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A cut-off value of plasma osteoprotegerin level may predict the presence of coronary artery calcifications in chronic kidney disease patients

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Abstract

Background. Expression of bone proteins resulting from transdifferentiation of vascular smooth muscle cells into osteoblasts suggests that vascular calcifications are a bioactive process. Osteoprotegerin (OPG) could play a key role in bone-vascular calcification imbalance and could be a marker of vascular calcification extent and progression. The purpose of this study was to evaluate relationships between vascular risk biomarkers (including classic risk factors and OPG) and coronary artery calcification (CAC) extent in chronic kidney disease (CKD) patients and to establish within the markers the appropriate cut-off value to predict CAC.

Methods. A total of 133 non-dialyzed CKD patients at various stages of kidney disease [75 males/58 females, median age: 69.9 (27.4–94.6)] were enrolled, excluding extrarenal replacement therapy patients. All underwent chest multidetector computed tomography for CAC scoring. Blood samples were collected for measurement of vascular risk markers (kidney disease, inflammation, nutrition, calcium phosphate and OPG). A potential relationship between CAC and these biological markers was investigated, and a

receiver-operating characteristic (ROC) curve was designed thereafter to identify a cut-off value of involved markers that best predicted the presence of CAC.

Results. After adjustment for age, diabetes, smoking and gender, among biological markers, only low-estimated glomerular filtration rate using Modification of Diet in Renal Disease [OR = 3.63 (1.10–12.02)], high $FEPO_4$ [OR = 3.99 (1.17–13.6)] and high OPG levels [OR = 8.54 (2.14–34.11)] were associated with the presence of CAC. A protective effect of $1.25(OH)_2$ vitamin D [OR = 0.20 (0.05–0.79)] and LDL cholesterol [OR = 0.27 (0.08–0.94)] on CAC was also observed. ROC curve analysis showed that the OPG best cut-off value predicting CAC was 757.7 pg/mL.

Conclusion. These results suggest that a CAC increase is strongly associated with a plasma OPG increase in CKD patients. The values of OPG >757.7 pg/mL allow us to predict the presence of CAC in these patients.

Keywords: chronic kidney disease; coronary artery calcification; osteoprotegerin

Introduction

Coronary artery calcifications (CAC) are fully recognized as a strong predictor of all-cause and cardiovascular mortality in haemodialysis (HD) patients [1]. Even though nearly all data about CAC relate to patients on dialysis therapy [2,3], CAC are already present in the early phase of chronic kidney disease (CKD) [4]. Their prevalence in patients with CKD is lower than that reported in chronic HD patients but greater than in controls. Russo *et al*. [5] showed that 40% of patients with CKD [with a mean glomerular filtration rate (GFR) of 33 mL/min] exhibited CAC compared with 13% in matched control subjects with no renal impairment.

The pathogenesis of vascular calcification in CKD is a complex and multifactorial process. Vascular calcification has long been considered as a passive phenomenon induced by the rise of calcium and phosphate concentrations in the serum leading to supersaturation and subsequent deposition in the form of hydroxyapatite. In CKD patients, several studies have found associations of both traditional risk factors such as older age, hypertension, hyperlipidaemia, diabetes [4], and uraemic-specific risk factors including abnormal mineral metabolism [6] with vascular calcification. Recently, similarities between vascular and skeletal calcification were evidenced suggesting a regulatory role for osteogenic and calcitropic factors in the development of cardiovascular disease [7]. The precise mechanisms driving vascular calcification and its clinical consequences are still unclear. However, new insights are emerging on a newly discovered group of bone-regulating molecules that belong to the TNF-related family including osteoprotegerin (OPG) [8]. Several epidemiological studies suggested that elevated serum levels of OPG were associated with the vascular risk in the general population [9–11]. In a recent work, we showed a prognostic value of OPG in HD patients [12]. Moreover, implication of OPG in the extent [13] and progression [14] of vascular calcifications was reported in this population [15].

CAC can be readily quantified non-invasively by radiographic imaging techniques such as multidetector computed tomography (MDCT). The purpose of this study was therefore (1) to evaluate the relationship between biomarkers of vascular risk (CKD, inflammation, malnutrition, calcium phosphate disorders and particularly a bone disease marker such as OPG) and the extent of CAC (measured by MDCT) in a population of CKD patients and (2) to establish within the markers involved the more appropriate cut-off value to predict the presence of CAC.

Methods

Patients

One hundred and thirty-three non-dialyzed CKD patients at various stages of kidney disease, issued from the outpatient general nephrology consultation of the Montpellier Lapeyronie University Hospital centre, entered this cross-sectional study. The inclusion criteria were age >18 years old and the presence of CKD defined by GFR in agreement with the National Kidney Foundation [16]. The study was conducted according to the principles of the Declaration of Helsinki and in compliance with International Conference on Harmonization/Good Clinical Practice regulations. According to the French Law, the study has been registered at 'Ministère de l'Enseignement Supérieur et de la Recherche' after approval by our institution ethical committee with the following number: DC-2008-417.

The CKD causes were as follows: glomerulonephritis ($n = 18$), cystic renal disease ($n = 9$), diabetic nephropathy ($n = 12$), diabetic and hypertensive nephropathy ($n = 10$), angiosclerosis and hypertensive nephropathy ($n = 60$), infectious/obstructive interstitial nephropathy ($n = 4$), renal neoplasia ($n = 1$), genetic/congenital cause ($n = 1$), necrotizing angiitis ($n = 1$), unknown cause ($n = 4$) and other cause ($n = 13$).

Detailed medical history including age, gender, weight, height, waist to hip ratio (defined as the waist girth/hip girth ratio), diabetes mellitus, hypertension, past or current smoking and the presence of atherosclerotic cardiovascular disease was recorded.

Existence of hypertension was defined by brachial blood pressure higher or equal to 140/90 mmHg and/or by a current antihypertensive treatment. A clinical examination was carried out.

The presence of atherosclerotic cardiovascular disease was defined by the presence of at least one of the three following manifestations: coronary heart disease, cerebrovascular disease or peripheral vascular disease. *Coronary heart disease* was defined as documented angina pectoris or a history of myocardial infarction. Angina pectoris was described as chest pain arising at exertion and disappearing with nitroglycerine or rest. The diagnosis may also have been made by a positive evaluation in nuclear medicine or a coronary stenosis >75% of luminal diameter evidenced by angiography. Myocardial infarction was defined as clinical symptoms, such as chest pain or dyspnoea, associated with a positive electrocardiogram and elevated cardiac markers (cardiac enzymes or troponins). *Cerebrovascular disease* was defined as a previous clinical cerebral disease (transient ischaemic attack or stroke) or the presence of atheromatous plaques on internal carotid arteries. *Peripheral vascular disease* included clinical symptoms such as intermittent claudication, abolished peripheral pulses or diminished arterial pulses or signs of atheromatous involvement of the lower limb.

Laboratory measurements

Blood samples were collected as part of our regular CKD patient follow-up, centrifuged and stored at −80°C for processing of the following parameters: serum creatinine, calcium, phosphate, alkaline phosphatase, $1.25(OH)_2$ vitamin D, intact PTH, total cholesterol (TC), LDL-cholesterol, HDL-cholesterol, serum albumin, transthyretin, hs-CRP, fibrinogen, insulin, glucose and OPG. All measurements were sequentially done with <1-year intervals after freezing.

Serum creatinine was measured using the enzymatic method (Olympus apparatus, Rungis, France) using reagents from Randox (Randox, Mauguio, France) and previously validated with the Roche enzymatic method

[17]. Glucose was measured using the enzymatic method (Olympus apparatus) using reagents from Olympus (Olympus). Calcium and phosphate were assessed using the colorimetric method (Olympus apparatus). Alkaline phosphatase was measured using the kinetic colour method based on the recommendations of the IFCC (Olympus apparatus). hs-CRP was determined by the immunoturbidimetry method (Olympus apparatus). Fibrinogen was measured by immunonephelometry using the von Clauss method (STA Fibrinogen, Diagnostica Stago, Asnières, France). Intact PTH and insulin were measured by an immunoradiometric assay (N-Tact PTH SP IRMA Kit, DiaSorin, MN, USA; Bi Ins IRMA, Cisbio International, Bagnols sur Cèze, France). $1.25(OH)_2$ vitamin D was measured by the radioimmunoassay (Immunodiagnostic Systems, Boldon, UK). Albumin and transthyretin were measured by the nephelometry technique (Immage Beckman Coulter, Villepinte, France). TC and HDL-cholesterol (HDL) levels were measured by the enzymatic method (Konelab DPC France, La Garenne Colombes, France). The LDL-cholesterol (LDL) rate was calculated using Friedwald's formula: [LDL] = [TC] − ([TG]/5) − [HDL]. OPG was determined by an enzyme-linked immunosorbent assay (Biovendor Laboratory Medicine, Brno, Czech Republic) [interassay CV = 6.5% (472.8 pg/mL) and 8.6% (1430.4 pg/mL); intraassay CV = 4.8% (477.0 pg/mL) and 3.7% (7074.0 pg/mL)] [18,19].

GFR was estimated using the reexpressed four-variable Modification of Diet in Renal Disease (MDRD) study equation (eGFR = 175 × standardized $S_{cr}^{-1.154}$ × $age^{-0.203}$ × 1.212 [if black] × 0.742 [if female]) [20].

Urinary phosphate excretion was expressed as Fractional excretion of PO_4 ($FEPO_4$) calculated as $FEPO_4$ (%) = urine[PO_4] × plasma[Creat] × 100/urine[Creat] × plasma[PO_4]. Same calculation was performed to determine urinary calcium excretion (FECa).

CAC imaging

All patients underwent MDCT for the purpose of the study.

Data acquisition. All MDCT scans derived from a multidetector-row spiral CT (Lightspeed VCT, General Electric Medical System, Milwaukee, WI, USA). Prospective ECG-triggered step-scan was performed using 2.5 mm collimation width ×64 detectors so that the centre of the temporal window corresponded to 70% of the R–R interval. The scanning parameters were a gantry rotation speed of 0.35 s per rotation, 120 kV and 300 mA 8 × 2.5 collimation and 20 mm table feed per rotation. The matrix size was 512 × 512 pixels, and the display field of view was 25 cm. The reconstruction kernel was standard. The temporal resolution was 250 ms.

Image evaluation. The calcium score was calculated using a semi-automatic software (SmartScore version 3.5, Advantage Window 4.4 workstation, General Electric, Milwaukee, WI, USA). Coronary calcification was defined as a plaque of ≥4 pixels (area = 1.37 mm^2) with a density of ≥130 Hounsfield units. Quantitative calcium scores were calculated according to the method described by Agatston *et al*. [21]. Coronary calcium scoring was performed by either a physician or computed tomography technician with specific training for the methodology described above.

Statistical analyses

Characteristics of the population were described by using proportions for categorical variables and the median and range for quantitative variables since their distributions were tested with the Shapiro–Wilk statistic and were skewed.

In order to evaluate the relationship between biomarkers of vascular risk and the extent of CAC, the patients were divided into two groups with respect to CAC, those with score <100 (considered as patients without CAC) and those with score ≥100 (presence of CAC) [22]. This calcium score has been chosen according to the latest guidelines from the American College of Cardiology and the American Society of Nuclear Cardiology endorsed by the American Heart Association (ACC/ASNC) [23,24].

Univariate logistic regression was used to determine differences in unadjusted clinical characteristics between patients with and without CAC. Associations between biological markers and CAC were tested using multivariate logistic regression. Odds ratios (OR) adjusted for age, gender, diabetes mellitus and smoking were obtained for each potential biological marker, using CAC (divided in two groups) as the dependent variable, and the biological marker (divided in tertiles, age, gender, diabetes mellitus and smoking) as the independent variable.

Thereafter, a receiver-operating characteristic (ROC) curve was designed to identify a cut-off value of OPG that best predicted the presence of CAC. The specificity and sensitivity were calculated (95% confidence interval, CI), as well as the positive predictive value (PPV) and the negative predictive value (NPV). The best possible cut point was defined as the highest Youden Index [(specificity + sensibility) − 1].

Significance was set at $P < 0.05$. All analyses were carried out with the SAS software, version 9.1 (SAS Institute, Cary, NC, USA) and the STATA software, version 9.2 (StataCorp, 2007).

Table 1. Characteristics of chronic kidney disease patients

Parameter	Value
No. of patients	133
Gender (male)	75 (56.4%)
Age (years)	69.9 (27.4–94.6)
BMI (kg/m^2)	26.3 (16.5–41.2)
Smoking habits	69 (51.9%)
Diabetes mellitus	33 (24.8%)
Hypertension	120 (90.2%)
Coronary heart disease	25 (18.8%)
Cerebrovascular disease	10 (7.5%)
Peripheral vascular disease	20 (15.0%)
eGFR (MDRD study equation) ($mL/min/1.73\ m^2$)	31.7 (6.5–91.9)
>60 $mL/min/1.73\ m^2$	15 (11.3%)
60–30 $mL/min/1.73\ m^2$	55 (41.4%)
<30 $mL/min/1.73\ m^2$	63 (47.4%)
Total cholesterol (mmol/L)	5.4 (3.1–9.2)
LDL-cholesterol (mmol/L)	3.0 (1.1–6.5)
HDL-cholesterol (mmol/L)	1.6 (0.7–3.4)
Hs CRP (mg/L)	2.2 (0.2–42.0)
Calcium (mmol/L)	2.4 (1.8–2.7)
Phosphate (mmol/L)	1.07 (0.58–2.34)
Calcium × Phosphate product ($mmol^2/L^2$)	2.49 (1.36–5.80)
PTH (pg/mL)	44.0 (6.0–493.0)
OPG (pg/mL)	896.2 (248.1–3383.5)
CAC	142 (0–2840)

Values were described by using proportions for categorical variables and median and range for quantitative variables.

Results

The clinical characteristics for the 133 CKD patients are summarized in Table 1.

The sex ratio of the patients was 75/58 (male/female), the median age was 69.9 with a range of (27.4–94.6) years old and the median BMI was 26.3 (16.5–41.2) kg/m^2.

Sixty-nine (51.9%) patients had ever smoked. Diabetes mellitus and hypertension were found in 33 (24.8%) and 120 (90.2%) patients, respectively. The history of coronary heart disease was present in 25 patients (18.8%), 10 (7.5%) patients had a history of cerebrovascular disease and 20 (15.0%) had a history of peripheral atherosclerotic disease.

Median eGFR using the MDRD study equation was 31.7 (6.5–91.9) $mL/min/1.73\ m^2$. The median TC content was 5.4 (3.1–9.2) mmol/L, the median LDL-cholesterol was 3.0 (1.1–6.5) mmol/L, the median HDL-cholesterol was 1.6 (0.7–3.4) mmol/L, the median hs-CRP level was 2.2 (0.2–42.0) mg/L, the median calcium × phosphate product was 2.49 (1.36–5.80) $mmol^2/L^2$, the median PTH was 44.0 (6.0–493.0) pg/mL and the median OPG level was 896.2

Table 2. Clinical predictors of increased coronary artery calcifications

	Coronary artery calcifications					
	Score <100 ($n = 61$)		Score ≥100 ($n = 72$)			
	n	%	*n*	%	OR (CI 95%)	*P*-value*
Gender						
Female	39	63.93	19	26.39	1	<0.0001
Male	22	36.07	53	73.61	**4.95 (2.36–10.37)**	
Median age (min–max), OR for 10 years increased	61.2 (27.4–85.1)		74.0 (45.7–94.6)		**2.76 (1.84–4.13)**	<0.0001
BMI						
<25	30	49.18	25	34.72	1	0.23
(25–30)	22	36.07	32	44.44	1.75 (0.82–3.73)	
≥30	9	14.75	15	20.83	2.00 (0.75–5.34)	
Waist to hip ratio						
<1	53	86.89	54	75.00	1	0.09
≥1	8	13.11	18	25.00	2.21 (0.88–5.51)	
Smoking						
No	40	65.57	24	33.33	1	0.0003
Yes	21	34.43	48	66.67	**3.81 (1.85–7.83)**	
Diabetes mellitus						
No	54	88.52	46	63.89	1	0.002
Yes	7	11.48	26	36.11	**4.36 (1.73–11.0)**	
Hypertension						
No	6	9.84	7	9.72	1	0.98
Yes	55	90.16	65	90.28	1.01 (0.32–3.19)	
Vitamin D analogues						
No	17	27.87	26	36.11	1	0.31
Yes	44	72.13	46	63.89	0.68 (0.33–1.43)	
Sevelamer HCl						
No	59	96.72	67	93.06	1	0.36
Yes	2	3.28	5	6.94	2.20 (0.41–11.8)	
Statins						
No	41	67.21	38	52.78	1	0.09
Yes	20	32.79	34	47.22	1.83 (0.90–3.72)	
Peripheral vascular disease						
No	59	96.72	54	75.00	1	0.003
Yes	2	3.28	18	25.00	**9.83 (2.18–44.37)**	
Coronary heart disease						
No	59	96.72	49	68.06	1	0.0006
Yes	2	3.28	23	31.94	**13.85 (3.11–61.7)**	
Cerebrovascular disease						
No	57	93.44	66	91.67	1	0.70
Yes	4	6.56	6	8.33	1.30 (0.35–4.82)	
Coronary heart disease or cerebrovascular disease or peripheral vascular disease						
No	55	90.16	35	48.61	1	<0.0001
Yes	6	9.84	37	51.39	**9.69 (3.71–25.3)**	

*P-value (for variables with more than two categories, the P-value of the test for trend is given).

(248.1–3383.5) pg/mL. The median CAC scoring was 142 (0–2840).

Characteristics of the patients with or without CAC

Among the 133 patients studied, CAC was present in 72 patients (54.1%) as defined by a score ≥100.

The presence of CAC was significantly associated with male gender [OR = 4.95 (2.36–10.37); $P < 0.0001$], age [OR = 2.76 (1.84–4.13) for 10 years increased; $P < 0.0001$], smoking habits [OR = 3.81 (1.85–7.83); $P = 0.0003$] and diabetes mellitus [OR = 4.36 (1.73–11.0); $P = 0.0018$]. Our results also showed that calcified patients (with score ≥100) more frequently had peripheral vascular disease [OR = 9.83 (2.18–44.37); $P = 0.0029$], coronary heart disease [OR = 13.85 (3.11–61.7); $P = 0.0006$] or at least one disease (coronary heart disease or cerebrovascular disease or peripheral vascular disease) [OR = 9.69 (3.71–25.3); $P < 0.0001$] (Table 2). No relationship between therapeutic interventions (vitamin D analogues, statins, sevelamer HCl) and CAC was evidenced.

Relationships between biological markers of vascular risk and the extent of CAC

Among biological markers of vascular risk, after adjustment for age, gender, diabetes and smoking habits, the main

associations with the presence of CAC were with low eGFR using the MDRD study equation [OR = 3.63 (1.10–12.02)], high $FEPO_4$ [OR = 3.99 (1.17–13.6)] and high OPG levels [OR = 8.54 (2.14–34.11)] (Table 3). Figure 1 displays the significant association between OPG values divided into tertiles and CAC ($P < 0.0001$). A protective effect of $1.25(OH)_2$ vitamin D [OR = 0.20 (0.05–0.79)] and LDL cholesterol [OR = 0.27 (0.08–0.94)] on CAC was also observed (Table 3). No significant difference was observed between the two groups regarding serum creatinine, calcium, FECa, phosphate, alkaline phosphatase, intact PTH, HDL-cholesterol, albumin, transthyretin, hs-CRP, fibrinogen, insulin and HOMA (Table 3).

In addition, after further adjustment for cardiovascular diseases, high OPG levels remained very strongly associated with CAC [OR = 10.12 (2.28–44.9); $P = 0.003$]. In patients without the history of cardiovascular diseases, elevated OPG concentrations were also associated with CAC even after adjustment for age, gender, diabetes and smoking habits [OR = 12.12 (2.06–71.3); $P = 0.01$].

ROC curve analysis for determination of the OPG cut-off value

ROC curve analysis (Figure 2) showed that plasma OPG level had a good prediction of CAC score, with an area under ROC curve of 0.79. The cut-off value best predicting CAC score, according to the maximum of the Youden Index, was 757.7 pg/mL (sensitivity = 91.7%; specificity = 59.0%). The diagnostic performance of OPG was further analysed using two cut-off values (a lower value to improve sensitivity and a higher value to improve specificity). Table 4 depicted different threshold values and the most relevant ones for CAC prediction were 709.9 and 963.7 pg/mL. The use of these two threshold values (CAC excluded <709.9 pg/mL and accepted >963.7 pg/mL) resulted in a 12.8% ($n = 17$) error rate and a grey area of diagnostic uncertainty of 32.3% ($n = 43$).

Discussion

In this study involving patients with CKD, we evaluated the relationship between established biomarkers of vascular risk (CKD, inflammation, nutrition, calcium phosphate disorders and particularly bone disease marker such as OPG) and the extent of CAC. We found that, among these factors, only eGFR, $FEPO_4$ and OPG were clearly associated with CAC among these 133 CKD patients. Thereafter, we established using a ROC curve a more appropriate cut-off value of OPG (equal to 757.7 pg/mL) to predict the presence of CAC with determination of a grey area (OPG levels between 709.9 and 963.7 pg/mL).

Only few studies have evaluated the extent of CAC in CKD patients in the predialysis state. The prevalence of CAC reported in the literature is in the range of 27–64% [5,25,26]. Our results, showing that 54.1% have vascular calcifications determined with a score >100, corroborated the high prevalence of CAC in this population, among which 9.7% of patients were at CKD stages 1+2, 38.9% at stage 3 and 51.4% at stages 4+5. It is noteworthy that coronary calcifications were directly determined here using MDCT in contrast to most of the studies that assessed vascular calcifications using a semi-quantitative plain radiological score based on peripheral arteries [27].

Expectedly, decline in GFR estimated with MDRD study equation is associated with an increase in vascular calcifications in agreement with previous reports [28]. This association may be due to kidney disease-induced metabolic abnormalities or comorbidities such as diabetes, hypertension, age, etc. Indeed, two recent studies [29,30] performed in a large population sample have shown that the relationship between estimated GFR evaluated by creatinine-based equation or cystatin determination is lost after adjustment for other cardiovascular risk factors. In order to further characterize the underlying mechanism of kidney disease-induced vascular calcifications, the relationship between renal or extra renal factors with CAC was evaluated. In agreement with previous reports, age [27,31], gender [32], smoking habits [33,34], diabetes [31] and cardiovascular diseases [1] were strongly associated with the CAC score.

Regarding biological markers of vascular risk and their association with the extent of CAC, neither inflammation, malnutrition nor calcium phosphate disorder markers (except for fractional excretion of PO_4) did predict the appearance of CAC. In contrast to our study, two other works in CKD patients revealed the involvement of mineral metabolism markers in the extent of vascular calcifications. The study from Tomiyama *et al*. reported that higher levels of phosphate were associated with the presence of severe CAC ($P = 0.013$) [26], while Sigrist *et al*. suggested the implication of Ca × P product (albeit with borderline significance) in the extent of vascular calcification of superficial femoral arteries [32]. However, our results are in total agreement with those from Russo *et al*. [5]. Very recently, Toussaint *et al*. also reported the same conclusions when examining such risk factors on calcifications of abdominal aorta and superficial femoral arteries [28]. Interestingly, among calcium phosphate disorder markers, only high $FEPO_4$ was associated with the presence of CAC, representative of a positive phosphate balance and subsequent unfavourable mineral metabolism environment in these patients. These results are consistent with several findings on the serum phosphate-regulating hormone, FGF-23. Indeed, Jean *et al*. very recently observed in a large group of HD patients that FGF-23 was the only mineral metabolism-related factor independently associated with vascular calcifications [35]. In the same manner, Gutierrez *et al*. reported increased FGF-23 levels to be independently associated with mortality among patients beginning HD treatment [36].

Finally, to our knowledge, this is the first study to demonstrate the relationship [OR = 8.54 (2.14–34.11)] between the extent of CAC and high OPG levels in a population of CKD patients before dialysis initiation [even after further adjustment for eGFR: OR = 5.59 (1.27–24.65) or cardiovascular diseases: OR = 10.12 (2.28–44.9)] with subsequent determination of a cut-off value that predicts the presence of CAC. The same association between OPG levels and the presence of CAC has been previously described in the general population [33], in uncomplicated type 2 diabetic subjects [34] and in HD patients [13]. Jean *et al*. in

Table 3. Biological predictors of increased coronary artery calcifications

	Coronary artery calcifications					
	Score <100 ($n = 61$)		Score ≥ 100 ($n = 72$)			
	n	%	*N*	%	OR (CI 95%)*	*P*-value*
Serum creatinine (μmol/L)						
<140	25	40.98	19	26.39	1	0.19
(140–204)	20	32.79	24	33.33	1.82 (0.62–5.33)	
≥ 204	16	26.23	29	40.28	2.85 (0.90–9.05)	
eGFR(MDRD study equation) (mL/min/1.73 m^2)						
<23.52	18	29.51	27	37.50	**3.63 (1.10–12.02)**	0.05
(23.52–41.31)	18	29.51	26	36.11	**3.16 (1.03–9.76)**	
≥41.31	25	40.98	19	26.39	1	
Calcium (mmol/L)						
<2.30	19	31.15	28	38.89	1	0.82
(2.30–2.41)	19	31.15	19	26.39	0.82 (0.27–2.49)	
≥2.41	23	37.70	25	34.72	1.20 (0.39–3.69)	
FECa (%)						
<0.80	21	35.00	21	32.81	1	0.46
(0.80–1.32)	23	38.33	17	26.56	0.88 (0.28–2.82)	
≥1.32	16	26.67	26	40.63	1.73 (0.57–5.27)	
Phosphate (mmol/L)						
<0.97	19	31.15	25	34.72	1	0.70
(0.97–1.15)	19	31.15	26	36.11	1.60 (0.52–4.92)	
≥1.15	23	37.70	21	29.17	1.41 (0.44–4.60)	
$FEPO_4$ (%)						
≤25.07	25	41.67	16	25.00	1	0.08
(25.07–38.42)	20	33.33	22	34.38	2.26 (0.74–6.93)	
>38.42	15	25.00	26	40.63	**3.99 (1.17–13.6)**	
Alkaline phosphatase (IU/L)						
<57	22	36.07	22	30.56	1	0.64
(57–80)	21	34.43	23	31.94	1.20 (0.40–3.63)	
≥80	18	29.51	27	37.50	1.69 (0.56–5.06)	
1.25 $(OH)_2$ vitamin D (pg/mL)						
≤22	20	32.79	25	34.72	1	0.06
(22–38)	20	32.79	23	31.94	**0.20 (0.05–0.79)**	
≥38	21	34.43	24	33.33	0.29 (0.08–1.06)	
Intact PTH (pg/mL)						
<32	21	34.43	22	30.56	1	0.52
(32–65)	24	39.34	21	29.17	0.64 (0.20–2.00)	
≥65	16	26.23	29	40.28	1.27 (0.42–3.81)	
HDL cholesterol (mmol/L)						
<1.41	15	24.59	30	41.67	2.77 (0.87–8.89)	0.17
(1.41–1.75)	23	37.70	19	26.39	1	
≥1.75	23	37.70	23	31.94	2.44 (0.79–7.60)	
LDL cholesterol (mmol/L)						
<2.68	24	39.34	20	27.78	**0.27 (0.08–0.94)**	0.12
(2.68–3.24)	13	21.31	31	43.06	1	
≥3.24	24	39.34	21	29.17	0.49 (0.15–1.61)	
Serum albumin (g/L)						
≤39.0	18	29.51	27	37.50	1	0.85
(39.0–42.7)	19	31.15	24	33.33	0.93 (0.30–2.87)	
≥42.7	24	39.34	21	29.17	0.73 (0.24–2.24)	
Transthyretin (g/L)						
<0.28	21	34.43	19	26.39	1	0.29
(0.28–0.36)	20	32.79	25	34.72	1.67 (0.51–5.44)	
≥0.36	20	32.79	28	38.89	2.57 (0.80–8.28)	
Hs-CRP (mg/L)						
<1.4	22	36.07	25	34.72	1	0.75
(1.4–3.6)	21	34.43	21	29.17	0.85 (0.28–2.60)	
≥3.6	18	29.51	26	36.11	0.65 (0.22–1.98)	
Fibrinogen (g/L)						
<3.8	23	37.70	28	38.89	1	0.71
(3.8–4.6)	22	36.07	22	30.56	1.44 (0.44–4.68)	
≥4.6	16	26.23	22	30.56	0.87 (0.28–2.73)	
Osteoprotegerin (pg/mL)						
<769.26	36	59.02	8	11.11	1	0.003
(769.26–1063.62)	16	26.23	29	40.28	**7.57 (2.06–27.85)**	
≥1063.62	9	14.75	35	48.61	**8.54 (2.14–34.11)**	

(Continued)

Table 3. (Continued)

	Coronary artery calcifications					
	Score <100 ($n = 61$)		Score ≥ 100 ($n = 72$)			
	n	%	*N*	%	OR (CI 95%)*	*P*-value*
Insulin (μU/mL)						
<4.3	25	41.67	15	23.44	1	0.15
(4.3–7.0)	22	36.67	20	31.25	1.45 (0.45–4.70)	
≥7.0	13	21.67	29	45.31	3.31 (0.95–11.5)	
HOMA						
≤0.99	24	40.00	18	28.13	1	0.82
(0.99–1.60)	21	35.00	19	29.69	1.09 (0.35–3.40)	
>1.60	15	25.00	27	42.19	1.45 (0.43–4.87)	

*Adjustment for age, diabetes mellitus, smoking and gender.
P-value (for variables with more than two categories, the *P*-value of the test for trend is given).

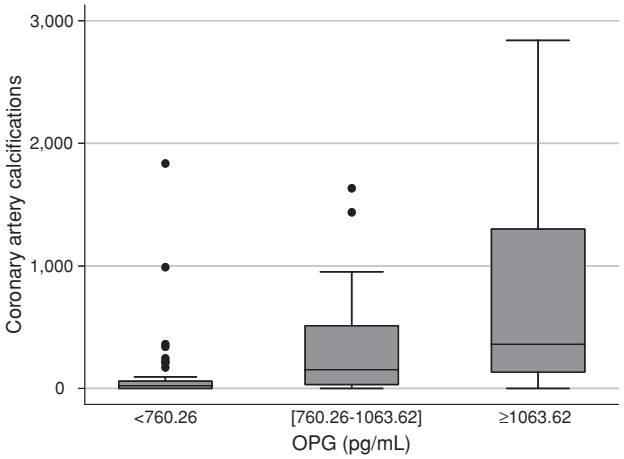

Fig. 1. Association between coronary artery calcification (CAC) score and plasma osteoprotegerin (OPG) levels. $P = 0.0003$*, 1st versus 2nd tertile of OPG. $P = 0.0003$*, 1st versus 3rd tertile of OPG. $P = 0.012$*, 2nd versus 3rd tertile of OPG. *Correction of Bonferroni done.

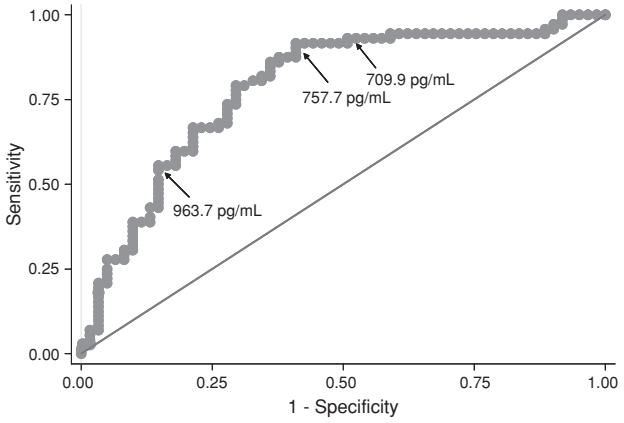

Fig. 2. Receiver-operating characteristic (ROC) curve of osteoprotegerin (OPG) plasma levels for the prediction of coronary artery calcifications (CAC) in 133 patients with chronic kidney disease.

their recent study showing FGF-23 as an independent factor associated with vascular calcifications also measured OPG. Interestingly, while OPG was not associated with severe vascular calcifications in logistic regression, it was demonstrated as an earlier marker of vascular calcification (being significantly increased in patients with score 1 versus score 0) compared to FGF-23 [35]. Our study also showed that, in patients without any history of cardiovascular diseases, OPG was strongly associated with CAC. These data suggest that the evaluation of OPG is of particular interest in these patients in predicting CAC and subsequently the occurrence of cardiovascular diseases.

The cut-off value of OPG to predict the presence of CAC (determined here for the first time using a ROC curve and equal to 757.7 pg/mL) is in total agreement with the levels of OPG reported in the literature. Indeed, in a previous work, our group found similar levels of OPG (equal to 737.1 pg/mL) above which non-uraemic diabetic patients had an enhancement of risk for silent myocardial ischaemia, this predictive value of OPG being observed both in males and females, in type 1 and 2 diabetic patients and regardless of the presence/absence of diabetic nephropathy determined by microalbumin level [37]. Similarly, in diabetic patients, Anand *et al*. depicted a relative risk of cardiac event equal to 5.76 ($P = 0.01$) for patients with OPG levels >856 pg/mL [34]. Interestingly, the cut-off value of OPG determined here in CKD patients up to stage 4 is lower (about half) than the predictive value of poor outcome previously observed in HD patients [12].

To date, the mechanism by which OPG levels might be related to CAC and further vascular events, is unknown. OPG, originally discovered as an inhibitor of osteoclastogenesis (by acting as a decoy receptor of the nuclear factor κ B ligand RANKL), has been proposed as a protective factor for vascular calcium deposition as reported in animal models [38,39]. Surprisingly, higher levels of OPG have been reported in patients with vascular damage [9–11], suggesting that an increase in OPG level may represent a compensatory self-defensive mechanism against factors that promote vascular calcification, atherosclerosis and other forms of vascular damage. Alternatively, OPG could be associated with endothelial dysfunction [40–42] and vascular stiffness [43–45] via its inflammatory role through binding to RANKL [40] or TNF-related apoptosis inducing ligand (TRAIL) [46].

Table 4. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of OPG to identify the presence of CAC

OPG (pg/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
709.86	**93.1**	**49.2**	**68.4**	**85.7**
757.70	**91.7**	**59.0**	**72.5**	**85.7**
805.58	86.1	63.9	73.8	79.6
856.80	76.4	70.5	75.3	71.7
898.92.0	68.1	73.8	75.4	66.2
950.00	62.5	78.7	77.6	64.0
963.72	**59.7**	**82.0**	**79.6**	**63.3**
998.82	54.2	85.2	81.3	61.2
1114.92	43.1	86.9	79.5	56.4

Our study acknowledged some limitations. Regarding biological parameters, (1) data on RANKL, previously reported as dramatically decreased in HD [12], are missing, but the measure of OPG may indirectly reflect the OPG/RANK/RANKL system [47]; (2) FGF-23 has not been evaluated here but could be represented by the measure of $FEPO_4$ (as an indirect indication of FGF-23 level); (3) Calcification inhibitor such as Fetuin-A, a negative acute phase protein, could be of interest, although no relationship between CAC and inflammation was observed here; (4) vitamin D status was only evaluated by the determination of $1.25(OH)_2$ vitamin D, and data on 25-OH vitamin D are missing. In our study, $1.25(OH)_2$ vitamin D has a tendency ($P = 0.06$) to protect against CAC. In agreement with other reports, 25-OH vitamin D that could appear as a better reflection of vitamin D status, has been recently proposed as a protective factor for arterial calcifications [48]. Finally, the relatively small sample size of this cross-sectional study may have prevented some of the detected associations from being statistically significant. The follow-up of this population could give in the future additive information on the prognostic value of OPG levels in CKD.

In conclusion, CKD patients are at increased risk for cardiovascular mortality; cardiovascular calcification is a risk factor for poor prognosis in this population. Therefore, management of biological markers of vascular risk is important in this population. Our present results demonstrate that among all risk factors examinated, OPG level is the strongest predictor of CAC in these patients that is in total agreement with its predictive value for mortality in HD patients. Thanks to the determination of an OPG cut-off value to predict the presence of CAC, OPG assessment should enable us to recognize patients at high risk of vascular calcifications, who should be managed aggressively.

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