

Independent Relationship of Osteoprotegerin Concentrations with Endothelial Activation and Carotid Atherosclerosis in Patients with Severe Rheumatoid Arthritis

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ABSTRACT. Objective. Osteoprotegerin (OPG) may contribute to the link between systemic inflammation and increased cardiovascular risk. We investigated the relationship of OPG concentrations with endothelial activation and carotid atherosclerosis in rheumatoid arthritis (RA).

Methods. OPG concentrations and those of endothelial activation molecules were measured by using ELISA in 34 patients who were treated with infliximab (IFX), both immediately before and after an IFX infusion. Carotid intima-media thickness (CMT) and plaque were determined by ultrasound in 27 of the study participants.

Results. Median (interquartile range) OPG concentrations decreased from 4.8 pmol/l (2.8–6.5) to 4.4 pmol/l (2.9–6.1; $p = 0.04$) upon IFX infusion. Baseline OPG concentrations were inversely associated with those of total and low-density lipoprotein (LDL) cholesterol (partial $R = -0.50$, $p = 0.004$, and $R = -0.48$, $p = 0.007$, respectively). Prior to IFX administration, OPG concentrations were associated with those of intercellular adhesion molecule (ICAM)-1 (partial $R = 0.34$, $p = 0.05$), CMT (partial $R = 0.51$ to 0.52 , $p < 0.009$), and plaque (OR = 1.52, 95% CI 1.01–2.29 to OR = 1.61, 95% CI 1.03–2.51; $p < 0.04$), independent of conventional risk factors and C-reactive protein concentrations or disease activity. Except for the OPG concentrations-plaque association ($p = 0.09$), these relationships remained significant subsequent to IFX administration ($p < 0.05$). Reductions in OPG levels related to those in vascular cell adhesion molecule (VCAM)-1 concentrations (partial $R = 0.35$, $p = 0.04$) and had borderline significance ($p = 0.09$) with those in ICAM-1 (partial $R = 0.29$) concentrations.

Conclusion. OPG concentrations are independently associated with endothelial activation and carotid atherosclerosis in RA. Reductions in OPG concentrations upon IFX administration are associated with decreased endothelial activation. OPG may be involved in increased cardiovascular disease risk and may improve its stratification in patients with RA. (First Release Feb 1 2014; J Rheumatol 2014;41:429–36; doi:10.3899/jrheum.131037)

Key Indexing Terms:

RHEUMATOID ARTHRITIS OSTEOPROTEGERIN TNF- α BLOCKADE
ENDOTHELIAL ACTIVATION ATHEROSCLEROSIS

Osteoprotegerin (OPG) is a member of the tumor necrosis factor (TNF) receptor superfamily, plays a central role in bone remodeling, and acts as a decoy receptor for the

receptor activator of nuclear factor- κ B ligand (RANKL)^{1,2}. OPG thereby reduces binding of RANKL to its receptor, RANK. The OPG/RANKL/RANK axis is strongly involved

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Supported by grants from “Fondo de Investigaciones Sanitarias” PI06/0024, PS09/00748, and PI12/00060 (Spain). This work was also partially supported by RETICS Program, RD08/0075 and RD12/0009/0013 (RIER) from “Instituto de Salud Carlos III” (ISCIII; Spain), and a Medical Research Council Grant (MRC2008_DES) from the National Research Foundation (South Africa; PH Dessen).

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Accepted for publication November 8, 2013.

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in osteoporosis and increasingly implicated in atherosclerosis^{1,2}. In addition, OPG was documented to act as a soluble neutralizing receptor for another member of the TNF superfamily, TNF-related apoptosis-inducing ligand (TRAIL), which is a molecule that exhibits antiinflammatory and antiatherosclerotic properties^{3,4,5}. OPG is produced by most human tissues and cells that include not only osteoblasts, but also endothelial and vascular smooth muscle cells. Proinflammatory cytokines enhance OPG production and OPG may also participate in the pathophysiology of rheumatoid arthritis (RA)^{6,7}. Moreover, C-reactive protein (CRP) can downregulate the expression of TRAIL⁴.

OPG knockout mice exhibit severe osteoporosis, as well as arterial media calcification, indicating a potential protective effect of OPG against calcification^{2,8}. However, this phenotype of arterial calcification occurs in the absence of fat deposition or atherosclerotic-like plaques and does not involve intimal calcification as observed in atherosclerosis. Further, cellular studies revealed that OPG can upregulate endothelial adhesion molecule production⁹ and indeed increase leukocyte adhesion to endothelial cells¹⁰. OPG is present in atherosclerotic plaques¹¹. Thus, OPG may have beneficial, detrimental, or dual effects on atherosclerosis development and progression².

Human studies have consistently revealed positive relationships between circulating OPG concentrations and an increased prevalence and severity of coronary artery disease^{12,13}, incident coronary artery disease^{14,15}, cerebrovascular disease¹⁶, and peripheral vascular disease¹⁷. In view of the findings in OPG knockout mice, it was postulated that the reported associations between OPG concentrations and cardiovascular disease (CVD) in humans may represent paradoxical and compensatory increases in OPG production in an attempt to attenuate atherogenesis in the presence of established CVD¹⁵.

Patients with RA experience a markedly enhanced traditional and nontraditional risk factor-mediated CVD burden^{18,19,20,21,22,23}. In RA, determination of increased OPG concentrations could be a promising candidate in enhancing CVD risk stratification as it may contribute to the link between high-grade inflammation and enhanced atherogenesis. A positive relationship between OPG concentrations and coronary artery calcification was recently documented in patients with RA²⁴. However, OPG-related increased coronary artery calcification may not necessarily represent atherosclerosis^{2,8}. Also, coronary artery calcification scores are far less sensitive than ultrasound (US)-determined carotid artery atherosclerosis in identifying patients at high risk of CVD^{25,26}. Recently, an age, sex, and CRP concentration-independent association between OPG levels and carotid artery plaque was documented in RA²⁷. Whether OPG concentrations associate with endothelial activation in RA is, to our knowledge, unknown at present. Taken together, the role of

OPG production in enhanced atherogenesis and CVD risk stratification in RA requires further elucidation. Therefore, in our study, we examined whether OPG concentrations are associated with those of endothelial activation molecules, as well as carotid intima-media thickness (CIMT) and plaque, independent of conventional cardiovascular risk factors, systemic inflammation, and disease activity in a cohort of patients with severe RA. We also determined whether upon the administration of the TNF- α blocker infliximab (IFX), potential reductions in OPG concentrations⁶ were related to decreased endothelial activation.

MATERIALS AND METHODS

Patients. Thirty-four patients who met the 1987 American College of Rheumatology²⁸ and 2010 American College of Rheumatology/European League Against Rheumatism criteria for RA²⁹ participated in our study. Each patient used IFX, 3 to 5 mg/kg, at 6 to 8 weekly intervals for at least 1 year, and oral methotrexate 15 to 25 mg weekly, with or without chloroquine (250 mg/day); prednisone 2.5 to 7.5 mg daily; and a nonsteroidal antiinflammatory agent. Also, 59.9% and 20.6% of participants had been treated with leflunomide and sulfasalazine, respectively, before the initiation of IFX therapy. The local institutional committee approved anti-TNF- α therapy and each patient gave informed consent to participate in the study.

Methods. We recorded demographic features, cardiovascular risk factors, cardiovascular agent use, disease characteristics, and markers of systemic inflammation. All measurements were taken in a fasting state. The Disease Activity Score in 28 joints (DAS28)³⁰ was calculated. Blood samples were taken at 8 a.m. to measure the erythrocyte sedimentation rate and concentrations of CRP (latex immunoturbidimetry), lipids (enzymatic colorimetry, plasma glucose, and insulin; DPC, Dipeasa). Insulin resistance was estimated by the homeostasis model of insulin resistance.

As reported³¹, the concentrations of the soluble endothelial activation molecules, including E-selectin, P-selectin, vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, and ICAM-3, were determined by ELISA (Bender MedSystems) before and 120 min after an infliximab infusion; their intraassay coefficients of variation and lower detection limits were 5.4% and 0.5 ng/ml, 2.4% and 1.06 ng/ml, 3.1% and 0.9 ng/ml, 4.1% and 3.3 ng/ml, and 2.5% and 0.58 ng/ml, respectively.

Carotid US was performed in 27 (79.4%) of participants. Our methodology used to assess carotid atherosclerosis was also reported previously³². The right-sided CIMT was evaluated using QLAB's CIMT-quantification software measurement plug-in (Philips Healthcare, DA Best). This instrumentation allows for highly consistent and reliable CIMT measurements, reducing the effort required to make assessments successfully and minimize the time needed to complete the respective investigations. For the purpose of our study, atherosclerotic plaque was defined as a distinct protrusion of > 1.5 mm into the vessel lumen³³. We used a high-resolution B-mode US (iE33 Philips ultrasound system, Philips Ultrasound) with a 10-MHz linear transducer. A single cardiologist (CG-J) performed all measurements. The reproducibility of CIMT measurements was evaluated in 17 patients and 17 controls within 1 week of the first US examination. The correlation coefficient for CIMT was 0.98.

Using an ELISA (Biomedica), we determined OPG concentrations before and 120 min after an IFX infusion. The intraassay coefficient of variation and lower detection limit were 10% and 0.14 pmol/l, respectively. As reported, we also measured serum concentrations of 3 adipokines including adiponectin³⁴, resistin³⁵, leptin³⁶, and ghrelin³⁷ before and after the IFX infusion.

Data analysis. Results are expressed as median [interquartile range (IQR)] and proportions for continuous and categorical variables, respectively.

Changes in OPG concentrations upon IFX infusion were assessed by the Wilcoxon Signed-Rank test. OPG concentrations in women and men were compared by the Mann-Whitney U test. Associations of OPG concentrations with cardiovascular risk factors, RA characteristics, markers of systemic inflammation, adipokine, ghrelin, and endothelial activation molecule concentrations, CIMT, and plaque were evaluated using potential confounder or/and mediating characteristics adjusted mixed linear or logistic regression models as appropriate.

Statistical computations were done using the GB Stat program (Dynamic Microsystems Inc.).

RESULTS

Recorded characteristics and changes in OPG concentrations upon IFX administration in 34 patients with RA. Table 1 shows the recorded characteristics in the 34 participants. Their median age was 57 years and 73.5% were women. Despite the use of conventional disease-modifying antirheumatic drug and TNF- α blockade in each patient, the overall disease activity was still in the moderate range with a median DAS28 of 4.3 (IQR: 3.3–5.1), and only 1 patient experienced disease remission (DAS28 < 2.4). Hence, patients included in our study had severe RA. The levels of each of the evaluated endothelial activation molecules decreased upon IFX administration; as previously recorded by us, these reductions were significant for ICAM-3 and P-selectin³¹. Importantly, these decreases were paralleled by a significant ($p = 0.04$) reduction in the median OPG concentrations from 4.8 (IQR: 2.8–6.5) to 4.4 (IQR: 2.9–6.1) pmol/l.

Age-adjusted and sex-adjusted associations of OPG concentrations. Concentrations were measured at time 0 and 120 minutes with cardiovascular risk factors, disease characteristics, systemic inflammation, and adipokine and ghrelin concentrations in patients with RA. Baseline and post-infusion IFX OPG concentrations were numerically higher in women than men [median = 5.1 (IQR: 2.9–6.9) vs 3.8 (IQR: 2.8–5.0) and 5.0 (IQR: 2.9–6.1) vs 3.7 (IQR: 2.8–4.4) pmol/l, respectively], but these differences were not significant ($p = 0.4$ and 0.2 , respectively). In the sex-adjusted analysis, age at disease onset and at the time of the study were associated with baseline and post-IFX infusion OPG concentrations [partial $R = 0.327$ ($p = 0.05$) and 0.390 ($p = 0.02$), and 0.344 ($p = 0.04$) and 0.419 ($p = 0.02$), respectively].

Table 2 gives the age at the time of the study (replaced by age at disease onset for model that included disease duration) and sex-adjusted associations of baseline OPG concentrations with cardiovascular risk factors, RA characteristics, systemic inflammation markers, and adipokine and ghrelin concentrations in the 34 participants. OPG concentrations were inversely associated with those of total and LDL cholesterol. These relationships were materially unaltered upon further adjustment for CRP concentrations or DAS28 (data not shown). Further, OPG concentrations related to those of CRP with borderline significance ($p =$

Table 1. Characteristics in 34 patients with rheumatoid arthritis.

Demographic characteristics	
Age at time of study, yrs	57 (46–65)
Age at disease onset, yrs	42 (37–55)
Female sex	73.5
Cardiovascular risk factors	
Current smoking	8.8
Body mass index, kg/m ²	24.1 (22.6–28.9)
Systolic blood pressure, mm Hg	120 (100–130)
Diastolic blood pressure, mm Hg	75 (70–80)
Total cholesterol, mg/dl	198 (171–212)
HDL cholesterol, mg/dl	62 (56–72)
LDL cholesterol, mg/dl	106.5 (90–122)
Triglycerides, mmol/l	102 (87–121)
Glucose, mg/dl	88 (78–97)
Insulin, μ U/ml	13.0 (8.6–19.3)
HOMA-IR, μ U.mmol/ml.l	2.9 (1.9–4.2)
Framingham score	2 (1–5)
Cardiovascular agents	
Antihypertensives	23.5
Statins	14.7
Glucose lowering agents	2.9
RA characteristics	
Disease duration, years	12 (5–16)
Rheumatoid factor–positive	91.2
Disease activity score in 28 joints	4.3 (3.3–5.1)
Tender joints, n	3 (1–6)
Swollen joints, n	3 (2–7)
VAS patient disease activity, mm	40 (25–50)
Cumulative prednisone use, g	27.5 (10.6–48.3)
Systemic inflammation	
Erythrocyte sedimentation rate, mm/hour	27 (14–39)
C-reactive protein, mg/l	5.5 (4.0–23.4)
Adipokines	
Adiponectin, ng/ml	15,683 (10,830–26,180)
Resistin, ng/ml	18.8 (14.8–26.8)
Leptin, ng/ml	10.7 (5.3–19.4)
Ghrelin, pg/ml	861.2 (700.5–979.9)
Endothelial activation molecules	
E-selectin at time 0 min, ng/ml	46.5 (33.5–67.4)
E-selectin at time 120 min, ng/ml	44.7 (28.0–66.2)
P-selectin at time 0 min, ng/ml	217.7 (133.1–315.5)
P-selectin at time 120 min, ng/ml	175.0 (96.9–248.1)
VCAM-1 at time 0 min, ng/ml	1015.5 (882.0–1317.0)
VCAM-1 at time 120 min, ng/ml	1009.5 (842.0–1230.0)
ICAM-1 at time 0 min, ng/ml	351.0 (288.4–409.9)
ICAM-1 at time 120 min, ng/ml	329.2 (257.3–380.4)
ICAM-3 at time 0 min, ng/ml	53.9 (48.6–65.2)
ICAM-3 at time 120 min, ng/ml	51.0 (42.8–62.1)
Carotid atherosclerosis, n = 27	
Intima-media thickness, mm	0.82 (0.59–1.02)
Plaque	40.7
OPG at time 0 min, pmol/l	4.8 (2.8–6.5)
OPG at time 120 min, pmol/l	4.4 (2.9–6.1)

Continuous and categorical variables are expressed as median (interquartile range) and proportions, respectively. HDL: high-density lipoprotein; LDL: low-density lipoprotein; RA: rheumatoid arthritis; HOMA-IR: homeostasis model of insulin resistance; VAS: visual analog scale; VCAM: vascular cell adhesion molecule; ICAM: intercellular adhesion molecule; OPG: osteoprotegerin.

Table 2. Age at time of the study and sex-adjusted associations of baseline osteoprotegerin (OPG) concentrations with cardiovascular risk factors, disease characteristics, systemic inflammation, and adipokine and ghrelin concentrations in patients with rheumatoid arthritis (RA).

Characteristics	Partial R (95% CI)	p
Cardiovascular risk factors		
Current smoking	0.28 (−0.07 to 0.63)	0.1
Body mass index	−0.15 (−0.55 to 0.22)	0.4
Systolic blood pressure	0.04 (−0.31 to 0.40)	0.8
Diastolic blood pressure	0.00 (−0.40 to 0.40)	1.0
Total cholesterol	−0.50 (−0.82 to −0.18)	0.004†
HDL cholesterol	−0.26 (−0.65 to 0.09)	0.1
LDL cholesterol	−0.48 (−0.80 to −0.16)	0.007†
Triglycerides*	0.06 (−0.31 to 0.43)	0.7
Glucose	−0.01 (−0.31 to 0.30)	1.0
Insulin*	−0.15 (−0.53 to 0.24)	0.4
HOMA-IR*	0.09 (−0.34 to 0.52)	0.7
Framingham score*	0.18 (−0.18 to 0.55)	0.3
RA characteristics		
Disease duration	0.14 (−0.22 to 0.51)	0.4
Rheumatoid factor–positive	−0.19 (−0.55 to 0.18)	0.3
Disease activity score in 28 joints	0.18 (−0.19 to 0.54)	0.3
Tender joints*	0.14 (−0.23 to 0.50)	0.5
Swollen joints	0.02 (−0.36 to 0.40)	0.9
VAS patient disease activity	0.13 (−0.23 to 0.49)	0.5
Cumulative prednisone use	−0.23 (−0.62 to 0.16)	0.2
Systemic inflammation		
Erythrocyte sedimentation rate	0.16 (−0.34 to 0.87)	0.4
C-reactive protein*	0.31 (−0.05 to 0.66)	0.09
Adipokines		
Adiponectin*	0.06 (−0.30 to 0.40)	0.8
Resistin*	0.13 (−0.27 to 0.57)	0.5
Leptin*	−0.25 (−0.61 to 0.11)	0.2
Ghrelin*	0.24 (−0.11 to 0.60)	0.2

Associations were assessed in mixed regression models (age at time of the study was replaced by age at disease onset in the model that included disease duration). † Statistically significant associations. * Non-normally distributed variables that were logarithmically transformed prior to entering them in the models. HDL: high-density lipoprotein LDL: low-density lipoprotein; HOMA-IR: homeostasis model of insulin resistance; VAS: visual analog scale.

0.09). OPG concentrations were not related to those of adipokines and ghrelin. Further adjustment for cardiovascular drug use in the models in Table 2 did not materially alter the findings (data not shown).

Independent relationships of baseline and post-IFX infusion OPG concentrations with endothelial activation and carotid atherosclerosis. Tables 3A and 3B show the independent relationships of OPG concentrations with endothelial activation and carotid atherosclerosis at baseline and after IFX administration, respectively. Independent of conventional risk factors as represented by the Framingham score and systemic inflammation as reflected by CRP concentrations, baseline OPG concentrations related to those of ICAM-1 and both CIMT and plaque; when the Framingham score and disease activity as estimated by the DAS28 were adjusted for, baseline OPG concentrations were associated

with those of both VCAM-1 and ICAM-1 concentrations, and CIMT, as well as plaque. Post-IFX OPG concentrations were related to those of ICAM-1, CIMT, and plaque independent of conventional risk factors and systemic inflammation. These relationships were materially unaltered upon adjustment for conventional risk factors and disease activity, although the post-infusion OPG-carotid plaque association was only borderline significant ($p = 0.09$). In our previous analysis, among the conventional risk factors, only total and LDL cholesterol concentrations were associated with those of OPG, and the DAS 28 was also not related to OPG concentrations (Table 2). Upon reassessing the OPG-endothelial relations in Table 3A with adjustment for only total and LDL cholesterol, the results were materially unaltered [partial $R = 0.35$ (95% CI 0.00 to 0.70), $p = 0.04$ and partial $R = 0.33$ (95% CI −0.02 to 0.68), $p = 0.06$, for the OPG-VCAM-1 and OPG-ICAM-1 relationships, respectively].

In view of our results as shown in Tables 3A and 3B, we determined whether changes in OPG concentrations were associated with changes in VCAM-1 and ICAM-1. Reductions in OPG levels related to those in VCAM-1 concentrations [partial $R = 0.35$ (95% CI 0.01 to 0.68), $p = 0.04$] and with borderline significance ($p = 0.09$) to those in ICAM-1 [partial $R = 0.29$ (95% CI −0.16 to 0.63)] concentrations.

Post-IFX infusion OPG concentrations were not related to those of post-infusion adipokines and ghrelin, and changes in OPG levels were also unrelated to those in adipokines and ghrelin^{34,35,36,37} (data not shown).

DISCUSSION

To our knowledge, our study shows for the first time that, in patients with severe RA, OPG concentrations relate to enhanced endothelial activation independent of well-established CVD risk factors, and that upon IFX infusion, reductions in OPG concentrations are associated with decreased endothelial activation. Patients with severe RA are particularly prone to increased cardiovascular risk³⁸. Our results also confirm previously reported findings of an association between OPG concentrations and carotid atherosclerosis²⁷ and that TNF- α blockade can reduce OPG concentrations in RA⁶. These findings indicate that increased OPG production is associated with markers of atherogenesis in RA. Finally, the favorable relationships between OPG concentrations and lipid measures, as produced by our data analysis, have been documented in subjects without RA³⁹, but not in those with RA, and may therefore constitute a severe disease-specific feature among patients with RA.

Our investigation was partly cross-sectionally designed, which precludes drawing inferences on the direction of causality. Therefore, our finding of an independent relationship between OPG concentrations and those of endothelial activation molecules, as well as carotid athero-

Table 3A. Independent relationships of osteoprotegerin (OPG) concentrations at time 0 minutes with endothelial activation at time 0 minutes and atherosclerosis in patients with rheumatoid arthritis.

Characteristics	Model 1		Model 2	
	Partial R	p	Partial R	p
Endothelial activation molecules				
E-selectin	−0.01 (−0.38 to 0.36)	1.0	0.02 (−0.34 to 0.40)	0.9
P-selectin*	0.18 (−0.18 to 0.54)	0.3	0.23 (−0.13 to 0.60)	0.2
VCAM-1	0.31 (−0.04 to 0.65)	0.08	0.36 (0.01 to 0.70)	0.04 [†]
ICAM-1*	0.34 (−0.01 to 0.69)	0.05 [†]	0.34 (−0.01 to 0.69)	0.05 [†]
ICAM-3	0.15 (−0.22 to 0.52)	0.4	0.16 (−0.21 to 0.53)	0.4
Carotid atherosclerosis				
Intima-media thickness*	0.52 (0.15 to 0.85)	0.008 [†]	0.51 (0.13 to 0.88)	0.009 [†]
	OR (95% CI)	p	OR (95% CI)	p
Plaque	1.61 (1.03–2.51)	0.03 [†]	1.52 (1.01–2.29)	0.04 [†]

OPG-endothelial activation and OPG-atherosclerosis relations were assessed in mixed regression models with adjustment for the log Framingham score and log C-reactive protein concentrations in model 1 and the log Framingham score and disease activity score in 28 joints in model 2. [†] Significant associations. * Characteristics that were non-normally distributed and were logarithmically transformed prior to entering them in linear regression models. VCAM: vascular cell adhesion molecule; ICAM: intercellular adhesion molecule.

Table 3B. Independent relationships of osteoprotegerin (OPG) concentrations at time 120 min* with endothelial activation at time 120 min and atherosclerosis in patients with rheumatoid arthritis.

Characteristics	Model 1		Model 2	
	Partial R	p	Partial R	p
Endothelial activation molecules				
E-selectin	0.08 (−0.29 to 0.45)	0.7	0.12 (−0.25 to 0.48)	0.5
P-selectin*	0.17 (−0.20 to 0.54)	0.3	0.24 (−0.13 to 0.60)	0.2
VCAM-1	−0.02 (−0.35 to 0.32)	0.9	0.04 (−0.31 to 0.37)	0.8
ICAM-1*	0.38 (0.03 to 0.72)	0.03 [†]	0.41 (0.07 to 0.75)	0.02 [†]
ICAM-3*	0.16 (−0.21 to 0.53)	0.4	0.18 (−0.18 to 0.55)	0.3
Carotid atherosclerosis				
Intima-media thickness*	0.53 (0.16 to 0.89)	0.007 [†]	0.50 (0.13 to 0.88)	0.01 [†]
	OR (95% CI)	p	OR (95% CI)	p
Plaque	1.52 (0.98–2.36)	0.05 [†]	1.42 (0.93–2.16)	0.09

OPG-endothelial activation and OPG-atherosclerosis relations were assessed in mixed regression models with adjustment for the log Framingham score and log C-reactive protein concentrations in model 1 and the log Framingham score and disease activity score in 28 joints in model 2. [†] Statistically significant associations. * Characteristics that were non-normally distributed and were logarithmically transformed prior to entering them in linear regression models. VCAM: vascular cell adhesion molecule; ICAM: intercellular adhesion molecule.

sclerosis, may represent a proatherogenic effect or a compensatory increase in OPG production in the presence of heightened CVD risk^{2,15}. Nevertheless, we also found that changes in OPG concentrations upon IFX administration were significantly associated with alterations in VCAM-1 concentrations. Taken together, our findings are congruent with those reported in cellular studies that assessed the effects of OPG on endothelial activation^{9,10}. Notably, we also found a borderline significant relationship between CRP concentrations and those of OPG. Because CRP can further downregulate TRAIL expression and OPG acts as a decoy receptor for TRAIL^{3,4,5}, the OPG-endothelial activation and OPG-atherosclerosis associations as found in the present investigation may also be partly attributable to an inhibition of TRAIL. Identification of the exact nature of the

OPG-cardiovascular risk relationship requires further investigation to determine the potential role of OPG targeting in reducing CVD risk in RA.

Paradoxical associations between adipokine concentrations and CVD risk were reported in subjects without and with RA and may represent a compensatory mechanism in the presence of enhanced CVD risk and in an attempt to reduce atherogenesis^{40,41,42}. Whether this phenomenon underlies the inverse association between OPG concentrations and those of total and LDL cholesterol in our investigation deserves further study. Indeed, OPG concentrations were significantly related to increased endothelial activation and atherosclerosis, even after adjusting for lipid measures, thereby arguing against an overall protective effect of OPG on CVD risk.

Patients with RA experience cytokine induced enhanced endothelial activation molecule production that further associates with prevalent and incident atherosclerosis^{43,44,45}. Conceptually, enhanced endothelial activation could reflect RA activity⁴⁶. However, disease activity and systemic inflammation were not associated with enhanced endothelial activation in the present investigation (data not shown) and the relationship between OPG concentrations and those of endothelial activation molecules were independent of both CRP concentrations and the DAS28.

Current recommendations on CVD risk stratification in RA³⁸ may result in a substantial proportion of patients at high or very high risk for cardiovascular events remaining unidentified and hence receiving inadequate CVD management²⁶. It was recently shown that carotid ultrasound may be particularly useful in improving CVD risk stratification in RA⁴⁷. Another promising tool in this context is the use of biomarkers with enhanced atherogenesis, especially prior to plaque development, in RA²⁶. Examples of this possibility include using resistin^{35,48} and angiopoietin-2⁴⁹ concentrations, as well as genetic markers^{50,51}. We found that OPG concentrations related not only to carotid atherosclerosis²⁷, but also endothelial activation independent of systemic inflammation and RA activity, as well as conventional CVD risk factors. Such an independent relationship was similarly shown between OPG concentrations and coronary artery calcification scores in RA²⁴. Thus, our results together with previously reported findings support a potential role of OPG concentrations in CVD risk stratification in RA. Such findings further suggest that, as applies to resistin⁴⁸, OPG may contribute to the link between systemic inflammation and increased CVD risk, and may assist in CVD risk stratification beyond information obtained through evaluation of routine systemic inflammatory markers.

In a previous study⁶, initiation of treatment with IFX in 21 patients with RA resulted in a gradual decrease in OPG concentrations that was significant after 6 weeks (but not within 2 weeks) after the first infusion and normalized after 14 to 22 weeks. Therefore, it is noteworthy that in our cohort of patients with severe RA that had already received IFX prior to the study, a significant reduction was observed within 2 h after a repeat infusion. We previously reported decreases in endothelial activation³¹ and resistin concentrations³⁵ and enhancement in insulin sensitivity⁵² in our cohort. Each of these processes^{43,44,45,48,53,54} reportedly contributes to enhanced atherogenesis in RA. Taken together, IFX administration may rapidly result in favorable effects on cardiovascular risk in patients on periodic treatment with this drug.

Systemic inflammation associates with high resistin^{35,48} and low adiponectin concentrations³⁴ and IFX therapy can also reduce ghrelin concentrations³⁶ in RA. In our investigation, OPG concentrations were consistently unrelated to

those of adipokines and ghrelin. If OPG on the one hand and adipokines and ghrelin on the other indeed contribute to altered cardiovascular risk in RA, they may effectuate this through different pathways.

Our present investigation has other limitations. Only 34 patients who had severe RA were included. Nevertheless, OPG concentrations were associated with both endothelial activation and carotid atherosclerosis, and this was consistent before and after an IFX infusion. Serum OPG levels do not necessarily reflect tissue concentrations. Future RA studies may also benefit from including patients in disease remission as inflammation in active RA can be expected to associate with increased OPG concentrations. Whether OPG levels predict progression of CIMT and plaque also merits further study.

OPG is likely involved in enhanced cardiovascular risk in RA. The direction of causality within the OPG concentrations-endothelial activation and OPG concentrations-atherosclerosis relations needs additional elucidation in future mechanistic and longitudinal studies prior to targeting OPG in CVD risk management in RA. Meanwhile, determination of OPG concentrations may be useful in improving CVD risk stratification in RA.

REFERENCES

- Hofbauer LC, Schoppet M. Clinical implications of the osteoprotegerin/RANKL/RANK system for bone and vascular diseases. *JAMA* 2004;28:490-5.
- Van Campenhout A, Golledge J. Osteoprotegerin, vascular calcification and atherosclerosis. *Atherosclerosis* 2009;204:321-9.
- Secchiero P, Corallini F, Beltrami AP, Ceconi C, Bonasia V, Di Chiara A, et al. An imbalanced OPG/TRAIL ratio is associated to severe acute myocardial infarction. *Atherosclerosis* 2010; 210:274-7.
- Secchiero P, Rimondi E, Di Lasio MG, Agnoletto C, Melloni E, Volpi I, et al. C-reactive protein downregulates TRAIL expression in human peripheral monocytes via an Egr-1-dependent pathway. *Clin Cancer Res* 2013;19:1949-59.
- Di Bartola BA, Cartland SP, Harith HH, Bobryshev YV, Schoppet M, Kavurma MM. TRAIL-deficiency accelerates vascular calcification in atherosclerosis via modulation of RANKL. *PLoS One* 2013;8:e74211.
- Ziolkowska M, Kurowska M, Radzikowska A, Luszczaykiewicz G, Wiland P, Dziewczopolski W, et al. High levels of osteoprotegerin and soluble receptor activator of nuclear factor κ B ligand and their normalization alter anti-tumor necrosis factor α treatment. *Arthritis Rheum* 2002;46:1744-53.
- Corallini F, Bossi F, Gonelli A, Tripodo C, Castellano G, Mohines TE, et al. The soluble terminal complement complex (SC5b-9) up-regulates osteoprotegerin expression and release by endothelial cells: implications in rheumatoid arthritis. *Rheumatology* 2009;48:293-8.
- Bucal N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, et al. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 1998;12:1260-8.
- Mangan SH, Van Campenhout A, Rush C, Golledge J. Osteoprotegerin up regulates endothelial cell adhesion molecule response to tumor necrosis factor- α associated with induction of angiopoietin-2. *Cardiovasc Res* 2007;76:494-505.
- Zauli G, Corallini F, Bossi F, Fischetti F, Durigutto P, Celeghini C,

- et al. Osteoprotegerin increases leukocyte adhesion to endothelial cells both in vitro and in vivo. *Blood* 2007;110:536-43.
11. Sandberg WJ, Yndestad A, Oie E, Smith C, Ueland T, Ovchinnikova O, et al. Enhanced T-cell expression of RANK ligand in acute coronary syndrome: possible role in plaque destabilization. *Arterioscler Thromb Vasc Biol* 2006;26:857-63.
12. Jono S, Ikari Y, Shioi A, Mori K, Miki T, Hara K, et al. Serum osteoprotegerin levels are associated with the presence and severity of coronary artery disease. *Circulation* 2002;106:1192-4.
13. Schopper M, Sattler AM, Schaefer JR, Herzum M, Maisch B, Hofbauer LC. Increased osteoprotegerin serum levels in men with coronary artery disease. *J Clin Endocrinol Metab* 2003;88:1024-8.
14. Semb AG, Ueland T, Aukrust P, Wareham NJ, Luben R, Gullestad L, et al. Osteoprotegerin and soluble receptor activator of nuclear factor- κ B ligand and risk for coronary events: a nested case – control approach in the Prospective EPIC-Norfolk Population Study 1993–2003. *Arterioscler Thromb Vasc Biol* 2009;29:975-80.
15. Kiechl S, Schett G, Wenning G, Redlich K, Oberhollenzer M, Mayr A, et al. Osteoprotegerin is a risk factor for progressive atherosclerosis and cardiovascular disease. *Circulation* 2004;109:2175-80.
16. Browner WS, Lui LY, Cummings SR. Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. *J Clin Endocrinol Metab* 2001;86:631-7.
17. Ziegler S, Kudlacek S, Lugar A, Minar E. Osteoprotegerin plasma concentrations correlate with severity of peripheral artery disease. *Atherosclerosis* 2005;182:175-80.
18. Dessein PH, Joffe BI, Veller MG, Stevens BA, Tobias M, Reddi K, et al. Traditional and nontraditional cardiovascular risk factors are associated with atherosclerosis in rheumatoid arthritis. *J Rheumatol* 2005;32:435-42.
19. Dessein PH, Norton GR, Woodiwiss AJ, Joffe BI, Wolfe F. Influence of nonclassical cardiovascular risk factors on the accuracy of predicting subclinical atherosclerosis in rheumatoid arthritis. *J Rheumatol* 2007;34:943-51.
20. del Rincón I, Freeman GL, Haas RW, O'Leary DH, Escalante A. Relative contribution of cardiovascular risk factors and rheumatoid arthritis clinical manifestations to atherosclerosis. *Arthritis Rheum* 2005;52:3413-23.
21. Solomon DH, Kremer J, Curtis JR, Hochberg MC, Reed G, Tsao P, et al. Explaining the cardiovascular risk associated with rheumatoid arthritis: traditional risk factors versus markers of rheumatoid arthritis severity. *Ann Rheum Dis* 2010;69:1920-5.
22. Kitas GD, Gabriel SE. Cardiovascular disease in rheumatoid arthritis: state of the art and future perspectives. *Ann Rheum Dis* 2011;70:8-14.
23. Solomon A, Woodiwiss AJ, Abdool-Carrim AT, Stevens BA, Norton GR, Dessein PH. The carotid artery atherosclerosis burden and its relation to cardiovascular risk factors in black and white Africans with established rheumatoid arthritis: a cross-sectional study. *J Rheumatol* 2012;39:1798-806.
24. Asanuma Y, Chung CP, Oeser A, Solus JF, Avalos I, Gebretsadik T, et al. Serum osteoprotegerin is increased and independently associated with coronary-artery atherosclerosis in patients with rheumatoid arthritis. *Atherosclerosis* 2007;195:e135-41.
25. Corrales A, Parra JA, González-Juanatey C, Rueda-Gotor J, Blanco R, Llorca J, et al. Cardiovascular risk stratification in rheumatic diseases: carotid ultrasound is more sensitive than Coronary Artery Calcification Score to detect subclinical atherosclerosis in patients with rheumatoid arthritis. *Ann Rheum Dis* 2013;72:1764-70.
26. Dessein PH, Semb AG. Could cardiovascular disease risk stratification and management in rheumatoid arthritis be enhanced? *Ann Rheum Dis* 2013;72:1743-6.
27. Asanuma YF, Shimada Y, Kouzu N, Yokota K, Nakajima K, Sato K, et al. Serum osteoprotegerin concentration is associated with carotid atherosclerotic plaque in patients with rheumatoid arthritis. *Mod Rheumatol* 2013;23:269-75.
28. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
29. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010;62:2569-81.
30. Prevoo ML, van't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995;38:44-8.
31. Gonzalez-Gay MA, Garcia-Unzueta MT, De Matias JM, Gonzalez-Juanatey C, Garcia-Porrúa C, Sanchez-Andrade A, et al. Influence of anti-TNF- α infliximab therapy on adhesion molecules associated with atherogenesis in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2006;24:373-9.
32. Gonzalez-Juanatey C, Llorca J, Martin J, Gonzalez-Gay MA. Carotid intima-media thickness predicts the development of cardiovascular events in patients with rheumatoid arthritis. *Semin Arthritis Rheum* 2009;38:366-71.
33. Gonzalez-Juanatey C, Llorca J, Testa A, Revuelta J, Garcia-Porrúa C, Gonzalez-Gay MA. Increased prevalence of severe subclinical atherosclerotic findings in long-term treated rheumatoid arthritis patients without clinically evident atherosclerotic disease. *Medicine* 2003;82:407-13.
34. Gonzalez-Gay MA, Llorca J, Garcia-Unzueta MT, Gonzalez-Juanatey C, De Matias JM, Martin J, et al. High-grade inflammation, circulating adiponectin concentrations and cardiovascular risk factors in severe rheumatoid arthritis. *Clin Exp Rheumatol* 2008;26:596-603.
35. Gonzalez-Gay MA, Garcia-Unzueta MT, Gonzalez-Juanatey, Miranda-Filloo JA, Vazquez-Rodriguez TR, De Matias JM, et al. Anti-TNF- α therapy modulates resistin in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2008;26:311-6.
36. Gonzalez-Gay MA, Garcia-Unzueta MT, Berja A, Gonzalez-Juanatey C, Miranda-Filloo JA, Vazquez-Rodriguez TR, et al. Anti-TNF- α therapy does not modulate leptin in patients with severe rheumatoid arthritis. *Clin Exp Rheumatol* 2009;27:222-8.
37. Gonzalez-Gay MA, Garcia-Unzueta MT, Berja A, Vazquez-Rodriguez TR, Gonzalez-Juanatey C, De Matias JM, et al. Anti-TNF- α therapy modulates ghrelin in patients with severe rheumatoid arthritis. *Ann Rheum Dis* 2008;67:1644-6.
38. Peters MJ, Symmons DP, McCarey D, Dijkmans BA, Nicola P, Kvien TK, et al. EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. *Ann Rheum Dis* 2010;69:325-31.
39. Gannage-Yared MH, Fares F, Semaan M, Khalife S, Jambart S. Circulating osteoprotegerin is correlated with lipid profile, insulin sensitivity, adiponectin and sex steroids in an ageing male population. *Clin Endocrinol* 2006;64:652-8.
40. Dessein PH, Woodiwiss AJ, Norton GR, Tsang L, Solomon A. Independent associations of total and high molecular weight adiponectin with cardiometabolic risk and surrogate markers of enhanced early atherogenesis in black and white patients with rheumatoid arthritis: a cross-sectional study. *Arthritis Res Ther* 2013;15:R128.
41. Dessein PH, Norton GR, Badenhorst M, Woodiwiss AJ, Solomon A. Rheumatoid arthritis impacts on the independent relationships

- between circulating adiponectin concentrations and cardiovascular metabolic risk. *Mediators Inflamm* 2013;2013:461849.
42. Sattar N, Nelson SM. Adiponectin, diabetes, and coronary heart disease in older persons: unraveling the paradox. *J Clin Endocrinol Metab* 2008;93:3299-301.
 43. Dessein PH, Joffe BI, Singh S. Biomarkers of endothelial dysfunction, cardiovascular risk factors and atherosclerosis in rheumatoid arthritis. *Arthritis Res Ther* 2005;7:R634-43.
 44. Dessein PH, Joffe BI. Suppression of circulating interleukin-6 concentrations is associated with decreased endothelial activation in rheumatoid arthritis. *Clin Exp Rheumatol* 2006;24:161-7.
 45. Sodergren A, Karp K, Boman K, Eriksson C, Lundstrom E, Smedby T, et al. Atherosclerosis in early rheumatoid arthritis: very early endothelial activation and rapid progression of intima media thickness. *Arthritis Res Ther* 2010;12:R158.
 46. Curtis JR, van der Helm-van Mil AH, Knevel R, Huizinga TW, Haney DJ, Shen Y, et al. Validation of a novel multibiomarker test to assess rheumatoid arthritis disease activity. *Arthritis Care Res* 2012;64:1794-803.
 47. Corrales A, González-Juanatey C, Peiró ME, Blanco R, Llorca J, González-Gay MA. Carotid ultrasound is useful for the cardiovascular risk stratification of patients with rheumatoid arthritis: results of a population-based study. *Ann Rheum Dis* 2013 Mar 16 (E-pub ahead of print).
 48. Dessein PH, Norton GR, Woodiwiss AJ, Solomon A. Independent relationship between circulating resistin concentrations and endothelial activation in rheumatoid arthritis. *Ann Rheum Dis* 2013;72:1586-8.
 49. López-Mejías R, Corrales A, Genre F, Hernández JL, Ochoa R, Blanco R, et al. Angiopoietin-2 serum levels correlate with severity, early onset and cardiovascular disease in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2013;31:761-6.
 50. Gonzalez-Gay MA, Gonzalez-Juanatey C, Lopez-Diaz MJ, Piñeiro A, Garcia-Porrúa C, Miranda-Filloo JA, et al. HLA-DRB1 and persistent chronic inflammation contribute to cardiovascular events and cardiovascular mortality in patients with rheumatoid arthritis. *Arthritis Rheum* 2007;57:125-32.
 51. Rodríguez-Rodríguez L, González-Juanatey C, Palomino-Morales R, Vázquez-Rodríguez TR, Miranda-Filloo JA, Fernández-Gutiérrez B, et al. TNFA -308 (rs1800629) polymorphism is associated with a higher risk of cardiovascular disease in patients with rheumatoid arthritis. *Atherosclerosis* 2011;216:125-30.
 52. Gonzalez-Gay MA, De Matias JM, Gonzalez-Juanatey C, Garcia-Porrúa C, Sanchez-Andrade A, Martin J, et al. Anti-tumor necrosis factor-alpha blockade improves insulin resistance in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2006; 24:83-6.
 53. Dessein PH, Tobias M, Veller MG. Metabolic syndrome and subclinical atherosclerosis in rheumatoid arthritis. *J Rheumatol* 2006;33:2425-32.
 54. Chung CP, Oeser A, Solus JF, Avalos I, Gebretsadik T, Shintani A, et al. Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. *Atherosclerosis* 2008;196:756-63.