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Serum osteoprotegerin and osteopontin levels are associated with arterial stiffness and the presence and severity of coronary artery disease

Dimitris Tousoulis [a,1,*], Gerasimos Siasos [a,b,1], Konstantinos Maniatis [a], Evangelos Oikonomou [a], Stamatios Kioufis [a], Marina Zaromitidou [a], Thodoris Paraskevopoulos [a], Stavroula Michalea [a], Christina Kollia [a], Antigoni Miliou [a], Eleni Kokkou [a], Athanasios G. Papavassiliou [b], Christodoulos Stefanadis [a]

[a] 1st Cardiology Department, University of Athens Medical School, "Hippokration" Hospital, Athens, Greece
[b] Department of Biological Chemistry, University of Athens Medical School, Athens, Greece

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ABSTRACT

Background: Osteopontin (OPN) and osteoprotegerin (OPG) have recently emerged as key factors in both vascular remodeling and development of atherosclerosis. Arterial stiffness has an independent predictive value for cardiovascular events. We evaluate the relationship between OPG, OPN serum levels and vascular function in coronary artery disease (CAD) patients.

Methods: The study population was consisted of 409 subjects (280 with CAD and 129 without CAD). Carotid-femoral pulse wave velocity (PWV) was measured as an index of aortic stiffness. OPG and OPN levels were measured, as markers of vascular remodeling and calcification, by ELISA. Gensini score was used to evaluate the extent of CAD.

Results: CAD patients, compared to those without CAD, had higher OPG (3.91 ± 1.87 pmol/l vs. 2.88 ± 1.32 pmol/l, $p<0.001$) and logOPN levels (1.81 ± 0.18 ng/ml vs. 1.71 ± 0.24 ng/ml, $p<0.001$) and impaired PWV (8.94 ± 2.21 m/s vs. 8.28 ± 1.91 m/s, $p=0.006$). Furthermore, PWV was associated with serum OPG levels ($r=0.19$, $p<0.001$) and with serum logOPN levels ($r=0.10$, $p=0.049$). Multivariate linear regression analysis revealed that increased OPG ($p=0.013$) and logOPN ($p=0.006$) levels are associated with 3-vessel CAD and Gensini score ($p=0.04$ for OPG and $p=0.09$ for OPN), independently of other known cardiovascular risk factors.

Conclusion: The present study revealed that serum OPG and OPN levels are positively associated with arterial stiffness, and with the extent of CAD. These preliminary results suggest that OPG and OPN levels are significantly correlated with vascular function contributing to the pathogenesis of atherosclerosis in CAD. Further studies are needed to explore the mechanisms of action of OPG and OPN in CAD.

1. Introduction

Recently, it has been found that parameters of vascular function and structure have important clinical impact in both healthy subjects and in coronary artery disease (CAD) patients. Arterial stiffness, as it can be estimated noninvasively by pulse wave velocity (PWV) has an independent predictive value for cardiovascular events [1]. Moreover, arterial stiffness is impaired in coronary atherosclerosis and is associated with the severity of CAD [2,3].

Osteopontin (OPN) and osteoprotegerin (OPG) have recently emerged as key factors in both vascular remodeling and development of atherosclerosis and arterial hypertension [4,5]. OPN is a phosphorylated glycoprotein originally found in bone and is known to be involved in the formation and calcification of bone [6]. Recently, OPN has been studied as a multifunctional protein that is upregulated in a variety of acute and chronic inflammatory conditions, such as wound healing, fibrosis, autoimmune disease, and atherosclerosis [7,8]. OPN is also found in human atherosclerotic plaques most strikingly associated with macrophages and foam cells infiltration [9]. Moreover is secreted by endothelial cells and vascular smooth muscle cells [10]. However, the role of OPN in vascular calcification, which is closely related to chronic and active inflammation, is that of a negative regulator because it is an inhibitor of calcification and an active inducer of decalcification [11].

OPG is also a secretory glycoprotein and is a member of the tumor necrosis factor alpha (TNF-α) receptor family. OPG was originally discovered as an inhibitor of bone resorption and its expression and production are regulated by various cytokines and hormones [12]. Moreover, OPG is produced by a variety of tissues such as heart, arteries, veins, lung, kidney, immune system and bone while is also expressed in coronary smooth muscle cells and endothelial cells in vitro [13,14].

* Corresponding author at: Vas. Sophias 114 str, 115 28, Athens, Greece. Tel.: +30 213 2088099; fax: +30 213 2088676.
E-mail address: drtousoulis@hotmail.com (D. Tousoulis).
[1] The first two authors (G.S., D.T.) equally contributed in this study.

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OPG and OPN levels are associated with the presence and severity of CAD [15–17]. However, to date no study evaluated the relationship between OPG, OPN serum levels and arterial wall properties in CAD patients. The purpose of this study was to investigate the association between arterial stiffness, a valid marker of early stage atherosclerosis and serum OPN and OPG levels in CAD patients and the role of OPN and OPG in vascular pathophysiology.

2. Methods

2.1. Study population

The study population consisted of 409 consecutive subjects undergoing diagnostic coronary angiography for suspected CAD. All patients fulfilled the criteria of stable chest pain and/or signs of myocardial ischemia on exercise electrocardiography for clinical indication for cardiac catheterization. CAD was defined as narrowing of more than 50% of at least one major coronary artery, and coronary angiographies were interpreted by at least two experienced cardiologists. On the basis of these coronary angiographies, the number of affected coronary arteries was determined, while patients with no angiography documented CAD were considered as healthy subjects.

Age and history of cigarette use were assessed through an interview preceding the physical examination. We defined as "smoker" the current smokers, who smoke at least one cigarette per day and as "no smoker" those who had never tried a cigarette in their life or those who had stopped smoking for at least 1 year. Blood pressure and a hematological and biochemical profile were determined. Diabetes was considered present if a patient was treated with insulin or oral agents or had a fasting glucose level ≥126 mg/dl. Hypertension was defined by systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, the current use of antihypertensive treatment, or a combination of the three. Hyperlipidemia was defined as total cholesterol level ≥200 mg/dl, the current use of lipid-lowering treatment, or both. All measurements, in this study were made by the same observer who was unaware of the disease status and treatment condition of the participants. Subjects with low ejection fraction (EF<50%, as estimated by echocardiography), valvulopathies, acute coronary syndromes or myocardial infarction in the last 6 months, chronic kidney disease, comorbidities such as malignancies, or with immunological diseases, osteoporosis and subjects receiving systemic glucocorticoids or immunosuppressants were excluded from the study.

The study (complied with the Declaration of Helsinki) was approved by the institutional ethics committee and an informed consent was given by each participant. The authors of this manuscript have certified that they comply with the principles of ethical publishing in the International Journal of Cardiology .

2.2. Evaluation of arterial stiffness

Arterial stiffness was evaluated in all patients before coronary angiography. Briefly, all subjects were fasting and abstained from coffee consumption for at least 12 h and were instructed to refrain from smoking at least six hours before the examination time and after 10 min rest carotid-femoral PWV, which is considered to be an index of aortic stiffness [19], was calculated from measurements of pulse transit time and the distance traveled between two recording sites (PWV = distance in meters divided by transit time in seconds) by using a well-validated noninvasive device (SphygmoCor; AtCor Medical, Sydney, Australia) [20] as previously described [21]. Two different pulse waves were obtained at 2 sites (at the base of the neck for the common carotid and over the right femoral artery) with the transducer. Distance was defined as the distance from the suprasternal notch to femoral artery minus the distance from the carotid artery to the suprasternal notch. The repeatability of the technique for determining PWV has been determined using the repeatability coefficient (British Standard Institution), which is given by the formula: $2\times(\Sigma d_i^2/n)$ (where n is the sample size and d_i is the difference between the two consecutive measurements, the lower the better). According to this formula the coefficient was found equal to 5.0%, which suggest good repeatability.

2.3. Coronary angiography

Selective coronary angiography was performed using a femoral or radial artery approach by experienced interventional cardiologists blinded to PWV measurements. Significant CAD was diagnosed visually if the narrowing of the luminal diameter of a major epicardial coronary artery was ≥50%. In CAD patients the severity of coronary artery disease was evaluated by:

1. Number of stenosed vessels: represented as the number of major stenosis in epicardial arteries with at least 1 obstructive lesion (≥50% reduction of lumen diameter), including left anterior descending artery (LAD), left circumflex artery (LCX), right coronary artery (RCA), and left main artery (LM).
2. Gensini score [22] for graded narrowing of the lumen: 1 for 1% to 25%, 2 for 26% to 50%, 4 for 51% to 75%, 8 for 76% to 90%, 16 for 91% to 99%, and 32 for total occlusion. This score is multiplied by a factor accounting for the importance of the lesion position in the coronary arterial tree; e.g., 5 for LM, 2.5 for proximal LAD, and 1 for proximal RCA. The severity of disease was expressed as the sum of the scores for individual lesions.

2.4. Biochemical measurements

Venous blood samples were centrifuged at 3000 rpm and serum/plasma was collected and stored at −80 °C until assayed. Serum levels of osteoprotegerin and plasma levels of osteopontin were measured, as markers of vascular remodeling and calcification, by commercially available ELISA kits (BioVendor GmbH, Germany and R&D Systems, Minneapolis, MN respectively). The sensitivity for osteoprotogerin was 0.13 pmol/l with an intra- and inter-assay CV of <4% and <6%, respectively. For osteopontin the sensitivity was 0.011 ng/ml with an intra- and inter-assay CV of <4% and <7%, respectively. Lipids and glucose levels were measured by using commercially enzymatic method.

2.5. Statistical analysis

All variables were tested for normal distribution of the data. The values of OPN and Gensini score were skewed and they were log-transformed to improve normality. Normally distributed data were expressed as means ± sd. A Student's t-test was used for normally distributed continuous data. A chi square test was used for categorical variables. For normally distributed data one way analysis of variance was performed to examine for intergroup differences. Differences in values between study subgroups were tested by using post hoc analysis after Scheffe correction. Pearson correlation coefficient was used to examine for relationships between normally distributed continuous variables. Univariate and multiple linear regression analysis were used to test for independent associations after adjustment for several confounders. P values

Table 1
Characteristics of Study population according to the extent of CAD.

Factor	Number of affected coronary arteries				p-values
	0	1	2	3	
Male/female (%)	60/40	90/10	97/3	94/6	<0.001
Age (years)	59 ± 13	59 ± 11	60 ± 9	61 ± 10	0.59
logOPN (ng/ml)	1.72 ± 0.24	1.82 ± 0.18*	1.76 ± 0.18	1.88 ± 0.22*	<0.001
	57.54 (38.22, 78.40)	66.55 (52.85, 83.68)	56.45 (45.31, 74.67)	71.42 (51.42, 105.36)	
OPG (pmol/l)	2.88 ± 1.32	3.75 ± 1.76*	3.41 ± 1.68	4.48 ± 2.15*	<0.001
PWV (m/s)	8.26 ± 1.92	8.69 ± 2.21	8.85 ± 2.23	9.42 ± 2.55*	0.016
Gensini score	2.2 ± 1.0	25.0 ± 16.0*	49.0 ± 29.2*	74.6 ± 40.5*	<0.001
Diabetes mellitus (%)	4	23	27	23	0.001
Arterial hypertension (%)	22	80	81	85	<0.001
Hypercholesterolemia (%)	10	40	28	22	<0.001
Current smokers (%)	28	26	22	16	0.43
Creatinine (mg/dl)	0.90 ± 0.16	0.93 ± 0.2	0.94 ± 0.23	0.96 ± 0.21	0.19

The number of affected coronary arteries was determined by coronary angiography. Categorical variables are expressed as valid percentages and continue variables as mean ± SD. In addition the medians with 1st and 3rd quartile of Osteopontin are expressed in their exponential form. Differences of normally distributed continuous data were tested with ANOVA. Differences of not normally distributed continuous data were tested with Kruskal–Wallis test. Chi square was used for the comparison between categorical variables. CAD: coronary artery disease; logOPN: logarithm of osteopontin to base 10; OPG: osteoprotogerin; PWV: pulse wave velocity.

* Revealed a statistical significant difference ($p<0.05$) compared to subjects with no CAD based on ANOVA post hoc with Scheffe correction and on Bonferroni correction for not normally distributed variables.

of <0.05 were considered to indicate statistical significance while, p values of <0.1 were considered to indicate statistical trend. All statistical calculations were performed using SPSS software (version 18.0; SPSS, Chicago, IL).

3. Results

On the basis of coronary angiographies, the 409 subjects (remained after the exclusion criteria) were categorized as subjects without CAD (n = 129), patients with 1-vessel disease (n = 124), patients with 2-vessel disease (n = 89), and patients with severe 3-vessel disease (n = 67). The characteristics of these four groups are listed in Table 1. The percentage of patients with diabetes mellitus, arterial hypertension and hypercholesterolemia were higher in patients with advanced CAD while there was no difference in age and serum creatinine levels. There was also a significant association between the number of affected coronary vessels and the extent of CAD as it can be calculated with Gensini score.

3.1. Arterial wall properties, OPN and OPG serum levels in patients with CAD

The whole population of CAD patients compared to those without CAD had significantly higher OPG levels (3.91 ± 1.87 pmol/l vs. 2.88 ± 1.32 pmol/l, p<0.001) and significantly higher logOPN levels (1.81 ± 0.18 ng/ml vs. 1.71 ± 0.24 ng/ml, p<0.001). Similarly, CAD patients compared to those without CAD had impaired PWV (8.94 ± 2.21 m/s vs. 8.28 ± 1.91 m/s, p = 0.006). Moreover, ANOVA reveled that OPG and OPN serum levels (p<0.001) as well as PWV values (p = 0.016) were associated with the severity of CAD (Table 1) (Fig. 1).

As shown in Table 2, OPG serum levels were higher in patients with 3-vessel CAD compared to patients without CAD (p<0.001) and in patients with diabetes mellitus compared to normoglycemic subjects (p = 0.01) while there was no difference in OPG serum levels between smokers and non smokers, subjects with arterial hypertension and subjects with normal arterial pressure and between patients with hypercholesterolemia and normocholesterolemic subjects (p = NS for all). As regards logOPN levels were higher in patients with 3-vessel CAD compared to patients without CAD (p<0.001), while there was no difference in logOPN levels between subjects with diabetes mellitus and normoglycemic subjects, between smokers and non smokers, between subjects with arterial hypertension and subjects with normal arterial pressure and between patients with hypercholesterolemia and normocholesterolemic subjects (p = NS for all).

In order to eliminate the impact of the aforementioned residual confounding we performed multiple linear regression analysis, after adjusting for sex, age, creatinine serum levels and the presence of diabetes mellitus, arterial hypertension and hypercholesterolemia, which revealed that patients with 3-vessel disease had significantly higher OPG serum levels compared to those without CAD (Table 2). Similarly, after adjustment for the aforementioned confounders, linear regression analysis revealed that patients with 3-vessel disease had significantly higher logOPN serum levels compared to those without CAD (Table 2).

As the severity of CAD disease was also estimated by Gensini score we applied the above described models after replacing the number of affected vessels by the base 10 logarithm of the estimated Gensini score. We also found that OPG serum levels are significantly higher in CAD patients with severe coronary atherosclerosis as it can be calculated with Gensini score (for logGensini score b = 0.509, 95%C.I. 0.026 to 0.992, p = 0.04). As concerning logOPN levels we found only a statistical trend concerning their association with the severity of CAD as calculated by Gensini score (for logGensini score b = 0.042, 95%C.I. −0.007 to 0.092, p = 0.09).

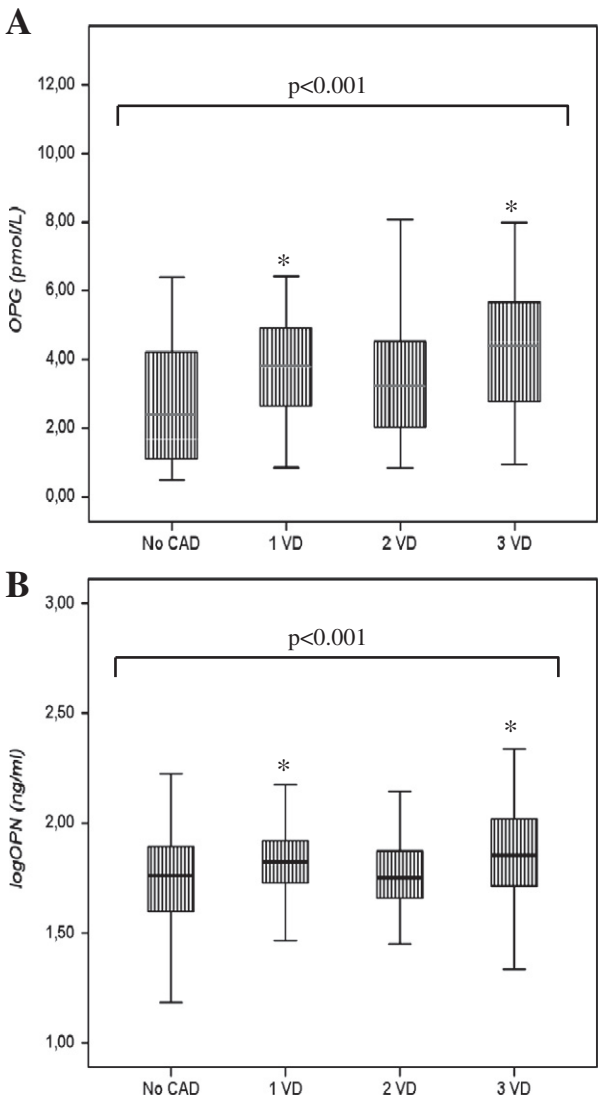

Fig. 1. A. Box-plots of OPG serum levels of subjects according to the extent of coronary artery disease. B. Box-plots of logOPN serum levels of subjects according to the extent of coronary artery disease. p values according to ANOVA; *: corresponds to significant difference (p<0.05) compared to subjects with no CAD; no CAD: subjects without coronary artery disease; 1 VD: subjects with one coronary artery disease; 2 VD: subjects with two coronary artery disease; 3 VD: subjects with three coronary artery disease.

3.2. Association of OPG and OPN serum levels with arterial wall properties

Pulse wave velocity were positively associated with serum OPG levels (r = 0.19, p<0.001) and with serum logOPN levels (r = 0.10, p = 0.049). As it is shown in Table 1 PWV was also associated with the extent of CAD. Linear regression analysis revealed that OPG serum levels were associated with PWV even after adjustment for the severity of CAD, gender, smoking habits and the presence of hypertension, diabetes mellitus, and hyperlipidemia (Table 3) while, after adjustment for the severity of CAD and the aforementioned confounders there was a statistical trend of association between logOPN and PWV levels (Table 4).

4. Discussion

In this study we found that OPG and OPN serum levels were significantly associated with the presence and severity of CAD. Moreover both serum OPG and OPN levels were positively associated with arterial stiffness.

Table 2
Univariate and multivariate linear regression analysis of osteoprotegerin (pmol/l) and logOsteopontin (ng/ml) respectively.

Variables	Osteoprotegerin (pmol/l)				logOsteopontin (ng/ml)			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	B (95% CI)	p	B (95% CI)	p	B (95% CI)	p	B (95% CI)	p
3-vessel disease[a]	1.59 (0.95, 2.23)	<0.001	1.10 (0.23, 1.96)	0.013	1.59 (0.95, 2.23)	<0.001	0.13 (0.04, 0.21)	0.006
2-vessel disease[a]	0.52 (−0.04, 1.08)	0.07	0.32 (−0.45, 1.10)	0.41	0.52 (−0.04, 1.08)	0.07	0.02 (−0.06, 0.10)	0.60
1-vessel disease[a]	0.86 (0.35, 1.38)	0.001	0.52 (−0.21, 1.26)	0.16	0.86 (0.35, 1.38)	0.001	0.08 (−0.01, 0.16)	0.035
Age (years)	0.04 (0.03, 0.06)	<0.001	0.04 (0.02, 0.07)	<0.001	0.03 (0.01, 0.04)	<0.005	0.002 (0.001, 0.005)	0.05
Sex[b]	0.11 (−0.38, 0.60)	0.67	−1.30 (−2.02, −0.58)	<0.001	0.11 (−0.38, 0.60)	0.67	−0.01 (−0.08, 0.06)	0.82
Arterial hypertension (y/n)	0.38 (−0.07, 0.84)	0.09	−0.16 (−0.74, 0.42)	0.57	0.027 (−0.02, 0.7)	0.22	−0.01 (−0.07, 0.05)	0.70
Diabetes mellitus (y/n)	0.71 (0.17, 1.24)	0.01	0.47 (−0.13, 1,07)	0.12	0.71 (0.17, 1.24)	0.43	0.01 (−0.06, 0.06)	0.96
Hyperlipidemia (y/n)	0.29 (−0.15, 0.73)	0.19	0.14 (−0.38, 0.66)	0.60	0.023 (−0.19, 0.06)	0.28	−0.004 (−0.06, 0.05)	0.87
Current smokers (y/n)	−0.09 (−0.35, 0.15)	0.45	0.27 (−0.28, 0.83)	0.33	0.02 (−0.01, 0.05)	0.11	0.04 (−0.02, 0.09)	0.17
Creatinine (mg/dl)	0.64 (−0.41, 1.69)	0.23	0.65 (−0.50, 1.81)	0.26	0.19 (0.09, 0.29)	<0.001	0.18 (−0.06, 0.29)	0.003

In arterial hypertension, diabetes mellitus and hyperlipidemia the presence of the disease was considered as reference category; current smokers were considered as reference category; (y/n): (yes/no); B: b regression coefficient; CI: confidence interval.
[a] Patients without coronary artery disease as reference category.
[b] Male sex was considered as reference category.

4.1. Osteopontin, osteoprotegerin and coronary artery disease

Previous studies have shown that vascular calcification, with its reduced compliance and altered mechanical properties, is a predisposing factor of plaque rupture, and a predictor of cardiovascular mortality [23]. In addition, the clinical coincidence of osteoporosis and vascular disease has long indicated that common mediators may adversely affect bone metabolism and vascular integrity [24]. Thus, OPG, act as key regulator of bone metabolism and the immune system. The production of OPG protein in endothelium and vascular smooth muscle cell exert anti-apoptotic effects by the binding to the TNF-related apoptosis-inducing ligand (TRAIL) and regulate vascular inflammation and immunity [25]. In a more recent study, OPG immunoreactivity were detected in the normal vascular wall and in early atherosclerotic lesions in humans, whereas OPG was expressed in advanced calcified lesions [26].

Consequently, even though OPG represents a protective factor for the vascular system through the prevention of vascular calcification and prevention of endothelial injury, paradoxically, increased serum levels of OPG in patients with vascular disease has been interpreted as an incomplete self defensive regulatory mechanism to counteract disease progression [17]. Published evidence suggests that the alterations of OPG serum levels may be associated with CAD [15,27]. Additionally, in a recent large population-based study, serum OPG was found to be associated with future risk of myocardial infarction, ischemic stroke, total mortality, mortality of ischemic heart disease, stroke and of non-vascular causes independent of traditional cardiovascular risk factors [28].

Furthermore, similarly OPN plays an important role in cardiovascular disease and atherosclerosis [29]. High levels of OPN mRNA and proteins were reported in atherosclerotic plaques [30]. Specifically, OPN in the plaque is believed to exert its effect through upregulation within, and in proximity to, activated cells, thereby becoming responsible for changes leading to instability, and inducing matrix metalloproteinase release, angiogenesis, hemorrhage, fibrous cap degradation, and thrombotic complications [29]. In addition, a considerable number of studies report that there is an association between serum OPN levels and the presence as well as the severity of CAD [16,31].

In this study we found that CAD patients, compared to those without CAD, had significantly higher OPG and OPN levels. Moreover both OPG and OPN serum levels were associated with the severity of CAD as it can be assessed by the numbers of affected coronary arteries. Importantly, we found that OPG and OPN serum levels were associated with Gensini score, a more meaningful and precise score to estimate the degree of coronary atherosclerosis. We have also found that OPG and OPN levels are positively correlated with age. These findings suggest that OPG and OPN are involved in the pathogenesis of atherosclerosis and may reflect certain stages of cardiovascular disease.

4.2. Osteopontin, osteoprotegerin and arterial stiffness

Evidence suggests that vascular function significantly contribute to pathogenesis of atherosclerosis [32]. Pulse wave velocity which are considered to be a measure of central vascular stiffness are significantly and independently associated with target organ damage as

Table 3
Univariate and multivariate linear regression analysis of OPG (pmol/l).

Variables	Univariate analysis		Multivariate analysis	
	B (95% CI)	p	B (95% CI)	p
logGensini score	0.68 (0.36, 1.00)	<0.001	0.70 (0.19, 1.21)	0.007
Gender	−0.12 (−0.64, 0.40)	0.643	−1.27 (−1.96, −0.58)	<0.001
Hypertension	0.38 (−0.07, 0.84)	0.098	0.32 (−0.56, 0.63)	0.914
Diabetes mellitus	0.70 (0.17, 1.24)	0.010	0.26 (−0.39, 0.92)	0.432
Smoking	−0.03 (−0.54, 0.47)	0.900	−0.01 (−0.59, 0.56)	0.969
Hyperlipidemia	0.29 (−0.15, 0.74)	0.193	0.026 (−0.54, 0.59)	0.929
PWV (m/s)	0.17 (0.08, 0.27)	<0.001	0.20 (0.09, 0.32)	0.001

PWV: pulse wave velocity; OPG: osteoprotegerin; B: b regression coefficient; CI: confidence interval. For the categorical variables in the equation women, non-smokers and the absence of hypertension, diabetes mellitus and of hyperlipidemia were used as the reference category.

Table 4
Univariate and multivariate linear regression analysis of logOPN (ng/ml).

Variables	Univariate analysis		Multivariate analysis	
	B (95% CI)	p	B (95% CI)	p
logGensini score	0.06 (0.03, 0.10)	<0.001	0.03 (−0.02, 0.08)	0.22
Gender	0.01 (−0.04, 0.06)	0.698	0.0003 (−0.06, −0.067)	0.99
Hypertension	0.03 (−0.02, 0.07)	0.228	−0.009 (−0.069, 0.050)	0.76
Diabetes mellitus	0.02 (−0.03, 0.07)	0.436	−0.03 (−0.09, 0.03)	0.33
Smoking	0.02 (−0.03, 0.07)	0.456	0.005 (−0.05, 0.06)	0.84
Hyperlipidemia	0.02 (−0.02, 0.07)	0.284	0.003 (−0.05, 0.06)	0.89
PWV (m/s)	0.01 (−0.01, 0.02)	0.057	0.011 (−0.002, 0.0226)	0.056

PWV: pulse wave velocity; OPG: osteoprotegerin; B: b regression coefficient; CI: confidence interval. For the categorical variables in the equation women, non-smokers and the absence of hypertension, diabetes mellitus and of hyperlipidemia were used as the reference category.

well as cardiovascular morbidity and mortality [33]. Moreover PWV is the most commonly applied non-invasive technique to monitor arterial stiffness related to vascular calcification.

Previous studies have shown a significant association between OPG and arterial stiffness in diabetic patients and subjects with established cardiovascular disease [34,35]. Moreover in a case control study involving male patients with peripheral artery disease (PAD) as well as clinically healthy controls reported an independent association between OPG and PWV in patients with PAD and in controls. These findings suggested that the calcification inhibitor OPG may influence aortic stiffening in atherosclerosis and in clinically healthy subjects [35]. In addition, elevated plasma OPN levels are associated with increased arterial stiffness in rheumatoid arthritis patients, suggesting that this protein might represent a bridge protein between inflammation and the consequent joint damage and cardiovascular risk in RA patients [36].

In our study, we found that arterial stiffness, as determined by PWV, is positively associated not only with serum OPG and OPN levels, but also with the presence and in some degree with the severity of CAD, introducing the hypothesis for a novel mechanism linking arterial stiffness with atherosclerosis in CAD. Furthermore, after adjustment for the severity of the CAD, the positive association between serum OPG levels and PWV remained and there was also a trend between logOPN levels and PWV. These findings suggest that OPG and OPN significantly affect vascular function and this predispose to CAD. Nevertheless, the precise mechanisms for these vascular effects as well as establishment of a role of OPG and OPN in vascular pathophysiology deserve further investigation.

4.3. Limitation

The cross-sectional design of the study does not allow for causal interpretations between OPG, OPN, arterial stiffness and coronary atherosclerosis. Moreover, the association between OPG, OPN and CAD disease is not linear in subjects with 1, 2 or 3-vessel CAD. Nevertheless, OPG and OPN where associated with the severity of CAD as it was assessed by a more precise score. We have also to mention that although we take into account most of the known risk factors of atherosclerosis progression in the association of OPG and OPN with arterial stiffness and CAD, we cannot definitely exclude residual confounding which may possibly affect our models.

5. Conclusion

The present study revealed that serum OPG and OPN levels were positively associated with arterial stiffness, as well as with the extent of CAD. These preliminary findings suggest another possible mechanism linking OPG and OPN serum levels with CAD progression through arterial wall stiffening and atherosclerosis progression and may have important clinical implications in the treatment of patients with atherosclerosis. Nevertheless, further studies are needed to conclude how surrogate markers of vascular calcification are implicated in the evolution of arterial stiffness, in atherosclerosis progression and in overt CAD.

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