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# Osteoprotegerin as a Predictor of Coronary Artery Disease and Cardiovascular Mortality and Morbidity

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**Osteoprotegerin (OPG) is a glycoprotein that acts as a decoy receptor for receptor activator of nuclear factor κB ligand (RANKL) and tumor necrosis factor-related apoptosis-inducing ligand. The OPG/RANKL/receptor activator of nuclear factor κB axis plays an important regulatory role in the skeletal, immune, and vascular systems. The protective role of OPG, in animal models, against vascular calcification has not been replicated in human trials; moreover, increased OPG levels have been consistently associated with the incidence and prevalence of coronary artery disease. There seems to be some dichotomy in the role of OPG, RANKL, and tumor necrosis factor-related apoptosis-inducing ligand in atherosclerosis and plaque stability. In this review, we integrate the findings from some of the important studies and try to draw conclusions with a view to gaining some insight into the complex interactions of the OPG/RANKL/receptor activator of nuclear factor κB axis and tumor necrosis factor-related apoptosis-inducing ligand in the pathophysiology of atherosclerosis. (J Am Coll Cardiol 2010;55: 2049–61) © 2010 by the American College of Cardiology Foundation**

Coronary artery disease (CAD) is the leading cause of mortality in the Western world. Myocardial infarction (MI) or death is the first presentation in a significant percentage of patients with CAD. With the technological advances being made in the detection of silent CAD, in terms of assessing atherosclerotic plaque burden with coronary artery calcium (CAC) score or identifying the percentage of myocardium at risk with myocardial perfusion imaging, stratification of these patients into low- and high-risk categories has become easier. These techniques have the drawback of using ionizing radiation, which has its attending risks. A biomarker specific enough to be able to identify a subset of patients at increased risk of CAD would indeed be invaluable. In this article, we focus on the evidence regarding the utility of osteoprotegerin (OPG) and its ligands, receptor activator of nuclear factor κB ligand (RANKL), and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) as potential biomarkers of cardiovascular disease.

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## Overview of Bone and Vascular Biology

A temporal link exists between the development of osteoporosis and vascular calcification, particularly marked in post-menopausal women and in the elderly (1–3). This link between the skeletal and cardiovascular systems can be traced back to the embryonic stage of human development when endochondral ossification is initiated by the penetration of the cartilaginous template by a vascular bud. The rapid proliferation of chondrocytes induces hypoxia resulting in inhibition of the hydroxylation of the hypoxia inducible factor 1α, which then stimulates increased production of vascular endothelial growth factor (4,5). Vascular endothelial growth factor induces endothelial cell proliferation, survival, and migration, resulting in the formation of a vascular network supplying oxygen and nutrients for further development (6).

During embryogenesis, hematopoietic cells and vascular cells are derived from a common ancestor, the hemangioblast, which is responsible for establishing the hematopoietic stem cell niche (7,8). Osteoblasts, endothelial cells, and bone marrow stromal cells are also derived from this niche (9).

Many of the bone regulatory proteins such as OPG (10), osteopontin (11), osteocalcin, bone morphogenetic protein 2 (12), and RANKL (10) have been shown to be present in atherosclerotic plaques in vitro and seem to be expressed by vascular cells in vivo as well. Osteopontin protein and mRNA levels are up-regulated in atherosclerotic plaques (13). Plasma osteopontin levels are also higher in patients

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**Abbreviations and Acronyms**

**AAA** = abdominal aortic aneurysm
**CAC** = coronary artery calcium
**CAD** = coronary artery disease
**EC** = endothelial cell
**MI** = myocardial infarction
**MMP** = matrix metalloproteinase
**OPG** = osteoprotegerin
**RANK** = receptor activator of nuclear factor κB
**RANKL** = receptor activator of nuclear factor κB ligand
**TNF** = tumor necrosis factor
**TRAIL** = tumor necrosis factor-related apoptosis-inducing ligand
**VSMC** = vascular smooth muscle cell

with CAD compared with those without (14). The role of osteopontin in atherosclerosis is unclear but is attributed to its role as a mediator of inflammatory response.

## Biochemistry of OPG

OPG is a cytokine of the tumor necrosis factor (TNF) receptor superfamily and is classed as an osteoclastogenesis inhibitory factor. Biochemically, OPG is a basic secretory glycoprotein composed of 401 amino acid residues with 7 distinct structural domains. It exists as either a monomer of 60 kDa or a disulfide bond linked homodimer of 120 kDa (15). The homodimeric form of OPG is biologically more active than the monomeric form. Although initial studies were done with monoclonal antibodies detecting only the homodimeric form of OPG, more recent studies use the enzyme-linked immunosorbent assay technique to detect total serum OPG levels—monomeric, homodimeric, and OPG bound to its ligands in the serum. The amino terminal domains 1 through 4 are cysteine rich and confer osteoclastogenesis inhibitory properties (15). Domains 5 and 6 at the carboxy terminal end of the protein contain apoptosis-mediating death domain homologous regions (15). Domain 7 contains a heparin-binding region as well as a free cysteine residue required for disulfide bond formation and dimerization (15) (Fig. 1).

In humans, the OPG gene is a single-copy gene extending over 29 kB of the genome in chromosome 8 and contains 5 exons (16). One major transcription initiation region is located upstream of the initiation ATG codon, and 2 other minor regions are noted further upstream.

## OPG/RANKL/Receptor Activator of Nuclear Factor κB (RANK) Axis and TRAIL

Osteoblasts are essential to the differentiation and proliferation of osteoclasts; together they play an important role in bone metabolism throughout life preserving the finely balanced dynamics of bone formation and resorption, but the exact nature of this regulatory control was not known until the late 1990s (17).

RANKL is expressed in vivo by osteoblasts, stromal cells, and T lymphocytes (18). RANK is expressed on the surface of osteoclast precursor cells such as monocytes, macrophages, and dendritic cells (19,20). Interaction of RANKL with RANK activates nuclear factor κ (a transcription factor) by degradation of IκB protein by IκB kinase; this degradation of IκB protein frees the nuclear factor κB complex, which then translocates to the nucleus initiating transcription of specific genes required for differentiation of osteoclasts (21).

OPG is expressed in vivo by endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and osteoblasts. Within ECs, OPG is associated with von Willebrand factor within secretory granules called Weibel-Palade bodies. Upon stimulation with TNF-α or interleukin-1β in vitro, the OPG-von Willebrand factor complex is secreted into the surrounding growth medium. This complex is also noted in human serum, indicating EC activation by proinflammatory cytokines as one of the possible sources of circulating OPG in patients with active atherosclerosis (22).

OPG acts as a decoy substrate to RANKL and competes with RANK, inhibiting RANKL-RANK interactions (23).

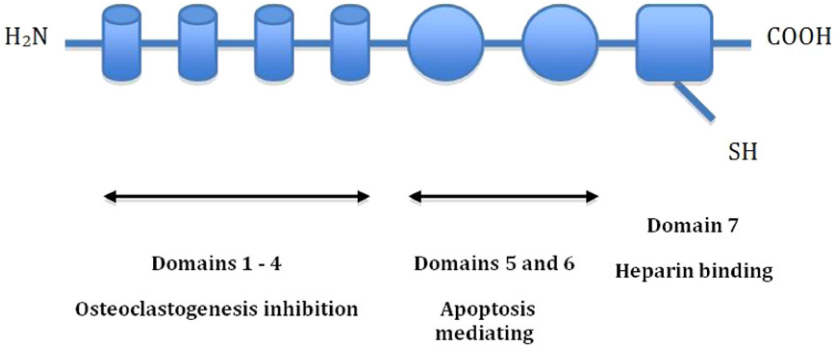


**Figure 1** Structural Domains of OPG

Domains 1 through 4 are cysteine-rich domains and express features similar to the extracellular domains of other tumor necrosis factor (TNF) receptors. Domains 5 and 6 contain 2 death domain homologous regions, similar to those found in the intracytoplasmic region of mediators of apoptosis such as TNF-related apoptosis-inducing ligand and tumor necrosis factor receptor 1. Domain 7 is a heparin-binding region with an unpaired cysteine residue requisite for disulfide bond formation.

A list of cells expressing OPG and various regulating factors/cytokines/hormones influencing the expression of OPG are listed in Table 1. Binding of OPG to RANKL prevents the proliferation and differentiation of osteoclasts and consequently bone resorption. OPG/RANKL/RANK also play an important role in vascular biology and adaptive immunity. OPG has been demonstrated in normal arteries, whereas RANK, RANK, and osteoclasts have been identified mainly in calcified arteries (23).

OPG has also been noted to bind to another member of the TNF superfamily, TRAIL. TRAIL functions as a homotrimer and is expressed as a type II transmembrane protein (24). The extracellular domain of this protein can be cleaved proteolytically from the cell surface to act as a soluble cytokine. TRAIL acts by binding to 4 TRAIL receptors. Two of these receptors (TRAIL receptor 1/death receptor 4 and TRAIL receptor 2/death receptor 5) contain cytoplasmic sequences homologous to the death domains of Fas and tumor necrosis factor receptor 1 and are able to mediate apoptosis via the caspase activation pathways. TRAIL receptor 3 and TRAIL receptor 4, along with OPG, are decoy receptors for TRAIL (25–27). ECs and VSMCs both express TRAIL receptors 1 and 2, but recombinant TRAIL conversely promotes proliferation and survival of these cells via the Akt and extracellular signal-regulated kinase/mitogen-activated protein kinase pathways (28).

**Table 1 Factors Regulating Osteoprotegerin**

| Cell Type | Cytokine/Hormone | Osteoprotegerin Expression |
|---|---|---|
| Endothelial | IL-1$\alpha$ | ↑ |
| | IL-1$\beta$ | ↑ |
| | TNF-$\alpha$ | ↑ |
| Smooth muscle cells | Estrogen | ↑ |
| | IL-1$\beta$ | ↑ |
| | TNF-$\alpha$ | ↑ |
| | PDGF | ↑ |
| | bFGF | ↑ |
| | TGF-$\beta$ | ↓ |
| | BMP-2 | ↓ |
| Osteoblasts | IL-1$\beta$ | ↑ |
| | IL-11 | ↑ |
| | IL-18 | ↑ |
| | TGF-$\beta$ | ↑ |
| | Vitamin D3 | ↑ |
| | Estradiol | ↑ |
| | Parathyroid hormone | ↓ |
| | Parathyroid hormone–related protein | ↓ |
| | PGE2 | ↓ |
| | Glucocorticoids | ↓ |
| Bone marrow stromal cells | IL-18 | ↑ |
| | TNF-$\alpha$ | ↑ |
| | BMP-4 | ↑ |
| | TGF-$\beta$ | ↑ |
| | IGF-1 | ↓ |
| | IL-1$\beta$ | ↓ |
| | IL-6 | ↓ |
| | IL-11 | ↓ |
| | IL-17 | ↓ |

bFGF = basic fibroblast growth factor; BMP = bone morphogenic protein; IGF = insulin-like growth factor; IL = interleukin; PDGF = platelet-derived growth factor; TGF = transforming growth factor; TNF = tumor necrosis factor.

## Role of OPG in Atherosclerosis and Vascular Calcification

**Animal models.** Selective deletion of OPG in mice results in early-onset severe osteoporosis as well as significant medial calcification of the aorta and renal arteries (17). When compared with $OPG^{+/+}$ mice, $OPG^{-/-}$ mice display increased calcification of the aortic media, particularly when given a high dose of phosphate or vitamin $D_3$. These arteries are also the sites of endogenous OPG expression in normal arteries, raising the possibility of a protective role of OPG. This protective role of OPG is particularly important for preventing vascular calcification occurring secondary to administration of warfarin and vitamin $D_3$ (29). However, inactivation of OPG in apoE knockout mice also resulted in augmented vascular calcification and increased size of atherosclerotic lesions compared with $OPG^{+/+}$ $apoE^{-/-}$ mice (30). This observation lends further credibility to the argument regarding the protective role of OPG.

Investigating this protective role of OPG in arterial calcification, Min et al. (31) observed that transgenic expression of OPG in $OPG^{-/-}$ mice prevents the onset of arterial calcification, whereas exogenous administration of high doses of recombinant human OPG in adult mice (>4 weeks old) did not affect a reversal of the aortic calcification, underlining the importance of the timing of OPG delivery. Notably, OPG seems to prevent calcification but is not able to reverse vascular calcification once it has occurred.

In a seminal study, Morony et al. (32) fed an atherogenic diet to low-density lipoprotein receptor knockout mice and subsequently treated them for 5 months with recombinant OPG (fc-OPG) or vehicle. The group of mice treated with fc-OPG had a significantly lower percentage of calcified plaque but, interestingly, without any difference in the number or size of the atherosclerotic lesions. Plasma OPG levels were measured in the vehicle-treated mice from the time of initiation of an atherogenic diet. A significant increase in plasma OPG levels was noted in the first month of starting the atherogenic diet. Over the course of the next few weeks, there was no further increase in the levels of OPG despite progression of atherosclerosis. There was also no decrease in the tissue levels of expression of the inflammatory cytokines such as interleukin-6, interleukin-1$\beta$, TNF-$\alpha$, and monocyte chemoattractant protein-1 in the Fc-OPG–treated mice. This leads us to believe that OPG protects against atherosclerotic calcification and may be a marker of the onset of atherosclerosis but not of its progression or its severity.

Conversely, serum levels of RANKL dropped below the baseline by 3-fold within a month of starting the diet. Subsequently, increasing serum levels of RANKL were shown to positively correlate with the number but not of size or area of atherosclerotic plaque lesions, whereas increasing vascular tissue RANKL mRNA levels from the second month correlated significantly with number, size, and area of plaque lesions.

Despite the growing evidence, the actual role of OPG in atherosclerotic calcification remains speculative. The cells of vascular origin suspected of osteogenic phenotypic transformation are adventitial myofibroblasts, pericyte-like calcifying vascular cells, and VSMCs (33–35). In view of the substantial role of VSMCs in atherosclerosis and intimal calcification, it is likely that along with ECs, they are the main source of increased levels of circulating OPG noted in cardiovascular disease.

A few of the possible explanations for the role of OPG in inhibiting arterial calcification are as follow:

1. RANKL is strongly expressed in the extracellular matrix of vessels with calcified plaque lesions and very weakly in the media in normal arteries (36). RANKL has been shown to initiate osteogenic phenotypic transformation of the VSMCs via the bone morphogenic protein 4 pathway (37) and increase alkaline phosphatase activity (33). OPG, being a natural decoy receptor for RANKL, inhibits this process and possibly arterial calcification as well.
2. Endothelial dysfunction leads to increased translocation of the inflammatory cells from the lumen into the intima. Increased production of proinflammatory cytokines such as TNF-$\alpha$ (38) and interferon gamma (39) by these cells results in initiation of arterial calcification as well as up-regulation of OPG expression in ECs and VSMCs, possibly as a counterprotective measure.
3. TRAIL is known to induce apoptosis of ECs and VSMCs in atherosclerotic plaque lesions. Binding of TRAIL to its decoy receptor OPG inhibits its proapoptotic activity, reducing the number of foci for calcification (27).
4. Deficiency of B lymphocytes in $ldlr^{-/-}$ mice resulted in a significant increase in atherosclerotic lesions ($p = 0.046$) (40). OPG regulates B-cell maturation, and in an $OPG^{-/-}$ model, immature B cells are noted, which could possibly have some direct role in regulating the immune response in atherosclerosis (41).

**Human studies.** Contrary to the apparent protective role of OPG observed in the animal models, there seems to be a distinct relationship between serum levels of OPG and severity of atherosclerosis in human studies. Some of the clinical studies conducted in humans and their principal findings are listed in Table 2.

**Association of OPG with other cardiovascular risk factors.** A few studies have explored the association between OPG levels and traditional cardiovascular risk factors (42,43). The only consistent direct association seems to be with increasing age and duration of diabetes (44). There is no consensus on the association of other established cardiovascular risk factors such as body mass index, serum low-density lipoprotein, high-density lipoprotein, or systolic hypertension.

Interestingly, premenopausal women (younger than 50 years of age) had higher OPG levels than men (younger than 50 years of age) ($p < 0.001$), whereas serum OPG levels were no different in post-menopausal women compared with men ($p = 0.179$) (45). We can thus conclude that there is a regulatory role for sex steroids in the expression of OPG. What this role is has seemingly evaded definition due to contradictory results from different studies. Rogers et al. (46) described a weak positive association of plasma OPG levels with estradiol, whereas Khosla et al. (45) showed that, in women, there is no significant association with either estradiol or testosterone. In men, one study showed a negative correlation with levels of testosterone (45), whereas another (47) showed a positive correlation with testosterone levels.

**OPG and vascular disease.** In human studies, a significant association between levels of circulating OPG and vascular calcification has been described. Clancy et al. (48) reported such an association with the presence of abdominal aortic calcification, a known risk factor in the development of abdominal aortic aneurysms (AAAs). Subsequently, a weakly significant association ($p = 0.04$, $r = 0.20$) was found with the progression of AAAs in a cohort of men with small AAAs followed for a period of 3 years with serial ultrasound studies (49). This correlation persisted on multivariate analysis controlling for traditional risk factors such as age, diabetes status, smoking, and lipid profile. The researchers also showed up-regulated OPG expression within the media of AAA biopsy specimens and more importantly demonstrated an attenuated secretion of OPG from the AAA biopsy specimens incubated with irbesartan in vitro, with the cytokine production being reduced by 50% within 4 days.

In a subsequent study (50), increased expression of angiotensin II type 1 receptor in aortic smooth muscle cells was observed in the presence of OPG. Human aortic smooth muscle cells incubated with angiotensin II showed a linear increase in OPG production. Reduced expression of angiotensin II type 1 receptor and tissue concentration of OPG and matrix metalloproteinase (MMP)-9 was noted upon adding the peroxisome proliferator-activated receptor-gamma ligand pioglitazone in aortic smooth muscle cells in vitro. Whether these results can be extrapolated to provide us with therapeutic targets in the management AAAs and/or a wider spectrum of cardiovascular disease remains to be seen.

In a cross-sectional study, Ziegler et al. (51) reported a positive correlation between serum levels of OPG and severity of peripheral artery disease, but there was no

**Table 2** Studies Evaluating the Association Between OPG/RANKL/RANK and Cardiovascular Disease

| First Author (Ref. #) | Target Population | Study Design | Primary Outcome Measure | Main Findings |
|---|---|---|---|---|
| Shin et al. (84) | Type 2 diabetes | Cross-sectional, n = 104 | FMAD as an indicator of endothelial function | Increased OPG levels were associated with endothelial dysfunction indicated by decreased FMAD |
| Xiang et al. (83) | Type 1 and 2 diabetes patients | Prospective, n = 50 (type 1); n = 86 (type 2) | Endothelial function measured as FMAD pre- and post-treatment with insulin | After 6 months of insulin therapy, FMAD was significantly increased, whereas serum OPG levels decreased significantly |
| Rasmussen et al. (113) | Type 1 diabetes patients | Cross-sectional, n = 291 | Cardiovascular disease prevalence | Higher OPG levels associated with cardiovascular disease, worse kidney function, glycemic control, and presence of nephropathy |
| Avignon et al. (86) | Asymptomatic patients with type 1 and 2 diabetes | Cross-sectional, n = 465 | Prevalence of silent myocardial ischemia by myocardial perfusion imaging | OPG levels were independently associated with presence of silent myocardial ischemia |
| Anand et al. (44) | Asymptomatic type 2 diabetes | Prospective, n = 510 | Presence of CAC and coronary events | Serum OPG levels associated with severity and progression of CAC and short-term cardiovascular events |
| Omland et al. (112) | Patients presenting with acute coronary syndromes | Prospective, n = 897 | Death, recurrent MI, HF hospitalization, and stroke | During an 89-month follow-up, higher baseline OPG levels were associated with mortality and HF hospitalization independent of C-reactive protein and troponin |
| Abedin et al. (114) | General population (unselected) | Cross-sectional, n = 3,386 | Prevalence of CAC and aortic plaque | Higher quartiles of OPG were independently associated with prevalence of CAC and aortic plaque |
| Sandberg et al. (56) | Patients with unstable and stable angina | Cross-sectional, n = 100 | OPG levels in stable vs. unstable angina | OPG was significantly higher in patients with unstable angina than in patients with stable angina |
| Helske et al. (115) | Patients with aortic stenosis | Cross-sectional, n = 131 | Prevalence of HF | HF was significantly associated with increasing levels of OPG that decreased after valve replacement |
| Omland et al. (116) | General population | Cross-sectional, n = 2,715 | Left ventricular structure and function | Higher OPG levels were associated with higher left ventricular mass and thickness and lower LVEF in male patients; higher LVESV and lower LVEF in female patients |
| Kiechl et al. (117) | General population | Prospective, n = 915 | Progression of carotid atherosclerosis and cardiovascular events | Over a 10-yr follow-up, higher OPG levels were associated with severity and progression of carotid atherosclerosis, vascular mortality, and incident cardiovascular disease |
| Semb et al. (64) | General population (nested case-control study) | Prospective, 951 cases and 1,705 controls | Coronary event | Higher baseline OPG but not RANKL levels were associated with coronary events |
| Kiechl et al. (65) | General population | Prospective, n = 909 | Cardiovascular disease incidence | Increasing serum RANKL levels were independently associated with increasing vascular risk but not atherosclerosis |
| Crisafulli et al. (66) | Patients with AMI (n = 58), asymptomatic CAD (n = 52), and normal controls (n = 52) | Cross-sectional, n = 162 | Serum OPG and RANKL levels in the different groups | OPG levels were significantly higher and RANKL levels significantly lower in AMI patients compared with asymptomatic CAD patients and normal subjects |

AMI = acute myocardial infarction; CAC = coronary artery calcium; CAD = coronary artery disease; FMAD = flow-mediated arterial dilation; HF = heart failure; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume; OPG = osteoprotegerin; RANK = receptor activator of nuclear factor κB; RANKL = receptor activator of nuclear factor κB ligand.

statistically significant difference in OPG levels between patients with documented peripheral artery disease and healthy controls. These findings were similar to those of Pennisi et al. (52).

**Role of OPG and RANKL in the pathogenesis of atherosclerosis.** The role of OPG in vascular disease prompted investigators to try to unravel the intertwined pathways by which the apparent proatherogenic action of

OPG in humans is mediated. Mangan et al. (53) demonstrated a linear increase in expression of adhesion molecules such as E-selectin, vascular cell adhesion molecule, and intercellular adhesion molecule when human umbilical vein endothelial cells are incubated with increasing concentrations of OPG and TNF-$\alpha$ in vitro. This causes increased binding of monocytes to these adhesion molecules on the ECs before their migration into the vascular intima, a key step in the pathogenesis of atherosclerosis (54). Furthermore, there were 47% more monocyte THP-1 cells bound per 1,000 ECs when incubated with OPG and TNF-$\alpha$ compared with no OPG, indicating its possible role in sensitizing ECs to the actions of TNF-$\alpha$ in vivo. In another study, human umbilical vein endothelial cells activated by exposure to TNF-$\alpha$ secreted significant amounts of OPG but not RANKL into the surrounding medium in vitro (55).

One of the hypotheses suggests that the association of increased OPG seen in cardiovascular disease is the result of an incomplete compensatory mechanism that immediately raises the question regarding the role of RANKL and its receptors in atherosclerosis and arterial calcification. RANKL mRNA levels are low in normal vessels but are much higher within calcified atherosclerotic plaques as well as thrombus of ruptured coronary plaque lesions, indicating their potential role in atherosclerotic plaque calcification and in plaque vulnerability and rupture (36,56). In the vessel wall, RANKL is likely expressed by ECs, smooth muscle cells, T lymphocytes, and mast cells, with its target cell membrane-bound receptor RANK being expressed on the surface of macrophages and dendritic cells (23). In the presence of RANKL, microvascular ECs are seen to promote adhesion and intimal migration of monocytes (57).

**Effect of OPG and RANKL on plaque stability.** Increased OPG levels and increased expression of RANKL on T lymphocytes and RANK on monocytes are noted in unstable plaque lesions (56). OPG levels were significantly higher in patients with unstable angina compared with healthy controls. In the same study, peripheral blood mononuclear cells were shown to have increased expression of RANKL after a percutaneous coronary intervention, which in essence is an iatrogenic plaque rupture. Interestingly, RANKL significantly increased the activity of MMP in VSMCs in patients with unstable angina. OPG supposedly neutralizes the effect of RANKL on induction of MMP activity in VSMCs by inhibiting its binding to RANK. Although this is true at higher OPG/RANKL ratios, at lower OPG/RANKL ratios, as are found in inflamed tissues, OPG enhances the MMP-inducing activity of RANKL. Binding of OPG with RANKL possibly inhibits the rapid clearance of RANKL from the serum, stabilizing its levels, thus seemingly augmenting its actions. This phenomenon has been observed with other soluble TNF-$\alpha$ receptors and their ligands (58).

This is a significant finding because MMPs seem to play a crucial role in the degradation of matrix in atherosclerotic plaques, rendering them vulnerable to plaque rupture (59–61). The clinical role of OPG and RANKL in atherosclerosis and plaque vulnerability is summarized in Figure 2.

**Clinical utility of OPG and RANKL as biomarkers.** In view of their considerable association with the prevalence and severity of CAD, there is significant interest in developing OPG and RANKL as biomarkers and probably as therapeutic targets for vascular disease. Their clinical role is currently limited due to their expression by numerous types of tissues in vivo. Identifying tissue isoforms of OPG/RANKL will potentially increase their clinical utility as a marker of CAD.

The ability of OPG to predict prevalence and severity of CAD was studied previously in a few studies with consistent results (42,62,63). The plasma levels of OPG were found to be higher in patients with acute coronary syndrome compared with those with stable angina or normal coronary arteries ($p = 0.032$). Serum OPG levels were associated with the severity of coronary stenosis and a modest correlation was also noted with the number of diseased vessels (62,63). These studies highlight the possible prognostic utility of OPG as a biomarker in a clinical setting. A 1-ng/ml increase in serum OPG levels relates to an odds ratio of 5.2 (95% confidence interval: 1.7 to 16.0, $p < 0.01$) for the presence of CAD.

These findings have been further strengthened by the results of the EPIC (European Prospective Investigation of Cancer)-Norfolk cohort, a longitudinal prospective study exploring the relationship between OPG levels and the risk of CAD (64). During the 6-year follow-up of 25,663 asymptomatic individuals with no history of CAD, a coronary event occurred in 951 participants. When these patients were compared with 1,705 age- and sex-matched healthy controls free of coronary events, based on age and sex, baseline OPG levels were found to be very good predictors of the development of CAD. Of note, there was also a linear relationship between serum OPG levels and the risk of CAD but no significant association has been noted in the levels of serum-soluble RANKL and coronary event risk over the follow-up period in the same cohort of patients.

In an earlier study by Kiechl et al. (65), plasma levels of uncomplexed soluble RANKL were significantly associated with vascular events with an adjusted hazard ratio per unit increase in level of RANKL being 1.27 (95% confidence interval: 1.16 to 1.4, $p < 0.001$). This prognostic ability was independent of the traditional cardiovascular risk factors as well as C-reactive protein, OPG, and carotid or femoral atherosclerosis as assessed by their intima-media thickness and the $\log_e$-transformed atherosclerosis score.

Another dimension to this complex process has been added by the finding of markedly lower levels of RANKL in patients presenting with ST-elevation MI compared with patients with stable CAD and age- and sex-matched healthy controls. Lower levels of uncomplexed RANKL have also been reported in patients with CAD compared

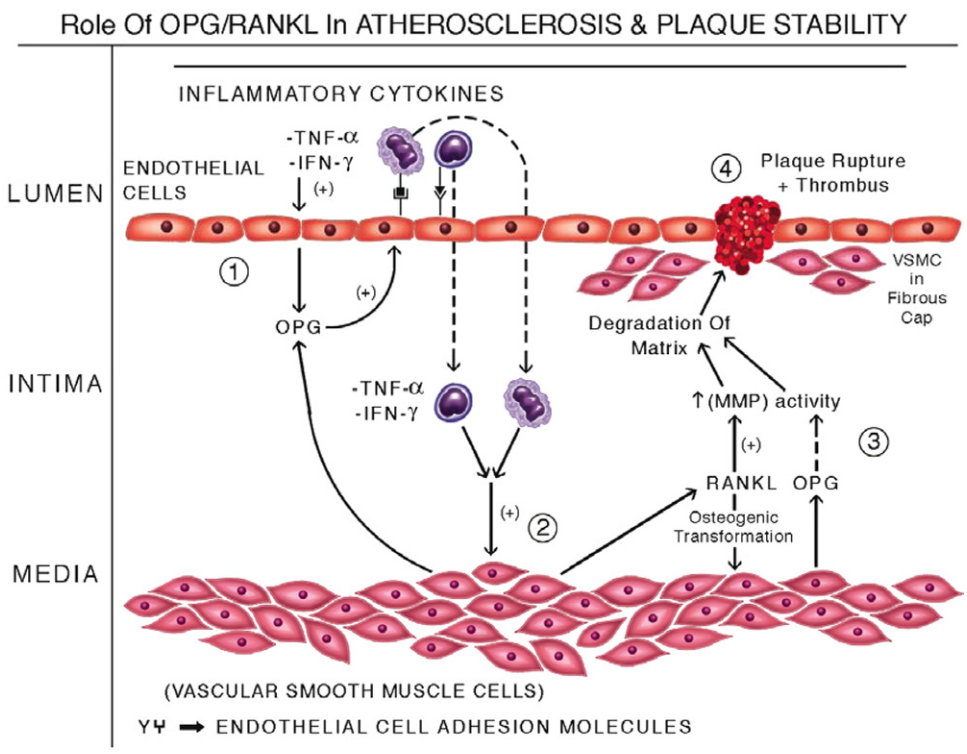


**Figure 2** **Role of OPG in Atherosclerosis**

1. In the presence of proinflammatory cytokines, expression of osteoprotegerin (OPG) is up-regulated in endothelial cells, which in turn increases the expression of endothelial cell adhesion molecules, helping in the transmigration of monocytes and lymphocytes into the intima of the vessel wall. 2. The inflammatory cells up-regulate expression of receptor activator of nuclear factor κB ligand (RANKL) and form vascular smooth muscle cells (VSMCs). 3. RANKL along with OPG at lower OPG/RANKL ratios increase activity of matrix metalloproteinase. 4. Increased matrix metalloproteinase (MMP) activity leads to degradation of the extracellular matrix and reduced thickness of the fibrous cap, the erosion of which causes thrombus formation. IFN = interferon; TNF = tumor necrosis factor.

with those without CAD, but RANKL levels were not associated with the severity of CAD (66,67).

It is possible that:

1. OPG is just a more reliable marker of OPG/RANKL/RANK axis activity than RANKL even though RANKL plays a more central role in the pathogenesis of atherosclerosis and plaque instability. Measurement of TNF-α superfamily of cytokines is difficult due to their inherent instability in biological fluids and their rapid clearance from the body, whereas the soluble TNF receptors are biologically more stable and also analytically more reliable. Production of TNF-α induces the shedding of the tumor necrosis factor receptors and closely reflects the TNF-α activity (i.e., the serum level of soluble TNF receptors reflects the background inflammatory state as the levels have been noted to increase significantly in inflammatory diseases) (68).
2. Assays of RANKL measure the levels of uncomplexed RANKL exclusively, whereas those of OPG measure total OPG levels in the serum (monomer, dimer, and bound). If the hypothesis that RANKL plays a pathogenic role in atherosclerosis as well as plaque instability were to be true, it would hold that as the levels of RANKL increased in the serum, it would get bound to OPG, its decoy receptor. The picture that emerges from this would be that of low RANKL levels but higher OPG levels in the serum.

The association of serum OPG levels with CAD seem consistent, but the same cannot be said for RANKL. More data from prospective studies are needed before a clearer hypothesis about the role of the OPG/RANKL/RANK axis in CAD can be formulated and its clinical utility considered.

## TRAIL, Atherosclerosis, and Cardiovascular Disease

The other ligand for OPG, TRAIL, also seems to participate significantly in atherosclerosis and cardiovascular disease. Normal arteries do not show any immunostaining for TRAIL protein and TRAIL mRNA. In arterial specimens of both Mönckeberg sclerosis and atherosclerosis, TRAIL was colocalized with OPG to the margins of calcification. In atherosclerosis, apoptosis was noted in the intima surrounding foam cell accumulations

and calcified lesions similar to where TRAIL immunoreactivity was also noted, indicating its role in cellular apoptosis and atherosclerosis (69).

There seems to be considerable confusion regarding the role of TRAIL in atherogenesis and plaque destabilization. Some studies suggest a role for TRAIL in increasing inflammatory gene expression on ECs (70,71), whereas others have demonstrated its role in the down-regulation of adhesion of monocytes to ECs (72). Similarly, there are conflicting reports regarding its role in the survival of ECs (28,73). TRAIL also promotes the migration and proliferation of the VSMCs from the media into the intima of the vessel wall, a key step in the propagation of atherosclerosis (74), but also in maintaining the stability of the advanced lesion being the main source of the extracellular matrix proteins and the fibrous cap.

In a small, cross-sectional study, Michowitz et al. (75) demonstrated that the serum level of soluble TRAIL was much lower in patients with acute coronary syndrome (acute coronary syndrome–MI/unstable angina) than in patients with either stable angina or normal coronary arteries ($p < 0.0001$). There was no significant difference noted in the level of TRAIL in the stable angina patients as well as those with normal arteries, pointing to the possibility of a defining role for TRAIL in plaque destabilization.

These findings were reproduced recently with a significantly higher serum OPG/TRAIL ratio noted in patients with acute MI compared with those with unstable angina and those with no CAD ($p < 0.01$) (76). Using competitive end point enzyme-linked immunosorbent assay in the presence of recombinant TRAIL and OPG, it was shown that the levels of OPG and TRAIL were not influenced by the presence of the other. Interestingly, a higher ratio of baseline OPG/TRAIL was noted in patients in whom heart failure subsequently developed at 6-month follow-up, post-MI compared with those with no heart failure. Measurement of OPG/TRAIL ratio seems to offer some indication regarding the severity of acute MI and short-term (6 months to 1 year) prognosis.

It is difficult to formulate one cohesive, comprehensive theory regarding the role of TRAIL in the initiation and progression of atherosclerosis as well as in plaque rupture. We can postulate that activation of apoptosis by TRAIL in an early plaque lesion is antiatherogenic by attempting to reduce the number of inflammatory cells and VSMCs within the intima. The findings that TRAIL promotes survival of ECs (28) and improves nitric oxide production (77), strengthen this hypothesis. In advanced plaque, TRAIL is localized to the shoulder region of the plaque (75), which is most prone to rupture. In response to the oxidized low-density lipoprotein or proinflammatory cytokines such as interferon gamma, TRAIL induces apoptosis of VSMCs/ECs (78), lymphocytes (79), and macrophages (80), rendering the plaque prone to rupture.

## OPG, Diabetes, and CAD

It is well established that patients with diabetes have a higher risk of accelerated atherosclerosis. One of the key steps in initiation of atherosclerosis is endothelial dysfunction characterized by decreased endothelial nitric oxide activity characterized by increased vascular production of reactive oxygen species and decreased nitric oxide synthase activity (81,82). In attempting to explain the role of OPG in atherosclerosis, the association of serum OPG levels with markers of endothelial dysfunction has been reported in patients with type 1 and 2 diabetes (83,84). In response to treatment with insulin for 6 months, OPG levels decreased significantly, coupled with a significant improvement in flow-mediated arterial dilation. This result seems to be contrary to the findings by Pritzker et al. (85), who elucidated the role of OPG and TRAIL in microvascular EC survival.

In a prospective study, Anand et al. (44) demonstrated the association between atherosclerotic plaque burden and OPG levels. In this study, 510 asymptomatic diabetic patients had CAC scans and were followed over a period of $18 \pm 5$ months after which a repeat scan was performed. Increased CAC scores were significantly associated with higher plasma levels of OPG in a multivariate model adjusted for other risk factors, statin use, and duration of diabetes (adjusted odds ratio: 2.84, $p < 0.01$) (Fig. 3). OPG levels were also significantly higher in subjects who experienced a cardiovascular event during the follow-up ($p < 0.0001$). In contrast, high-sensitivity C-reactive protein and interleukin-6 levels neither correlated with CAC score nor predicted cardiovascular events in the short term.

These results were similar to those of Avignon et al. (86), who demonstrated the significant association of OPG levels and silent CAD, diagnosed by myocardial perfusion imaging in patients with type 2 diabetes. In a multivariate analysis, OPG levels of >8 pmol/l were shown to be independently associated with abnormal myocardial perfusion imaging (odds ratio: −4.2, $p < 0.01$) Interestingly, plasma OPG levels were significantly lower in patients treated with thiazolidinediones. The findings were attributed to the role of OPG in EC dysfunction (55) and the endothelial protective role of thiazolidinediones (87,88).

These findings are of significant clinical importance. The American Diabetes Association recommends screening for CAD in patients with type 2 diabetes with ≥2 additional cardiovascular risk factors with an exercise stress test. This guideline encompasses a large population of diabetic patients. The ability to identify the subset of diabetic patients at increased risk of silent CAD based on plasma OPG levels could be of enormous benefit in risk stratifying these patients. Better risk stratification will allow more appropriate focus of resources and could also avoid unnecessary investigations in a large segment of patients.

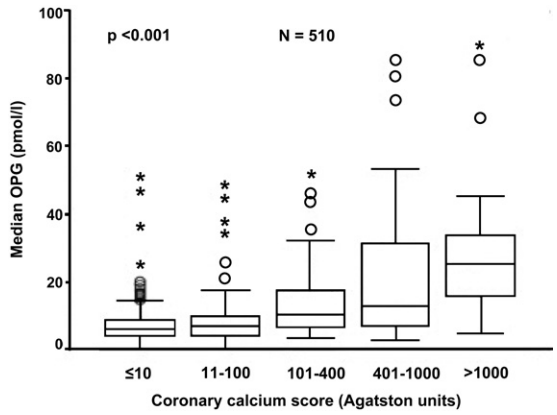


**Figure 3** OPG and Prevalence of Coronary Artery Calcium

Box and whisker plots demonstrating the median and interquartile ranges of osteoprotegerin (OPG) in patients with minimal, mild, moderate, severe, and extensive coronary artery calcification. **Circles** = outliers (1.5 to 3 box lengths from **upper edge of box**); **asterisks** = extreme outliers (>3 box lengths). From Anand et al. (44).

## OPG and Cardiovascular Disease in Chronic Kidney Disease

The life expectancy of patients with end-stage renal disease is significantly reduced due to early-onset CAD accounting for >50% of all causes of mortality (89). CAD and vascular calcification develop faster in patients with chronic kidney disease than in the general population (90). Apart from the traditional cardiovascular risk factors, several nontraditional risk factors specific to end-stage renal disease patients have been identified for this accelerated atherosclerosis and vascular calcification. The prevalence of chronic inflammation, altered mineral metabolism (higher serum Ca X phosphate product), hyperhomocysteinemia, increased oxidative stress, fetuin A, matrix Gla protein, and OPG are some that have been implicated (91–93). It is beyond the scope of this review to comment on the role of each of these factors.

Coronary artery and vascular calcification is strongly associated with cardiovascular disease and mortality (94,95). OPG levels are significantly higher in patients with chronic kidney disease compared with age- and sex-matched controls, and increasing OPG levels have a linear relationship with worsening renal function (96). Similar findings are noted in patients with chronic kidney disease on hemodialysis (97). In the study by Nitta et al. (98), 102 patients on regular hemodialysis were assessed for aortic calcification by a noncontrast computed tomography scan. Serum OPG levels correlated significantly with the presence and severity of aortic calcification in a multivariate analysis.

In prospective studies, higher serum OPG levels are significantly associated with cardiovascular events (99,100). Morena et al. (101) used receiver operator characteristic curve analysis to obtain the cutoff level of OPG best able to predict the presence of significant coronary calcification. This was identified at 757.7 pg/ml with 91.7% sensitivity and 59% specificity.

It has been suggested that increased OPG levels in chronic kidney disease patients could just be accumulation due to impaired renal clearance of OPG. The decrease in OPG levels within 14 days of renal transplantation supports this hypothesis (102). It is also possible that OPG levels are higher as a compensatory/protective mechanism against the confluence of the "perfect storm" of proatherogenic and procalcification factors seen in this group of patients or the persistent low-grade inflammation induces increasing OPG expression from vascular cells in a vicious cycle of inflammation-endothelial dysfunction-atherosclerosis-calcification.

## Role of OPG in Left Ventricular Dysfunction

Left ventricular dysfunction is one of the key prognostic indicators of cardiovascular mortality and morbidity (103). The role of TNF-$\alpha$ in the development of left ventricular dysfunction has come to the forefront in recent years (104,105). Up-regulated expression of members of the cytokines of the TNF family, including TRAIL, by peripheral blood mononuclear cells in heart failure patients has been reported (106). Similarly, inflammatory cytokines, specifically, plasma soluble TNF receptor 1, levels were found to be independent predictors of left ventricular hypertrophy (107).

In a mouse model of heart failure, significantly increased mRNA expression of OPG was noted in both the ischemic and nonischemic myocardium compared with those without heart failure. Human subjects with heart failure had significantly increased serum levels of OPG compared with healthy controls. Furthermore, there was a clear-cut relationship between New York Heart Association functional class, cardiac index, and N-terminal pro–B-type natriuretic peptide levels with p values consistently <0.01. Serum levels of RANKL correlated in patients with New York Heart Association functional class IV heart failure, but correlated well with the cardiac index ($p < 0.05$) (108). The role of RANKL/RANK and OPG in heart failure is attributed partly to the induction in activity of MMP by RANKL in human fibroblasts (MMP-2 and -9 in particular), resulting in matrix degradation, adverse ventricular remodeling, and worsening myocardial function (109,110).

The prognostic utility of serum OPG levels in heart failure patients was investigated in 2 prospective studies (11,112). Ueland et al. (111) recruited a subset (n = 234) of patients from the original OPTIMAAL (Optimal Therapy in Myocardial Infarction with the Angiotensin II Antagonist Losartan) cohort of patients with a known history of MI and left ventricular dysfunction. During a 27-month follow-up, there were 32 deaths (14%), and there was a significant difference in baseline OPG levels in those who survived compared with those who experienced increased all-cause mortality and specific cardiovascular events (2.8

ng/ml vs. 4.2 ng/ml, $p < 0.001$ in both cases). In a subsequent study, Omland et al. (112) followed a cohort of 897 patients with acute coronary syndrome for a median period of 89 months. A total of 83 patients were hospitalized with heart failure during the follow-up. The baseline level of OPG correlated significantly with the incidence of heart failure (Fig. 4) (hazard ratio for every 1-SD increase in logarithm-transformed level of OPG being 2, $p < 0.001$). This association remained significant on adjusting for conventional risk factors including left ventricular ejection fraction.

## Conclusions

Considerable controversy still exists regarding the role of OPG/RANKL/RANK/TRAIL in cardiovascular disease. There is as yet no hypothesis unifying the apparent dichotomy in the nature of OPG/RANKL/TRAIL noted in animal and human studies. It is possible that serum OPG levels are increased in response to the vascular insult and ongoing process of inflammation within an atherosclerotic plaque lesion as the component of a complex compensatory mechanism, placing RANKL and/or TRAIL at the center of the pathogenesis atherosclerotic plaque lesions.

We have also described various studies highlighting the apparent proatherogenic role of increased OPG itself. It seems that OPG plays a role right from EC activation in the presence of proinflammatory cytokines to plaque instability with its inherent up-regulation of MMP activity. The serum OPG levels could probably indicate ongoing EC injury as well as activation of the VSMCs, which have been observed in progressing plaque lesions. An increased OPG level could be an indicator of a proinflammatory milieu responsible for propagation of atherosclerosis. OPG as a marker of inflammation is supported by the reduction in expression of OPG in response to insulin, angiotensin II receptor blockers, and peroxisome proliferator-activated receptor-gamma ligands, which have all been shown to attenuate background inflammation.

The more interesting theory is that OPG does indeed have a dichotomous role in humans. In healthy individuals, the proatherogenic and antiatherogenic effects are being held in a fine balance, but in the face of persistent positive induction by various risk factors associated with congestive heart disease, similar to the majority of immune mechanisms, the proatherogenic pathway becomes predominant to the detriment of the subject.

Although the clinical prognostic utility of OPG seems to be awhile away yet, it does hold a great deal of promise in helping the clinician risk stratify patients with cardiovascular disease more accurately. It may come to pass that measurement of a host of biomarkers such as OPG/RANKL/TRAIL/OPN in combination may be recommended to provide more robust and clinically relevant information.

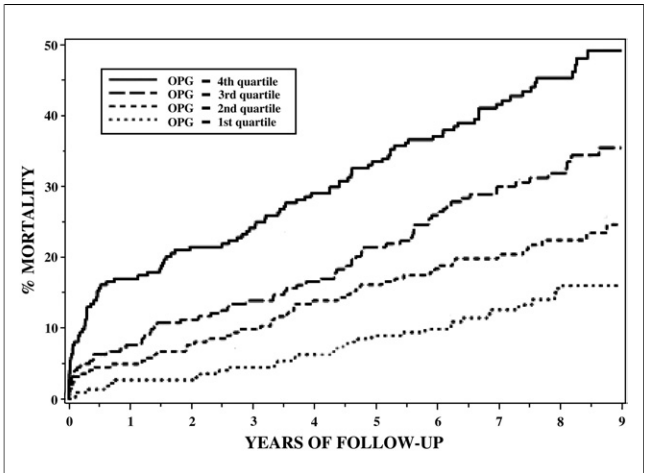


**Figure 4** **Increasing OPG Levels Post-Myocardial Infarction Predict Long-Term Mortality**

Baseline OPG levels were significantly associated with all-cause mortality at median follow-up of 89 months. Hazard ratio for 1-SD increase in log transformed OPG at baseline was 1.7 (95% confidence interval: 1.5 to 1.9, $p < 0.0001$). Comparing the first and fourth quartiles of OPG, the hazard ratio was 4.2 (95% confidence interval: 2.8 to 6.3, $p < 0.0001$). From Omland et al. (112). Abbreviation as in Figure 2.

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**Key Words:** atherosclerosis ■ osteoprotegerin ■ receptor activator of nuclear factor κB ligand ■ tumor necrosis factor–related apoptosis-inducing ligand ■ vascular calcification.