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Original Research Article

Osteoprotegerin and osteoprotegerin/TRAIL ratio are associated with cardiovascular dysfunction and mortality among patients with renal failure

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ABSTRACT

Purpose: The high prevalence of cardiovascular morbidity and mortality among patients with chronic kidney disease (CKD) is observed especially in those undergoing dialysis. Osteoprotegerin (OPG) and its ligands, receptor activator of nuclear factor kappa-B ligand (RANKL) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) have been associated with cardiovascular complications. Our aim was to study their role as cardiovascular risk factors in stage 5 CKD patients.

Patients and methods: OPG, RANKL and TRAIL concentrations were measured in 69 hemodialyzed CKD patients and 35 healthy volunteers. In CKD patients, cardiovascular dysfunction was assessed with aortic pulse wave velocity (AoPWV), carotid artery intima-media thickness (CCA-IMT), coronary artery calcium score (CACS) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) serum concentrations. Cardiovascular and overall mortality data were collected during a 7-years follow-up.

Results: OPG plasma concentrations were higher in CKD patients comparing to controls. Total soluble RANKL was lower and OPG/RANKL ratio higher in patients. Soluble TRAIL concentrations did not differ between the groups and OPG/TRAIL ratio was higher in CKD patients. OPG and OPG/TRAIL positively predicted long-term mortality (all-cause and cardiovascular) in CKD patients. OPG positively correlated with AoPWV, CCA-IMT and NT-proBNP whereas OPG/TRAIL with AoPWV and NT-proBNP. Described relationships were independent of classical and non-classical cardiovascular risk factors, with exception of age.

Conclusions: Our study confirmed the role of OPG as a biomarker of cardiovascular dysfunction and a predictor of mortality in stage 5 CKD. OPG/TRAIL ratio can be proposed as a predictor of cardiovascular dysfunction and mortality.

1. Introduction

Cardiovascular complications are the main cause of mortality among chronic kidney disease (CKD) patients treated with dialysis, with the rate about 10–20-times higher comparing to general age- and sex-matched population [1]. Accelerated atherosclerosis in these patients is accompanied by Mönckeberg calcification of vascular *media* [2]. In addition to classical cardiovascular risk factors, atherosclerosis and vascular calcification are accelerated by chronic inflammation, malnutrition (malnutrition-inflammation-atherosclerosis syndrome), bone and mineral metabolism disorders (both with low and high bone turnover), elevated serum homocystein and oxidative stress. These abnormalities are well recognized in stage 3–5 CKD patients [3,4].

Osteoprotegerin (OPG) is a secretory glycoprotein belonging to tumor necrosis factor receptor superfamily. It is involved in bone metabolism regulation as well as vascular calcification, inflammation and apoptosis (reviewed in [5,6]). In bones, OPG is secreted by

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osteoblasts and acts as a soluble decoy receptor for receptor activator of nuclear factor kappa-B (RANK) ligand (RANKL), leading to reduced osteoclastogenesis. OPG/RANKL/RANK triad is also involved in cellular and humoral immune responses, including T cell proliferation and B cell maturation [6]. Besides RANKL, OPG has been shown to bind specifically the tumor necrosis factor related apoptosis inducing ligand (TRAIL) and neutralize its pro-apoptotic functions [7]. Both OPG ligands are present in blood in a soluble form (sRANKL, sTRAIL).

Despite numerous studies, the role of OPG in atherosclerosis and vascular calcification is not clear. In rodents, OPG seems to be protective against vascular calcification and, less consistently, atherosclerosis [8,9]. In humans, however, high OPG concentrations are repeatedly associated with cardiovascular pathology. In general population, high serum OPG have been shown to predict the incidence and severity of cardiovascular disease [10–13] and related mortality [10,11]. In patients with CKD, OPG concentrations are higher comparing to general population [14], negatively correlate with GFR [15] and have been associated with adverse cardiovascular outcomes [16–18].

Also, several epidemiological studies connect OPG ligands with cardiovascular diseases. In general population, serum RANKL concentrations have been shown to positively predict the incidence of cardiovascular diseases (especially myocardial infarction and ischemic stroke) [19], but not atherosclerotic plaque burden [19,20]. However, in another population-based study [12], sRANKL concentrations have not been associated with new cardiovascular events. Recently, low sTRAIL has been shown to predict mortality in older patients with cardiovascular disease [21] and in CKD patients [22,23].

The aim of our study was to assess the relationships between plasma concentrations of OPG and serum concentrations of sRANKL and sTRAIL as well as the molar ratios of OPG to its soluble ligands, and the extend of cardiovascular dysfunction as well as long-term cardiovascular and all-cause mortality in hemodialyzed CKD patients. Cardiovascular dysfunction was assessed with the use of widely accepted, non-invasive tests, i.e. aortic pulse wave velocity (AoPWV), carotid artery intima-media thickness (CCA-IMT), coronary artery calcium score (CACS) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) serum concentrations.

2. Patients and methods

2.1. Patients and study protocol

Sixty-nine CKD patients (39 men, 30 women, aged 31–90 years, mean 60 ± 12 years) treated with maintenance hemodialysis at our Nephrology Department were recruited for the study between October and December 2004. The inclusion criteria were stable clinical course for at least 3 months. Patients with acute inflammatory states, neoplastic diseases, hepatitis or HIV infections were excluded. The patients were dialyzed three times a week for 4–5 h, with the use of reutilized polysulphone dialyzers, bicarbonate dialysate and low molecular weight heparin anticoagulation. The median period of dialysis treatment was 60 months (range 11–360 months). The causes of end-stage renal disease were: chronic glomerulonephritis (34 patients), pyelonephritis (16), polycystic kidney disease (10), diabetic nephropathy (3), unknown (6). Clinical characteristics of patients and most important comorbidities are listed in Table 1.

Thirty-five healthy volunteers (i.e. with no signs or symptoms of cardiovascular disease, kidney diseases, acute inflammation or malignancy), in age and sex comparable with CKD patients (Table 1), were recruited in order to obtain control values for key laboratory tests.

Table 1
Clinical and epidemiological characteristics of patients.

	CDK patients ($n = 69$)	Control group ($n = 35$)
Age, years	60 ± 12	57 ± 9[NS]
Men, *n* (%)	39 (57)	19 (54)[NS]
Dialysis therapy duration, months	60 (36–100), range 11–360	–
Observation period, months	56 (29–84), range 4–84	–
Median survival, months	56.6	–
All-cause mortality, *n* (%)	39 (57)	–
Cardiovascular mortality, *n* (%)	31 (45)	–
BMI, kg/m^2	23.6 (21.0–26.7)	23.3 (21.8–23.9)[NS]
Systolic blood pressure, mmHg	137 ± 15	126 ± 8*
Diastolic blood pressure, mmHg	81 ± 7	74 ± 6*
Mean arterial pressure, mmHg	100 ± 9	91 ± 6*
Pulse pressure, mmHg	56 ± 10	52 ± 7[NS]
Hypertension, *n* (%)	55 (80)	0
Diabetes, *n* (%)	8 (12)	0
Current smoking, *n* (%)	15 (22)	4 (11)[NS]
Ischemic heart disease, *n* (%)	35 (51)	0

[NS] No statistically significant difference between CKD patients and controls.
Abbreviations: BMI, body mass index; *n*, number.
* $P < 0.001$ in comparison between CKD patients and controls.

The study was a priori approved by the local Bioethical Committee (approval number KBET/127/B/2006). All the participants gave the written informed consent for the study.

At the start of the study, CKD patients were subjected to clinical examination, followed by venous blood collection for laboratory tests. The measurements of AoPWV, CACS and CCA-IMT were performed within two months from recruitment. AoPWV was measured between carotid and femoral arteries with the use of Complior device (Colson, France), after 10 min rest in supine position, and was arithmetic mean of 10 measurements. CACS was measured with multislice spiral computed tomography using Somatom Sensation 64 Cardiac equipped with calcium scoring software, as previously described [24]. CCA-IMT was measured by one examinator during diastolic phase of heart cycle with the use of an Aloka 5500 SV ultrasonograph with a 7.5 MHz head for vascular examination. Two-three measurements were taken at the level of the bulb, 1 cm from a bifurcation and at half length of right and left common carotid artery. The final result was an arithmetic mean of the measurements.

The mortality data of CKD patients were recorded over a 7-year (84-month) period. The causes of death were verified based on the disease histories of patients who died in hospital and medical records from general practitioners for outside-hospital deaths. Cardiovascular mortality was defined as death from acute myocardial infarction, left-ventricular failure, or cerebral stroke. A follow-up interviewer was not aware of laboratory or imaging tests' results.

2.2. Laboratory tests

Fasted blood samples were collected before middle-week dialysis session. The routine laboratory tests: complete blood count, serum albumin, glucose, calcium (Ca), inorganic phosphate (Pi), intact parathormone (iPTH), total cholesterol and cholesterol fractions, NT-proBNP and C-reactive protein (CRP) were performed on the day of collection. Biochemical tests were performed with Modular P analyzer (Roche Diagnostics), NT-proBNP was measured by electrochemiluminescence immunoassay with Modular P Analyzer (Roche, Germany) and CRP with BN II Nephelometer (Siemens, Germany). K_2-EDTA plasma samples (for OPG determination) and sera (for sRANKL and sTRAIL determination) from CKD patients and healthy volunteers were aliquoted and stored in

−70 °C until assayed. OPG was assessed with Human Osteoprotegerin kit (BioVendor, Czech Republic). The limit of detection for the test was 0.03 pmol/L. The reference values for OPG as determined by manufacturer were 4.1 ± 2.3 pmol/L. Soluble TRAIL was measured with Human TRAIL/TNFSF10 Quantikine ELISA (R&D Systems, Minneapolis, USA) with the limit of detection of 2.86 pg/mL (0.04 pmol/L). The reference interval established for sTRAIL was 28–135 pg/mL (i.e. 0.39–1.88 pmol/L; manufacturer's information). The results were reported in pmol/L assuming the molecular weight of sTRAIL (72 kDa for the trimer). Total sRANKL was measured with total sRANKL (human) ELISA Kit (Immundiagnostik, Germany). The lower detection limit for sRANKL was 1.56 pg/mL (0.026 pmol/L; manufacturer's information). The intra-assay and inter-assay coefficients of variation for OPG and sTRAIL were below 6% and for sRANKL below 9.5%.

2.3. Statistical analysis

Categorical data were reported as number (percent) and analyzed with Chi-squared test. Continuous data were reported as mean ± standard deviation (SD) or median (lower-upper quartile), according to distribution. Normality was assessed with Shapiro–Wilk test. Groups were compared using *t*-test or Mann–Whitney test, as appropriate. Simple and multiple linear regression models were computed after log10-transformation of right-skewed variables. Survival times were calculated with the start at the date of patient's initial assessment, and the end at death, renal transplantation or the end of 7-year follow-up. Survival curves were estimated with Kaplan–Meier method and compared with log-rank test. Simple and multiple Cox proportional hazard regression models were used to show associations of studied variables with mortality. Multiple linear and Cox regression models were adjusted for the factors known to influence outcomes in CKD, chosen a priori and listed in Section 3. All statistical tests were two-tailed and the results were considered significant at $P \leq 0.05$.

3. Results

3.1. Comparison of OPG, sRANKL and sTRAIL concentrations between CKD patients and healthy volunteers

The results of selected biochemical and imaging tests in the studied group of 69 CKD patients are presented in Table 2. OPG, sRANKL and sTRAIL concentrations as well as OPG/sRANKL and OPG/sTRAIL molar ratios observed in 35 healthy volunteers are also presented in Table 2. Median OPG concentrations in CDK patients were more than 2-times higher comparing to healthy controls ($P < 0.0001$); roughly half of the studied group had OPG above the upper value obtained in a control group (13.12 pmol/L). Median sRANKL was about 5-times lower in CKD patients ($P = 0.0002$) and OPG/sRANKL ratios were 7-times higher in CKD patients ($P < 0.0001$) than in controls. Soluble TRAIL concentrations were comparable in patients and controls ($P = 0.1$) and OPG/sTRAIL molar ratios were about 2-times higher in CKD patients ($P < 0.0001$).

3.2. Analysis of OPG, sTRIAL and sRANKL concentrations with respect to cardiovascular and all-cause mortality

During 7 years of observation, 39 CKD patients (56%) died, mostly due to cardiovascular causes (31 patients). Other causes of death included cancer (6 patients) and infections (2 patients). Four patients underwent successful renal transplantation during the study period. At the end of the study, 26 patients were still treated with hemodialysis. Median observation period was 56 (29–84) months (Table 1). Median overall survival was 56.6 (lower quartile 31.0) months.

Table 2
The results of selected biochemical and imaging tests.

	HD patients ($n = 69$)	Reference values
Glucose, mmol/L	4.89 ± 1.08	3.3–5.6
Total cholesterol, mmol/L	4.97 ± 1.17	3.2–5.2
LDL cholesterol, mmol/L	2.69 ± 0.87	0.2–3.4
HDL cholesterol, mmol/L	1.15 ± 0.34	0.9–3.0
Triglycerides, mmol/L	2.30 ± 1.07	0.2–2.3
Albumin, g/L	38.9 ± 3.4	35.0–50.0
Hemoglobin, g/dL	11.6 ± 1.6	F: 11–15; M: 12–17
iPTH, pg/mL	378 (132–1035)	12–72
Ca, mmol/L	2.30 ± 0.22	2.02–2.61
Pi, mmol/L	1.87 ± 0.53	0.87–1.45
Ca x Pi, mg^2/dL^2	52.7 ± 15.1	≤55
CRP, mg/L	5.71 (2.17–10.80)	0.16–3.30
OPG, pmol/L	13.33 (10.53–17.38)	5.72 (4.45–6.94)[C,*]
sRANKL, pmol/L	1.79 (1.14–2.96)	8.71 (4.14–14.82)[C,*]
OPG/sRANKL	7.03 (3.60–11.63)	0.61 (0.26–1.15)[C,*]
sTRAIL, pmol/L	1.58 ± 0.49	1.45 ± 0.29 [C,NS]
OPG/sTRAIL	7.40 (5.50–11.90)	3.96 (2.18–4.78)[C,*]
NT-proBNP, ng/L	6850 (3570–2620)	F: <222; M: <194
AoPWV, m/s	13.9 (11.7–16.6)	–
CACS, Agatston units	487 (109–1853)	0–100
CCA-IMT, mm	0.90 (0.80–1.05)	–

[C] Values obtained in a control group of 35 healthy volunteers.
[NS] No statistically significant difference between CKD patients and controls.
Abbreviations: AoPWV, aortic pulse wave velocity; Ca, serum calcium; CACS, coronary artery calcium score; Ca x Pi, calcium-phosphate product; CCA-IMT, common carotid artery intima-media thickness; CRP, C-reactive protein; HDL, high density lipoproteins; iPTH, intact parathormone; LDL, low density lipoproteins; NT-proBNP, N-terminal pro-B-type natriuretic peptide; OPG, osteoprotegerin; Pi, serum phosphate; sRANKL, soluble receptor activator of nuclear factor kappa B; sTRAIL, soluble tumor necrosis factor-related apoptosis-inducing ligand.
[*] $P < 0.001$ in comparison between CKD patients and controls.

The survival was significantly better in CKD patients with OPG concentrations below the median value of 13.33 pmol/L (i.e. values comparable to healthy controls) than in patients with higher OPG (Fig. 1). In a subgroup with OPG below the median, 14 (41%) patients died (including 9 from cardiovascular causes), as compared with 25 (71%) patients (22 from cardiovascular causes) among those with higher OPG concentrations ($P = 0.02$ for all-cause and $P = 0.005$ for cardiovascular mortality). Moreover, OPG

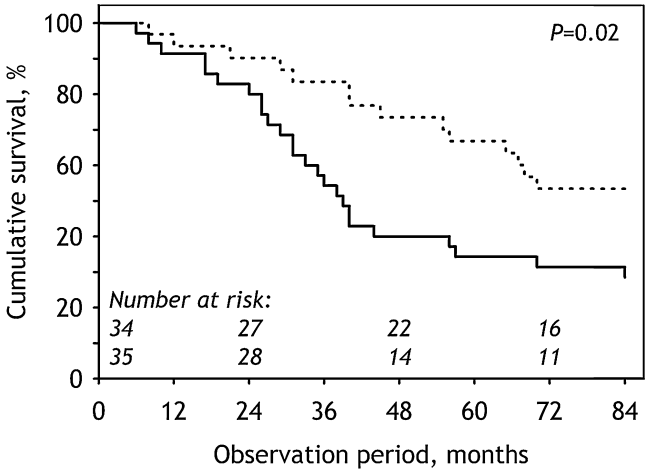

Fig. 1. Kaplan–Meier curves for all-cause mortality in patients with OPG concentration above or equal to median value of 13.33 pmol/L (solid line) versus patients with OPG below median (dashed line). Numbers at risk in both groups at the beginning of the study and after 24, 48 and 72 months and *P* value for the difference between the two groups in log-rank test are shown at the graph.

concentrations (continuous variable) were significantly correlated with both all-cause and cardiovascular mortality (Table 3). Neither sRANKL, OPG/sRANKL, nor sTRAIL predicted all-cause or cardiovascular mortality in the studied group, however, OPG/sTRAIL ratios were significantly positively associated with all-cause and cardiovascular mortality in simple Cox regression (Table 3).

Multiple regression analysis was performed to study whether the associations of OPG and OPG/sTRAIL with mortality depend on age, dialysis therapy duration and the variables representing classical and non-classical risk factors for cardiovascular morbidity and mortality in CKD. However, OPG concentrations and consequently, OPG/sTRAIL ratios were strongly correlated with age in the studied group ($r = 0.63$; $P < 0.0001$ and $r = 0.61$; $P < 0.0001$, respectively). Thus, in the first step, we constructed the multiple Cox models adjusted for dialysis therapy duration, sex, diabetes, hypertension, smoking, LDL-cholesterol, CRP, albumin, iPTH and Ca x Pi, and not including age, in order to avoid redundancy of predictors. OPG concentrations as well as OPG/sTRAIL ratios significantly predicted long-term all-cause and cardiovascular mortality independently of these variables (Table 3). In the second step, age was added to the models mentioned above. After addition of age, the associations of OPG and OPG/sTRAIL with mortality became insignificant.

3.3. Analysis of OPG, sTRIAL and sRANKL concentrations with respect to cardiovascular dysfunction

Serum OPG was positively associated with NT-proBNP concentrations as well as with AoPWV, CACS and CCA-IMT results in CKD patients, as shown with simple regression after log-transformation of the variables (Fig. 2, Table 4). The strongest correlation was noted between log(OPG) and log(NT-proBNP). Also, OPG/sTRAIL ratios were positively correlated with NT-proBNP, AoPWV, CACS and CCA-IMT after log-transformation of the variables (Table 4). Soluble RANKL was not correlated with NT-proBNP, AoPWV, CACS or CCA-IMT. OPG/sRANKL ratios were positively correlated only with NT-proBNP ($r = 0.32$, $P = 0.03$ after log-transformation of both variables). The concentrations of sTRAIL negatively correlated with log(CACS) ($r = -0.28$; $P = 0.04$) and log(NT-proBNP) ($r = -0.27$; $P = 0.047$) and did not correlate with AoPWV or CCA-IMT. Of note, CACS was itself significant positive predictor of all-cause ($P = 0.005$) and cardiovascular mortality ($P = 0.0007$), and AoPWV positively predicted all-cause mortality ($P = 0.02$) in studied patients.

Multiple regression analysis was performed in two steps, as described for multiple Cox models. The correlations of log(OPG) with log(NT-proBNP), log(AoPWV) and log(CCA-IMT) were confirmed in multiple models adjusted for log(dialysis duration), sex, diabetes, hypertension, smoking, LDL-cholesterol, log(CRP), albumin, log(iPTH) and Ca x Pi. Similarly, log(OPG/sTRAIL) significantly predicted log(NT-proBNP) and log(AoPWV) independently of the above mentioned confounders (Table 4). Other correlations significant in simple regression were not confirmed by multiple models. None correlations were confirmed by the models that were additionally adjusted for age.

4. Discussion

In the present study, higher OPG plasma concentrations as well as higher OPG/sTRAIL ratio predicted the long-term risk of death in CKD patients on hemodialysis. OPG positively correlated with variables related to dysfunction of cardiovascular system, namely, AoPWV, CACS, CCA-IMT and NT-proBNP. Additionally, OPG/sTRAIL ratio positively correlated with NT-proBNP concentrations and AoPWV. All these findings were independent of classical and non-classical cardiovascular risk factors, with exception of age of patients.

In CKD patients, Mönckeberg-type calcification of vascular *media* cause increased arterial stiffness and accelerated pulse wave velocity [17]. Increased arterial stiffness results in higher cardiac after-load, leading to left-ventricular heart failure. Several studies have shown correlations of increased OPG concentrations with the extend and progression of vascular calcifications (expressed as calcium score of coronary arteries or the aorta) as well as heart valve calcifications in CKD patients, including those treated with dialysis [18,25–27]. Also, in such population, OPG concentrations positively correlated with AoPWV [15,17,25] as well as with CCA-IMT [27,28]. These pathological findings are consistent with higher numbers of cardiovascular complications (coronary artery disease, acute myocardial infarction and unstable angina) in CKD patients, especially in stage 5 of the disease. Also, increased vascular stiffness, hypertension, together with prevalent anemia, may lead to left-ventricular hypertrophy and heart failure with increased NT-proBNP concentrations, although we have not found any previous studies showing OPG correlation with NT-proBNP in CKD patients. Consequently, higher serum OPG have been linked with (cardiovascular) mortality in CKD [17,26,29,30].

Our results are generally consistent with these findings, however, the associations between OPG and vascular dysfunction and mortality in our analysis were lost after adjustment for age. In our study, OPG concentrations (and, consequently, OPG/sTRAIL ratios) were strictly correlated with the age of patients despite severe kidney disease. Such correlation was also noted in other studies recruiting CKD patients treated with maintenance hemodialysis [16–18,31,32], although its strength varied between the studies (from $r = 0.34$ in [18] to $r = 0.60$ in [31] and $r = 0.68$ in [32]). The strong correlation of OPG with age in our study group resulted in significant redundancy in multiple models adjusted for age, leading to loss of significance of OPG and OPG/sTRAIL correlations with outcome variables. Consistently with our results, the authors who reported strong correlation between OPG and age were unable to confirm the associations between OPG and CACS [31] or

Table 3
Cox regression results for OPG or OPG/sTRAIL ratio as an independent variable. The multiple models ([a]) were adjusted for dialysis therapy duration, sex, diabetes, hypertension, smoking, LDL-cholesterol, CRP, albumin, iPTH and Ca x Pi.

Independent variable	All-cause mortality		Cardiovascular mortality	
	HR (95% CI)	*P*	HR (95% CI)	*P*
OPG, per 1 pmol/L				
Simple model	1.07 (1.03–1.13)	0.0007	1.09 (1.04–1.15)	0.0002
Multiple model[a]	1.08 (1.02–1.14)	0.011	1.07 (1.01–1.14)	0.025
OPG/sTRAIL, per 1 unit				
Simple model	1.07 (1.03–1.10)	0.0002	1.07 (1.04–1.11)	0.0001
Multiple model[a]	1.06 (1.02–1.11)	0.007	1.06 (1.01–1.11)	0.007

Abbreviations: Ca x Pi, calcium-phosphate product; CRP, C-reactive protein; iPTH, intact parathormone; LDL, low density lipoproteins; OPG, osteoprotegerin; sTRAIL, soluble tumor necrosis factor-related apoptosis-inducing ligand; CI, confidence interval; HR, hazard ratio.

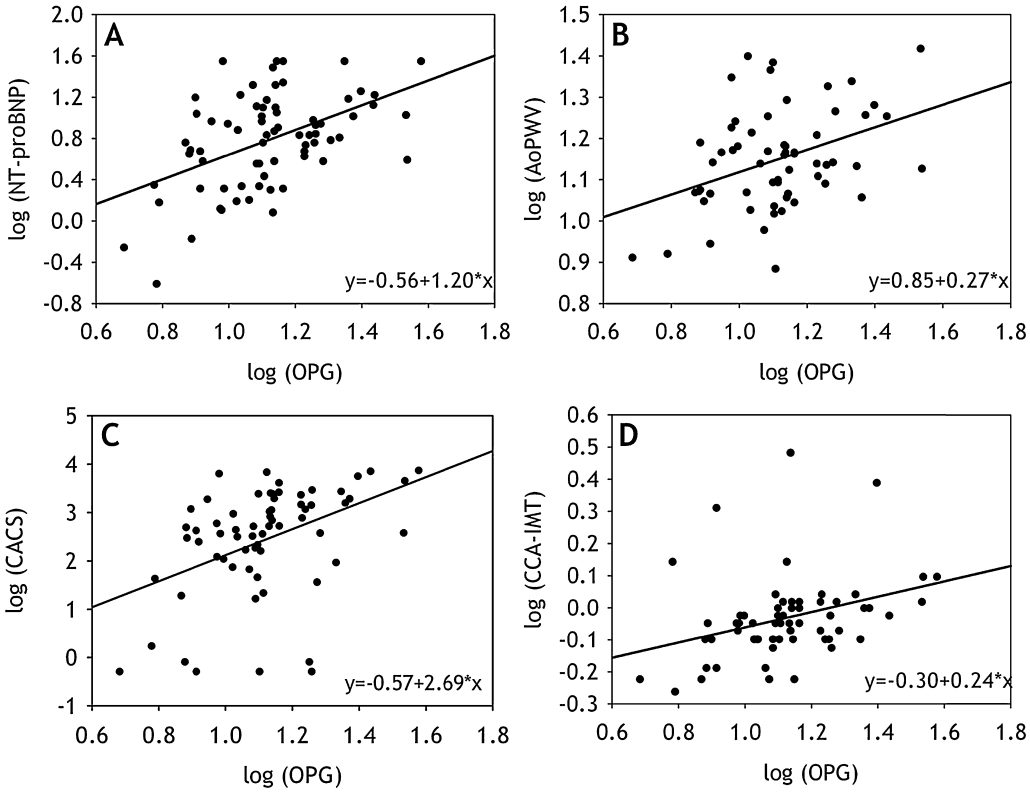

Fig. 2. The interrelations between OPG and NT-proBNP (A), AoPWV (B), CACS (C) and CCA-IMT (D) (data are log-transformed). Regression equations are presented at the bottom of the graphs. *Abbreviations*: AoPWV, aortic pulse wave velocity; CACS, coronary artery calcium score; CCA-IMT, common carotid artery intima-media thickness; NT-proBNP, N-terminal pro-B-type natriuretic peptide; OPG, osteoprotegerin.

Table 4
Linear regression results for log(OPG) or log(OPG/sTRAIL) as an independent variable. The multiple models ([a]) were adjusted for log(dialysis therapy duration), sex, diabetes, hypertension, smoking, LDL-cholesterol, log(CRP), albumin, log(iPTH) and Ca x Pi. Pearson correlation coefficients are shown for simple models and partial correlation coefficients for multiple models.

Independent variable	Dependent variable							
	log(NT-proBNP)		log(AoPWV)		log(CACS)		log(CCA-IMT)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
log(OPG)								
Simple model	0.49	<0.0001	0.39	0.002	0.44	0.0003	0.33	0.009
Multiple model[a]	0.45	0.002	0.45	0.002	0.18	0.2	0.30	0.047
log(OPG/sTRAIL)								
Simple model	0.44	0.0009	0.34	0.01	0.42	0.001	0.37	0.006
Multiple model[a]	0.42	0.006	0.38	0.01	0.17	0.2	0.19	0.2

Abbreviations: AoPWV, aortic pulse wave velocity; CACS, coronary artery calcium score; Ca x Pi, calcium-phosphate product; CCA-IMT, common carotid artery intima-media thickness; CRP, C-reactive protein; iPTH, intact parathormone; LDL, low density lipoproteins; NT-proBNP, N-terminal pro-B-type natriuretic peptide; OPG, osteoprotegerin; sTRAIL, soluble tumor necrosis factor-related apoptosis-inducing ligand.

CCA-IMT [32] in multiple analysis adjusted for age. Moreover, although the association between OPG and AoPWV was independent of age in the study of Pateinakis et al. [32], it was much weaker in multiple than in simple analysis ($P = 0.041$ versus $P < 0.001$).

In our study, the correlation of OPG with CACS was not confirmed by multiple analysis. Similar results were reported by Mesqiuta et al. [18]: OPG predicted CACS in stage 4 CKD patients while it did not in chronically hemodialyzed patients with stage 5 CKD. Also, a recent study on Polish CKD patients treated with maintenance hemodialysis showed similar results (CACS was independently predicted only by age and hemodialysis therapy duration) [31]. It may be hypothesized that the association between OPG and vascular calcifications may be masked or disrupted in end-stage renal failure due to pronounced abnormalities of mineral metabolism (including secondary parathyroidism) or the influence of chronic inflammation stimulated by hemodialysis.

To our knowledge, the correlation of OPG with NT-proBNP has not been previously reported in CKD patients, although it has been demonstrated in type 2 diabetic patients with microalbuminuria [33]. The association observed in our study may in part reflect renal retention of both compounds, which is well-known in case of NT-proBNP, and has also been shown for OPG [34]. NT-proBNP in hemodialyzed patients has been associated with inflammation, poor nutritional status, advanced age and comorbidity, as well as fluid overload; nevertheless, it remains correlated with cardiovascular mortality in such patients [35]. The association between OPG and NT-proBNP observed in our patients may reflect the association between atherosclerosis or vascular calcification and

left-ventricular hypertrophy and heart failure. However, further studies are needed to verify the hypothesis.

In contrast to OPG, the evidence linking sTRAIL and sRANKL concentrations with cardiovascular dysfunction in CKD are less numerous and, especially in case of sRANKL, less consistent. Some authors did not report a difference in RANKL concentrations between CKD patients and controls [36,37], some showed higher [14] while some lower concentrations in CKD stage 5 patients [26]. RANKL has been inversely associated with coronary calcium score in CKD patients treated with hemodialysis [26], however, in another study, low sRANKL in hemodialyzed patients has been linked with reduced risk for all-cause mortality [30]. The discrepancy between the results may be, to some extent, due to variability in laboratory methods, designed to measure either total or free sRANKL. Our patients had lower total sRANKL comparing to control subjects, however, we were unable to show significant correlations of sRANKL or OPG/sRANKL ratio with cardiovascular dysfunction or mortality.

Low concentrations of sTRAIL, a negative acute phase protein, have been shown to predict mortality in CKD patients, including those undergoing hemodialysis [22,23]. In our study, sTRAIL itself was not correlated with mortality, however, it was negatively correlated with NT-proBNP concentration. In comparison, OPG/sTRAIL ratio was positively correlated with cardiovascular dysfunction and predicted all-cause and cardiovascular mortality after adjustment for multiple cardiovascular risk factors. However, it was not a better predictor of cardiovascular dysfunction than OPG alone, although its association with mortality was statistically more significant.

The precise role of OPG/RANK/RANKL system and TRAIL in cardiovascular disease is not fully elucidated. High OPG concentrations are usually considered as incomplete compensatory mechanism against vascular calcification [5,26,29], where OPG is mainly released by vascular smooth muscle cells and endothelial cells [5,6,38]. OPG may inhibit osteogenic differentiation of vascular smooth muscle cells, induced by RANKL [39]. OPG may also reduce the number of foci for calcification, by blocking TRAIL-mediated apoptosis of vascular smooth muscle cells and endothelial cells [2,40]. However, in CKD patients osteoblasts seem to be an important source of OPG, reflecting the attempt to reduce excessive bone resorption [1]. In hemodialyzed men, serum OPG has been shown to correlate with the markers of bone turnover [15]. Also, in end-stage renal disease OPG may rise due to renal retention, given fast decrease after renal transplantation [5]. Some authors, however, point to possible adverse effects of OPG on vasculature, including up-regulation of endothelial cells' adhesion molecules, facilitating transmigration of inflammatory cells into *intima*, as well as up-regulation of matrix metalloproteinase release by vascular smooth muscle cells, leading to atherosclerotic plaque instability [5,6].

The limitations of our study include relatively low number of participants, thus our negative findings regarding lack of correlations of sRANKL, OPG/sRANKL ratio or sTRAIL with cardiovascular dysfunction or mortality may be questioned. However, recruitment of 69 patients enabled us to detect correlations with rho ≥ 0.33 at $P \leq 0.05$ with the statistical power of 80%.

5. Conclusions

Summarizing, our study confirms the usefulness of OPG and OPG/sTRAIL ratio as biomarkers of cardiovascular dysfunction and predictors of mortality in end-stage renal disease.

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Conflict of interests

The authors declare no conflict of interests.

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