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# Osteoprotegerin and Vascular Calcification: Clinical and Prognostic Relevance

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## ABSTRACT

*Osteoprotegerin (OPG) is a key regulator in bone metabolism, that also has effect in vascular system. Studies suggest that osteoprotegerin is a critical arterial calcification inhibitor, and is released by endothelial cells as a protective mechanism for their survival in certain pathological conditions, such as diabetes mellitus, chronic kidney disease, and other metabolic disorders. That has been shown in studies in vitro and in animal models. The discovery that OPG deficient mice (OPG  $-/-$  mice) develop severe osteoporosis and arterial calcification, has led to conclusion that osteoprotegerin might be molecule linking vascular and bone system. Paradoxically however, clinical trials have shown recently that OPG serum levels is increased in coronary artery disease and correlates with its severity, ischemic cardiac decompensation, and future cardiovascular events. Therefore it is possible that osteoprotegerin could have a new function as a potential biomarker in early identification and monitoring patients with cardiovascular disease. Amongst that osteoprotegerin is in association with well known atherosclerosis risk factors: undoubtedly it is proven its relationship with age, smoking and diabetes mellitus. There is evidence regarding presence of hyperlipoproteinemia and increased serum levels of osteoprotegerin. Also the researches have been directed in genetic level, linking certain single nucleotide genetic polymorphisms of osteoprotegerin and vascular calcification appearance. This review emphasises multifactorial role of OPG, presenting numerous clinical and experimental studies regarding its role in vascular pathology, suggesting a novel biomarker in cardiovascular diseases, showing latest conclusions about this interesting topic that needs to be further explored.*

**Key words:** osteoprotegerin, vascular calcification, coronary artery disease, bone system, vascular system, atherosclerosis, biomarker

## Introduction

Since its initially discovered in 1997 as a key regulator in bone turnover<sup>1</sup>, osteoprotegerin (OPG) has become subject of intense research in its role as a common mediator of bone metabolism and vascular calcification and vascular diseases<sup>2-6</sup>. Extracellular matrix calcification is normal physiological process, necessary for proper development of tissues like bone, teeth and cartilage. However when it occurs in tissues that normally do not mineralize, calcification can lead to serious consequences. Researches in vitro, and in animal models show that OPG inhibits vascular calcification<sup>7-12</sup>. Paradoxically, clinical researches have shown that OPG serum levels is increased in patients with progressive cardiovascular disease<sup>13</sup>, correlates with presence and severity of coronary artery disease (CAD)<sup>14</sup>, is associated with left ventricular hypertrophy and C-reactive

protein<sup>15</sup>, is increased in chronic haemodialysis patients<sup>16,18</sup>, is increased in type 2 diabetic patients with microvascular complications<sup>19</sup> and is increased in heart failure after acute myocardial infarction<sup>20</sup>. This has led to interesting debate about potential role of OPG as a vascular disease biomarker. Exact mechanism by which OPG affects cardiovascular pathology is still unclear. The need for complete picture is directed to valuable researches, that show OPG is not only marker but mediator in vascular pathology that modulates osteogenic, inflammatory and apoptotic response<sup>7,8</sup>. Integrating results above shown of recent experimental research, in animal models, and human studies till year 2010 this review demonstrates role of OPG in vascular pathology.

## OPG/RANK/RANKL/TRIAL System

OPG known as a factor that inhibits osteoclastogenesis (OCIF) is a glycoprotein, member of tumor necrosis factor superfamily (TNF). It is a basic glycoprotein comprising 401 amino acid residues arranged into 7 structural domains. It is found as either a 60 kDa monomer or 120 kDa dimer linked by disulfide bonds<sup>1</sup>. It has a pleiotropic effect in bone metabolism, endocrine and immunological system. Osteoprotegerin (OPG) and receptor activator for nuclear factor kappaB ligand (RANKL) are cytokines traditionally associated with bone turnover. RANKL activates osteoclasts and bone resorption, while OPG acts as a decoy receptor for RANKL, inhibiting bone resorption. The net of these cytokines regulates differentiation and activation of osteoclasts and holds balance between bone osteoclasts and osteoblasts<sup>4</sup>. RANKL is expressed at osteoblasts, stromal cells and T cells, binding RANK at the surface of osteoclasts, monocytes and dendritic cells<sup>5,21</sup>. OPG, a soluble glycoprotein is widely expressed on most human tissues, including bone (osteoblasts) and vasculature (endotel and vascular smooth muscle cells (VSMV)). Acting as a soluble decoy receptor competing for RANKL, OPG prevents RANK-RANKL interaction, thus inhibiting osteoclastic differentiation and bone resorption<sup>4,5,8</sup>. As a member of tumor necrosis factor superfamily (TNF) OPG modulates inflammatory response and has antiapoptotic effect. OPG as well neutralizes pro-apoptotic activity induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), that is expressed at VSMC and T cells<sup>22,23</sup>.

## Vascular Calcification

Vascular calcification often occurs in process of aging, progression of atherosclerosis, and in specific metabolic disorders, such as diabetes mellitus, and end stage renal disease, leading to severe clinical consequences. Such calcification can appear in various places such as heart valves, arterial intima and media, capillaries, forming localised or diffuse calcification, causing active immunological response and mineral homeostasis disbalance. Just recently discovered RANKL/RANK/OPG system, are domain of vascular biology widely explored as factors essential in regulation of vascular calcification, immune response, and bone turnover<sup>8</sup>. From a histological perspective, vascular calcification may be classified based on its location, association with plaque, and mode of formation<sup>2</sup>. Whereas a minor form of widespread nonspecific organ and soft tissue calcification derives from abnormal calcium/phosphate products, more common types of vascular calcification may occur by actively regulated processes in the absence of raised calcium/phosphate levels. Morphologically, these types of calcification are distinguishable by whether calcification primarily takes place in cardiac valves, in arterial intimal layers in association with macrophages and lipid and vascular smooth muscle cells (VSMCs) as in atherosclerosis, or in arterial media layers because of elastin fiber mineralization as in end-stage renal disease or diabetes mellitus. Like most

biological processes, vascular calcification is actively regulated by the networks that involve positive and negative regulators, temporal expression or activation of modulators, and multiple amplification or suppressive feedback loops that orchestrate cell recruitment, differentiation, function, survival, and interactions with other cells or matrix molecules<sup>2,3,24</sup>. Mounting evidence suggests that RANK, RANKL, and OPG may participate in multiple aspects of these processes governing vascular calcification.

Traditionally it is assumed that vascular calcification is passive process of calcium relocation from bone tissue to vasculature. However complex interactions between inhibitors and promoters of vascular calcification are observed. In normal vasculature, different group of regulatory factors such as an extracellular pyrophosphate (PPi), muscle glycogen phosphorylase (MGP), fetuin, osteopontin and OPG inhibiting process of calcification in the vasculature<sup>25</sup>. In atherosclerosis, as in calcific aortic valve stenosis inflammatory markers including activated macrophages and T cells initialize active bone tissue formation. Increased expression of RANKL and decreased expression of OPG are found at the cells of localized calcific areas<sup>26</sup>. As opposed to calcification of artery media, OPG expression is increased in calcific areas, while RANKL expression is decreased<sup>27</sup>. In vasculature with endothelial dysfunction (diabetes mellitus, end-stage renal disease), earlier mentioned inhibitory factors (for vascular calcification) may become weak, and therefore make vasculature submissive for actions of vascular calcification promoters such as toxic effect of hyperglycaemia, endothelin-1, alkaline phosphatase (ALP), bone morphogenetic proteins 2 and 4 (BMP-2, BMP-4), transforming growth factor-beta (TGF-beta), RANKL<sup>25</sup>. Macrovascular complications such as cardiovascular diseases and peripheral artery diseases are leading cause of increased mortality and morbidity, especially in diabetes mellitus patients. Macrovascular impairment etiopathogenesis in diabetes mellitus is multifactorial, and differs in diabetes type 1 or 2. Endothelial cell dysfunction is early vascular alteration in diabetes mellitus type 2, and is associated with poor regulation of glycemic status. Chronic hyperglycemia may cause damage of vascular milieu leading in long term to early apoptosis of endothelial cells. Apoptotic cells existence in vascular lumen may trigger reaction cascade between promoters and inhibitors of arterial calcification<sup>25</sup>.

Medial artery calcification is a typical feature in diabetes mellitus, and an important predictor of cardiovascular diseases<sup>8,25</sup>. It occurs mainly independently of atherosclerosis. Medial artery calcification occurs in normal calcium and phosphate serum levels, which is opposite to vascular calcification, a form that occurs in high serum calcium-phosphate product levels, and make widespread tissue deposition into elastic fibers<sup>3,25</sup>. Intimal calcification (another form of calcification) is commonly associated with atherosclerosis, characterized by lipid accumulation, inflammation, fibrosis and development of focal plaques<sup>8</sup>.

Medial artery calcification in diabetes mellitus is associated with OPG, that has been detected in calcified

regions of atherosclerosis and of medial artery calcification, associated with enhanced apoptosis, suggesting a significant role for the regulators of bone metabolism in the pathogenesis of medial artery calcification in patients with diabetes<sup>27</sup>. Therefore, OPG is an important modulator of bone metabolism, and manifests its effect not only in bone but in arteries<sup>28</sup>. Considering all this it seems that osteoprotegerin is a key inhibitor of artery calcification, and is released by endothelial cells as a protective mechanism in certain conditions (diabetes mellitus, end stage renal disease)<sup>12</sup>. It is therefore a potential marker for early identification and monitoring of impaired mineral metabolism and vasculopathy in diabetes mellitus patients<sup>27,29</sup>.

### Osteoprotegerin and Atherosclerosis Risk Factors

Present studies identified high OPG serum levels as an independent risk factor for progressive atherosclerosis and incidence of cardiovascular diseases in general population<sup>7,13</sup>. These findings might be relevant in routine assessment of vascular risk. However it can not be completely rule out the possibility that high OPG serum levels are barely an epiphenomena of inflammatory process in atherosclerotic tissue. Nevertheless number of experimental studies confirm active role of OPG in vascular pathology<sup>3-6,8,10,11</sup>. It is yet to explore whether OPG acts as a causative risk factor or it has a contraregulatory protective mechanism in atherosclerosis.

It is found arguably that OPG strongly correlates with age<sup>30,31</sup>. In patients stratified by gender and age OPG was higher in patients with diabetes mellitus<sup>29,30</sup>. It is associated with smoking<sup>30</sup>.

The prevalence and severity of atherosclerosis on carotid artery (carotid artery disease) significantly increased with higher OPG levels. After adjustment for age and gender, correlation decreased but was still significant<sup>30</sup>.

Age, female gender, black race, smoking, personal and family anamnesis of coronary artery disease, diabetes mellitus, hyperlipoproteinemia, significantly correlates with higher OPG serum levels<sup>30</sup>.

In the study based on 3,386 subjects aged 30–65 years, Abedin et al. found high OPG levels to be independently associated to coronary artery calcification and aortic plaque, and to correlate to its severity, after adjustment for age, gender, smoking anamnesis, diabetes mellitus, hyperlipoproteinemia, and family anamnesis of early coronary artery disease onset<sup>30</sup>. These findings suggest its role as a novel atherosclerosis biomarker.

In accordance with previous and present researches, Szulc et al.<sup>32</sup> demonstrated that serum OPG levels positive correlate to age in the study including 252 healthy men age 19–85, also Fahrleitner-Pammer et al.<sup>33</sup> in a study including 177 healthy women showed positive correlation to age, but not to bone mass or bone turnover markers.

### Protective Role of OPG on Vascular Calcification: Possible Underlying Mechanism

OPG and RANKL have an important role in osteogenic modulation of vasculature. OPG is produced by various tissues, mainly endothelial cells-vascular smooth muscle cells (VSMCs) with high levels especially in aortic and renal arteries<sup>34</sup>. As opposed to OPG, RANKL is not usually detected in normal vasculature<sup>5,34</sup>. OPG has indirect anti-apoptotic and protective effect on endothelial cells, acting as a decoy receptor for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)<sup>12,22</sup>.

In bone milieu, RANKL has a function of central osteoclasts maturation factor, promoting osteoclastic activity and bone resorption, while OPG oppose RANKL action<sup>34</sup>. RANKL promotes, and OPG protects against vascular calcification<sup>27,34</sup>.

High OPG levels seen in endothelial dysfunction, may be barely compensatory mechanism by endothelial cells to protect against TRAIL apoptosis. In relation to that, high OPG levels may be survival factor for endothelial cells<sup>12,35</sup>. In context of endothelial cell dysfunction these vascular cells may overexpress OPG<sup>12</sup>. This process may continue to calcification process, in fact, OPG level may continue increasing as calcification progresses<sup>27</sup>. According to that, vascular calcification inhibition processes are tightly regulated by protective pathways as a response to metabolic and inflammatory stimulation.

High OPG levels correlate to high inflammatory markers (CRP, interleukine 6 (IL-6), and fibrinogene), as well with HbA1c level and insulin resistance<sup>35</sup>. It seems that OPG expression on vascular cells has significant protective effect against vascular calcification, thus against calcification of artery media<sup>28</sup>. The presence of hyperglycaemia, oxidative stress, activated cytokine net and other effective stimulus can eventually let vascular calcification promoters prevailed this protective mechanism thus accelerate calcification process<sup>29</sup>. Preventing calcification progression, osteoblast-like cells and vascular cells near matrix can further increase OPG production. These processes of inhibition and promotion may be progressive and continuing till forming bone like tissue in the vasculature wall<sup>28,29</sup>.

### Animal Model Overview

In the mouse model, OPG deficient mice (OPG <sup>-/-</sup> mice) resulted in severe osteoporosis, but also unexpected vascular calcification phenotype<sup>10</sup>. There is endogenous OPG expression on aorta and renal arteries, and exactly these arteries develop calcification lesions in OPG <sup>-/-</sup> mice<sup>10</sup>.

Studies have shown these calcification include bone matrix such as hydroxiapatite, bone proteins, as well as growth factors, and adhesive molecules<sup>36</sup>.

This dual phenomena of osteoporotic bone loss and arterial calcification is proven in the OPG <sup>-/-</sup> mice model. These arteries (aorta and renal arteries) are precisely the places with the most common calcification development in

humans with severe atherosclerosis. Although artery calcification in OPG  $-/-$  mice develop in absence of lipid deposition and other vascular lesion normally presented in patients with atherosclerosis. In addition, the difference is in the fact that, artery calcification in atherosclerosis is a late event, whereas artery calcification in OPG  $-/-$  mice is an early event<sup>10</sup>.

Mouse rescue experiments show that expression of OPG as a transgene on an OPG null background prevented the onset of arterial calcification, indicating that systemic OPG plays a protective role in major arteries. However, injection of a high dose of recombinant OPG into adult OPG $^{-/-}$  mice did not affect the incidence of arterial calcification, indicating that OPG cannot reverse the calcification process. It is not clear from these data whether the size of calcified lesions stopped increasing in the OPG-injected group. Delivery of OPG to younger animals, or treatment for >4 week, will be necessary to determine whether OPG can stop further progression of vascular calcification<sup>9</sup>.

Calcified vascular lesion contain various bone matrix proteins such as collagen type I, osteocalcin and bone morphogenic protein 2<sup>37–39</sup>.

In another animal model study, male OPG ( $-/-$ ), OPG ( $+/-$ ), and OPG ( $+/+$ ) mice were fed a high phosphate diet from 6 to 10 weeks after birth, and then 1 $\alpha$ ,25-dihydroxyvitamin D3 (calcitriol) was injected for 3 days. They found that severe calcification developed in the media of the aorta in OPG ( $-/-$ ) mice. Under electron microscopy, calcium deposits were observed in the cytoplasm and extracellular matrix of vascular smooth muscle cells (VSMCs). Neither apoptosis of VSMCs nor infiltration of macrophages was observed. ALP activity of aortic tissue correlated with the calcified lesion area. Mouse aorta and bone extracts revealed an identical pattern by ALP electrophoresis<sup>40</sup>. That demonstrated that OPG had anticalcification activity in the aorta, probably through the down-regulation of ALP activity, since in this study authors clearly indicated that ALP activity in the aorta is increased and may contribute to aortic calcification.

### Vascular Calcification and OPG Serum Concentration in Humans

As already previously shown high serum OPG levels correlate to age, diabetes mellitus, hypertension, high cardiovascular mortality, presence and severity of coronary artery disease, end stage renal disease<sup>4,5,17–19,41</sup>. Serum OPG levels decreasing in patients on immunosuppressive or glucocorticoid therapy<sup>42–44</sup>.

Intimal vascular calcification is objective marker of atherosclerosis, and its severity is independent risk factor for cardiovascular disease development<sup>45</sup>. Coronary or aortic calcification assessment demand CT scanning, analyses and evaluation data, to provide their accuracy<sup>46</sup>. Potential blood marker used for vascular calcification quantification, could be used as a coronary artery disease risk factor, and its advantage would be accessibility, non-

exposure to irradiation, noninvasive testing, and lower price comparing to invasive examination.

According to the finding that serum OPG is increased in conditions that are supported by atherosclerosis<sup>4,5,17–19</sup>, there is a possibility of using serum osteoprotegerin in predicting vascular calcification and cardiovascular risk, thus serve as a marker for vascular calcification. Further studies are needed to evaluate its exact clinical usage.

### Human Studies on OPG and Vascular Calcification

In the study on 102 subjects both men and women undergoing haemodialysis, was found that serum OPG levels were higher in haemodialysis patients and increased with the grade of aortic calcification. In this study multiple regression analyses indicated that serum OPG levels were independently associated with the severity of aortic calcification<sup>16</sup>. However, the mechanism whereby serum OPG was related to the progression of vascular calcification in dialysis patients is unknown.

The abdominal aorta was studied by non-contrast CT scanning in consecutive sequential 8 mm slices and the aortic calcification index (ACI) was estimated as the proportion of aortic circumference covered by calcification. By this method, arteriosclerosis was quantified morphometrically in the cross section with the most extensive arteriosclerosis. The arithmetic mean values of three measurements were calculated and used for analysis. The patients were divided into four groups according to their ACI: group I (ACI 0–10%), group II (ACI 11–30%), group III (ACI 31–50%) and group IV (ACI 51–100%)<sup>16</sup>. Multiple regression analyses were performed to adjust for the roles of different pathogenic factors on vascular calcification (serum levels of albumin, total cholesterol, triglyceride, diastolic and systolic blood pressure or the calcium x phosphate (Ca x P) product). Serum OPG and CRP levels were independently associated with the extent of vascular calcification<sup>16</sup>. These findings suggest not only the possibility that serum OPG could serve as a biomarker for vascular calcification, but also suggesting that OPG may be involved in the development of vascular calcification in haemodialysis patients.

As it is established in the research on 515 patients undergoing haemodialysis vascular calcification severity impaire the outcome in these patients, and is a predictor for cardiovascular mortality<sup>47</sup>. Considering this, the significance of serum osteoprotegerin in these patients might be great for determination of their prognosis among other known risk factors.

Other research with peripheral artery disease patients shown connection between serum OPG levels and infrarenal and abdominal aortic calcification severity. The infrarenal arteries and abdominal aorta were studied by non-contrast CT scanning for calcification assessment<sup>48</sup>. These connection provides potential value of serum OPG in predicting infrarenal and aortic calcification in these patients, proving value of serum OPG in another population of patients.

## Osteoprotegerin and Coronary Artery Disease

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<sup>49</sup>. In previous studies has been shown that serum OPG levels significantly increased with coronary artery disease severity. Serum OPG levels increased in the presence of one, two or three vessel disease<sup>13–15</sup>. Overall, these researches show that serum OPG levels increase in conditions with vascular calcification, and is connected with acutisation of coronary artery disease, due to plaque instability<sup>49</sup>.

In the study regarding association of OPG with the presence and severity of CAD, serum OPG levels was measured in 201 patients to whom was performed coronarography, because of the chest pain. The severity of CAD was assessed in coronarography and was classified by the number of stenotic coronary arteries. Serum OPG levels were measured by ELISA, and were significantly higher in patients with CAD, then those without, and significance increased by increasing severity of CAD<sup>14</sup>. These insights show that altered serum OPG levels may reflect the status and severity of CAD, thus the status of vascular calcification. These findings are based on the opinion that OPG play an important role in pathophysiology of atherosclerosis<sup>7</sup>. The exact mechanism by which serum OPG is altered in these patients is still unclear, but there is a possibility that increased serum OPG present insufficient compensatory mechanism to prevent initiated vascular calcification cascade.

The study regarding relationship between serum OPG levels with CAD severity, left ventricular hypertrophy and C-reactive protein, shown as well that serum OPG levels were higher in patients with three vessel disease comparing to those with two, one or no vessel disease. The same was shown for the CRP. Correlation was positive even after adjustment for age.

Age and LV mass index also positive correlated to serum OPG levels, but this significance was lost after adjustment for age. Two-dimensional echocardiography and M-mode echocardiography was used to measure the wall thickness and the internal diameter, and both were measured at end-diastole. There was no significant gender difference in OPG levels<sup>15</sup>. Interestingly, in this study the association of OPG with inflammatory markers is shown. OPG functions as a soluble decoy receptor for receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL or OPG ligand). RANKL is produced by osteoblastic lineage cells and activated T lymphocytes and stimulates its receptor, RANK, which is located on osteoclasts and dendritic cells<sup>21</sup>. OPG, RANKL, and RANK act as key regulators of bone metabolism and the immune system. Because vascular diseases are promoted by immune-mediated mecha-

nisms, OPG may be involved in the progression of atherosclerosis<sup>7</sup>. OPG is also a receptor for the cytotoxic ligand TNF-related apoptosis-inducing ligand (TRAIL), a potent activator of apoptosis. One possibility is that OPG influences vascular disease by inhibiting TRAIL-induced apoptosis of vascular cells<sup>22,23</sup>.

Although the mechanism for the vascular effects of OPG is unknown, emerging evidence indicates OPG may act as a protective factor for vascular diseases. One hypothesis is that increased serum OPG levels may be a compensatory self-defensive response to the progression of atherosclerosis<sup>14</sup>.

## Osteoprotegerin and Aortic Valve Stenosis

Over the past 10 to 15 years, calcific aortic valve disease, which includes aortic sclerosis and aortic stenosis, has become recognized as an active process. Calcification and fibrosis are the main feature of aortic valve stenosis. Calcification contributes to the rigidity of the valve, thereby increases LV outflow obstruction. Numerous studies prove that calcific aortic valve stenosis is an active, rather than passive process, as was thought earlier<sup>50–52</sup>. Valvular calcificated deposits contain calcium and phosphates in the form of hydroxiapatite, calcium-phosphate mineral, that is present in calcified arterial tissue and in bone formation<sup>37,50,51</sup>. Proteins involved in tissue calcification regulation detected in calcific valvular tissue, include bone morphogenetic proteins (BMPs) 2 and 4<sup>26,52</sup>, RANKL<sup>52</sup>, and osteopontin<sup>53–55</sup>. RANK is expressed on normal valve cusps, but downregulated on aortic valve lesions<sup>26</sup>.

In aortic valve lesions, calcific noduli appears to develop their formation first in lesions of lipid deposition, especially those with oxidized lipids<sup>56</sup>. Aortic valve lesions contain as well tenascin C, an extracellular matrix glycoprotein in developing bone<sup>57</sup>. Recently a subgroup of valvular fibroblasts have been discovered, that express osteoblast markers and forming hydroxiapatite spontaneously-containing calcified noduli in vitro<sup>26,51</sup>. In response to oxidized cholesterol<sup>51</sup>, transforming growth factor beta 1 (TGF beta 1)<sup>51</sup>, BMP 2<sup>51</sup> and RANKL<sup>26</sup> increase their expression of osteoblast markers, thereby increasing the extent of calcified noduli formation. In addition, tenascin C increasing expression of matrix metalloproteinase 2 (MMP 2) in those cells<sup>57</sup>.

As well, it was shown that hyperphosphatemia induces calcified vesicular formation in the miofibroblasts, thus suggesting possible mechanism linking chronic renal disease and valvular calcification<sup>52,58,59</sup>.

Recent study shown that by immunohistochemistry using human aortic valves, RANKL was not expressed at relevant levels in controls but detectable in aortic valve stenosis<sup>60</sup>. OPG expression was marked in controls but significantly lower in aortic valve stenosis.

Areas containing focal calcification exhibited significantly less OPG-positive cells as compared to non-calcified regions. Stimulation with RANKL lead to a significant

rise in matrix calcification, nodule formation, alkaline phosphatase activity, expression of the bone-type isoenzyme of alkaline phosphatase, and expression of osteocalcin in cultured human aortic valve myofibroblasts.

In conclusion RANKL and OPG are differentially expressed in calcific aortic stenosis. In cultured human aortic valve myofibroblasts, RANKL promotes matrix calcification and induces the expression of osteoblast-associated genes, indicating a transition towards an osteogenic phenotype<sup>60</sup>. These results suggest that the RANKL-OPG pathway may regulate valvular calcification in calcific aortic valve stenosis.

### OPG Gene Polymorphism and Vascular Calcification

There are several studies on the subject if and how OPG gene polymorphism, affect vascular calcification<sup>61,62</sup>. All together, polymorphism among four single nucleotide are explored.

In the research that contains 468 men without coronary artery disease, and with one, two, or three vessel disease diagnosed by coronarography, OPG gene polymorphism was performed. Although single polymorphisms were not associated with coronary artery disease, linkage of polymorphisms 950 and 1181 revealed that haplotypes were overrepresented in men with coronary artery disease with an increased risk of CAD in carriers of genotypes 950 TC/1181 GC and 950 CC/1181 CC.

Furthermore, serum OPG levels were correlated with the presence of a C allele at position 950. In summary, linkage of genetic variations of the OPG gene at positions 950 and 1181 may confer an increased risk of coronary artery disease in Caucasian men<sup>61</sup>. Certainly it would be interesting to extend these genetic researches on OPG gene polymorphism to wider population of patients, such as patients who develop heart failure after myocardial infarction, as well in patients who develop heart failure in severe aortic valve stenosis.

In research on Koreans, genotyping of four polymorphisms, A163G, G209A, T245G and T950C, in the promoter region of the OPG gene was performed in 251 healthy Korean women (mean age 51.3±6.9 years) and in a second study population consisting of 100 patients who underwent coronary angiography (mean age 57.0±11.9 years), by allelic discrimination using the 5' nuclease polymerase chain reaction assay. Cardiovascular risk factors and serum OPG levels were measured and aortic calcification in thoracic and abdominal aorta was examined by simple radiological methods. That research shown prevalence of aortic calcification increased significantly as the subjects grew older. The frequencies of mutant alleles were

significantly higher in the subjects with aortic calcification compared with those without aortic calcification in G209A and T950C polymorphisms, although these significances were lost after adjustment for age. No significant relationship was found between OPG gene polymorphisms and serum OPG levels or cardiovascular risk factors. In the second study group, there were no associations between OPG promoter genotypes and aortic calcification, serum OPG levels, or coronary artery disease<sup>62</sup>. As we have shown, there's no unique conclusion on the subject how OPG gene polymorphism affect vascular calcification, thus more researches in that area are needed to clarify this connection.

### Conclusion

Obviously there is much excitement regarding osteoprotegerin as a linking molecule between bone and vascular system, although controversy still exists on the role of OPG/RANKL/RANK/TRAIL system in cardiovascular diseases. There is yet no joint opinion unifying dichotomy of OPG in animal models and human studies. It is possible that serum OPG levels is increased in response to the vascular injury and ongoing process of inflammation within an atherosclerotic lesions. The high OPG levels probably, as shown by various studies, indicate endothelial cells injury, so an increased OPG levels could be an indicator of a proinflammatory milieu in propagation of atherosclerosis. One hypothesis is that increased serum OPG levels may be a compensatory self-defensive response to the progression of atherosclerosis. Serum OPG levels is increased in the presence of one, two or three vessel disease, so it is promising as a risk stratification biomarker amongst patients with coronary artery disease. It is demonstrated that OPG provide impressive independent prognostic information in patients who develop heart failure after acute myocardial infarction, representing a noninvasive tool for monitoring morbidity and mortality in these patients. Future studies must determine if these results apply solely to post-infarction heart failure or if OPG can provide prognostic information for a wider group of heart failure and acute myocardial infarction patients. Studies also suggest that the RANKL-OPG pathway may regulate valvular calcification in calcific AS, since the RANKL and OPG are differentially expressed in calcific AS. There is yet to explore whether OPG gene polymorphism play any role in the presence and severity of cardiovascular diseases, indicating that it might be genetically regulated. Nevertheless, the identification of OPG as a novel cardiovascular risk marker suggests an association between mediators of bone homeostasis and cardiovascular diseases and supports a link between bone and vascular calcification.

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## OSTEOPROTEGERIN I VASKULARNE KALCIFIKACIJE: KLINIČKA I PROGNOŠTIČKA VAŽNOST

### SAŽETAK

Osteoprotegerin (OPG) je važan modulator koštanog metabolizma, koji ujedno manifestira svoj učinak i na krvožilni sustav. Pretpostavlja se da je osteoprotegerin ključni inhibitor arterijskih vaskularnih kalcifikacija, a otpuštaju ga endotelne stanice kao protektivni mehanizam za svoje preživljenje u određenim patološkim stanjima, kao u prvom redu šećernoj bolesti, kroničnoj renalnoj insuficijenciji, te metaboličkim premećajima. Navedeno je pokazano u dosadašnjim studijama na animalnim modelima. Suprotno tome, mnoge kliničke studije posljednjih godina povezuju serumsku razinu osteoprotegerina sa postojanjem i težinom koronarne bolesti, ishemijskom kardijalnom dekompenzacijom, te budućih kardiovaskularnih događaja. Zbog navedenoga sve se više spominje njegova uloga kao potencijalnog biomarkera u ranoj identifikaciji i praćenju kardiovaskularnih bolesti. Između ostaloga OPG se na osnovu kliničkih istraživanja dovodi u vezu i sa poznatim faktorima rizika ateroskleroze; nedvojbeno je dokazana njegova pozitivna korelacija sa dobi, te pušenjem. Dokazana je i pozitivna korelacija sa komplikacijama od kardiovaskularnih bolesti u pacijenata sa šećernom bolesti. Postoje i dokazi vezani uz povišenu razinu serumskog OPGa i hiperlipoproteinemije. Istraživanja su vršena i na genetskoj razini, vežući određene genske polimorfizme pojedinih nukleotida osteoprotegerina uz pojavu vaskularnih kalcifikacija. Ovaj članak naglašava multifaktorijalnu ulogu OPGa, prikazujući brojne kliničke i eksperimentalne studije glede njegove uloge u vaskularnoj patologiji, predlažući novi biomarker u kardiovaskularnim bolestima, pokazujući nove zaključke u vezi ove zanimljive teme, koja treba daljnja istraživanja.