Osteoprotegerin Is a Risk Factor for Progressive Atherosclerosis and Cardiovascular Disease

Stefan Kiechl, MD; Georg Schett, MD; Gregor Wenning, MD; Kurt Redlich, MD; Martin Oberhollenzer, MD; Agnes Mayr, MD; Peter Santer, MD; Josef Smolen, MD; Werner Poewe, MD; Johann Willeit, MD

Background — Osteoprotegerin is a novel member of the tumor necrosis factor receptor superfamily and a soluble decoy receptor of the receptor activator of nuclear factor-κB ligand. Recent experimental research has implicated osteoprotegerin in atherogenesis, but epidemiological confirmation of this concept is sparse.

Methods and Results — As part of the prospective, population-based Bruneck Study, severity, initiation, and progression of atherosclerosis were assessed in carotid arteries. Cases of incident cardiovascular disease and vascular mortality were carefully recorded over a 10-year period (1990 to 2000). Osteoprotegerin levels were measured in samples obtained at baseline and during follow-up. Serum osteoprotegerin showed a strong association with numerous vascular risk factors, including age, diabetes, markers of systemic inflammation, chronic infection, and smoking. In multivariate analyses, osteoprotegerin was significantly related to severity and 10-year progression of carotid atherosclerosis. Furthermore, a high level of osteoprotegerin was an independent risk factor for incident cardiovascular disease (adjusted relative risk for the top versus bottom tertile group for osteoprotegerin 2.2 [1.3 to 3.8]; \( P=0.001 \)) and vascular mortality (adjusted relative risk for the top versus bottom tertile group for osteoprotegerin 3.1 [1.2 to 8.2]; \( P=0.010 \)) but not for mortality due to nonvascular causes.

Conclusions — Osteoprotegerin is an independent risk factor for the progression of atherosclerosis and onset of cardiovascular disease. (Circulation. 2004;109:2175-2180.)

Key Words: atherosclerosis • risk factors • myocardial infarction • inflammation • immune system
Clinical History and Examination

Body mass index was calculated as weight divided by height squared (kg/m²). Smoking status and alcohol consumption were recorded as detailed previously. The activity score was composed of the scores for work (3 categories) and sports/leisure activities (0, >2 hours per week). Socioeconomic status was defined on a 3-category scale (low, medium, or high) based on information about occupational status and educational level of the person with the highest income in the household. Diabetes was diagnosed according to World Health Organization criteria. Chronic infections were assessed by means of an extensive screening procedure, as detailed previously.

Assessment of incident (fatal and nonfatal) cardiovascular disease was based on the patient’s medical history, a detailed review of the Bruneck Hospital databases and death certificates, and the results of clinical and various laboratory examinations. Myocardial infarction was defined as a relative increase in the atherosclerosis score (1990 to 2000) that exceeded twice the measurement error (κ-coefficients for this categorization >0.8 [reproducibility sample, n=100]). Intima-media thickness (IMT) was quantified at the far wall of plaque-free sections of the common, carotid arteries as the distance between the lumen-intima and media-adventitia interface (intraobserver coefficient of variation, 7.9% [n=100]).

Statistical Analysis

Associations between OPG tertile groups and vascular risk factors, lifestyle and demographic variables, IMT, and presence and severity of atherosclerosis were assessed with generalized linear models and logistic regression analysis. The association between OPG tertile groups and 10-year changes in carotid atherosclerosis was tested by logistic regression. Initiation of atherosclerosis was analyzed in subjects free of carotid plaques at study baseline (n=500), whereas calculations that addressed the progression of atherosclerosis focused on subjects with preexisting carotid plaques (n=326). The relation between OPG tertile groups and incident cardiovascular disease and mortality from vascular and nonvascular causes was analyzed by means of Cox regression analysis, with OPG entered as a time-dependent covariate. We performed tests for linear trend by treating the medians in each category of OPG as a continuous variable. Analyses were repeated after exclusion of subjects who had experienced cardiovascular disease before study baseline. Multivariate equations were adjusted for age, sex, established vascular risk factors, and variables found to be associated with OPG in the correlation analyses. We assigned values for missing data (C-reactive protein, E-selectin, and intracellular adhesion molecule-1) using multiple linear regression. Regression models were fitted for each variable with the covariates selected from among those listed in Table 1 (forward stepwise selection with standard inclusion and exclusion criteria). The main findings were replicated in separate analyses that treated log-transformed OPG levels as a continuous variable.

Results

Distribution, tertile groups, and main descriptive characteristics of baseline serum OPG levels are depicted in the Figure. OPG emerged as relatively stable over time, as indicated by the strong nonparametric correlation between levels measured in 1990 and 1995 (r=0.71) and a considerable consistency in the attribution to OPG tertile groups in both assessments. In brief, approximately two thirds of study participants remained in the same tertile group, and more than 90% of the remaining subjects changed to a neighboring one.

OGP and Risk Factors

In agreement with previous studies, OPG was strongly correlated with age in both the 1990 and the 1995 evaluation (Spearman rank correlation coefficients 0.52 and 0.58, respectively). In age- and sex-adjusted models, OPG was also related to diabetes and some features of the associated metabolic syndrome, various markers of endothelial activation, and systemic inflammation and major proinflammatory conditions such as chronic infection or smoking (Table 1).

OGP and Atherosclerosis

At study baseline, prevalence and severity of carotid atherosclerosis (atherosclerosis score) increased substantially with higher levels of OPG (23.3%, 37.0%, and 66.6%, and 0.87, 1.60, and 3.81 mm in tertile groups for OPG, P<0.001 for each comparison). After controlling for sex and for age, a potential confounder in this analysis, the associations decreased in strength but remained significant (P<0.001 each). Age- and sex-adjusted mean values of the atherosclerosis
score in tertile groups for OPG were 1.68, 1.72, and 2.89 mm. In fully adjusted models, probability values amounted to 0.09 (presence of atherosclerosis) and 0.03 (atherosclerosis score), respectively. Analysis focusing on the association between IMT, a precursor lesion of atherosclerosis, and OPG fell short of statistical significance after adjustment for age and sex (adjusted means in tertile groups for OPG: 943, 947, and 959 \( \mu m \); \( P = 0.45 \)).
In the prospective risk analysis, OPG was seen to be significantly related to the initiation and progression of carotid atherosclerosis. Whereas the former association disappeared after adjustment for age and sex, the latter maintained significance in multivariate models that controlled for age, sex, major vascular risk factors, and variables correlated with OPG (Table 2). All findings emerged independently of whether OPG was analyzed as a continuous or categorized (tertile groups) variable (Table 2).

**OPG and Vascular Disease**

OPG was found to be a significant and independent risk factor for 10-year incidence of cardiovascular disease and for vascular mortality but not for mortality due to nonvascular causes (Table 3). Its predictive significance applied to both men and women and persisted after multivariate adjustment (Table 3). Results were virtually identical after the exclusion of subjects who had experienced cardiovascular disease before study baseline \( n=65 \); Table 3). To lessen the weight of age, supplementary analyses were performed in population subgroups in a lower age range. In these equations, OPG gained relevance and maintained significance in spite of the substantially reduced number of subjects and associated loss in calculational power. For example, adjusted relative risks of incident cardiovascular disease and vascular mortality for the top versus bottom tertile group for OPG were 3.3 (1.7 to 6.7) and 4.0 (1.2 to 13.2) in subjects aged 60 years and over at study baseline (models such as those in Table 2; \( P<0.001 \) and \( P=0.013 \), respectively).

**Discussion**

OPG, a secreted basic glycoprotein and member of the TNF receptor superfamily, was initially described by Simonet et al.\(^5,12\) as a key regulator of bone homeostasis and vessel calcification in animals. It is produced by various tissues, including main components of human vasculature such as smooth muscle cells and the endothelium.\(^5,13-15\) The latter cell lines, apart from constituting a source for OPG, may also be a target of OPG action (paracrine regulation). Functionally, OPG binds to RANKL and TRAIL,\(^6\) thereby inhibiting ligation of these mediators to their cognate receptors and subsequent activation of specific proinflammatory and pro-apoptotic signaling pathways.

The significance of OPG for vascular biology has recently been given its first epidemiological support. A cohort study of elderly women showed that those who died of stroke or any vascular cause had higher OPG levels at study baseline.\(^7\) Two subsequent cross-sectional evaluations in selected patient groups at high vascular risk (subjects undergoing coronary

**Baseline distribution, tertile groups, and main descriptive characteristics of serum OPG.**

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**TABLE 2. OR for Initiation and Progression of Carotid Atherosclerosis Calculated for OPG Tertile Groups and for a 1-SD Unit Increase in OPG Levels (1990–2000, n=826)*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
<th>( P ) for Trend</th>
<th>OR (95% CI)</th>
<th>1-SD Unit Change in OPG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initiation of atherosclerosis‡</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.0</td>
<td>1.4 (0.9–2.0)</td>
<td>1.8 (1.1–2.9)</td>
<td>0.018</td>
<td>1.3 (1.1–1.7)</td>
<td>0.011</td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.0</td>
<td>1.0 (0.6–1.5)</td>
<td>0.9 (0.5–1.7)</td>
<td>0.82</td>
<td>1.0 (0.8–1.3)</td>
<td>0.82</td>
</tr>
<tr>
<td>Multivariate adjustment</td>
<td>1.0</td>
<td>0.8 (0.5–1.3)</td>
<td>0.7 (0.3–1.2)</td>
<td>0.19</td>
<td>0.9 (0.7–1.1)</td>
<td>0.33</td>
</tr>
<tr>
<td>Multivariate adjustment†</td>
<td>1.0</td>
<td>0.8 (0.5–1.3)</td>
<td>0.7 (0.3–1.2)</td>
<td>0.19</td>
<td>0.9 (0.7–1.1)</td>
<td>0.33</td>
</tr>
<tr>
<td><strong>Progression of atherosclerosis‡</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.0</td>
<td>1.2 (0.5–2.6)</td>
<td>2.5 (1.1–5.5)</td>
<td>0.014</td>
<td>1.4 (1.0–1.8)</td>
<td>0.036</td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.0</td>
<td>1.2 (0.5–2.6)</td>
<td>2.8 (1.2–6.8)</td>
<td>0.011</td>
<td>1.4 (1.0–1.9)</td>
<td>0.034</td>
</tr>
<tr>
<td>Multivariate adjustment</td>
<td>1.0</td>
<td>1.2 (0.5–2.9)</td>
<td>2.9 (1.1–7.3)</td>
<td>0.017</td>
<td>1.5 (1.1–2.1)</td>
<td>0.021</td>
</tr>
<tr>
<td>Multivariate adjustment†</td>
<td>1.0</td>
<td>1.2 (0.5–2.9)</td>
<td>2.7 (1.1–6.9)</td>
<td>0.027</td>
<td>1.5 (1.0–2.0)</td>
<td>0.031</td>
</tr>
</tbody>
</table>

*ORs and 95% CIs were derived by logistic regression analysis. Multivariate adjustment; see Table 3.
†Analysis was further adjusted for levels of C-reactive protein, E-selectin, and intracellular adhesion molecule-1.
‡The initiation of atherosclerosis was studied in subjects free of carotid plaques at study baseline \( n=500 \), and progression of atherosclerosis was studied in those with preexisting plaques \( n=326 \).
angiography) revealed a strong positive association between OPG serum level and advanced coronary artery disease.8,9

The present study is the first prospective, population-based survey addressing the association between OPG and atherosclerotic vascular disease. In brief, OPG was found to be significantly and independently related to severity and 10-year progression of carotid artery disease, as well as to incident cardiovascular disease and vascular mortality (Tables 2 and 3). Emergence of strong associations in the multivariate models is remarkable given the prominent correlation between OPG and age. These equations should be expected to produce conservative risk estimates, because they did not consider the possibility that effects of age and other risk factors for atherosclerosis are mediated in part by OPG.

Of note, the OPG level was not independently related to nonvascular mortality (Table 3) or to many other diseases such as cancer, autoimmune diseases, or asthma (data not presented). Thus, OPG appears not to be a nonspecific marker of disease or poor health.

How OPG may operate in vascular pathophysiology is not known precisely. However, several clues from experimental studies suggest a role of OPG in vascular calcification, regulation of apoptosis, and immune defense.

Vascular Calcification
Vascular calcification was previously assumed to be entirely passive owing to a shift of calcium from the bone to the arterial wall and is now increasingly recognized to underlie a complex regulation by calcification inhibitors.16–18 OPG counteracts calcification by its well-established capacity to inhibit bone resorption and is considered to be a candidate calcification inhibitor.17,18 Notably, OPG knockout mice develop severe hypercalcemia and early calcium deposits in the media and subintima of the aorta, accompanied by the formation of typical bone matrix.12,19 In this and other murine models of arterial calcification, a normal phenotype can be rescued by cross-breeding with OPG transgenic mice and/or by early OPG substitution.19,20 Whether or not these intriguing findings can be extrapolated to human atherosclerosis remains to be elucidated, because murine and human arterial calcification differ in terms of time course, histological appearance, and some associated circumstances.19

Apoptosis and Immune Defense
As anticipated, OPG is a decoy receptor for TRAIL, thus exerting antiapoptotic effects on endothelial and other susceptible cells and acting as a “survival factor.”6,15 Furthermore, OPG participates in the vascular cytokine network.13

### TABLE 3. Relative Risk of Cardiovascular Events and Mortality Due to Vascular and Nonvascular Causes Calculated for OPG Tertile Groups and for a 1-SD Unit Increase in OPG Levels (1990–2000, n=915)

<table>
<thead>
<tr>
<th></th>
<th>Hazard Ratio (95% CI)</th>
<th>1-SD Unit Change in OPG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tertile 1</td>
<td>Tertile 2</td>
</tr>
<tr>
<td>Incident cardiovascular disease</td>
<td>n=18</td>
<td>n=24</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.0</td>
<td>1.5 (0.8–2.8)</td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.0</td>
<td>1.2 (0.6–2.2)</td>
</tr>
<tr>
<td>Multivariate adjustment</td>
<td>1.0</td>
<td>1.2 (0.6–2.2)</td>
</tr>
<tr>
<td>Multivariate adjustment†</td>
<td>1.0</td>
<td>1.2 (0.7–2.2)</td>
</tr>
<tr>
<td>Multivariate adjustment‡</td>
<td>1.0</td>
<td>1.0 (0.5–2.0)</td>
</tr>
<tr>
<td>Mortality due to vascular causes</td>
<td>n=5</td>
<td>n=8</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.0</td>
<td>1.9 (0.6–5.7)</td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.0</td>
<td>1.6 (0.5–4.8)</td>
</tr>
<tr>
<td>Multivariate adjustment</td>
<td>1.0</td>
<td>1.5 (0.5–4.8)</td>
</tr>
<tr>
<td>Multivariate adjustment†</td>
<td>1.0</td>
<td>1.5 (0.5–4.7)</td>
</tr>
<tr>
<td>Mortality due to nonvascular causes</td>
<td>n=18</td>
<td>n=22</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.0</td>
<td>1.3 (0.7–2.4)</td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.0</td>
<td>0.9 (0.5–1.6)</td>
</tr>
<tr>
<td>Multivariate adjustment</td>
<td>1.0</td>
<td>0.8 (0.4–1.6)</td>
</tr>
<tr>
<td>Multivariate adjustment†</td>
<td>1.0</td>
<td>1.1 (0.6–2.1)</td>
</tr>
</tbody>
</table>

n values refer to number of events. Hazard ratios and 95% Cs were derived by Cox regression analyses with OPG treated as a time-dependent covariate. Cardiovascular disease subsumes the following events: transient ischemic attacks, ischemic stroke, myocardial infarction, peripheral artery disease, revascularization procedures, and vascular deaths. The multivariate model included variables for age, sex, social status, smoking, systolic blood pressure, diabetes, body mass index, HDL and LDL cholesterol, lipoprotein(a), fibrinogen, white blood cell count, ferritin, and chronic infection.

*Median (range) values for tertiles of OPG: tertile 1, 2.96 (0.30–9.9) pmol/L; tertile 2, 3.54 (3.17–3.95) pmol/L; and tertile 3, 4.34 (3.96–24.12) pmol/L.

†Analysis was further adjusted for levels of C-reactive protein, E-selectin, and intracellular adhesion molecule-1.

‡Analysis after exclusion of subjects who had experienced cardiovascular disease before study baseline (n=65).
Proinflammatory cytokines such as interleukin-1 or TNF-α and growth factors such as platelet-derived growth factor inhibit OPG expression in endothelial and vascular smooth muscle cells, and OPG, in turn, interferes with various inflammatory signaling pathways. In murine models, OPG is crucial to B-cell maturation and a sufficient antibody response, whereas the OPG ligand RANKL augments dendritic cell survival and modulates T-cell activity. Finally, peroxisome proliferator-activated receptor-γ, an important nuclear transcription factor, which on activation exerts anti-inflammatory and atherogenic effects, has recently been shown to downregulate OPG expression by approximately one fifth of normal levels. Given all these potential links between OPG and vascular inflammation and immunity, it is not unexpected that OPG was significantly related to high \( C \)-reactive protein and various other acute-phase reactants, as well as to proinflammatory conditions such as smoking and chronic infections, in the present study (Table 1).

All these considerations suggest an active role of OPG in vascular pathophysiology, but it is not immediately apparent whether OPG is beneficial or injurious to the vasculature or whether effects differ depending on the stage of atherosclerotic vessel disease. The most convenient interpretation of an epidemiological association between high OPG and high vascular risk would be that of a causal risk factor. Alternatively, however, OPG has been proposed to be a compensatory vascular defense system that is upregulated in athero-epidemiological association between high OPG and high vascular risk. Although it ultimately cannot be ruled out that OPG is a response to atherosclerosis was first put forth by Browner et al and reinforced by others.

In conclusion, the present study identifies high serum OPG as an independent risk factor of progressive atherosclerosis and incident cardiovascular disease in the general community. This finding may be relevant to routine assessment of vascular risk. Although it ultimately cannot be ruled out that high levels of OPG are entirely an epiphenomenon of inflammatory processes harbored in atherosclerotic tissue, a number of experimental studies advocate an active role of OPG in vessel pathology. High OPG may act as a causal risk factor of or represent a counterregulatory protective response to atherosclerosis. Now that genetically engineered OPG analogs have been given to humans in small pilot trials testing the effects of OPG on bone turnover, urgent clarification of the precise nature of the association between OPG and atherosclerosis is warranted.

Acknowledgments

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References

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