BIOMARKERS ASSOCIATED WITH VASCULAR CALCIFICATION IN PERITONEAL DIALYSIS

Juan C. Ramirez-Sandoval,¹ Ivan Casanova,² Alejandro Villar,¹ F. Enrique Gomez,³ Cristino Cruz,¹
and Ricardo Correa-Rotter¹

*Departments of Nephrology and Mineral Metabolism,¹ Radiology,² and Physiology of Nutrition,³
National Institute of Medical Sciences and Nutrition Salvador Zubiran, Mexico City, Mexico*

◆ **Background:** Vascular calcification is strongly associated with cardiovascular disease and mortality. However, some factors related to vascular calcification in patients with end-stage renal disease receiving peritoneal dialysis (PD) remain unknown. This study aimed to evaluate the associations of osteoprotegerin (OPG), osteopontin (OPN), osteocalcin (OCN), fibroblast growth factor 23 (FGF-23), magnesium, and phosphate clearance with vascular calcification in PD subjects, assessed by plain radiographs.

◆ **Methods:** Simple vascular calcification scores (SVCS) obtained from plain X-rays of the pelvis and hands, and the Kauppila Index (KI) from lateral lumbar X-rays were assessed in 76 adults receiving PD for ≥ 6 months (43 women, median age 39 years, median time on PD 1.4 years). Levels of OPG, OPN, OCN, and FGF-23 were determined by luminometry.

◆ **Results:** Serum OPG levels were higher in subjects with vascular calcification ($n = 22$ with SVCS > 3 ; $n = 19$ with KI > 7) compared with those with less calcification ($p < 0.001$). Spearman's correlation coefficients between OPG and SVCS and KI were $r = 0.49$ and $r = 0.51$, respectively (both $p < 0.001$). Subjects with vascular calcification had significantly lower renal phosphate clearance. Multiple regression analysis showed that vascular calcification assessed by SVCS was associated with age ($r = 0.2$, $p = 0.042$), diabetes mellitus ($r = 2.4$, $p < 0.001$), body mass index (BMI) ($r = 0.09$, $p = 0.037$), and OPG ($r = 0.22$, $p = 0.001$). Vascular calcification assessed by KI was associated with age ($r = 0.16$, $p < 0.001$), time on PD ($r = 0.54$, $p = 0.001$) and OPG ($r = 0.08$, $p = 0.04$). Osteocalcin, OPN, FGF-23, and magnesium were not associated with vascular calcification.

◆ **Conclusions:** Higher levels of OPG were consistently associated with vascular calcification in subjects on PD.

non-traditional risk factors associated with cardiovascular mortality, and it is closely related to age, arterial stiffening, inflammation, protein-energy wasting syndrome, mineral metabolism disorders, time on dialysis, and mortality, among other factors (3).

Patients on PD may have different mineral metabolism disorders than patients on hemodialysis, including a higher frequency of adynamic bone disease (4) or a reduced risk of cardiac valve calcification over time, probably related to the higher prevalence of residual renal function (RRF) (5). Observational studies of small numbers of patients on PD have described a prevalence of vascular calcification in the range of 32 – 60% (6–8).

There is an urgent need to identify diagnostic and causal factors related to the process of vascular calcification, some of which may be amenable to medical intervention. Osteogenic markers that play an important role in mineralization of ectopic sites have recently been identified and linked to vascular calcification (9), including osteoprotegerin (OPG) (10), osteopontin (OPN) (11), osteocalcin (OCN) (12), and fibroblast growth factor 23 (FGF-23) (13). These complement previously-known factors such as hypomagnesemia (14) or diminished peritoneal and renal phosphate clearance (15), which have been poorly studied in PD patients in clinical settings. Phosphate clearance represents a modifiable determinant of serum phosphate control (16) which is strongly linked to arteriolar calcification and mortality.

The purpose of this study was to investigate the associations between vascular calcification, assessed by standardized radiologic indexes that employ plain radiographs, and levels of OPG, OPN, OCN, FGF-23, magnesium, and phosphate clearance, in patients on PD. We also examined the associations between these factors and cardiac valve calcification assessed by echocardiography.

PATIENTS AND METHODS

This cross-sectional study was conducted at a tertiary National Health Institution in Mexico City. A total of 104 consecutive patients receiving PD at an outpatient clinic, seen between September 2012 and October 2013, were initially enrolled. The inclusion criteria were: age > 18 years, treatment

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Cardiovascular disease is the major cause of death in patients with end-stage renal disease on peritoneal dialysis (PD) (1,2). Vascular calcification is one of the most important

Correspondence to: Juan C. Ramirez-Sandoval, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, Sección XVI, PC 14000, Mexico City, DF, Mexico.

carlos.ramirez@incmnsz.mx

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with PD for ≥ 6 months, and a stable clinical course for ≥ 3 months before inclusion in the study. Subjects with episodes of infection within the previous 3 months (including peritonitis, tunnel and peritoneal catheter exit-site infection, urinary tract infection, pneumonia, or others), pregnancy, or a history of previous hemodialysis or kidney transplant were excluded.

Thirteen subjects had received PD for < 6 months, 6 refused to participate, 5 had a history of previous hemodialysis and 4 had recent admissions for other medical issues. A total of 76 subjects were included in the analysis. Informed consent was obtained from all subjects and the study protocol was approved by our hospital Institutional Biomedical Research Review Board (approval number 598).

All PD patients were seen once at the clinic to deliver 24-hour effluent dialysate volume and 24-hour urinary volume (if there was RRF); on the same day, we performed a peritoneal equilibration test, recorded clinical data, took plain radiographs and collected blood samples for osteogenic biomarkers, calcium, blood cell count, 25(OH) vitamin D, and C-reactive protein (CRP). Other routine laboratory tests such as phosphorus, intact parathyroid hormone (iPTH, immunoradiometric assay, reference value 9–55 ng/L), alkaline phosphatase, and serum lipid levels were taken from clinical records. In this case, all patients had data from at least 3 laboratory tests within 3 months before or after assessment of vascular calcification.

VASCULAR CALCIFICATION

Vascular calcification was assessed using the simple vascular calcification score (SVCS) standardized by Adragao and obtained from plain X rays of the pelvis and hands (17), and by the semi-quantitative Kauppila Index (KI), obtained from abdominal aortic calcification observed in lateral lumbar X rays (18). The total SVCS ranged from 0–8; scores > 3 have been associated with increased cardiovascular risk (19). The KI ranged from 0–24; KI > 7 has been associated with increased coronary calcification, as evaluated by computed tomography (20). All x-ray images were evaluated by the same expert radiologist who was blinded to the patients' clinical and laboratory data. The intra-observer coefficient of variation was below 2%. The κ coefficients differentiating between KI ≤ 7 and > 7 , and SVCS ≤ 3 and > 3 were 0.92 and 0.98, respectively.

MINERAL METABOLISM PROTEINS

Serum samples were collected and stored at -80°C until assays were performed. The concentrations of selected bone and mineral metabolism proteins (OPG, OCN, OPN, intact FGF-23) were assessed using the Luminex/Magpix system (HBNMAG-51K-04, Austin, TX, USA). Minimum detectable concentrations for OPG, OCN, OPN, and FGF-23 were 1.9 pg/mL, 68.5 pg/mL, 37.7 pg/mL, and 9.2 pg/mL respectively; intra and inter-assay coefficients of variability were 5% and 11% for OPG, 5% and 12% for OCN, 2% and 12% for OPN, and 8% and 12% for FGF-23, respectively. Samples were analyzed individually and levels were estimated using a 5-parameter polynomial

curve with Xponent(r) software (Millipore, Billerica, MA, USA). All samples were analyzed simultaneously at the end of the recruitment period by an observer blinded to laboratory data.

PERITONEAL TRANSPORT AND PHOSPHATE CLEARANCE

Standard parameters of dialysis adequacy were determined by measuring total (renal and peritoneal) weekly urea clearance (Kt/V) and creatinine clearance using standard methods (21). Phosphate clearance was calculated according to Twardowsky (22): peritoneal phosphate clearance ($\text{L/week}/1.73 \text{ m}^2$) was calculated as (dialysate phosphate in $\text{mmol/L} \div \text{plasma phosphate in mmol/L} \times 24 \text{ h effluent dialysate volume (L)} \times 7$ (corrected for 1.73 m^2 body surface area)). Renal phosphate clearance ($\text{L/week}/1.73 \text{ m}^2$) was calculated as (urine phosphate in $\text{mmol/day} \div \text{plasma phosphate in mmol/L} \times 7$ (corrected for 1.73 m^2 body surface area)).

CARDIAC VALVE CALCIFICATION

All subjects underwent 2-dimensional echocardiography using a 3.3 MHz phased-array transducer (Philips Mod IE33, Philips Medical Systems, Service Hardware Rev D.0, Bothell, WA, USA). Heart valve calcification was defined as bright echoes of 1 mm on ≥ 1 cusp of the aortic valve or mitral valve or mitral annulus or both. The echocardiographic studies were interpreted by 2 cardiologists who were blinded to all clinical details.

STATISTICAL ANALYSIS

Continuous variables were assessed for normality using the Kolmogorov-Smirnov test. Results are presented as mean $\pm$ standard deviation for normally-distributed data, or median and interquartile range (IQR) for non-normally-distributed data. We divided the subjects based on the presence of calcification in X rays (KI ≤ 7 or > 7 , and SVCS ≤ 3 or > 3). Comparison of continuous variables between groups was performed using Student's *t*-test or non-parametric Mann-Whitney U test. Categorical variables were compared using χ^2 or Fisher's exact tests. Spearman coefficient or Pearson's correlation tests were employed to assess correlations between continuous variables. We calculated a total sample size of 62 patients on PD, considering a relevant simple correlation of 0.4 for each mineral metabolism protein, with a type I error of 0.05 and a type II error of 0.10 (23). Multivariate linear regression was used to identify variables that contributed significantly to the variability in vascular calcification as a continuous variable. To examine the ability of OPG to detect vascular calcification, we plotted a receiver operating characteristics (ROC) curve to detect SVCS > 3 or KI > 7 . Statistical analysis was performed with SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) and graphics were analyzed using Graphpad prism 5 (San Diego, CA, USA). The results were considered statistically significant when $p < 0.05$.

RESULTS

SUBJECT CHARACTERISTICS

A total of 76 subjects was included. Their median age was 39 years (IQR 30–59 years), 43 (57%) were female, 19 (25%) were diabetic, 50 (66%) had RRF, 28 (37%) were on continuous ambulatory PD and 48 (63%) on automated PD, and the median time on PD was 1.4 years (IQR 0.9–2.5 years). The main causes of end-stage renal disease were diabetes mellitus in 19 (25%) and lupus nephropathy in 13 (17%) subjects, while the etiology was unknown in 22 (29%) patients. The mean 25(OH) vitamin D level was 15 ± 6.6 ng/mL, and all subjects had levels < 30 ng/mL.

Vascular calcification was absent in 37 (47%) and 45 (59%) subjects according to the SVCS and KI, respectively. The clinical and laboratory characteristics of subjects with SVCS ≤ 3 and > 3 , and with KI ≤ 7 and > 7 are shown in Table 1. Subjects with higher calcification scores according to both indices were older, had a higher body mass index (BMI), a higher prevalence of diabetes mellitus and tobacco use, and lower levels of 25(OH) vitamin D. Subjects with KI > 7 had lower levels of albumin and cholesterol, whereas subjects with SVCS > 3 had lower total weekly Kt/V urea

associated with loss of RRF (kidney Kt/V 0.6 ± 0.3 vs 0.9 ± 0.2 , $p > 0.001$). There were no differences between the groups in terms of serum levels of calcium, phosphorus, iPTH, or CRP, or in use of calcium phosphate binders or calcitriol.

OSTEOGENIC MARKERS AND PHOSPHATE CLEARANCE IN RELATION TO VASCULAR CALCIFICATION

Osteoprotegerin serum levels were significantly higher in subjects with SVCS > 3 and those with KI > 7 compared with those with lower calcification scores (Table 2, Figure 1). Spearman's correlation coefficient revealed positive correlations between OPG levels and both calcification scores (SVCS, $r = 0.49$, $p < 0.001$; KI, $r = 0.51$, $p < 0.001$; Figure 1).

Low renal phosphate clearance was also associated with higher calcification scores in both groups, but total phosphate clearance was only lower in patients with KI > 7 . Although the association between peritoneal phosphate clearance and calcification was not significant, it displayed a trend to be lower only in patients with KI > 7 (Table 2). Compared with the relationship between OPG and calcification, renal phosphate clearance showed lower and negative correlations with both calcification scores (SVCS, $r = -0.35$, $p < 0.001$; KI, $r = -0.30$, $p = 0.006$).

TABLE 1
Relationships Between Subject Characteristics, SVCS and KI

Characteristic ^a	SVCS			KI		
	≤ 3 (n=54)	> 3 (n=22)	p	≤ 7 (n=57)	> 7 (n=19)	p
Age, years	41 $\pm$ 18	50 $\pm$ 16	0.037	34 (28–46)	68 (60–70)	< 0.001
Women	32 (59)	11 (50)	0.54	33 (59)	10 (53)	0.78
BMI, kg/m ²	23 (19–24)	27 (23–31)	0.042	22 (20–27)	27 (25–29)	0.001
Smokers	2 (4)	5 (23)	0.019	1 (2)	6 (32)	< 0.001
Diabetes	10 (19)	9 (41)	0.041	10 (18)	9 (47)	0.02
Time on dialysis, years	0.9 (0.6–1.6)	1.5 (0.7–2.0)	0.63	2.3 (1.4–3.8)	7.9 (1.4–11.6)	0.64
APD ^b	23 (43)	5 (23)	0.10	22 (39)	6 (32)	0.58
Anuric	15 (28)	11 (50)	0.018	20 (35)	6 (32)	0.78
Pulse pressure, mmHg	42 (35–55)	45 (39–55)	0.94	42 (35–50)	50 (40–66)	0.15
Hemoglobin, g/L	108 $\pm$ 18	106 $\pm$ 23	0.78	105 $\pm$ 20	111 $\pm$ 15	0.31
Albumin, g/L	36 $\pm$ 6	36 $\pm$ 7	0.79	37 $\pm$ 6	34 $\pm$ 5	0.03
Total cholesterol, mmol/L	4.7 $\pm$ 1.0	4.5 $\pm$ 1.0	0.42	4.8 $\pm$ 1.0	4.2 $\pm$ 0.8	0.02
Calcium, mmol/L	2.2 $\pm$ 0.3	2.2 $\pm$ 0.38	0.68	2.3 $\pm$ 0.3	2.2 $\pm$ 0.2	0.79
Phosphate, mmol/L	1.7 $\pm$ 0.6	1.8 $\pm$ 0.67	0.31	1.8 $\pm$ 0.6	1.6 $\pm$ 0.6	0.43
iPTH, ng/L	347 (138–722)	260 (164–478)	0.87	363 (144–814)	289 (166–418)	0.37
Magnesium, mmol/L	1.0 $\pm$ 0.2	1.0 $\pm$ 0.2	0.81	1.0 $\pm$ 0.2	1.0 $\pm$ 0.2	0.59
25(OH)vitD, nmol/L	40.4 $\pm$ 14.0	28.7 $\pm$ 16.7	0.007	39.9 $\pm$ 15.7	30.0 $\pm$ 14.5	0.047
CRP, nmol/L	13 (0–66)	28 (10–86)	0.18	10 (0–48)	37 (10–95)	0.69
Use of phosphate binders Ca	32 (59)	12 (55)	0.70	36 (63)	8 (42)	0.12
Use of calcitriol or paricalcitol	16 (31)	9 (41)	0.34	20 (35)	5 (26)	1.00
Kt/V urea+	2.1 $\pm$ 0.5	1.8 $\pm$ 0.4	0.008	2.0 $\pm$ 0.4	2.0 $\pm$ 0.4	0.81
Creatinine clearance	59 (42–86)	52 (34–60)	0.057	54 (42–70)	61 (46–86)	0.17
nPNA, g/kg	1.2 $\pm$ 0.26	1.0 $\pm$ 0.22	0.049	1.1 $\pm$ 0.2	1.1 $\pm$ 0.2	0.78

SVCS = simple vascular calcification score; KI = Kaupilla index; BMI = body mass index; APD = automated peritoneal dialysis; iPTH = intact parathyroid hormone; CRP = C-reactive protein; nPNA = total nitrogen appearance normalized to body weight.

^a Values are given as n (%) for categorical variables, mean $\pm$ standard deviation for normally-distributed data, and median (interquartile range) for non-normally-distributed data.

^b Automated peritoneal dialysis (APD) defined as ≥ 3 exchanges using a cyclor with at least 1 daytime dwell.

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TABLE 2
Relationships Between Osteogenic Biomarkers and Phosphate Clearance, SVCS and KI

	≤3 (n=54)	SVCS >3 (n=22)	p	≤7 (n=57)	KI >7 (n=19)	p
OPG, pg/mL	641 (477–998)	1,110 (709–1,569)	0.04	896 (604–1,341)	1,656 (1,118–1850)	<0.001
OPN, ng/mL	18.5 (5.3–36.4)	12.4 (5.2–28.8)	0.52	14.8(5.0–34.4)	25.9 (9.3–40.1)	0.14
OCN, ng/mL	53.6 (28.8–131.3)	54.8 (25.2–91.1)	0.67	55.2 (29.5–116.1)	42.3 (21.0–127.2)	0.58
FGF-23, ng/mL	1.1 (0.2–9.1)	3.2 (0.4–8.7)	0.22	1.3 (0.3–10.4)	1.7 (0.2–7.0)	0.37
Magnesium, mmol/L	0.9 (0.8–1.2)	1.0(0.9–1.2)	0.62	1.0 (0.8–1.2)	0.9 (0.8–1.2)	0.66
Total phosphate clearance, L/wk per 1.73m ²	44 (25–47)	39 (30–49)	0.07	47 (35–51)	40 (30–49)	0.041
Peritoneal phosphate clearance, L/wk per 1.73m ²	34 (25–39)	34 (29–43)	0.39	35 (24–42)	33 (26–39)	0.058
Renal phosphate clearance, L/wk per 1.73m ²	12 (0–27)	4 (0–14)	0.02	11 (2–35)	6 (0–11)	0.045

SVCS = simple vascular calcification score; KI = Kaupilla index; OPG = osteoprotegerin; OPN = osteopontin; OCN = osteocalcin; FGF-23 = fibroblast growth factor 23.

Values are given as median (interquartile range).

Figure 1 — Relationships between OPG levels and vascular calcification scores. SVCS = simple vascular calcification score; KI = Kaupilla index; OPG = osteoprotegerin.

Levels of 25(OH) vitamin D were also associated with vascular calcification. The Spearman's correlation coefficients between 25(OH) vitamin D and SVCS and KI were $r = -0.28$

($p = 0.089$) and $r = -0.21$ ($p = 0.049$), respectively. Osteoprotegerin levels were also correlated with 25(OH) vitamin D levels ($r = -0.19$, $p = 0.04$). When subjects were divided into tertiles based on OPG levels, 25(OH) vitamin D levels were 39.9 ± 7.5 , 34.9 ± 12.5 and 32.4 ± 10.0 nmol/L for tertiles 1, 2, and 3, respectively ($p = 0.034$).

There were no differences between the groups in terms of OCN, OPN, FGF-23, and Mg levels. Serum phosphorus and FGF-23 displayed a strong correlation (Pearson's correlation between log FGF-23 and serum phosphorus, $r = 0.6$, $p < 0.0001$), but neither was significantly associated with calcification scores.

Osteoprotegerin was the only osteogenic marker that was consistently associated with vascular calcification according to the multivariate analysis. Age, BMI, presence of diabetes mellitus, and OPG independently predicted SVCS in the best-fit multivariate regression model, adjusted for major cardiovascular risk factors, while age, duration on PD, and OPG were independent predictors of KI (Table 3). According to the multivariate analysis, each 1,000 pg/mL increase in OPG levels could explain almost 2 points in SVCS and almost 1 point in KI, independently of presence of diabetes and of other inflammatory markers such as CRP. There were no associations between vascular calcification and total phosphate clearance, renal phosphate clearance, or other osteogenic or inflammatory markers (CRP) according to the multivariate analysis.

OSTEOGENIC MARKERS AND PHOSPHATE CLEARANCE AND THEIR RELATION TO CARDIAC VALVE CALCIFICATION

Aortic valve calcification was detected in 10 of 76 subjects (13%), 2 of whom also had mitral valve calcification. The median OPG levels were 1,415 pg/mL (IQR 949 – 2,316) and 896 pg/mL (IQR 633 – 1,351) in subjects with and without cardiac valve calcification, respectively, detected by echocardiography ($p = 0.044$). There were no differences in terms of the other tested biomarkers.

TABLE 3
Independent Predictors of SVCS and KI Scores According to
Multiple Linear Regression Analysis

Parameter	Coefficient		
	B	95% CI	p
Linear regression with SVCS ^a			
Age	0.20	0.026–0.4	0.042
Gender masculine	0.48	–0.6–1.6	0.37
Diabetes mellitus	2.42	1.1–3.7	<0.001
BMI	0.09	0.1–0.2	0.037
OPG/100 ²	0.22	0.1–0.3	0.001
R ² =0.51, <i>p</i> < 0.001 for the model			
Linear regression with KI			
Age	0.16	0.09–0.23	<0.001
Male Gender	1.66	–0.05–3.37	0.06
Diabetes mellitus	–0.47	–2.6–1.7	0.66
Duration on PD (log months)	0.54	0.19–0.72	0.001
CRP	2.72	–0.51–5.9	0.097
OPG/100 ^b	0.08	0.01–0.11	0.048
R ² =0.78, <i>p</i> < 0.001 for the model			

SVCS = simple vascular calcification score; KI = Kaupilla index; CI = confidence interval; BMI = body mass index; OPG = osteoprotegerin; PD = peritoneal dialysis; CRP = C-reactive protein.

^a Coefficient B and 95% CI for variables with a statistically significant contribution to explain the magnitude of SVCS or abdominal aortic calcification measured by KI.

^b Osteoprotegerin levels were divided by 100 for convenient analysis.

OPG AND VASCULAR CALCIFICATION IN PLAIN RADIOGRAPHS

We analyzed the ability of OPG to detect vascular calcification (SVCS > 3 and/or KI > 7) using an ROC curve. The area under the ROC curve was 0.84 (95% confidence interval [CI] 0.75 – 0.94, *p* < 0.001). The sensitivities and specificities of different OPG values are presented in Table 4. Using 2 cut-off points (OPG < 750 pg/mL excluded the diagnosis of calcification and OPG > 1,350 pg/mL accepted it), 5 subjects were classified erroneously (error rate of 7%) and 24 subjects had an undetermined classification (a gray area of poor accuracy of 32%). The area under the ROC curve for OPG to detect SVCS > 3 was 0.70 (95% CI 0.56 – 0.90, *p* = 0.046) and to detect KI > 7 was 0.765 (95% CI 0.647 – 0.884, *p* = 0.002).

DISCUSSION

This study examined the associations between several non-traditional biomarkers and vascular calcification in subjects on PD. Of the tested markers, only OPG was consistently associated with the presence and severity of vascular calcification according to 2 different calcification scores, independently of other risk factors. In addition, OPG values were also elevated in patients with cardiac valve calcification.

TABLE 4
Sensitivities, Specificities, and Likelihood Ratios
for Different Values of OPG to Identify Vascular
Calcification, Defined as SVCS > 3 or KI > 7

OPG pg/mL	Sensitivity (%)	Specificity (%)	Likelihood ratio
656	97	39	1.5
750	93	51	2.0
993	83	66	2.4
1,350	62	90	6.4
1,592	55	98	7.5

SVCS = simple vascular calcification score; KI = Kaupilla index; OPG = osteoprotegerin.

Osteoprotegerin is a decoy receptor for the receptor activator of nuclear factor κ B ligand (RANKL) (24) and has been appointed as a mediator of inflammation, being closely associated with malnutrition, cardiovascular events, and mortality in patients on PD (25). Given that OPG is an inhibitor of calcification (26), it seems contradictory that OPG levels should be higher in patients with calcification (27); however, evidence suggests that OPG levels reflect the synthesis and secretion by vascular wall cells, mainly smooth muscle cells and endothelial cells (28), and it might thus amplify the adverse effects of inflammation in endothelial cell dysfunction (29). Several epidemiological studies have shown a positive association between OPG levels and vascular calcification (30), but few have included PD patients. Higher OPG levels have been independently associated with cardiovascular events in this population (31) and have been identified as a risk factor related to the *de novo* development of mitral valve calcification (32), as well as being positively correlated with the area of the aorta affected by vascular calcification, as assessed by computed tomography (33) and with elevated aortic pulse wave velocity (34). To the best of our knowledge, the current study is the first to demonstrate an association between vascular calcification defined by validated x-ray scores and OPG.

The proposed OPG value of > 1,350 pg/mL to detect a high degree of vascular calcification on X rays is in agreement with mean values of 1,308 pg/mL described in PD patients with an area of abdominal aortic calcification $\geq$ 15% (33) and the median value of 1,254 pg/mL (62.71 pmol/L; 1 pg/mL = 0.05 pmol/L) associated with cardiovascular mortality (31).

Vascular calcification is a complex process. Cross-sectional studies of patients on hemodialysis or with advanced chronic kidney disease have shown simple correlations ranging from *r* = 0.3 – 0.6 (35) between vascular calcification and age, loss of renal function, or the presence of diabetes. Other factors such as iPTH, calcium, and phosphorus have shown inconsistent correlations with vascular calcification in several studies (36).

Hyperphosphatemia is a strong pathogenic risk factor for vascular calcification in patients on dialysis (37). Phosphorus clearance has been proposed to be related to vascular calcification and is a variable that might be improved in PD patients

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in a clinical setting. In anuric patients, the adequacy of PD appeared to be the most important factor in phosphorus control (38). We found a slight inverse correlation between phosphorus clearance and vascular calcification, in particular renal phosphate clearance, though this association was not significant in the multivariate analysis.

Fibroblast growth factor 23 increases phosphate excretion and is a counter-regulatory hormone of $1,25(\text{OH})_2$ vitamin D synthesis (39). Fibroblast growth factor 23 is found in high concentrations in patients on dialysis and shows positive relationships with mortality in hemodialysis (40) or incident PD patients (41). Fibroblast growth factor 23 is associated with vascular calcification on X rays in hemodialysis patients (42), or in stage 3 and 4 kidney disease patients (43). Intriguingly, FGF-23 levels were positively correlated with renal phosphate clearance in the present study, as in other reports (44), but not with peritoneal clearance or vascular calcification index. We expected FGF-23 to be positively related to the degree of vascular calcification, though it is possible that this association was overshadowed by other factors.

A high prevalence of hypovitaminosis D (< 75 nmol/L) was observed in all subjects; this was unexpected in Mexico City, which has a latitude associated with sun exposure every day of the year. However, uremia, diabetes mellitus, inflammation, and ongoing effluent loss may contribute to the development of hypovitaminosis D (45,46). Some authors have suggested that supplementing the circulating $25(\text{OH})$ vitamin D pool can ameliorate vascular calcification through effects on PTH, cytokines, the inflammatory milieu, and the calcification processes (47), without the risk of vitamin D excess reportedly associated with the use of $1,25(\text{OH})_2$ vitamin D. The observed association between higher OPG levels and hypovitaminosis D allows us to speculate that supplementation of $25(\text{OH})$ vitamin D could reduce OPG levels and slow the calcification process.

Different methods can be used to identify vascular calcifications in PD patients involving different individual arterial regions. Different susceptibilities of these regions to calcification in chronic kidney disease have been described and the underlying mechanisms are only starting to emerge (48). In the multivariate analysis, we found that vascular calcification in peripheral sites was strongly associated with diabetes and BMI, whereas age and longer dialysis vintage were associated with calcification in central sites. We can speculate that arterial type, arterial size, shear stress, and paracrine mechanisms, and others, may be involved in these differences, and further studies focusing on the mechanisms of regional calcification are needed. Nevertheless, calcification in both regions is related to mortality, and OPG is useful in a similar way to diagnose the presence of vascular calcification in both peripheral and central arteries.

This study has limitations. Its cross-sectional design precluded the establishment of causality between OPG and vascular calcification. Furthermore, there was an absence of information regarding vascular calcification status prior to the initiation of PD therapy; however, all subjects had been on PD for at least 6 months, which would have theoretically

exposed them to the variables associated with PD to greater or lesser degrees, depending on their conditions. Another factor to consider is that the diagnosis of calcification was based on x-ray examination rather than coronary tomography. Nevertheless, the semi-quantitative assessment of vascular calcification using plain X rays has the advantages of being simple and cheap. It also has been validated in several studies, is applicable in daily clinical practice, and has a lower radiation risk than that associated with tomography, making it a useful tool recommended by international consensus.

In conclusion, higher OPG levels were consistently associated with vascular calcification and could thus provide a more useful biomarker of vascular calcification and cardiac valve calcification than other osteogenic proteins or phosphate clearance in subjects on PD.

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DISCLOSURES

The authors have no financial conflicts of interest to declare.

REFERENCES

1. Krediet RT, Balafa O. Cardiovascular risk in the peritoneal dialysis patient. *Nat Rev Nephrol* 2010; 6:451–60.
2. Wang AY. Vascular and other tissue calcification in peritoneal dialysis patients. *Perit Dial Int* 2009; 29(Suppl 2):S9–14.
3. Wang Y. Vascular and valvular calcification in chronic peritoneal dialysis patients. *Int J Nephrol* 2011; 2011:198045.
4. Levy R, Gal-Moscovici A. Renal osteodystrophy in peritoneal dialysis: special considerations. *Perit Dial Int* 2008; 28(Suppl 2):S12–9.
5. Rroji M, Seferi S, Cafka M, Petrela E, Likaj E, Barbullushi M, et al. Is residual renal function and better phosphate control in peritoneal dialysis an answer for the lower prevalence of valve calcification compared to hemodialysis patients? *Int Urol Nephrol* 2014; 46:175–82.
6. Stompór T, Pasowicz M, Sulłowicz W, Dembińska-Kieć A, Janda K, Wójcik K, et al. An association between coronary artery calcification score, lipid profile, and selected markers of chronic inflammation in ESRD patients treated with peritoneal dialysis. *Am J Kidney Dis* 2003; 41:203–11.
7. Ammirati AL, Dalboni MA, Cendoroglo M, Draibe SA, Santos RD, Miname M, et al. The progression and impact of vascular calcification in peritoneal dialysis patients. *Perit Dial Int* 2007; 27:340–6.
8. Lee H, Hwang YH, Jung JY, Na KY, Kim HS, Son MJ, et al. Comparison of vascular calcification scoring systems using plain radiographs to predict vascular stiffness in peritoneal dialysis patients. *Nephrology (Carlton)* 2011; 16:656–62.
9. Stompór T. An overview of the pathophysiology of vascular calcification in chronic kidney disease. *Perit Dial Int* 2007; 27(Suppl 2):S215–22.
10. Quercioli A, Montecucco F, Bertolotto M, Ottonello L, Pende A, Mach F, et al. Coronary artery calcification and cardiovascular risk: the role of RANKL/OPG signalling. *Eur J Clin Invest* 2010; 40:645–54.
11. Wang KX, Denhardt DT. Osteopontin: role in immune regulation and stress responses. *Cytokine Growth Factor Rev* 2008; 19:333–45.
12. Renneberg RJ, de Leeuw PW, Kessels AG, Schurgers LJ, Vermeer C, van Engelshoven JM, et al. Calcium scores and matrix Gla protein levels: association with vitamin K status. *Eur J Clin Invest* 2010; 40:344–9.

13. Larsson TE. FGF23 beyond mineral metabolism: a bridge to cardiovascular disease. *Clin J Am Soc Nephrol* 2011; 6:2735–7.
14. Wei M, Esbaei K, Bargman J, Oreopoulos DG. Relationship between serum magnesium, parathyroid hormone, and vascular calcification in patients on dialysis: a literature review. *Perit Dial Int* 2006; 26:366–73.
15. Yavuz A, Ersoy FF, Passadakis PS, Tam P, Evaggelos DM, Katopodis KP, *et al.* Phosphorus control in peritoneal dialysis patients. *Kidney Int Suppl* 2008; 108:S152–8.
16. Schmitt CP, Borzych D, Nau B, Wühl E, Zurowska A, Schaefer F. Dialytic phosphate removal: a modifiable measure of dialysis efficacy in automated peritoneal dialysis. *Perit Dial Int* 2009; 29(4):465–71.
17. Adragao T, Pires A, Lucas C, Birne R, Magalhaes L, Gonçalves M, *et al.* A simple vascular calcification score predicts cardiovascular risk in haemodialysis patients. *Nephrol Dial Transplant* 2004; 19:1480–8.
18. Kauppila LI, Polak JF, Cupples LA, Hannan MT, Kiel DP, Wilson PW. New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. *Atherosclerosis* 1997; 132:245–50.
19. Adragão T, Pires A, Birne R, Curto JD, Lucas C, Gonçalves M, *et al.* A plain X-ray vascular calcification score is associated with arterial stiffness and mortality in dialysis patients. *Nephrol Dial Transplant* 2009; 24:997–1002.
20. Bellasi A, Ferramosca E, Muntner P, Ratti C, Wildman RP, Block GA, *et al.* Correlation of simple imaging tests and coronary artery calcium measured by computed tomography in hemodialysis patients. *Kidney Int* 2006; 70:1623–8.
21. Nolph KD, Moore HL, Twardowski ZJ, Khanna R, Prowant B, Meyer M, *et al.* Cross-sectional assessment of weekly urea and creatinine clearances in patients on continuous ambulatory peritoneal dialysis. *ASAIO J* 1992; 38:M139–42.
22. Twardowski ZJ, Nolph KD, Khanna R, Gluck Z, Prowant BF, Ryan LP. Daily clearances with continuous ambulatory peritoneal dialysis and nightly peritoneal dialysis. *ASAIO Trans* 1986; 32:575–80.
23. Lachin JM. Introduction to sample size determination and power analysis for clinical trials. *Control Clin Trials* 1981; 2:93–113.
24. Nakamura M, Udagawa N, Matsuura S, Mogi M, Nakamura H, Horiuchi H, *et al.* Osteoprotegerin regulates bone formation through a coupling mechanism with bone resorption. *Endocrinology* 2003; 144:5441–9.
25. Huang JW, Lien YC, Yang CY, Liu KL, Wu CF, Yen CJ, *et al.* Osteoprotegerin, inflammation and dyslipidemia are associated with abdominal aortic calcification in non-diabetic patients on peritoneal dialysis. *Nutr Metab Cardiovasc Dis* 2014; 24:236–42.
26. Min H, Morony S, Sarosi I, Dunstan CR, Capparelli C, Scully S, *et al.* Osteoprotegerin reverses osteoporosis by inhibiting endosteal osteoclasts and prevents vascular calcification by blocking a process resembling osteoclastogenesis. *J Exp Med* 2000; 192(4):463–74.
27. Van Campenhout A, Golledge J. Osteoprotegerin, vascular calcification and atherosclerosis. *Atherosclerosis* 2009; 204:321–9.
28. Collin-Osdoby P. Regulation of vascular calcification by osteoclast regulatory factors RANKL and osteoprotegerin. *Circ Res* 2004; 95:1046–57.
29. Collin-Osdoby P, Rothe L, Anderson F, Nelson M, Maloney W, Osdoby P. Receptor activator of NF- κ B and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis. *J Biol Chem* 2001; 276:20659–72.
30. Montagnana M, Lippi G, Danese E, Guidi GC. The role of osteoprotegerin in cardiovascular disease. *Ann Med* 2013; 45:254–64.
31. Koo HM, Do HM, Kim EJ, Lee MJ, Shin DH, Kim SJ, *et al.* Elevated osteoprotegerin is associated with inflammation, malnutrition and new onset cardiovascular events in peritoneal dialysis patients. *Atherosclerosis* 2011; 219:925–30.
32. Avila-Díaz M, Mora-Villalpando C, Prado-Urbe Mdel C, Orihuela-Rodriguez O, Villegas-Antelo E, Gómez-Noriega AM, *et al.* De novo development of heart valve calcification in incident peritoneal dialysis patients. *Arch Med Res* 2013; 44:638–44.
33. Huang JW, Lien YC, Yang CY, Liu KL, Wu CF, Yen CJ, *et al.* Osteoprotegerin, inflammation and dyslipidemia are associated with abdominal aortic calcification in non-diabetic patients on peritoneal dialysis. *Nutr Metab Cardiovasc Dis* 2014; 24:236–42.
34. Krzanowski M, Janda K, Dumnicka P, Dubiel M, Stompór M, Kuśnierz-Cabala B, *et al.* Relationship between aortic pulse wave velocity, selected proinflammatory cytokines, and vascular calcification parameters in peritoneal dialysis patients. *J Hypertens* 2014; 32:142–8.
35. Smith ER, Ford ML, Tomlinson LA, Bodenham E, McMahon LP, Farese S, *et al.* Serum calcification propensity predicts all-cause mortality in predialysis CKD. *J Am Soc Nephrol* 2014; 25:339–48.
36. Inaba M, Okuno S, Imanishi Y, Yamada S, Shioi A, Yamakawa T, *et al.* Role of fibroblast growth factor-23 in peripheral vascular calcification in non-diabetic and diabetic hemodialysis patients. *Osteoporos Int* 2006; 17:1506–13.
37. Kendrick J, Chonchol M. The role of phosphorus in the development and progression of vascular calcification. *Am J Kidney Dis* 2011; 58:826–34.
38. Wang AY, Woo J, Sea MM, Law MC, Lui SF, Li PK. Hyperphosphatemia in Chinese peritoneal dialysis patients with and without residual kidney function: what are the implications? *Am J Kidney Dis* 2004; 43:712–20.
39. Kuro-o M. Klotho, phosphate and FGF-23 in ageing and disturbed mineral metabolism. *Nat Rev Nephrol* 2013; 9:650–60.
40. Gutiérrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, *et al.* Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med* 2008; 359:584–92.
41. Kim HJ, Park M, Park HC, Jeong JC, Kim DK, Joo KW, *et al.* Baseline FGF23 is associated with cardiovascular outcome in incident PD patients. *Perit Dial Int* 2014. In press.
42. Jean G, Bresson E, Terrat JC, Vanel T, Hurot JM, Lorriaux C, *et al.* Peripheral vascular calcification in long-haemodialysis patients: associated factors and survival consequences. *Nephrol Dial Transplant* 2009; 24:948–55.
43. Craver L, Dusso A, Martinez-Alonso M, Sarro F, Valdivielso JM, Fernández E. A low fractional excretion of phosphate/FGF23 ratio is associated with severe abdominal aortic calcification in stage 3 and 4 kidney disease patients. *BMC Nephrol* 2013; 14:221.
44. Dominguez JR, Shlipak MG, Whooley MA, Ix JH. Fractional excretion of phosphorus modifies the association between fibroblast growth factor-23 and outcomes. *J Am Soc Nephrol* 2013; 24:647–54.
45. Shah N, Bernardini J, Piraino B. Prevalence and correction of 25(OH) vitamin D deficiency in peritoneal dialysis patients. *Perit Dial Int* 2005; 25(4):362–6.
46. Ellam T. Confounded complexity: vitamin D, parathyroid hormone, and metabolic syndrome in peritoneal dialysis. *Perit Dial Int* 2014; 34(4):333–7.
47. Zitterman A, Schleithoff SS, Koerfer R. Vitamin D and vascular calcification. *Curr Opin Lipidol* 2007; 18:41–6.
48. Schlieper G. Vascular calcification in chronic kidney disease: not all arteries are created equal. *Kidney Int* 2014; 85(3):501–3.