Original Article

Serum Osteoprotegerin as a Screening Tool for Coronary Artery Calcification Score in Diabetic Pre-Dialysis Patients

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Although cardiovascular disease is a principal cause of death in patients with chronic kidney disease (CKD), it is often asymptomatic in diabetic patients. The coronary artery calcification score (CACS) measured by multidetector computed tomography (MDCT) is useful for screening ischemic heart disease in the general population. We investigated which clinical parameters predict high CACS in predialysis diabetic nephropathy (DN). Participants were 85 patients with DN. Nobody had any history of coronary angioplasty or coronary bypass surgery. We measured blood counts, blood chemistry, bone alkaline phosphatase, intact-PTH, interleukin-6, osteoprotegerin (OPG), hemoglobin A1c, 25-hydroxyvitamin D (25(OH)D) and fetuin-A. CACS and bone mineral density (BMD) were measured by a single 16-slice MDCT and DEXA, respectively. The median value of CACS equaled 256 Agatston units (range 0–4494 units). Stepwise increase in CACS with CKD stage progression was observed ($p<0.01$ for trend). Simple regression analyses showed that Log (CACS+1) was positively correlated with age, systolic blood pressure, phosphorus and OPG. In addition, it was negatively correlated with nutritional parameters, such as body mass index, albumin, total-cholesterol and 25(OH)D. Fetuin-A and BMD had no impact on CACS. Multiple regression analyses showed that low albumin and high OPG were associated with high CACS. The sensitivity of OPG for detecting CACS>200 was 80%, when the cut-off value was 1.2 ng/mL. In conclusion, CACS increased with CKD stage progression in predialysis DN patients. Serum OPG was positively associated with high CACS and can be a useful screening tool for severe coronary calcification, whereas no association between fetuin-A and CACS was found. (*Hypertens Res* 2008; 31: 1163–1170)

***Key Words*: coronary artery calcification score, diabetic nephropathy, osteoprotegerin, fetuin-A/α2 HS-glycoprotein**

Introduction

For predialysis chronic kidney disease (CKD) patients, the risk for cardiovascular death has been reported to be higher than the risk for end-stage renal disease requiring renal replacement therapy (*1*). However, coronary artery disease (CAD) has not yet been thoroughly studied in these subjects. Given the high prevalence of asymptomatic CAD in diabetic patients, it might be better to screen CAD by some other method than invasive and expensive coronary angiography.

Evaluation of coronary artery calcification score (CACS)

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by MDCT (multidetector spiral computed tomography) has been shown to be useful as a screening tool for ischemic heart disease in the general population (*2*). With respect to CKD, higher CACS by MDCT has been used to successfully identify a history of cardiovascular disease in hemodialysis patients (*3*), and Block *et al*. revealed that CACS measured just after hemodialysis initiation predicted a future hard outcome in the dialysis period (*4*). These data imply the clinical significance of measuring CACS in asymptomatic predialysis CKD patients.

Dyslipidemia and chronic inflammation, including the inflammation associated with rheumatoid arthritis, are known to accelerate vascular calcification. Other diseases such as osteoporosis (*5*) and diabetes mellitus (DM) (*6*) are also associated with vascular calcification. In addition to these conditions, CKD also contributes to vascular calcification, possibly by disturbing calcium and phosphate metabolism (*7*). In 2006, the KDIGO (Kidney Disease Improving Global Outcome) group advocated a new diagnostic concept called CKD-MBD (mineral and bone disorder), which combined extraosseous calcification, laboratory abnormalities, and bone disease (*8*). However, the association of vascular calcification and mineral metabolism was confirmed only in dialysis patients (*9*, *10*).

With respect to the molecules associated with vascular calcification, matrix Gla protein (MGP), osteoprotegerin (OPG), and fetuin-A are reported to act locally or systemically to inhibit calcification (*11*). However, regarding fetuin-A, consistent results have not been obtained in predialysis patients (*12–14*).

The aims of our study were to study the association between CACS and CKD stages in predialysis diabetic CKD patients and to determine the clinical parameters that are associated with high CACS for the use of screening.

Methods

Patient Recruitment

This was a cross-sectional observational study on CACS in a cohort of patients with diabetic nephropathy (DN). We enrolled 85 DN patients who were recruited from the outpatient services of Osaka University Hospital in Japan between April 2005 and April 2007. Individuals who had progressed to end-stage renal disease requiring dialysis or who had a history of myocardial infarction, coronary angioplasty or coronary bypass surgery were excluded. Those who were receiving, or had received glucocorticoid or active vitamin D were excluded from the study. Three patients receiving calcium carbonate were included in this study. The study protocol was approved by the ethical committee of Osaka University Hospital and all the subjects provided written informed consent. In this study, the diagnosis and classification of CKD stages were established according to the criteria from the Clinical Practice Guidelines for Chronic Kidney Disease from the National Kidney Foundation–Kidney Disease Outcomes Quality Initiative (*15*). The glomerular filtration rate (GFR) was estimated (=eGFR) using the reexpressed Modification of Diet in Renal Disease equation (MDRD) (*16*):

$$\text{eGFR} = 175 \times \text{Cr}^{-1.154} \times \text{Age}^{-0.203} \ (\times 0.742 \text{ if female}).$$

Data Collection

We measured physical parameters, biochemical parameters, bone markers, inflammation markers, bone mineral density (BMD) and CACS. Blood pressure (BP) was measured in duplicate using a size-appropriate cuff after the subject had been seated in a chair for about 5–10 min. The blood sample was taken on the day of the consultation visit and analyzed for the followings: blood counts, blood urea nitrogen (BUN), serum creatinine (Cr) measured by the enzymatic method, albumin (Alb), calcium (Ca), phosphorus (P), intact parathyroid hormone (i-PTH), bone alkaline phosphatase (BAP), 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), 25-hydroxyvitamin D (25(OH)D), total cholesterol (T-chol), triglyceride (TG), glycosylated hemoglobin (HbA1c), C-reactive protein (CRP) and interleukin-6 (IL-6). Intact PTH and BAP were assayed using an Allegro two-site intact PTH immunoradiometric assay (IRMA) kit (Nichols Institute Diagnostics, San Juan Capistrano, USA), and an Osteolinks-BAP high-sensitivity diagnostic enzyme immunoassay (EIA) kit (Sumitomo Pharmaceuticals Co., Osaka, Japan), respectively. $1,25(OH)_2D_3$ and 25(OH)D were measured using a 1,25-hydroxyvitamin D RIA kit (TFB; Immunodiagnostic Systems Ltd., Boldon, UK) and a ^{125}I RIA kit (DiaSorin Inc., Stillwater, USA), respectively. Serum OPG and fetuin-A were measured using a Human Osteoprotegerin enzyme-linked immunoadsorbent assay (ELISA) kit (Biovendor Laboratory Medicine Inc., Brno, Czech Republic) and a Human Fetuin-A ELISA kit (Epitope Diagnostics Inc., San Diego, USA), respectively. The serum Ca level was corrected for Alb by the Payne formula (Ca; serum corrected Ca = Ca + (4 − Alb), if Alb <4.0). BMD was measured by Dual Energy X-ray Absorptiometry (DEXA) (Discovery A system; Hologic Inc., Bedford, USA) at the lumbar spine (L2–4), femoral neck and distal end of radius.

Multidetector Computed Tomography

CACS was evaluated using multidetector computed tomography (MDCT) (16-slice technique on the model Light Speed Ultra 16; GE Yokogawa Medical Systems, Tokyo, Japan). The timing of image acquisition was coordinated with the diastolic phase of the cardiac cycle at 70% or thereabout (total three phases) of the R wave to R wave interval (RR interval) as determined through electrocardiographic monitoring with a 2.5 mm gap between slices. Scanning time was about 30 s for the entire zone of interest that encompassed the whole

Table 1. Baseline Distribution of Demographics, Clinical Characteristics and Laboratory Parameters among CKD Stages

	CKD stage				*p* value
	1+2	3	4	5	
n (female %)	18 (16.7%)	26 (26.9%)	25 (32.0%)	16 (37.5%)	n.s.
Age (years)	63 (34–75)	68 (43–84)	68 (35–80)	60 (35–83)	n.s.
DM duration (years)	12 (5–40)	19 (5–37)	20 (5–44)	13 (6–37)	n.s.
Body mass index	25.2±3.51	24.3±3.67	24.5±4.16	23.6±3.11	n.s.
Systolic BP (mmHg)	132.9±13.3	137.9±19.1	147.7±18.2	157.3±18.3	<0.005
Diastolic BP (mmHg)	80.9±9.3	74.4±11.1	77.6±11.4	82.6±13.3	n.s.
Pulse pressure (mmHg)	52.0±12.0	63.5±14.8	70.1±15.7	74.6±10.7	<0.001
Lumbar spine (*T* score)	0.09±1.43	−0.26±1.23	−0.31±1.36	−0.41±2.28	n.s.
Femoral neck (*T* score)	−1.29±0.86	−1.39±0.85	−1.33±0.82	−1.73±1.70	n.s.
Distal end of radius (*T* score)	−1.00±1.10	−1.43±1.54	−0.48±1.21	−1.60±2.18	n.s.
Hemoglobin (g/dL)	14.9±1.45	12.2±1.86	10.8±1.49	10.2±1.47	<0.01
Creatinine (mg/dL)	0.88±0.19	1.67±0.29	2.76±0.54	6.51±2.40	<0.001
eGFR (mL/min/1.73 m^2)	83.4 (63.1–144.4)	37.6 (30.3–58.6)	21.8 (15.4–29.7)	8.07 (4.67–14.9)	<0.001
BUN (mg/dL)	14.8±3.76	27.0±8.30	45.0±13.99	61.1±12.50	<0.01
Albumin (g/dL)	4.15±0.25	3.93±0.50	3.66±0.53	3.31±0.37	<0.01
Calcium (mg/dL)	9.44±0.33	9.39±0.60	9.04±0.59	8.43±0.94	<0.01
Corrected Ca (mg/dL)	9.50±0.31	9.61±0.40	9.40±0.45	9.12±0.81	<0.03
Phosphorus (mg/dL)	3.15±0.60	3.40±0.58	3.98±0.62	5.05±1.04	<0.01
T-chol (mg/dL)	200.4±28.0	191.7±29.9	179.6±30.7	186.1±43.8	n.s.
Triglyceride (mg/dL)	183 (63–290)	118 (41–297)	147 (76–243)	116 (44–214)	n.s.
HbA1c (%)	6.69±1.04	6.70±1.11	6.66±0.94	6.03±0.92	n.s.
BAP (U/L)	28.0 (17.5–48.7)	27.0 (10.3–50.6)	23.5 (13.4–56.6)	24.5 (12.7–68.6)	n.s.
i-PTH (pg/mL)	48.3 (26.9–216.4)	66.0 (16.8–141.7)	104.2 (47.3–277.4)	216.3 (73.6–470.2)	<0.001
$1,25(OH)_2D_3$ (pg/mL)	50.2±19.9	39.7±14.7	23.0±11.7	13.4±14.8	<0.001
25(OH)D (ng/mL)	23.2±4.82	22.2±5.59	21.6±5.62	16.4±3.78	<0.01
CRP (mg/dL)	0.1 (0.1–0.5)	0.1 (0.1–0.6)	0.1 (0.1–1.0)	0.1 (0.1–4.3)	n.s.
Interleukin-6 (pg/mL)	10.8 (0.5–68.2)	3.9 (0.7–45.9)	4.2 (1.2–26.4)	6.7 (2.2–68.0)	n.s.
Fetuin-A (g/L)	0.755 (0.213–3.852)	0.950 (0.536–3.036)	0.696 (0.434–2.325)	0.822 (0.175–2.200)	n.s.
Osteoprotegerin (ng/mL)	0.965±0.433	1.259±0.479	1.500±0.560	1.846±0.594	<0.001

Bone mineral densities of lumbar spine, femoral neck and distal end of radius are shown as *T* score. Corrected Ca = calcium + (4 − albumin), if albumin <4.0. CKD, chronic kidney disease; DM, diabetes mellitus; BP, blood pressure; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; T-chol, total cholesterol; HbA1c, glycosylated hemoglobin; BAP, bone alkaline phosphatase; i-PTH, intact parathyroid hormone; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; CRP, C-reactive protein.

heart. This equipment was capable of detecting lesions of a density of at least 130 Hounsfield units (HU) and a minimum area of 0.4882 mm^2. Total CACS was calculated using Smartscore software (GE Yokogawa Medical Systems) in modified Agatston units (*17*). Scanning was performed at 120 kV and 300 mAs. The average radiation dose was 11.85 mGy as CTDIvol (volume CT dose index), 142.15 mGy cm as DLP (dose product length) and 97.4% as effective dose.

Statistical Analyses

First, subject characteristics were compared across CKD stages using χ^2 tests for categorical variables, analysis of variance (ANOVA) and Dunnett's test for normally distributed continuous variables, and Kruskal-Wallis tests for non-normally distributed variables. Data are expressed as the mean±SD or median [range]. Normality was assessed for all variables, with CACS, eGFR, CRP, IL-6, fetuin-A, i-PTH and BAP requiring logarithmic transformation. In order to log-transform the CACS, the conventional method of Log (CACS+1) was used.

Second, simple linear regression analysis was performed with CACS as the dependent variable and the others as independent variables.

Finally, we performed multiple linear regression analysis with forward selection, enrolling all variables with a *p*-value of <0.05 in univariate analysis, with or without major risk factors such as age, gender, BP and eGFR that were selected a priori. Multicollinearity diagnostics were performed to ensure the adequacy of the model produced. Values of $p<0.05$ were considered to indicate statistical significance. JMP version 5.1.2J for Windows (SAS Institute Inc., Cary,

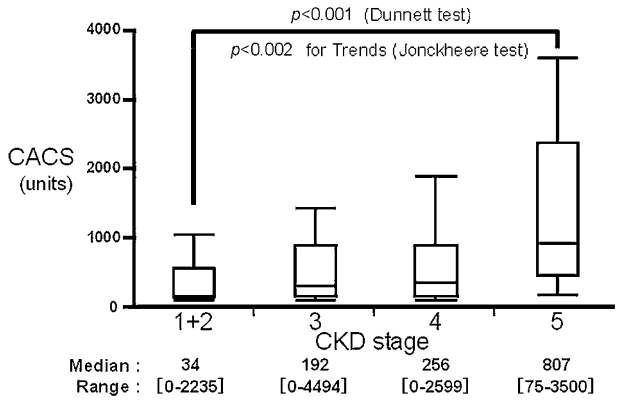

Fig. 1. *Stepwise increase in CACS with CKD progression. As CKD stage progressed, the CACS (coronary artery calcification score) tended to increase ($p<0.002$, Jonckheere-Terpstra test). CACS in patients with stage 5 disease was significantly higher than those in patients with stage 1+2 by Dunnett's test ($p<0.001$). For the logarithmic transformation, the conventional method of Log (CACS+1) was used.*

USA) and Dr SPSS version 11.0.1J for Windows (SPSS Inc., Chicago, USA) were used to assist with the analysis.

Results

The clinical characteristics of the 85 patients (61 male) are summarized in Table 1. Systolic BP (SBP), pulse pressure (PP), BUN, phosphate, i-PTH, and OPG were positively correlated with CKD stage, whereas Hb, Alb, Ca, serum $1,25(OH)_2D_3$ and 25(OH)D were negatively correlated with CKD stage. In particular, a stepwise increase in PP was observed with CKD stage progression (average 52.0, 63.5, 70.1 and 74.6 mmHg in CKD stage 1+2, 3, 4, and 5 patients, respectively). A similar relationship with CKD stage was observed regarding SBP. There was no significant difference in age, gender, diastolic BP (DBP), or DM duration between CKD stages. Dyslipidemia (statin users or abnormal-range lipid parameters) was observed in 57.1% of all patients.

The median value of CACS was 256 Agatston units (range, 0–4494). There was a trend toward higher CACS with higher CKD stage (Fig. 1) ($p<0.002$, Jonckheere-Terpstra test). This was especially true for CKD stage 5 patients, in whom CACS was significantly higher than the values in stage 1+2 patients ($p<0.001$, Dunnett's test).

Coronary Artery Calcification and Risk Factors: Univariate Analyses

In simple regression analyses, CACS was significantly positively correlated with age, DM duration, SBP, PP, P, corrected Ca×P product and OPG, and significantly negatively correlated with body mass index (BMI), Hb, Alb, T-chol and 25(OH)D (Table 2). No significant correlation was observed between CACS and gender, TG, HbA1c, fetuin-A, inflammation markers, or any of several bone markers other than 25(OH)D and OPG. It was unexpected that there was no significant association between fetuin-A and CACS, although OPG, which has also been reported to be a calcification inhibitory factor, was significantly correlated with CACS (Fig. 2).

Simple regression analysis revealed that increased OPG was associated with higher SBP, greater CKD stages, and older age, whereas fetuin-A did not have these associations (data not shown). These data might account for the link between OPG and CACS.

Table 2. Simple Regression Analysis between CACS and Independent Variables

Independent variables	*r*	*p* value
Gender	—	n.s.
Age (year)	0.287	<0.01
DM duration	0.337	<0.005
Body mass index	−0.210	0.05
Systolic BP (mmHg)	0.336	<0.005
Diastolic BP (mmHg)	—	n.s.
Pulse pressure (mmHg)	0.335	<0.005
Lumbar spine (*T* score)	—	n.s.
Femoral neck (*T* score)	—	n.s.
Distal end of radius (*T* score)	—	n.s.
Log (eGFR)	−0.324	<0.005
Hemoglobin (g/dL)	−0.367	<0.001
BUN (mg/dL)	0.314	<0.005
Albumin (g/dL)	−0.295	<0.01
Calcium (mg/dL)	—	n.s.
Corrected Ca (mg/dL)	—	n.s.
Phosphorus (mg/dL)	0.239	<0.05
Corrected Ca×P	0.220	<0.05
T-chol (mg/dL)	−0.217	<0.05
Triglyceride (mg/dL)	—	n.s.
HbA1c (%)	—	n.s.
Log (BAP)	—	n.s.
Log (i-PTH)	—	n.s.
$1,25(OH)_2D_3$ (pg/mL)	—	n.s.
25(OH)D (ng/mL)	−0.286	<0.05
Log (CRP)	—	n.s.
Log (IL-6)	—	n.s.
Log (Fetuin-A)	—	n.s.
Osteoprotegerin (ng/mL)	0.418	<0.001

CACS, coronary artery calcification score; DM, diabetes mellitus; BP, blood pressure; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; T-chol, total cholesterol; HbA1c, glycosylated hemoglobin; BAP, bone alkaline phosphatase; i-PTH, intact parathyroid hormone; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; CRP, C-reactive protein; IL-6, interleukin-6.

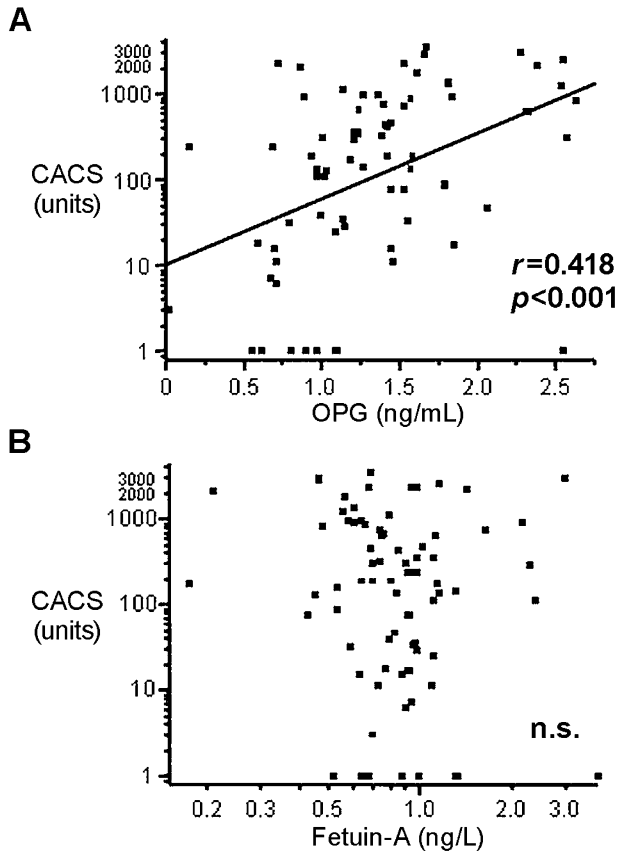

Fig. 2. *Simple regression analysis for CACS by bone associated cytokines. The association between CACS and OPG (A) and that between CACS and fetuin-A are shown (B). Simple regression analysis revealed that CACS was positively correlated with OPG but not fetuin-A.*

Coronary Artery Calcification and Risk Factors: Multivariate Analyses

We selected potential independent variables in multivariate analysis from significant contributors in simple regression analyses. We constructed two models in multivariable regression analysis with Log (CACS+1) as a dependent variable. Model 1 was constructed using the stepwise forward method and model 2 was adjusted for eGFR and traditional risk factors such as age, BP, and gender.

Potential explanatory variables in model 1 were age, sex, SBP, BMI, Log (eGFR), Alb, T-chol, 25(OH)D, and OPG. In model 1, OPG proved to be positively associated and Alb negatively associated with CACS (Table 3). Even after adjusting for eGFR and traditional risk factors such as age, gender, and SBP, OPG was still positively correlated with CACS (model 2; Table 4).

Regarding OPG, which fit in both models, the receiver operating characteristic (ROC) curve analysis for CACS>200 revealed that the sensitivity and specificity of OPG were 79.4% and 66.7%, respectively, when the cut-off value was set at 1.207 ng/mL. The area under the curve (AUC) was 0.716 (95% confidence interval: 0.594–0.837). Regarding Alb, which also proved to be a significant contributor to CACS in model 1, ROC analysis revealed a similar result. The AUC for Alb was 0.679 (95% confidence interval: 0.549–0.809) and the Youden's index was the highest when the cut-off value was 4.0 g/dL (Fig. 3).

Table 3. Multiple Linear Regression Analysis for CACS by Stepwise Method (Model 1)

Independent variables	β-coeffient	*t* value	*p* value
Albumin (g/dL)	−1.033379	−2.16	0.0342
Osteoprotegerin (ng/mL)	1.9989543	4.38	<0.0001

r^2=0.302, p<0.0001. CACS, coronary artery calcification score.

Table 4. Multiple Linear Regression Analysis for CACS, Adjusting for Age, Sex, Systolic BP and eGFR (Model 2)

Independent variables	β-coeffient	*t* value	*p* value
Albumin (g/dL)	−0.845862	−1.48	0.1442
Osteoprotegerin (ng/mL)	1.4094878	2.16	0.035
Age (year)	0.0264413	0.95	0.3454
Systolic BP (mmHg)	0.0171023	1.11	0.2722
Log (eGFR)	−0.202159	−0.46	0.6483
Gender (female)	−0.104371	−0.35	0.7242

r^2=0.329, p<0.001. CACS, coronary artery calcification score; BP, blood pressure; eGFR, estimated glomerular filtration rate.

Discussion

Our study on predialysis DN patients showed that CACS increased with the progression in CKD stages and OPG could be a marker for severe CACS, whereas fetuin-A failed was not a significant contributor to CACS.

Several studies have reported that CKD has a detrimental effect on CACS. Cozzolino *et al*. revealed that CACS in patients with stage 5 CKD was significantly higher than CACS in other stages (*18*), and Kramer *et al*. showed that patients with lower eGFR were more likely to have a CACS>400 than those with higher eGFR (*19*). Our study was compatible with their study and suggested that early evaluation of CACS and intervention might be necessary for predialysis DN patients.

OPG is one of the cytokines affecting bone metabolism, in addition to MGP and the bone morphogeneic protein (BMP). OPG inhibits activity and differentiation of osteoclasts, by acting as a decoy for the receptor activator of nuclear factor κ-B ligand (RANKL). OPG is expressed not only on osteoblasts but also on vascular smooth muscle cells and endothelial cells (*20*). OPG-deficient mice are characterized by osteoporosis and arterial calcification. These phenomena have been pre-

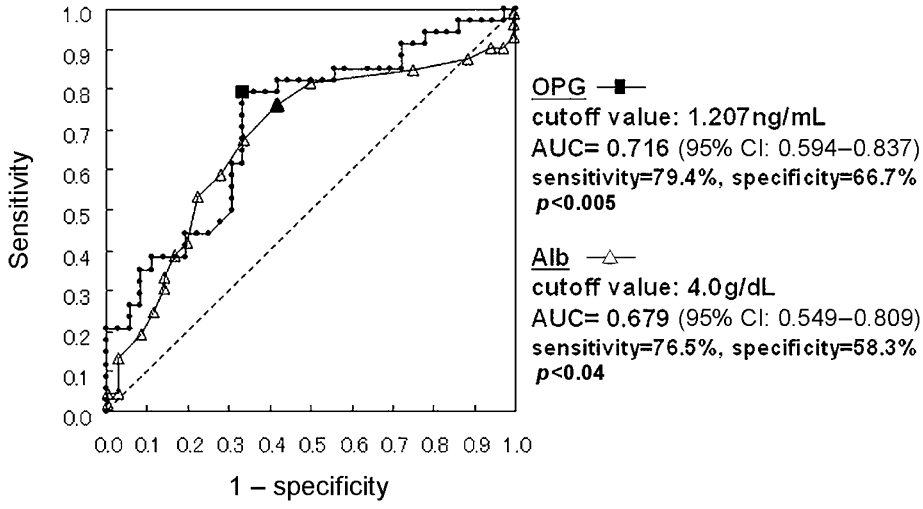

Fig. 3. *ROC analysis for high CACS (> 200 units) regarding osteoprotegerin (OPG) and albumin (Alb). The ROC curves were drawn to determine the cut-off level of OPG and Alb for high CACS (> 200 units). Both OPG and Alb were found to be suitable markers for high CACS. AUC, area under the curve.*

vented by gene therapy using OPG (*21*), indicating that the OPG/RANK/RANKL system is involved in the regulation of bone metabolism and vascular calcification (*22*).

Some clinical researchers have already reported an association between cardiovascular disease and serum OPG (*7*). Kiechl *et al*. reported that high OPG was a risk factor for cardiovascular disease (*23*). Anand *et al*. reported that elevated OPG level was a predictor for high CACS in DM patients without renal failure (*24*). However, the clinical settings of their studies were completely different from ours. In addition, they did not include patients with renal failure, whereas our cohort consisted of diabetic patients with CKD. The specificity of OPG for detecting CACS > 200 was not very high in our study, but the sensitivity was high enough for OPG to be used as a serum biomarker in screening. These findings were completely different from those in former animal studies. The elevation of serum OPG in patients with high CACS in the present study might be attributable to a protective adaptation to calcification stress. Since this study was a cross-sectional study, the direct causal relationship between OPG and CACS is unknown.

Older age, high BP and CKD are risk factors for high CACS. In our study, however, they did not remain as significant factors for high CACS after adjusting for serum OPG. The reason for this might be that older age and higher stages of CKD are reflected in high serum OPG. In fact, in our study, serum OPG was positively correlated with CKD stages, BP, and age. These data are compatible with previous clinical studies reporting that OPG was high in aged people, osteopenic patients (*25*) and CKD patients (*26*).

Previous studies have revealed that fetuin-A/α2 HS-glycoprotein (Ahsg) is specifically accumulated in not only bone but also ectopic calcified tissues (*27*, *28*). Schafer *et al*. reported that Ahsg-deficient mice develop severe calcification of various organs on a mineral and vitamin D–rich diet and on a normal diet when the deficiency is combined with a DBA/2 genetic background (*29*). Reynolds *et al*. reported that fetuin-A is an inhibitor of spontaneous precipitation of hydroxyapatite containing Ca and P in vascular smooth muscle cells (*30*). That is, fetuin-A confers protection against calcification *in vivo*, and thus we expected that serum fetuin-A and CACS would be negatively correlated. In fact, the results of some clinical studies have suggested that low serum fetuin-A levels may contribute to the progression of vascular and soft tissue calcification (*31*, *32*).

However, in our study, we did not observe a significant association between fetuin-A and CACS. This is possibly because the anti-calcification effect of fetuin-A was masked by its ability to exacerbate insulin resistance (*33*). Insulin resistance worsens the proatherogenic milieu among diabetic patients. This may, in turn, promote atherosclerosis and lead to intimal calcification of the coronary arteries. In this context, the clinical observation by Mehrotra *et al*. that high fetuin-A was associated with high CACS (*13*) is easy to understand.

According to previous studies, these molecules seem to inhibit calcification by different mechanisms. Fetuin-A accelerates the solubility of minerals by forming a fetuin mineral complex (FMC) containing calcium and phosphate (*27*, *34*), to participate in the inhibition of ectopic calcification. Measuring FMC instead of fetuin-A might allow us to detect a significant association with CACS. On the other hand, the expression of OPG in the media of great arteries (*20*), and in different vascular cell types such as coronary smooth muscle cells (*35*) and endothelial cells (*36*), suggests the possible involvement of local autocrine or paracrine system. In fact, it

has been reported that OPG acts as a survival factor for endothelial cells (*36*). And one recent study demonstrated that OPG deficiency decreased aortic expression of the parathyroid hormone–related protein, which is another endogenous inhibitor of vascular calcification, leading to increased aortic tissue activity of bone-type alkaline phosphatase (*37*). In this sense, OPG can be considered a "vasculoprotegerin" that acts locally to inhibit calcification.

The positive association between T-chol and CACS has been well documented in the general population. Unexpectedly in our study, however, simple regression analysis revealed that T-chol was negatively correlated with CACS. Given that serum 25(OH)D and BMI, both nutritional parameters in CKD patients, were also negatively associated with CACS and that serum Alb was a independent negative determinant for CACS in multivariate analysis (model 1), this negative association might imply the importance of nutritional state in determining the extent of coronary artery calcification. In fact, Avram *et al*. reported that the mortality of hemodialysis patients was worse in those who had lower T-chol or lower serum Alb when introduced to hemodialysis (*38*). In another study from Japan, Nishizawa *et al*. reported that low cholesterol was a risk for cardiovascular mortality in hemodialysis patients (*39*). Together with our present findings, these data would seem to suggest the involvement of malnutrition inflammation atherosclerosis (MIA) syndrome in the pathogenesis of arterial calcification. However, we could not find any positive associations between CACS and inflammation markers, such as IL-6 and CRP. This is partly because the levels of these acute reactant markers varied so widely over the observation period that an inflammation state could not be evaluated by only a single blood sampling.

In conclusion, in the present cohort we found coronary artery calcification even in the early stages of DN, and showed that CACS increased with CKD stage progression. Serum OPG, unlike fetuin-A, was independently and positively correlated with CACS and can be used as a biomarker for screening CACS in DN. Further studies will be needed to elucidate whether OPG is only an indicator of CACS, or whether any intervention that decreases serum OPG is actually cardioprotective.

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