BMI), rheumatoid factor (RF) positivity, anticyclic citrullinated peptide (anti-CCP) antibody positivity, and joint erosion status were associated with OPG concentrations [partial R (p) = 0.175 (0.03), –0.277 (0.0009), 0.323 (< 0.0001), 0.217 (0.008), and 0.159 (0.05), respectively]. Median (interquartile range) OPG concentrations increased from 6.38 (3.46–9.31) to 7.07 (5.04–10.65) and 8.64 (6.00–11.52) ng/ml in controls and patients with RA who had CVD and those who did not, respectively (p = 0.0002). Upon adjustment for age, sex, traditional risk factors, and BMI in mixed regression models, OPG concentrations remained lower in controls compared to patients with RA without CVD (p = 0.05) and in the latter compared to those with CVD (p = 0.03); the association of OPG concentrations with CVD among patients with RA also persisted after additional adjustment for RF and anti-CCP antibody positivity, and erosion status (p = 0.04).

Conclusion. OPG concentrations are associated with disease severity and CVD prevalence in patients with RA. Whether consideration of OPG concentrations can improve CVD risk stratification in RA merits future longitudinal investigation. (First Release Nov 1 2014; J Rheumatol 2015;42:39–45; doi:10.3899/jrheum.140690)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
DISEASE SEVERITY
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The osteoprotegerin (OPG)/receptor activator of nuclear factor-κB ligand (RANKL)/tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) system regulates bone homeostasis and is increasingly recognized to be related to

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cardiovascular disease (CVD)[1,2,3,4,5,6,7]. OPG reflects the overall activity of this cytokine network in which it further acts as a decoy receptor for RANKL, which can enhance atherosclerotic plaque vulnerability to rupture[3], and TRAIL, which has antiinflammatory and anti-atherosclerotic properties[8,9,10,11,12]. OPG also directly upregulates endothelial cell adhesion molecule expression and increases leukocyte adhesion to endothelial cells[10,11]. In human studies, OPG concentrations were consistently associated with an increased prevalence, severity, and incidence of CVD[1,2,13].

The heightened traditional and nontraditional risk factor-mediated CVD burden in rheumatoid arthritis (RA) is now well documented[14,15,16,17,18,19]. However, atherogenesis remains poorly understood and current CVD risk stratification is reportedly suboptimal in RA[18,19]. Patients with RA experience cytokine-mediated, increased OPG production[10]. Indeed, OPG is reportedly involved in the pathophysiology of RA[10]. Importantly, in the present context, OPG concentrations were also recently found to be independently related to endothelial activation[20], subclinical carotid atherosclerosis[20,21], and coronary artery calcification[22], and hence could aid in improving cardiovascular risk assessment in RA. Further, tumor necrosis factor (TNF)-α blockade resulted in reduced endothelial activation that was associated with decreased OPG concentrations[20]. OPG is implicated in the reported link between systemic inflammation and increased CVD risk in RA[20,21,22]. In our present study, we determined whether OPG concentrations are independently related to prevalent-established CVD among patients with RA.

MATERIALS AND METHODS

Patients. We enrolled 151 white Spanish patients who met the 1987 American College of Rheumatology[23] and the 2010 American College of Rheumatology/European League against Rheumatism criteria[24] for RA at the Hospital Universitario Marques de Valdecilla (Santander, Spain). These included 54 consecutive patients with established CVD and 97 age-matched and sex-matched cases without CVD. OPG concentrations were also determined in 62 control subjects without CVD who were among the community-based, randomly recruited attendees at family physician healthcare centers of the Cantabria region. They were matched by age, sex, and race to the 151 participants with RA. Controls had no family history of RA, polymyalgia rheumatica, psoriatic arthritis, or any connective tissue disease. The Ethics Committee of Cantabria for Hospital Universitario Marques de Valdecilla in Santander approved the study. The participating patients and controls gave the necessary informed written consent to participate and for publication of the results.

Assessments. At the time of our study, we calculated the Disease Activity Score in 28 joints (DAS28)[25] and measured the erythrocyte sedimentation rate, concentrations of C-reactive protein (latex immunoturbidimetry), lipids (enzymatic colorimetry), glucose, rheumatoid factor (RF; nephelometry), anticyclic citrullinated peptide (anti-CCP) antibody (ELISA), and creatinine (colorimetry) in patients with RA. Chronic kidney disease was diagnosed when the Modification of Diet in Renal Disease equation[26] was less than 60 ml/min/1.73 m^2. We recorded the presence of extraarticular manifestations, including nodular disease, Felty's syndrome, pulmonary fibrosis, rheumatoid vasculitis, and Sjögren syndrome that were defined as previously reported[27], and the use of cardiovascular drugs and antirheumatic agents.

The presence of traditional risk factors including hypertension, dyslipidemia, smoking, and diabetes as well as CVD-composing ischemic heart disease (IHD), cerebrovascular disease, and peripheral artery disease was determined as previously described[28,29] in patients as well as controls. Dyslipidemia was identified when a previous clinical diagnosis of hypercholesterolemia or/and hypertriglyceridemia was present or total cholesterol or/and triglyceride concentrations were ≥ 240 mg/dl and 160 mg/dl, respectively. Hypertension was considered present when there was a previous diagnosis of the respective comorbidity or the systolic blood pressure was ≥ 140 mmHg or/and diastolic blood pressure ≥ 90 mmHg. Diabetes was identified on the basis of a previous diagnosis or when 2 fasting plasma glucose levels on different days were > 125 mg/dl. Participants were stratified as smokers when they had smoked during the previous 10 years. Apart from traditional risk factors, we also calculated body mass index (BMI) and diagnosed obesity when the obtained value was ≥ 30 kg/m^2.

The definition of IHD included acute coronary syndrome with or without persistent ST-segment elevation and chronic coronary heart disease. IHD was diagnosed when any of the following criteria were met: a recorded diagnosis of acute coronary syndromes composed of acute myocardial infarction or unstable angina, the presence of pathological Q waves on an electrocardiogram, and coronary imaging showing > 50% stenosis of at least 1 coronary vessel. Ischemic dilated cardiomyopathy was also included in this category if systolic function was impaired and the left ventricle dilated with evidence of IHD on electrocardiography or/and catheterization studies. Cerebrovascular disease was diagnosed when a patient had sustained a stroke or/and transient ischemic attack (TIA). Strokes were confirmed by computer tomography or/and magnetic resonance imaging. TIA were identified when the symptom duration was self-limited to less than 24 h without residual neurological damage. A peripheral artery disease diagnosis required confirmation by Doppler and arteriography.

Human OPG serum levels were determined by ELISA. Briefly, 96-well microplates were precoated with anti-human OPG antibody (PeproTech). Recombinant human OPG (PeproTech) was used to prepare the standard curve. The standard dilution series ranged from 0.313 to 20 ng/ml. First, 50 µl of each standard or sample was added to the appropriate wells and incubated for 3 h at room temperature. After discarding the solution and washing 4 times, 50 μl of prepared biotinylated anti-human OPG antibody (PeproTech) was added to each well and incubated for 1 h. After washing away unbound biotinylated antibody, 50 µl of horseradish peroxidase-conjugated avidin (eBioscience) was pipetted into the wells and incubated for 30 min. Finally, plates were developed with ABTS Liquid Substrate (PeproTech) and read at 405 nm and 600 nm (as reference wavelength).

Statistical analysis. Results were expressed as mean (SD) and median [interquartile range (IQR)] for normally and non-normally distributed continuous characteristics, respectively, and as proportions for categorical variables. Baseline characteristics between patients and controls, and patients without and with CVD were compared by the Student t test, Mann-Whitney U test, or in univariate logistic regression models as appropriate. Associations of baseline characteristics with OPG concentrations were assessed in age-adjusted and sex-adjusted mixed regression models. OPG concentrations among controls and patients without and with CVD were first compared by the Kruskal-Wallis test, and subsequently in mixed regression models with adjustment for age; sex; traditional cardiovascular risk factors including hypertension, dyslipidemia, smoking, and diabetes; and the baseline non-RA characteristic of BMI and RA features including RF, anti-CCP, and joint erosion status as these were associated with OPG concentrations in the preceding analysis, as appropriate.

Statistical computations were made using the GB Stat program (Dynamic Microsystems Inc.). Significance was set at a p value of ≤ 0.05.

RESULTS

Baseline characteristics and CVD in study participants. Women composed 67.7% and 62.3% of the control and RA groups, respectively (p = 0.5 for comparison). As shown in Table 1, traditional cardiovascular risk factor profiles and their burden, as indicated by the number of the respective risk factors, were more favorable in controls compared to patients without CVD and in the latter compared to those with CVD. BMI did, however, not differ among the 3 subgroups. Compared to patients without CVD, those with CVD also had earlier disease onset and experienced more prevalent RF and anti-CCP positivity and extraarticular manifestations, and increased inflammatory markers. Thirty-two of the patients (72.3%) with CVD had experienced their first cardiovascular event subsequent to RA symptom onset.

Associations between baseline characteristics and OPG concentrations in patients with RA. Table 2 shows that age at time of the study was associated with OPG concentrations. Obesity and BMI were related inversely to OPG concentrations whereas RA severity markers, including RF and anti-CCP positivity and erosive disease, were associated with high OPG concentrations.

OPG concentrations in controls and patients with RA. As given in Figure 1, the median (IQR) OPG concentrations increased from 6.38 (3.46–9.31) to 7.07 (5.04–10.65) and 8.64 (6.00–11.52) ng/ml in controls and patients with RA who had and did not have CVD, respectively (p = 0.004).

OPG concentrations among controls and patients without and with CVD in multivariable analysis. As also given in Figure 1, OPG concentrations remained lower in controls compared to RA patients without CVD, and when comparing patients without CVD to those with CVD (upon adjustment for demographic variables, traditional cardiovascular risk factors, and BMI). The association of OPG concentrations with CVD among patients with RA also persisted after additional adjustment for RA severity markers including RF, anti-CCP, and erosion status; further adjustment for disease activity, systemic inflammation, or triglycerides/high-density lipoprotein (HDL) cholesterol ratio did not materially alter these results (data not shown).

DISCUSSION

To the best of our knowledge, our present investigation shows for the first time that OPG concentrations are increased in patients with RA who have CVD compared to those who do not, independently of demographic features, traditional cardiovascular risk factors, adiposity, and disease characteristics. This confirms our primary research hypothesis. In addition, we found that disease severity markers relate to large OPG concentrations, and adiposity is associated with reduced OPG concentrations in RA. Our results also confirm the previously reported finding of increased OPG concentrations associated with RA[7].

Our investigation was prompted by our recent findings[20] that OPG concentrations were independently related to surrogate markers of early atherogenesis and atherosclerosis in 34 patients with severe RA who had not experienced cardiovascular events. The cross-sectional design of our study precludes drawing inferences on the direction of causality and hence the identified OPG-CVD relation requires elucidation in future longitudinal and mechanistic investigations. OPG may have beneficial, detrimental, or dual effects on cardiovascular risk[1]. In support of a protective effect, OPG knockout mice experience enhanced arterial media calcification[1]. It was, therefore, postulated that the positive OPG-CVD association in non-RA subjects is likely to originate in the process of reverse causality, in which a compensatory increase in OPG production occurs in the presence of enhanced cardiovascular risk[13]. Additionally, the reported interaction of OPG with RANKL would be expected to result in reduced CVD risk[3]. However, in keeping with a proatherogenic effect, OPG also reduces the effects of TRAIL[4,5,6] and can by itself induce endothelial activation[11,12]. Finally, because OPG is overexpressed in vulnerable atherosclerotic lesions, increased OPG concentrations could conceptually, at least in part, also represent atherosclerosis extent[2].

Ziolkowska, *et al*[7] documented that cytokines enhance OPG production in mononuclear cells and fibroblast-like synoviocytes from patients with RA, and that TNF-α blockade reduces OPG concentrations. Congruent with these findings, Asanuma, *et al*[22] showed that systemic inflammation and disease activity relate to OPG concentrations in RA. These associations were not found in our present analysis, probably because disease activity as estimated by DAS28 was lower in our study and a substantial proportion of patients were treated with biological agents, including TNF-α inhibitors. However, we found that several disease severity markers including RF, anti-CCP, and erosive disease are related to OPG concentrations. Disease severity variables are associated with increased atherogenesis in RA[14,15,16,17,18,19]. Taken together, previously reported data with our current findings suggest that OPG may contribute to the link between disease activity or/and severity and increased CVD risk in RA.

Classic cachexia, defined as reduced muscle mass and fat mass, is uncommon in RA, but is associated with a high inflammatory burden and severe joint damage in patients with a poor cardiovascular prognosis. In our present investigation, we observed that obesity related inversely to OPG concentrations. An inverse relation between adiposity and OPG concentrations was previously also found in non-RA subjects[30,31]. However, to our knowledge, a definite plausible explanation for this awaits further study. In our present investigation, the adiposity-OPG associations remained significant after adjusting for RA characteristics (data not shown).

Table 1. Baseline characteristics and cardiovascular events in study participants. Results are expressed as mean (SD), median interquartile range, or proportions.

Characteristic	Controls, n = 62	Patients with RA without CVD, n = 97	Patients with RA with CVD, n = 54	p[a]	p[b]	p[c]
Age at time of study, yrs	69.2 (11.4)	70.5 (9.7)	70.3 (10.0)	0.5	0.6	0.9
Women	67.7	63.9	59.3	0.6	0.3	0.6
Prevalence of CVD						
Ischemic heart disease	—	—	50	—	—	—
Cerebrovascular accident	—	—	48	—	—	—
Peripheral artery disease	—	—	17	—	—	—
No. CVD events	—	—	1.2 (0.4)	—	—	—
Traditional CVD risk factors						
Any	51.6	86.6	96.3	< 0.0001	< 0.0001	0.03
Hypertension	33.9	65.3	77.8	0.0003	< 0.0001	0.08
Dyslipidemia	12.9	47.4	63.0	< 0.0001	< 0.0001	0.05
Smoking	21.0	28.9	51.9	0.3	0.0007	0.006
Diabetes	21.0	16.8	35.2	0.5	0.09	0.01
No. risk factors	0.9 (1.0)	1.6 (1.0)	2.3 (1.0)	0.0001	< 0.0001	< 0.0001
Body mass index, kg/m^2	28.6 (5.3)	28.5 (5.6)	28.4 (5.3)	—	—	0.9
Obesity	32.3	36.7	31.4	0.8	0.8	0.6
Blood pressure, mmHg						
Systolic	—	141 (22)	138 (17)	—	—	0.4
Diastolic	—	78 (9)	75 (10)	—	—	0.05
Lipid variables, mg/dl						
Total cholesterol	—	199 (37)	192 (38)	—	—	0.3
LDL cholesterol	—	118 (30)	109 (34)	—	—	0.1
HDL cholesterol	—	59 (47–73)	57 (46–65)	—	—	0.2
Total-HDL cholesterol ratio	—	3.5 (11)	3.6 (0.9)	—	—	0.7
Triglycerides	—	92 (73–130)	103 (80–152)	—	—	0.1
Triglycerides/HDL cholesterol ratio	—	1.5 (1.1–2.6)	1.8 (1.4–2.9)	—	—	0.05
Chronic kidney disease	—	8.0	13.9	—	—	0.6
Cardiovascular drugs						
Antihypertensives	—	65.3	77.8	—	—	0.08
Statins	—	38.9	57.4	—	—	0.02
Glucose-lowering agents						
Any	—	15.1	29.6	—	—	0.06
Oral hypoglycemic agents	—	9.7	20.4	—	—	0.03
Insulin	—	7.4	9.3	—	—	0.7
Age at RA onset, yrs	—	62.4 (13.4)	57.0 (12.4)	—	—	0.02
RA disease duration, yrs	—	6 (2–13)	8 (3–21)	—	—	0.1
RF-positive	—	41.2	57.4	—	—	0.05
Anti-CCP–positive	—	35.1	44.4	—	—	0.3
Extraarticular manifestation(s)	—	14.4	29.6	—	—	0.03
Joint erosion(s) in hands and/or feet	—	22.9	27.8	—	—	0.5
C-reactive protein, mg/l	—	3.0 (1.3–7.0)	4.7 (2.0–11.0)	—	—	0.03
Erythrocyte sedimentation rate, mm/h	—	14 (6–25)	20 (9–39)	—	—	0.01
Disease Activity Score in 28 joints	—	3.1 (1.4)	3.2 (1.4)	—	—	0.7
Clinical Disease Activity Index	—	7.5 (2.0–12.5)	5.0 (3.0–12.0)	—	—	0.6
Antirheumatic agents						
Any DMARD	—	88.5	87.0	—	—	0.9
Synthetic DMARD						
Any	—	84.5	83.3	—	—	0.8
Methotrexate	—	68.0	70.4	—	—	0.8
Chloroquine	—	29.9	26.9	—	—	0.6
Leflunomide	—	7.2	5.7	—	—	0.7
Sulfasalazine	—	3.0	6.0	—	—	0.5
No.	—	1.1 (0.6)	1.1 (0.7)	—	—	1.0
Biologic DMARD						
Any	—	28.1	25.9	—	—	0.6
TNF-α inhibitor	—	14.6	11.1	—	—	0.6
Non TNF-α inhibitor	—	13.5	14.8	—	—	0.8
Prednisone	—	46.4	53.7	—	—	0.4

[a] p for comparison between controls and patients without CVD. [b] p for comparison between controls and patients with CVD. [c] p for comparison between patients without and with CVD. RA: rheumatoid arthritis; CVD: cardiovascular disease; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CCP: cyclic citrullinated peptide antibody; DMARD: disease-modifying antirheumatic drug; TNF-α: tumor necrosis factor-α; RF: rheumatoid factor.

Table 2. Age-adjusted and sex-adjusted associations of baseline characteristics with OPG concentrations[a] among patients with RA. Relationships were assessed in mixed regression models. Age at disease onset (rather than age at time of study) was entered in the model that included disease duration.

Characteristic	Partial R	p
Age at time of study	0.175	0.03
Age at RA onset	–0.011	0.9
Women	0.079	0.3
Traditional cardiovascular risk factors		
Any risk factors	0.021	0.8
Hypertension	0.079	0.3
Dyslipidemia	0.038	0.7
Smoking	0.108	0.2
Diabetes	0.032	0.7
Obesity	–0.218	0.01
No. risk factors	0.023	0.8
Body mass index	–0.277	0.0009
Blood pressure		
Systolic	0.049	0.8
Diastolic	0.035	0.7
Lipid variables		
Total cholesterol	0.060	0.5
LDL cholesterol	–0.068	0.4
HDL cholesterol[a]	0.074	0.4
Total-HDL cholesterol ratio	–0.060	0.5
Triglycerides[a]	0.142	0.1
Triglycerides/HDL cholesterol ratio[a]	0.085	0.3
Chronic kidney disease	0.019	0.8
Cardiovascular drugs		
Antihypertensives	0.079	0.3
Statins	0.073	0.4
Glucose lowering agents		
Any	0.034	0.7
Oral hypoglycemic agents	–0.014	0.9
Insulin	–0.007	0.9
RA disease duration[a]	0.131	0.1
RF-positive	0.323	< 0.0001
Anti-CCP–positive	0.217	0.008
Extraarticular manifestation(s)	0.091	0.3
Joint erosion(s) in hands or/and feet	0.159	0.05
C-reactive protein[a]	0.123	0.1
Erythrocyte sedimentation rate[a]	0.067	0.4
Disease Activity Score in 28 joints	–0.017	0.9
Antirheumatic agents		
Any DMARD	0.046	0.6
Synthetic DMARD		
Any	0.013	0.9
Methotrexate	–0.030	0.7
Chloroquine	0.117	0.2
Leflunomide	0.030	0.7
Sulfasalazine	0.132	0.1
No.	0.106	0.2
Biologic DMARD		
Any	0.023	0.8
TNF-α inhibitor	0.040	0.6
Non TNF-α inhibitor	0.063	0.5
Prednisone	0.084	0.3

[a] Logarithmically transformed. OPG: osteoprotegerin; RA: rheumatoid arthritis; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CCP: cyclic citrullinated peptide antibody; DMARD: disease-modifying antirheumatic drug; TNF-α: tumor necrosis factor-α; RF: rheumatoid factor.

Our study has other limitations. OPG concentrations are reportedly associated with insulin resistance as reflected by homeostasis model assessment[32], which was not recorded in the current investigation. Insulin resistance also relates to carotid and coronary atherosclerosis in RA[33,34]. The triglycerides-HDL cholesterol ratio represents another potential surrogate marker of insulin resistance[35,36]. Our analysis revealed that triglycerides-HDL cholesterol ratios were indeed larger in patients with CVD compared to those without CVD, but were not related to OPG concentrations. Upon adjusting for the triglycerides-HDL ratio, the OPG-CVD relationship persisted among patients with RA. Future studies on the potential effect of OPG on cardiovascular risk in RA should include insulin sensitivity evaluation. Finally, serum OPG levels do not necessarily reflect its tissue concentrations.

OPG concentrations are associated with disease severity and independently relate to prevalent-established CVD in RA. Our current results support previously reported findings that indicate a potential role of OPG in increased cardiovascular risk and its stratification in RA. This merits confirmation in a future longitudinal investigation.

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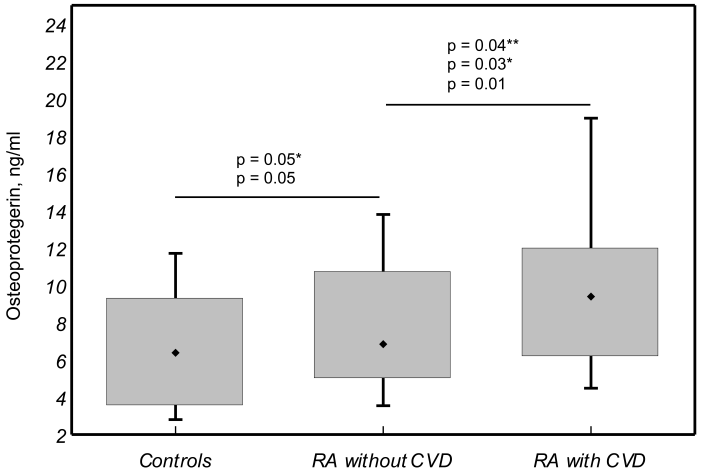

Figure 1. Box plots showing median and 25–75 and 10–90 percentiles of osteoprotegerin concentrations among controls and patients with RA who have and do not have CVD. F ratio = 8.9. P = 0.004 for comparison among the 3 groups (Kruskal-Wallis). * p adjusted for age at time of study, sex, BMI, and major traditional cardiovascular risk factors (hypertension, dyslipidemia, smoking, and diabetes). ** p additionally adjusted for anticyclic citrullinated peptide antibody, rheumatoid factor, and erosive disease. RA: rheumatoid arthritis; CVD: cardiovascular disease; BMI: body mass index.

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