# Osteoprotegerin as a possible novel predictor of cardiovascular dysfunction

## Osteoprotegeryna – proponowany nowy wskaźnik występowania chorób układu krążenia

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## Abstract

Osteoprotegerin (OPG) is a prime regulator of bone remodelling under both physiological and pathological conditions. Its role is strictly related to the receptor activator of nuclear-factor (NF)-κB ligand (RANKL), which is also produced by osteoblasts. RANKL is a main activator of osteoclasts' differentiation during bone resorption, and OPG is a decoy receptor for RANKL, which inhibits osteoclast differentiation by interrupting interactions between RANKL and its receptor (RANK).

OPG is produced by osteoclasts as well as by stromal, haematopoietic (megakaryocytes) or endothelial cells. Lymphocyte-derived cytokines also play a critical role during bone metabolism and OPG-regulated immunological stimulation. IL-4 and IL-13, T helper 2 (Th2) cytokines produced by antigen-activated T cells induce OPG production by osteoblasts. The RANK/RANKL system is also implicated in dendritic cell-T lymphocyte interactions. Thus, dendritic cell survival, T lymphocyte activation, as well as B lymphocyte development, maturation and function are regulated by OPG. On the other hand, some clinical data indicate that OPG levels are strong and independent predictors of cardiovascular disease (coronary artery calcification and hypertension), which suggests that OPG may play a regulatory role in cardiac pathology and vascular remodelling.

In this article, the current view of the role of OPG as a new biomarker or prognostic parameter in cardiovascular disease will be presented.

**Key words:** dendritic cells, osteoprotegerin, bone remodelling biomarkers, inflammation, cardiovascular diseases.

## Streszczenie

Osteoprotegeryna (OPG) jest głównym regulatorem przebudowy kości w warunkach fizjologicznych i stanach chorobowych. Jej aktywność jest regulowana przez układ aktywatora receptora dla czynnika jądrowego (NF)-κB i jego liganda (RANKL). RANKL jest głównym aktywatorem różnicowania i osteoklastogenezy podczas resorpcji kości, a OPG jest receptorem-atrapą, który hamuje różnicowanie osteoklastów przez blokowanie oddziaływań między ligandem RANKL a jego receptorem (RANK). Osteoprotegeryna jest produkowana przez komórki macierzy, komórki hematopoetyczne (megakariocyty) i komórki śródbłonka. Zależne od limfocytów cytokiny również odgrywają krytyczną rolę w metabolizmie kości i regulowanej przez OPG stymulacji układu odpornościowego. Interleukina 4 (IL-4) i IL-13, T helper 2 (Th2) są to cytokiny produkowane przez aktywowane antygenem komórki T, które indukują w osteoblastach ekspresję OPG. Oś RANK/RANKL jest również związana z odziaływaniami między komórkami dendrytycznymi a limfocytami T. Aktywność komórek dendrytycznych, aktywacja limfocytów T i dojrzewanie oraz funkcja limfocytów B są potencjalnie regulowane przez OPG.

Z drugiej strony, najnowsze badania kliniczne pokazują, że OPG jest silnym i niezależnym wskaźnikiem prognostycznym chorób układu krążenia (zwapnień tętnic wieńcowych i nadciśnienia), co sugeruje, że OPG może również odgrywać rolę regulatora w patologii serca i przebudowie naczyń.

Niniejszy artykuł komentuje aktualny stan wiedzy na temat zastosowania OPG jako biomarkera i parametru prognostycznego chorób układu krążenia.

**Słowa kluczowe:** biomarkery przebudowy kostnej, choroby układu krążenia, komórki dendrytyczne, osteoprotegeryna, zapalenie.

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The first reference to the role of osteoprotegerin (OPG) in cardiovascular disease was made by Jono et al. in 2002 [1]. They documented that serum OPG levels are associated with the progression and the severity of coronary artery disease evaluated by coronary angiography. In the past, there was no information about the main sources and regulatory mechanism of OPG on the cardiovascular system. Our view about its role has significantly changed since then [2, 3].

## The OPG/RANK/RANKL triad

OPG is a prime regulator of bone remodelling and may exert a substantial influence on the severity of cardiovascular disease [4, 5]. OPG operates as a decoy receptor by blocking the interaction between the receptor activator of nuclear-factor-κB ligand (RANKL) and TNF-related apoptosis-inducing ligand (TRAIL), as well as their related receptors: RANK and TRAIL-R1/TRAIL-R2 [6, 7]. OPG regulates the RANK/RANKL system and protects against bone loss. In opposition to its protective role, OPG was shown to block TRAIL-induced apoptosis, binding of OPG to TRAIL, abolishing its anti-osteoclastogenic activity [8]. It was demonstrated in a mouse model that OPG-knockout mice (OPG -/-) developed early-onset osteoporosis and arterial calcification [9]. Additionally the restoration of this gene prevented osteoporosis progression and arterial calcification [10].

## OPG and other cardiovascular risk factors

What is important is that serum OPG levels positively correlate with age [1, 4]. Additionally, Vik et al. observed in the general population that age and sex have a differential impact on the association between OPG and carotid intima media thickness (CIMT) [11]. CIMT analysis of carotid plaque prevalence showed that after adjustment for age, gender and the "classical" risk factors (i.e. smoking, systolic blood pressure, BMI, total cholesterol, HDL cholesterol) the strength of association between OPG and *de novo* plaque formation or their area progression is significantly reduced (mainly in men) [11]. The distribution of male sex across tertiles of OPG is significantly reduced in the general population; however, age distribution among female and male subjects is similar [11, 12]. Taking into consideration that OPG can act both as an inhibitor or activator of atherosclerosis, these findings may suggest that increased serum OPG may inhibit progression of early atherosclerosis in younger female subjects.

Similarly to adiponectin, in non-diabetic subjects OPG was significantly decreased in obese as opposed to lean ones [13]. In a general population, OPG negatively correlated with body weight, BMI, waist circumference and fasting plasma insulin, while positively correlating with insulin sensitivity, and glycated haemoglobin levels [12, 13]. Moreover, the distribution of BMI values across OPG tertiles is significantly reduced [12]. The relationship between OPG and BMI is observed also in loan subjects, where the loss of height, mainly associated with age, and caused by changes of posture, lower muscle strength, decrease in size of the intervertebral discs and the development of osteoporosis, is positively associated with OPG levels [14]. These data suggest that both bone remodelling, loss of muscle strength and mass, and the disease presentation may influence OPG levels.

In diabetic patients, OPG was significantly elevated in patients with increased coronary artery calcification (CAC) [15]. OPG levels were also higher in hypertensive patients with retinopathy, patients with a high probability of 10-year cardiovascular risk, three or more damaged target organs (heart, vessels, kidneys) and those with previous episodes of ischaemic cardiomyopathy or hypercholesterolaemia (odds ratio: 3.33 and 2.91 respectively) [16]. In apparently healthy individuals, plasma OPG levels were significantly associated with inflammation and arterial hypertension [17]. OPG predicts the premature state of CAC in asymptomatic normotensive individuals and renal function significantly contributes to this process both in hypertensive and normotensive subjects [18]. Thus OPG can be used as an indicator of diabetes- and hypertension-associated vascular pathologies as well as a predictor of endothelial dysfunction and cardiovascular risk.

## Predictive value of OPG levels

A number of prospective studies (the Bruneck Study and the Tromsø Study) have shown that the serum OPG increase per standard deviation (1.13 ng/mL or 1.38 pmol/L) was associated with an increased incidental risk of myocardial infarction, ischaemic stroke or "vascular mortality" in crude and adjusted models over a 10-year follow-up period [4, 12]. Moreover, in the Tromsø Study ($n$ = 6265) hazard ratios with 95% confidence intervals per SD of death due to ischaemic heart disease or nonvascular causes were as follows: 1.20 (1.11–1.31) and 1.31 (1.22–1.41).This finding supports the concept that OPG serum levels may serve as a prerequisite in the prognosis of cardiovascular risk.

## Protective or pathogenic role of OPG

OPG is produced by osteoclasts as well as by stromal, haematopoietic (megakaryocytes) or arterial wall cells (endothelial cells and vascular smooth muscle cells – VSMC) [19, 20]. Very recent studies have shown that osteocytes are also an important source of RANKL and OPG, with levels similar (or exceeding) to those in osteoblasts; thus they exhibit a greater capacity to control osteoclastogenesis than osteoblasts by the canonical Wnt signalling pathway [21]. The deleterious effect of OPG on the vascular wall has been shown *in vitro* and *in vivo*. OPG can promote the adherence of neutrophils to endothelial cells [22]. Moreover, angiotensin II, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and proinflammatory cytokines may stimulate OPG expression in VSMC [23, 24], thus providing evidence for the role of OPG in VSMC senescence and development of vascular calcification [25]. This non-specific ligand-independent biological activity occurs due to its heparin-binding domain [26]. The possible interactions between vascular, bone and immune cells are schematically presented in Figure 1.

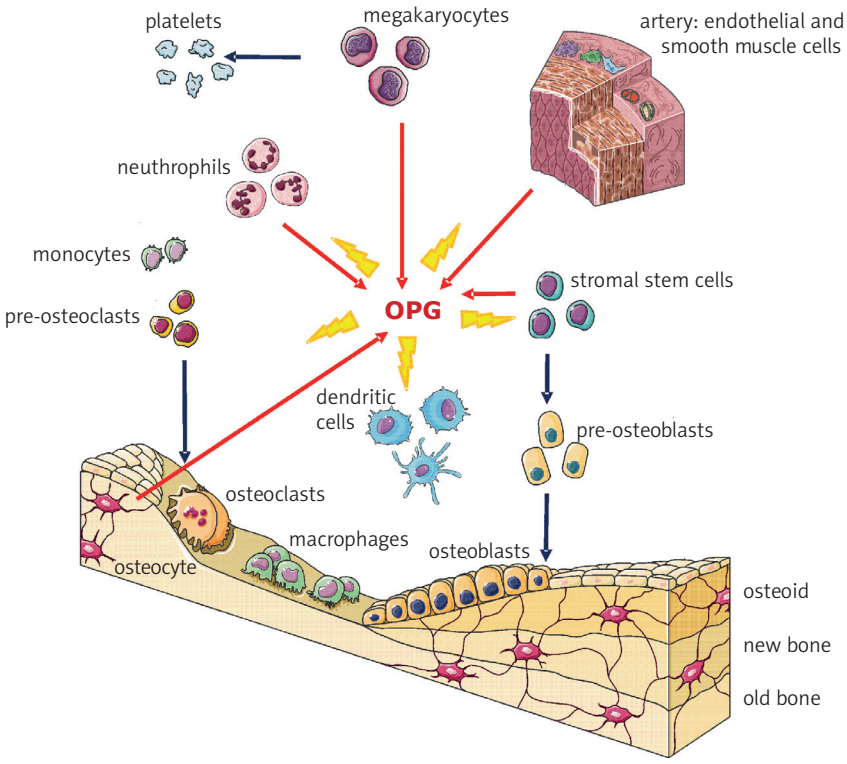


**Fig. 1.** The central role of osteoprotegerin (OPG) in vascular and bone biology is presented. Red arrows represent the pathways of OPG release by different cell populations and tissue. Yellow lightning symbols represent the regulatory role of OPG. Black arrows – OPG-induced cell differentiation. The figure was produced using Servier Medical Art

Lymphocyte-derived cytokines also play a critical role during bone metabolism and the OPG-regulated stimulation. Interleukin-4 (IL-4), a pleiotropic immune cytokine produced by T lymphocytes, mast cells, basophils and natural killer cells, induces OPG production by osteoblasts, and inhibits osteoclast activity [27]. The RANK/RANKL system is also implicated in dendritic cell-T lymphocyte interactions. Dendritic cell (DC) survival, T lymphocyte activation and B lymphocyte development, maturation and function are regulated by OPG. RANKL is a dendritic cell-stimulating agent, which prolongs DC survival and stimulates T cell maturity, thereby regulating DC proliferation [28].

Despite animal studies generally favouring the protective role of OPG, predominantly in terms of vascular calcification [9], the latest data support the pathogenic role of OPG in the development and progression of atherosclerotic lesions [28]. *In vivo* treatment of ApoE-/- mice with human OPG induced signs of fibrosis, and up-regulated the arterial expression of TGF-β1, increasing collagen content [29].

Recently, it was demonstrated that OPG regulates not only DC survival but also the nature of DC-dependent inflammatory responses. OPG treatment reduced the survival and cytokine production of DCs obtained from wild-type (WT) mice. On the other hand, OPG deficient (OPG KO – knockout) mice, developed osteoporosis. Nevertheless, their DCs survived better than WT DCs, and produced more TNF-α, IL-12p40, and IL-23 cytokines than WT DCs in response to *Escherichia coli* LPS [30].

## OPG as a therapeutic target

Intervention studies on animal models suggest that OPG deficiency promotes atherogenesis and arterial calcification [9, 31]. However, exogenous OPG supplementation does not change atherosclerosis progression but influences plaque morphology and collagen content [28, 32]. Angiotensin II, which influences OPG expression in VSMC, may be used as a potential target in OPG reducing treatment. However, in a previous study on serum OPG levels in hypertensive subjects ($n$ = 68 and $n$ = 259), in which about 60% of hypertensives were treated with angiotensin-converting enzyme (ACE) inhibitors, there was no significant difference in OPG levels between treated and non-treated subjects [16, 17]. Thus, the potential role of ACE inhibitors, the most frequently used drugs in standard hypertensive treatment, in OPG expression must be revised in larger prospective studies.

In conclusion, the OPG/RANK/RANKL triad is a promising system to be investigated as a marker of calcification-related cardiovascular risk and a therapeutic target [33].

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