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Relationship between serum sclerostin, vascular sclerostin expression and vascular calcification assessed by different methods in ESRD patients eligible for renal transplantation: a cross-sectional study

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Abstract

Purpose Vascular calcification (VC) is known to be prevalent in patients with end-stage renal disease (ESRD). Sclerostin has been identified to be involved in the cross-talk between the kidney, vasculature, and bone. The aims of the present study were to evaluate vessel sclerostin expression and its correlation with VC, as well as serum sclerostin levels.

Methods A total of 51 adult ESRD patients undergoing living donor renal transplant (RT) were enrolled in this study. Serum sclerostin levels were measured by enzyme-linked immunosorbent assays. The thoracic aorta calcification (TAC) was measured by computed tomography (CT). The aortic calcification area index (ACAI) was used to evaluate the severity of TAC. During the RT surgery, the internal iliac arteries were collected and paraffin-embedded in 40 patients, followed by immunohistochemical staining for sclerostin expression and von Kossa-staining for vascular medial calcification degree.

Results The prevalence rate of TAC detected by CT was 58.82%. The positive rates of the internal iliac arterial calcification and vessel sclerostin expression were both 45%. Vessel sclerostin was strongly co-localized with medial calcification. Multivariate analyses revealed that only serum sclerostin was significantly associated with the presence of TAC, the severity of TAC and the positive expression of vessel sclerostin. Kappa test showed that the consistency of the two different calcification assessment methods, as well as the consistency of vessel sclerostin expression and von Kossa-staining were high. Furthermore, the cutoff points of serum sclerostin for vessel sclerostin expression, the presence of VC evaluated by CT and that evaluated by pathology were 1599.92 pg/mL, 2475.52 pg/mL, and 2116.23 pg/mL, respectively.

Conclusions The two methods, namely CT and pathology, to evaluate VC were highly consistent. Serum sclerostin was an independent determinant of positive expression of vessel sclerostin and VC in ESRD patients eligible for RT.

Keywords End-stage renal disease · Vessel sclerostin · Serum sclerostin · Vascular calcification

Introduction

Vascular calcification (VC) is known to be one of the driving forces for the remarkably elevated cardiovascular morbidity and mortality, with a prevalence rate of nearly 90% in end-stage renal disease (ESRD) patients [1]. Nevertheless, data regarding predictors of VC are still limited.

Sclerostin, a glycoprotein (22.5 kDa) produced by the SOST gene, is a mainly osteocyte-derived soluble inhibitor of the canonical Wnt-β-catenin signaling pathway [2]. Cumulative evidence implies that serum sclerostin may play a crucial role in the cross-talk between the kidney, vasculature, and bone [3]. Recent experimental and clinical evidence demonstrates expression of extraosseous sclerostin in calcifying vascular smooth muscle cells (VSMCs), in aortic extracts and in aortic valves [4–7]. In addition, it was validated that in hemodialysis patients, sclerostin strongly co-localized with areas of calcification in aortic valve tissue [8]. These data corroborate the fact that sclerostin may play a critical role in the development of VC. However, the studies on the association of serum sclerostin with VC in ESRD patients are inconsistent. While some studies have suggested a positive correlation between elevated sclerostin and VC

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[9–13], other studies have shown no [8] or even negative correlation between them [14–16]. To date, there was no clear explanation for these discrepant findings. Additionally, it remains unknown whether extraosseous sclerostin can spill over to the circulation and whether sclerostin will be studied as a candidate risk factor for VC in ESRD patients.

To our knowledge, there have been few reports about the relationship between the vessel sclerostin expression, the circulating sclerostin levels and VC. Regarding the hypothesis that changes in the levels of sclerostin may influence the process of VC, this study aimed to investigate the internal iliac arterial sclerostin expression, serum sclerostin levels and VC in different parts by different detection methods in ESRD patients undergoing living donor renal transplantation (RT).

Methods

Study design

Between April 2016 and August 2017, 51 adult ESRD patients undergoing living donor RT at the Department of Urology, the Third Affiliated Hospital of Soochow University, Changzhou, China were enrolled in this cross-sectional observational study. Of these patients, 7 were pre-dialysis, 37 were undergoing hemodialysis (HD), and 7 were undergoing peritoneal dialysis (PD). Inclusion criteria were as follows: (1) patients ranged in age from 18 to 75; (2) patients were able to provide consent; (3) PTH ≤ 800 pg/mL; and (4) patients had no history of parathyroidectomy. Patients were excluded if they had any severe infection, malignancy, primary hyperparathyroidism, active autoimmune disease at the time of blood sampling, had any acute cardiovascular event in the preceding 3 months, or refused to provide an informed consent.

Baseline data including the demographic characteristics, initial kidney disease, prescribed medications, smoking status, comorbidities including diabetes mellitus (DM), and prior history of cardiovascular disease (CVD) were collected from the medical records. CVD was defined as history of coronary death, myocardial infarction, coronary insufficiency, angina, ischemic stroke, hemorrhagic stroke, transient ischemic attack, peripheral artery disease or heart failure.

The study protocol was performed according to the Ethics Committee of the Third Affiliated Hospital of Soochow University, China. All participants provided written informed consent before entering the study.

Biochemical measurement

In pre-dialysis patients and PD patients, blood samples were collected in the morning, after overnight fasting; and in HD patients, fasting morning blood samples were harvested after the long dialysis interval prior to dialysis. All of the samples were immediately centrifuged and analyzed for serum creatinine (SCr), blood urea nitrogen (BUN), uric acid, calcium (Ca), phosphate (P), serum albumin, glucose, C-reactive protein (CRP), total cholesterol (TC), triglyceride (TG), hemoglobin (Hb), alkaline phosphatase (AKP) (UniCel AU5800 immunoassay systems, Beckman Coulter, USA), and intact parathyroid hormone (iPTH) (Beckman Coulter's UniCel DxI 800 immunoassay systems, Beckman Coulter, USA) via standard laboratory procedure using an automated analyzer. Corrected serum Ca was adjusted to serum albumin level, according to Payne's formula [17].

In addition, serum was stored at − 80 °C until further analyses. Commercially available enzyme-linked immunosorbent assays were used to determine levels of serum bone-specific alkaline phosphatase (BSAP) (Beckman Coulter, USA), osteoprotegerin (OPG) (eBioscience, USA), human soluble α-Klotho (IBL-Immuno, Japan), human fibroblast growth factor-23 (FGF23) (EMD Millipore, USA), and serum sclerostin (BioMedica, Austria; intra- and inter-assay coefficients of variation were < 7% and 10%, respectively).

Vascular calcification assessment

Non-enhanced computed tomography (CT) was performed on a 64-detector CT scanner (Optima CT660, General Electric Healthcare) on the day of blood sampling to measure the thoracic aorta calcification (TAC). Foci of the TAC were detected by an experienced radiologist, blinded to clinical and biological data.

TAC was defined as the presence of calcium within the boundaries of the descending thoracic aorta visualized on CT, measuring from the lower edge of the pulmonary artery bifurcation inferiorly to the cardiac apex. The ascending thoracic aorta is also visualized on these scans. However, previous studies suggested that ascending thoracic aorta calcium was uncommon in a cohort free of clinical CVD at baseline and descending aorta concentrated most of the calcifications [18, 19]. Moreover, descending thoracic aortic calcification was confirmed to be associated with CVD and non-CVD morbidity and mortality [20, 21]. Given these, we only included the descending aorta in this analysis.

We used the aortic calcification area index (ACAI), which was derived using a method described in a prior paper, to evaluate the severity of TAC [22]. During imaging, ten slices of the descending thoracic aorta were obtained at 1-cm intervals. The area of each aortic cross section and calcification was measured using NIH Image software (NIH: National Institutes of Health, United State of America). The calcification area was then divided by the aortic cross-sectional area and expressed as a percentage (%). ACAI was taken as the mean value of the ten slices.

During the living donor RT surgery, the internal iliac arteries were collected in 40 patients. Samples were immediately fixed in 4% phosphate-buffered formalin, and subsequently embedded in paraffin. The degree of vascular medial calcification was examined by the von Kossa-staining.

Cardiovascular risk assessment

If patients had an age range of 30–74 year and had no history of CVD at the baseline examination, their cardiovascular risk was estimated by Framingham risk scores, basing on age, gender, smoking status, systolic blood pressure, treatment for hypertension, diabetes, and body mass index (BMI) [23]. Due to the non-Gaussian Framingham risk score distribution, we dichotomized it into two groups, below 10 (low risk) and equal or above 10 (intermediate/high risk), according to the International Atherosclerosis Society.

Immunohistochemistry

The internal iliac arteries were collected during RT operations with end-to-end anastomosis, and then the arteries were paraffin-embedded and cut prior to immunohistochemical (IHC) staining for sclerostin (Abcam, Cambridge, UK).

Statistical analyses

Statistical analyses were conducted using SPSS 17.0 software. All continuous variables were checked for normal distribution and non-normally distributed variables were transformed as logarithms or reciprocals. Continuous factors are expressed as mean ± standard deviation. Student's *t* test was used to compare the differences between two groups. Categorical factors are presented by frequencies and percentage. The Chi-square test was applied to evaluate differences in prevalence. To establish parameters associated with vessel sclerostin expression and TAC, we studied all exploratory factors in the first instance in a univariate logistic regression model. Factors which showed a P-value < 0.1 in the univariate analyses were studied simultaneously in a multivariate logistic regressions. Receiver operating characteristics (ROC) were plotted and areas under the curve (AUCs) were calculated. Sensitivity/specificity for the cutoff point of serum sclerostin levels for discriminating positive vessel sclerostin expression, positive internal iliac artery calcification examined by von Kossa-staining and positive TAC measured by CT was calculated. Kappa test was employed to determine the consistency of different calcification assessment methods, as well as the consistency of vessel sclerostin expression and VC. Statistical significance was defined at the level of $P < 0.05$.

Results

Characteristics of the study population

The demographics, clinical characteristics, biochemical data, and comorbidities of the patients are summarized in Table 1. A total of 51 adult ESRD patients undergoing living donor RT were recruited for the present study. Twenty-three patients had chronic glomerulonephritis, 2 had hypertensive nephrosclerosis, 2 had polycystic kidney disease, 1 had IgA nephropathy, 1 had diabetic kidney disease, and 22 had unknown causes. Twelve patients used calcium-containing phosphate binders. No one used calcium-free phosphate binders or Cinacalcet. The calcification and sclerostin expression of the internal iliac arteries were detected in 40 patients and the positive rates were both 45%. Only 35 patients were considered eligible for estimating their cardiovascular risk by using Framingham risk scores. Forty-nine percent of patients had a severe cardiovascular risk with a Framingham risk score equal or above 10.

Parameters associated with TAC

Table 1 compares the clinical and biochemical parameters between patients with and without TAC. In the present study, 58.82% (30 of 51) of the patients had detectable calcifications on CT. Patients with TAC were older, more often diabetic, had higher BMI, and had a higher prevalence of CVD. Moreover, patients with TAC had significantly higher glucose, CRP, serum sclerostin, and lower diastolic blood pressure when compared with patients free of TAC. In addition, the positive rate of sclerostin staining was significantly higher in the TAC group than in the non-TAC group. Treatment differences were not observed except for more use of calcium-containing phosphate binders in patients with TAC versus non-calcified thoracic aorta. Patients with TAC exhibited a tendency toward higher Framingham score than patients without TAC, although this was not significant. There were no additional parameters with significant differences between the two groups.

In univariate logistic regression, older age, diabetes, CVD history, higher BMI, higher glucose, higher sclerostin levels, lower the reciprocal of CRP, and lower diastolic blood pressure were all associated with the presence of TAC (Table 2).

Relying on the P-values obtained in the univariate analyses ($P \leq 0.1$ in Table 2), age, diabetes history, CVD history, BMI, diastolic blood pressure, calcium-containing phosphate binders use, glucose, the reciprocal of CRP and

Table 1 Characteristics of ESRD patients in positive and negative thoracic aorta calcification groups

	All patients ($n=51$)	TAC(+) ($n=30$)	TAC(−) ($n=21$)	P value
Clinical parameters				
Age (years)	54.63 ± 12.84	60.63 ± 9.55	46.05 ± 12.2	0.000
Males, *n* (%)	35 (68.63%)	20 (66.67%)	15 (71.43%)	0.718
DM, *n* (%)	15 (29.41%)	14 (46.67%)	1 (4.76%)	0.001
CVD, *n* (%)	16 (31.37%)	13 (43.33%)	3 (14.29%)	0.028
BMI (kg/m^2)	22.07 ± 3.32	22.92 ± 3.41	20.87 ± 2.86	0.029
Smoking, *n* (%)	13 (25.49%)	6 (20%)	7 (33.33%)	0.282
Systolic blood pressure (mmHg)	143.1 ± 24.72	139.97 ± 24.51	147.57 ± 24.9	0.284
Diastolic blood pressure (mmHg)	87.45 ± 15.45	82.33 ± 13.68	94.76 ± 15.17	0.004
Drug prescription				
ESA use, *n* (%)	45 (88.24%)	25 (83.33%)	20 (95.24%)	0.194
Calcitriol use, *n* (%)	23 (45.1%)	16 (53.33%)	7 (33.33%)	0.158
Calcium-containing phosphate binders use, *n* (%)	12 (23.53%)	10 (33.33%)	2 (9.52%)	0.049
Anti-hypertensive agents use, n (%)	45 (88.24%)	26 (86.67%)	19 (90.48%)	0.678
Metabolic and inflammation biomarkers				
Hb (g/L)	104.72 ± 20.04	105.19 ± 20.38	104.06 ± 20.03	0.845
TG (mmol/L)	1.93 ± 1.11	2.13 ± 1.22	1.66 ± 0.9	0.139
TC (mmol/L)	4.2 ± 1.18	4.12 ± 1.15	4.32 ± 1.24	0.556
Glucose (mmol/L)	6.23 ± 2.19	6.84 ± 2.25	5.36 ± 1.82	0.016
SCr (μmol/L)	787.43 ± 217.98	751.77 ± 186.56	838.39 ± 252.36	0.165
BUN (mmol/L)	17.66 ± 4.81	17.42 ± 4.75	18 ± 4.98	0.675
Uric acid (μmol/L)	356.7 ± 98.53	364.96 ± 99.42	344.9 ± 98.45	0.48
1/ (CRP) (ml/mg)	129.32 ± 88.54	91.03 ± 78.65	176.72 ± 77.67	0.001
Albumin (g/L)	29.49 ± 5.76	28.64 ± 5.87	30.72 ± 5.52	0.207
Vascular calcification scoring by the pathologist and cardiovascular risk				
Internal iliac artery calcification, *n* (%)[a]	18 (45%)	18 (78.26%)	0 (0.00%)	0.000
Framingham score ≧ 10, *n* (%)[b]	17 (48.57%)	11 (64.71%)	6 (33.33%)	0.063
Mineral-bone disease biomarkers				
Log (iPTH) (pg/mL)	2.28 ± 0.43	2.35 ± 0.46	2.18 ± 0.38	0.152
Corrected Ca (mmol/L)	2.58 ± 0.2	2.6 ± 0.2	2.56 ± 0.2	0.472
P (mmol/L)	1.74 ± 0.61	1.68 ± 0.63	1.84 ± 0.6	0.377
Log (FGF-23) (pg/mL)	3.12 ± 0.92	3.18 ± 0.92	3.03 ± 0.94	0.591
Klotho (pg/mL)	386.03 ± 162.73	375.8 ± 164.34	400.62 ± 163.3	0.597
Log (AKP) (μ/L)	1.96 ± 0.21	2.00 ± 0.23	1.91 ± 0.19	0.16
BSAP (ng/mL)	38.27 ± 27.55	40.16 ± 25.81	35.64 ± 30.24	0.572
OPG (ng/mL)	320.45 ± 86.07	331.93 ± 97.77	308.42 ± 72.28	0.377
Serum sclerostin (pg/mL)	2365.97 ± 1537	2944.52 ± 1711	1567.02 ± 730.8	0.000
Positive sclerostin staining, *n* (%)[a]	18 (45%)	17 (73.91%)	1 (5.88%)	0.000

ESRD end-stage renal disease, *TAC* thoracic aorta calcification, *DM* diabetes mellitus, *CVD* cardiovascular disease, *BMI* body mass index, *ESA* erythropoietin stimulating agents, *Hb* hemoglobin, *TG* triglyceride, *TC* total cholesterol, *SCr* serum creatinine, *BUN* blood urea nitrogen, *CRP* C-reactive protein, *iPTH* intact parathyroid hormone, *Ca* calcium, *P* phosphate, *FGF-23* fibroblast growth factor-23, *AKP* alkaline phosphatase, *BSAP* bone-specific alkaline phosphatase, *OPG* osteoprotegerin

[a]Number = 40

[b]Number = 35

serum sclerostin were included in the multivariate regression analyses. Among these, age ($P=0.009$), the reciprocal of CRP ($P=0.014$) and serum sclerostin concentrations ($P=0.048$) were the only parameters that remained significantly associated with TAC. Patients with TAC showed an increased prevalence of diabetes when compared with patients without TAC, although insignificant ($P=0.066$; Table 2). Collinearity was excluded in all regression analyses.

Table 2 Univariate and multivariate logistic regression analyses between thoracic aorta calcification and clinical characteristics

Variables	Univariate 95% CI	*P*	Multivariate 95% CI	*P*
Age	1.118 (1.051, 1.19)	0.000	1.112 (1.026, 1.206)	0.009
DM	17.5 (2.074, 147.645)	0.009	–	0.066
CVD	4.588 (1.109, 18.975)	0.035	–	0.266
BMI	1.25 (1.015, 1.541)	0.036	–	0.171
Smoking	2 (0.559, 7.151)	0.286	–	–
Diastolic blood pressure	0.938 (0.895, 0.984)	0.009	–	0.421
Calcium-containing phosphate binders use	4.75 (0.919,24.558)	0.063	–	0.259
Glucose	1.443 (1.053, 1.978)	0.023	–	0.260
1/(CRP)	0.986 (0.978, 0.995)	0.003	0.985 (0.974, 0.997)	0.014
Log (iPTH)	2.755 (0.681, 11.147)	0.155	–	–
Corrected Ca	2.938 (0.164, 52.589)	0.464	–	–
P	0.654 (0.258, 1.66)	0.371	–	–
Log (FGF-23)	1.19 (0.639, 2.22)	0.583	–	–
Klotho	0.999 (0.996, 1.003)	0.589	–	–
Log (AKP)	9.052 (0.389, 210.756)	0.17	–	–
BSAP	1.006 (0.985, 1.028)	0.565	–	–
OPG	1.003 (0.996, 1.011)	0.372	–	–
Serum sclerostin	1.001 (1, 1.002)	0.005	1.001 (1, 1.002)	0.048

DM diabetes mellitus, *CVD* cardiovascular disease, *BMI* body mass index, *iPTH* intact parathyroid hormone, *Ca* calcium, *P* phosphate, *FGF-23* fibroblast growth factor-23, *AKP* alkaline phosphatase, *BSAP* bone-specific alkaline phosphatase, *OPG* osteoprotegerin

Based on the extent of descending thoracic aortic calcification, patients were divided into two groups according to whether the ACAI was below or above the mean value. Severe calcification was present in 43.33% (13 of 30) of the patients with detectable TAC according to the ACAI being higher than the mean value. In this group, the utilization rate of calcium-containing phosphate binders was higher whereas the levels of serum sclerostin were significantly lower. Patients with higher ACAI appeared to have more severe hypoalbuminemia, although insignificant (Table 3).

As shown in Table 4, there was a significant association between severe TAC and higher utilization rate of calcium-containing phosphate binders ($P=0.045$) and lower sclerostin levels ($P=0.011$) in the univariate analyses. In multivariate regression analyses, only serum sclerostin was significantly associated with severe TAC ($P=0.011$).

Determinants of positive sclerostin expression in the internal iliac arteries

Among the internal iliac arteries from the ESRD patients, 18 (45%) vessels had positive sclerostin staining. The patients with positive sclerostin staining were more likely to have (1) higher mean age, BMI, and incidences of DM; (2) decreased levels of diastolic blood pressure and usage rate of erythropoietin stimulating agents (ESA); (3) significantly higher rate of VC measured by two different methods; and (4) higher glucose, CRP and serum sclerostin levels. However, no obvious differences were found in other variables between the two groups (Table 5).

According to univariate logistic regression analyses, positive vessel sclerostin expression was significantly correlated with old age, diabetes, higher BMI, higher glucose, higher sclerostin levels, lower the reciprocal of CRP and lower diastolic blood pressure. In multivariate analyses, only BMI ($P=0.032$), the reciprocal of CRP ($P=0.034$) and sclerostin levels ($P=0.008$) were identified as independent determinants of positive vessel sclerostin expression (Table 6). Collinearity was excluded in all regression analyses.

Relationship between sclerostin and vascular calcification

As shown in Fig. 1, von Kossa-staining and sclerostin staining of the internal iliac arteries, and calcification of thoracic aorta detected by CT were all positive in 17 patients, and all negative in 16 patients. Only one patient had a positive sclerostin expression but negative VC measured by the above two methods, while another patient had the opposite result. In addition, TAC were identified by CT in five patients while there was no sclerostin expression in the non-calcified internal iliac arteries. IHC staining showed that the sclerostin was localized in the artery media layer and strongly co-localized with areas of calcification.

Besides, Kappa test showed that the consistency of the two different calcification assessment methods, as well as

Table 3 Characteristics of ESRD patients with different thoracic aorta calcification severity according to the aortic calcification area index

	ACAI > 9.3% ($n=13$)	ACAI ≤ 9.3% ($n=17$)	P value
Clinical parameters			
Age (years)	60.54 ± 10.93	60.71 ± 8.7	0.963
Males, *n* (%)	9 (69.23%)	11 (64.71%)	0.794
DM, *n* (%)	7 (53.85%)	7 (41.18%)	0.491
CVD, *n* (%)	6 (46.15%)	7 (41.18%)	0.785
BMI (kg/m^2)	23.95 ± 4.27	22.13 ± 2.43	0.151
Smoking, *n* (%)	2 (15.38%)	4 (23.53%)	0.58
Systolic blood pressure (mmHg)	139 ± 27.01	140.71 ± 23.25	0.854
Diastolic blood pressure (mmHg)	81.31 ± 12.49	83.12 ± 14.85	0.726
Drug prescription			
ESA use, *n* (%)	12 (92.31%)	13 (76.47%)	0.249
Calcitriol use, *n* (%)	7 (53.85%)	9 (52.94%)	0.961
Calcium-containing phosphate binders use, *n* (%)	7 (53.85%)	3 (17.65%)	0.037
Anti-hypertensive agents use, *n* (%)	11 (84.62%)	15 (88.24%)	0.773
Metabolic and inflammation biomarkers			
Hb (g/L)	98.06 ± 12.87	110.64 ± 23.57	0.094
TG (mmol/L)	2.48 ± 1.6	1.89 ± 0.84	0.204
TC (mmol/L)	3.91 ± 1.2	4.26 ± 1.13	0.416
Glucose (mmol/L)	6.83 ± 2.04	6.84 ± 2.47	0.991
SCr (μmol/L)	806.22 ± 198.06	710.12 ± 171.5	0.166
BUN (mmol/L)	17.59 ± 3.79	17.29 ± 5.49	0.865
Uric acid (μmol/L)	374.49 ± 100.15	357.68 ± 101.31	0.654
1/(CRP) (ml/mg)	88.13 ± 80.3	93.16 ± 80.17	0.876
Albumin (g/L)	26.4 ± 5.1	30.35 ± 5.98	0.067
Mineral-bone disease biomarkers			
Log (iPTH) (pg/mL)	2.4 ± 0.56	2.32 ± 0.38	0.632
Corrected Ca (mmol/L)	2.6 ± 0.18	2.6 ± 0.21	0.942
P (mmol/L)	1.74 ± 0.51	1.64 ± 0.72	0.663
Log (FGF-23) (pg/mL)	3.26 ± 1.12	3.11 ± 0.76	0.666
Klotho (pg/mL)	389.76 ± 163.39	365.12 ± 169.26	0.691
Log(AKP) (μ/L)	2.05 ± 0.27	1.96 ± 0.19	0.3
BSAP (ng/mL)	44.51 ± 26.66	36.63 ± 25.39	0.423
OPG (ng/mL)	363.97 ± 43.73	299.89 ± 126.1	0.137
Serum sclerostin (pg/mL)	1890.76 ± 962.38	3800.7 ± 1725.4	0.001

the consistency of sclerostin expression and von Kossa-staining were high (Kappa value = 0.754, $P=0.000$; Kappa value = 0.899, $P=0.000$, respectively). However, the consistency of sclerostin expression and VC detected by CT was common (Kappa value = 0.655, $P=0.000$).

Furthermore, ROC curve analyses showed that the cutoff point of serum sclerostin levels to discriminate patients with and without vessel sclerostin expression, patients with and without VC evaluated by CT, and patients with and without VC evaluated by pathology were 1599.92 pg/mL with 88.9% sensitivity and 71.4% specificity, 2475.51 pg/mL with 54.5% sensitivity and 87.5% specificity, and 2116.23 pg/mL with 82.4% sensitivity and 81.8% specificity, respectively. The AUCs of the serum sclerostin levels for predicting the above indicators were 0.884 (95% confidence interval (CI) 0.716–0.972, $P=0.000$), 0.69 (95% CI 0.519–0.862, $P=0.048$), and 0.85 (95% CI 0.72–0.98, $P=0.000$), respectively (Fig. 2).

Discussion

The major findings of this study were (1) that the positive rates of sclerostin expression and von Kossa-staining in the internal iliac arteries were highly consistent, meanwhile, sclerostin strongly co-localized with areas of calcification; (2) that the consistency of the two different calcification assessment methods, CT and pathology, was high; and (3)

Table 4 Parameters associated with severe thoracic aorta calcification (the aortic calcification area index above 9.3%), in univariate and multivariate analyses

Variables	Univariate 95% CI	*P*	Multivariate 95% CI	*P*
Age	0.998 (0.924, 1.078)	0.961	–	–
DM	0.6 (0.14, 2.575)	0.492	–	–
CVD	0.817 (0.19, 3.505)	0.785	–	–
BMI	1.19 (0.933, 1.516)	0.16	–	–
Smoking	1.692 (0.259, 11.065)	0.583	–	–
Calcium-containing phosphate binders use	0.184 (0.035, 0.963)	0.045	–	0.226
Albumin	0.874 (0.751, 1.016)	0.079	–	0.638
Log (iPTH)	1.507 (0.299, 7.589)	0.619	–	–
Corrected Ca	0.866 (0.02, 36.714)	0.94	–	–
P	1.312 (0.404,4.255)	0.652	–	–
Log (FGF-23)	1.2 (0.541, 2.661)	0.654	–	–
Klotho	1.001 (0.996, 1.005)	0.68	–	–
Log (AKP)	6.095 (0.201, 184.528)	0.299	–	–
BSAP	1.013 (0.983, 1.043)	0.414	–	–
OPG	1.009 (0.996, 1.023)	0.173	–	–
Serum sclerostin	0.999 (0.998, 1)	0.011	0.999 (0.998, 1)	0.011

DM diabetes mellitus, *CVD* cardiovascular disease, *BMI* body mass index, *iPTH* intact parathyroid hormone, *Ca* calcium, *P* phosphate, *FGF-23* fibroblast growth factor-23, *AKP* alkaline phosphatase, *BSAP* bone-specific alkaline phosphatase, *OPG* osteoprotegerin

that serum sclerostin was a relatively reliable and sensitive predictor to discriminate the expression of vessel sclerostin and the presence and severity of VC.

In our study, the presence of TAC was influenced by age, diabetes, CVD history, BMI, serum sclerostin levels, serum CRP levels and diastolic blood pressure in univariate logistic regression. Nevertheless, only age, CRP and serum sclerostin concentrations remained statistically significant in the multivariate regression analyses. Additionally, serum sclerostin was the only parameter to be significantly associated with the severity of TAC in the multivariate regression analyses. As previously reported, CRP might reflect the damage of inflammatory cells and cytokines to blood vessels, which might be important in the development of VC [24]. Furthermore, a previous study showed that VC in CKD occurred before the elevation of serum phosphorus [25]. The above studies may explain our results that serum sclerostin and CRP, but not serum phosphate levels, were positive associated with VC. Besides, the present study did not confirm an independent association of high levels of OPG with VC in contrast to a previous study by Morena et al. [11]. Similarly, the link between FGF23 and VC was not obtained in our study, which was in accordance with several previous studies [12, 26], whereas was contrary to other observations [27, 28].

Additionally, our study revealed that the results of vessel sclerostin expression and calcification staining were highly consistent. Moreover, vessel sclerostin expression was strongly co-localized with areas of medial calcification. Previous animal and in vitro studies have indeed shown increased expression of sclerostin both in calcified VSMCs as well as in a mouse model of medial calcification [4, 29]. More specifically, sclerostin was reported in aortic tissue adjacent to areas of calcification in 15 HD patients [8], which was in accordance with our study. However, a recent observational study by Qureshi et al. refuted our view [10]. We speculate that preanalytical sclerostin stability, assay characteristics, heterogeneity in enrolled patients, cohort size, differences in sensitivity of the methods used to measure VC, variable regions in arterial territories examined, and statistical issues involving power may contribute to the discrepancies. Although in our study, the consistency between CT and pathological examination was high, CT images were unable to discriminate between intimal and medial calcification, otherwise stated, to some extent, pathological examination was better than CT in assessing VC.

It was worth noting that our study failed to find a relation between CVD and VC detected by CT in multivariate analyses or vessel sclerostin expression in univariate and multivariate analyses, which was in accordance with a previous study [30]. We speculate that this is because the medial wall calcification is not a detector of atherosclerosis, the major cause of CVD.

In the present study, ROC curve analyses were used to detect the cutoff point of serum sclerostin levels to predict positive VC evaluated by CT or by the pathologists. We found that in ESRD patients undergoing RT, when serum sclerostin concentration was > 2475.51 pg/mL, it strongly indicated that there may be aortic calcification.

Table 5 Characteristics of ESRD patients in positive and negative vessel sclerostin expression groups

	All patients ($n=40$)	IHC staining for sclerostin		*P* value
		Positive ($n=18$)	All patients ($n=40$)	
Clinical parameters				
Age (years)	55.25 ± 12.69	61.11 ± 10.84	50.45 ± 12.27	0.007
Males, *n* (%)	28 (70%)	15 (83.33%)	13 (59.09%)	0.096
DM, *n* (%)	13 (32.5%)	10 (55.56%)	3 (13.64%)	0.005
CVD, *n* (%)	12 (30%)	8 (44.44%)	4 (18.18%)	0.071
BMI (kg/m^2)	22.18 ± 3.46	23.68 ± 3.45	20.95 ± 3.02	0.011
Smoking, *n* (%)	11 (27.5%)	5 (27.78%)	6 (27.27%)	0.972
Systolic blood pressure (mmHg)	139.45 ± 23.73	134.06 ± 20.37	143.86 ± 25.78	0.197
Diastolic blood pressure (mmHg)	85.33 ± 15.72	79.44 ± 12.47	90.14 ± 16.71	0.03
Drug prescription				
ESA use, *n* (%)	36 (90%)	14 (77.78%)	22 (100%)	0.02
Calcitriol use, *n* (%)	19 (47.5%)	8 (44.44%)	11 (50%)	0.726
Calcium-containing phosphate binders use, *n* (%)	10 (25%)	4 (22.22%)	6 (27.27%)	0.714
Anti-hypertensive agents use, *n* (%)	34 (85%)	15 (83.33%)	19 (86.36%)	0.789
Metabolic and inflammation biomarkers				
Hb (g/L)	103.97 ± 19.8	104.84 ± 23.7	103.25 ± 16.49	0.805
TG (mmol/L)	1.92 ± 1.19	2.14 ± 1.52	1.75 ± 0.83	0.307
TC (mmol/L)	4.2 ± 1.15	4.01 ± 1	4.36 ± 1.27	0.354
Glucose (mmol/L)	6.26 ± 2.32	7.25 ± 2.49	5.44 ± 1.86	0.012
SCr (μmol/L)	765.34 ± 206.28	798.83 ± 162.38	737.94 ± 236.5	0.36
BUN (mmol/L)	17.2 ± 4.82	16.53 ± 4.36	17.74 ± 5.19	0.437
Uric acid (μmol/L)	349.11 ± 97.01	338.83 ± 97.14	357.52 ± 98.35	0.551
1/(CRP) (ml/mg)	103.89 ± 85.64	65.16 ± 65.07	135.58 ± 88.62	0.008
Albumin (g/L)	29.23 ± 5.94	29.89 ± 6.54	28.68 ± 5.5	0.526
Vascular calcification and cardiovascular risk				
Internal iliac artery calcification, *n* (%)	18 (45%)	17 (94.44%)	1 (4.55%)	0.000
TAC, *n* (%)	23 (57.5%)	17 (94.44%)	6 (27.27%)	0.000
Framingham score ≧ 10, *n* (%)[a]	13 (46.43%)	6 (60%)	7 (38.89%)	0.714
Mineral-bone disease biomarkers				
Log (iPTH) (pg/mL)	2.32 ± 0.45	2.27 ± 0.41	2.36 ± 0.49	0.539
Corrected Ca (mmol/L)	2.58 ± 0.2	2.59 ± 0.17	2.57 ± 0.23	0.705
P (mmol/L)	1.71 ± 0.66	1.61 ± 0.59	1.8 ± 0.71	0.38
Log (FGF-23) (pg/mL)	3.17 ± 0.96	3.21 ± 0.78	3.13 ± 1.11	0.815
Klotho (pg/mL)	381.53 ± 153.23	354.86 ± 124.74	403.35 ± 172.92	0.326
Log(AKP) (μ/L)	1.98 ± 0.23	1.94 ± 0.21	2.01 ± 0.25	0.372
BSAP (ng/mL)	35.71 ± 28.39	40.15 ± 35.14	31.72 ± 20.71	0.382
OPG (ng/mL)	322.4 ± 77.18	327.13 ± 97.58	319.33 ± 63.2	0.782
Serum sclerostin (pg/mL)	2252.2 ± 1278	3038.13 ± 1334	1578.56 ± 747.5	0.000

ESRD end-stage renal disease, *IHC* immunohistochemical, *DM* diabetes mellitus, *CVD* cardiovascular disease, *BMI* body mass index, *ESA* erythropoietin stimulating agents, *Hb* hemoglobin, *TG* triglyceride, *TC* total cholesterol, *SCr* serum creatinine, *BUN* blood urea nitrogen, *CRP* C-reactive protein, *TAC* thoracic aorta calcification, *iPTH* intact parathyroid hormone, *Ca* calcium, *P* phosphate, *FGF-23* fibroblast growth factor-23, *AKP* alkaline phosphatase, *BSAP* bone-specific alkaline phosphatase, *OPG* osteoprotegerin

[a]Number = 28

Our results showed unequivocally that both serum sclerostin and vessel sclerostin were involved in VC, suggesting that sclerostin may be implicated in the pathophysiology of VC via a systemic and local effect, be it direct or indirect. Furthermore, our study indicated that the patients with more severe TAC had significantly lower sclerostin levels. Similarly, both Kirkpantur et al. [31] and Balci et al. [32] discovered that serum sclerostin was significant predictor of

Table 6 Univariate and multivariate logistic regression analyses between vessel sclerostin expression and clinical characteristics

Variables	Univariate 95% CI	*P*	Multivariate 95% CI	*P*
Age	1.084 (1.017, 1.154)	0.013	–	0.555
DM	7.917 (1.711, 36.633)	0.008	–	0.919
CVD	3.6 (0.864, 15.008)	0.079	–	0.569
BMI	1.326 (1.043, 1.687)	0.021	1.478 (1.035, 2.112)	0.032
Diastolic blood pressure	0.949 (0.902, 0.998)	0.042	–	0.473
Glucose	1.472 (1.062, 2.041)	0.02	–	0.826
1/(CRP)	0.988 (0.979, 0.998)	0.013	0.982 (0.966, 0.999)	0.034
Serum sclerostin	1.002 (1.001, 1.003)	0.002	1.002 (1.001, 1.004)	0.008

DM diabetes mellitus, *CVD* cardiovascular disease, *BMI* body mass index, *CRP* C-reactive protein

arteriovenous fistula calcification in hemodialysis patients according to univariate analyses; nevertheless, this correlation became inverse following multivariate analyses, which were in accordance with a previous study in CKD patients not yet receiving dialysis [5]. In addition, previous studies confirmed that sclerostin could inhibit the Wnt/β-catenin canonical signaling pathway [33], which may be involved in VSMCs proliferation and migration, both in vivo and in vitro [34, 35]. Therefore, we put forward the hypothesis that high sclerostin levels or sclerostin overexpression in calcifying vasculature might be vasculoprotective and anti-calcific, most probably by attenuate the upregulation of the canonical Wnt pathway that aimed to retard further VC [4, 36]. We expect that sclerostin, superior to OPG, FGF23, and serum phosphorus, may be used as a promising blood marker in assessing VC in ESRD patients undergoing RT, and prevention of VC may be possible through the modulation of sclerostin levels.

Previous literature data confirmed that serum sclerostin levels in ESRD patients were remarkably increased [37, 38]. However, the source of elevated circulating sclerostin remained elusive. The present study showed a significant correlation of peripheral serum sclerostin and vascular sclerostin levels, supporting the data from our previous observation [39]. Cejka et al. [40] confirmed an increase in urinary sclerostin excretion with declining kidney function. Both animal and human experiments revealed that sclerostin osteocyte expression decreased in more severe CKD stages [41, 42]. Together with these published data, we hold the opinion that the increase of serum sclerostin may be due to increased vascular production, being independent of diminished renal clearance and increased bone production.

Apart from serum sclerostin levels, serum CRP concentrations were also independent determinants of positive vessel sclerostin expression, suggesting inflammatory cytokines may paly a role in sclerostin regulation, which was compatible with previous studies [43, 44]. It was note worthy that a link between iPTH and sclerostin had not been obtained in our study, corroborating with the results of other previous analyses in predialysis CKD patients [11, 38]. Conversely, data from both rodent models and CKD1-5D patients demonstrated an inverse correlation between iPTH and sclerostin [45, 46]. We hypothesized that differences in the applied assays, low levels of iPTH observed in our study and low sample size may partially be responsible for the discrepancies.

This study is the first to jointly explore the relationship between serum sclerostin, vessel sclerostin and VC in a cohort of Chinese ESRD patients. Several particular strengths of the study are the use of both histological assessment and imageological diagnosis to evaluate the presence of VC and the use of ACAI to evaluate the severity of TAC. Limitations of this study include the small sample size, the cross-sectional and single-center design, the lack of bone histomorphometry data to detect sclerostin of osteocytic origin, the lack of evaluating the molecular expression of sclerostin in the internal iliac segments, the lack of assessing the tissue expression of FGF-23, a-klotho, OPG and other biomarkers, and the weak association between serum sclerostin levels and positive VC. Additionally, the study participants are relatively younger, without severe secondary hyperparathyroidism or severe comorbidities, suggesting that they are not representative of the overall ESRD population. Finally, the trial design does not allow drawing firm conclusions whether sclerostin is within the aetiological pathway of uremic VC and whether baseline sclerostin is a risk factor for VC progression.

Conclusions

In conclusion, we found that 58.82%, 45%, and 45% of ESRD patients undergoing living donor RT showed positive TAC detected by CT, positive VC detected by the pathologists, and positive expression of sclerostin in the internal iliac artery media layer, respectively. The results of VC assessed by different methods were highly consistent. Serum sclerostin levels are the co-determinants of positive vessel sclerostin expression and the presence as well as the severity of VC. Circulating sclerostin concentrations may

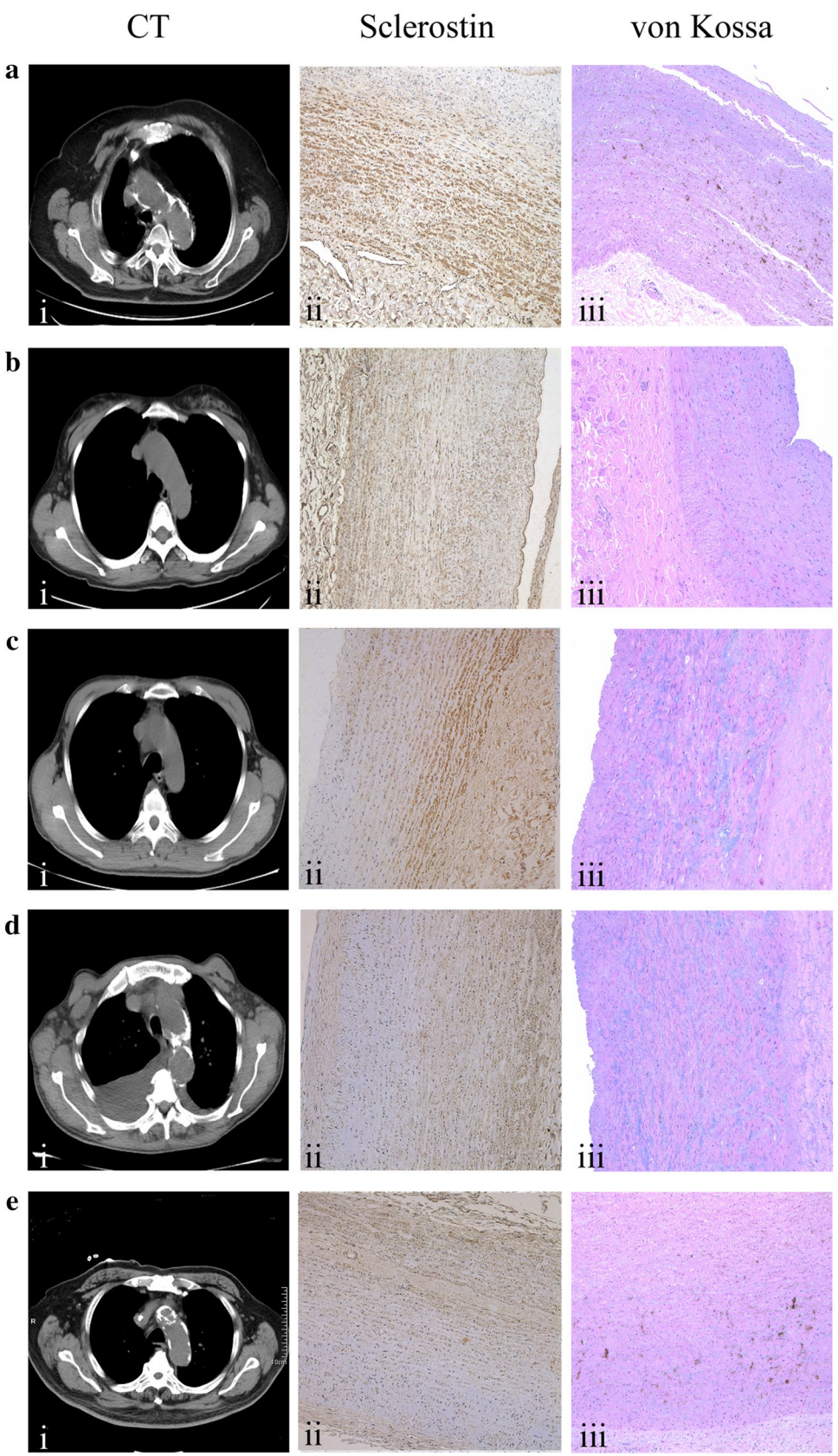

Fig. 1 Thoracic AoC evaluated by CT, von Kossa-staining in internal iliac arteries, and IHC staining of sclerostin staining in internal iliac arteries from ESRD patients. **a** positive expression of the above three indicators (×100); **b** negative expression of the above three indicators (×100); **c** positive expression of sclerostin while negative expression of VC measured by CT as well as by von Kossa-staining (×100); **d** positive expression of thoracic AoC while negative expression of sclerostin and von Kossa-staining in internal iliac arteries (×100); **e** positive expression of VC measured by CT and by von Kossa-staining while negative expression of sclerostin in internal iliac arteries (×100)

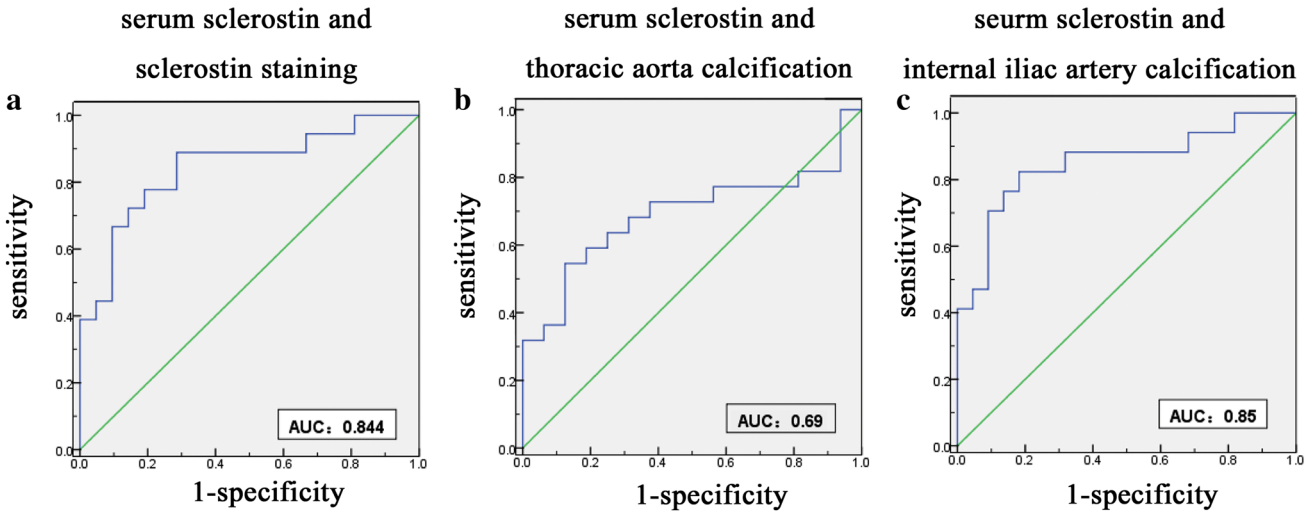

Fig. 2 ROC analyses of vessel positive sclerostin expression, positive thoracic AoC evaluated by CT, and positive internal iliac arterial calcification evaluated by von Kossa-staining with respect to serum sclerostin levels. **a** ROC analysis of vessel positive sclerostin expression with serum sclerostin levels; **b** ROC analysis of thoracic AoC with serum sclerostin levels; **c** ROC analysis of internal iliac arterial calcification with serum sclerostin levels

partly reflect production and secretion by the cells of the vasculature.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual part in the study.

References

1. Budoff MJ, Rader DJ, Reilly MP, Mohler ER 3rd, Lash J, Yang W, Rosen L, Glenn M, Teal V, Feldman HI, Investigators CS (2011) Relationship of estimated GFR and coronary artery calcification in the CRIC (Chronic Renal Insufficiency Cohort) Study. Am J Kidney Dis 58(4):519–526. https://doi.org/10.1053/j.ajkd.2011.04.024
2. Lewiecki EM (2014) Role of sclerostin in bone and cartilage and its potential as a therapeutic target in bone diseases. Ther Adv Musculoskelet Dis 6(2):48–57. https://doi.org/10.1177/1759720x13510479
3. Viaene L, Behets GJ, Claes K, Meijers B, Blocki F, Brandenburg V, Evenepoel P, D'Haese PC (2013) Sclerostin: another bone-related protein related to all-cause mortality in haemodialysis? Nephrol Dial Transplant 28(12):3024–3030. https://doi.org/10.1093/ndt/gft039
4. Zhu D, Mackenzie NC, Millan JL, Farquharson C, MacRae VE (2011) The appearance and modulation of osteocyte marker expression during calcification of vascular smooth muscle cells. PLoS ONE 6(5):e19595. https://doi.org/10.1371/journal.pone.0019595
5. Claes KJ, Viaene L, Heye S, Meijers B, d'Haese P, Evenepoel P (2013) Sclerostin: another vascular calcification inhibitor? J Clin Endocrinol Metab 98(8):3221–3228. https://doi.org/10.1210/jc.2013-1521
6. Koos R, Brandenburg V, Mahnken AH, Schneider R, Dohmen G, Autschbach R, Marx N, Kramann R (2013) Sclerostin as a potential novel biomarker for aortic valve calcification: an in-vivo and ex-vivo study. J Heart Valve Dis 22(3):317–325
7. Didangelos A, Yin X, Mandal K, Baumert M, Jahangiri M, Mayr M (2010) Proteomics characterization of extracellular space components in the human aorta. Mol Cell Proteom MCP 9(9):2048–2062. https://doi.org/10.1074/mcp.M110.001693
8. Brandenburg VM, Kramann R, Koos R, Kruger T, Schurgers L, Muhlenbruch G, Hubner S, Gladziwa U, Drechsler C, Ketteler M (2013) Relationship between sclerostin and cardiovascular calcification in hemodialysis patients: a cross-sectional study. BMC Nephrol 14:219. https://doi.org/10.1186/1471-2369-14-219
9. Lv W, Guan L, Zhang Y, Yu S, Cao B, Ji Y (2016) Sclerostin as a new key factor in vascular calcification in chronic kidney disease stages 3 and 4. Int Urol Nephrol 48(12):2043–2050. https://doi.org/10.1007/s11255-016-1379-8
10. Qureshi AR, Olauson H, Witasp A, Haarhaus M, Brandenburg V, Wernerson A, Lindholm B, Soderberg M, Wennberg L, Nordfors L, Ripsweden J, Barany P, Stenvinkel P (2015) Increased circulating sclerostin levels in end-stage renal disease predict biopsy-verified vascular medial calcification and coronary artery calcification. Kidney Int 88(6):1356–1364. https://doi.org/10.1038/ki.2015.194
11. Morena M, Jaussent I, Dupuy AM, Bargnoux AS, Kuster N, Chenine L, Leray-Moragues H, Klouche K, Vernhet H, Canaud B, Cristol JP (2015) Osteoprotegerin and sclerostin in chronic kidney disease prior to dialysis: potential partners in vascular calcifications. Nephrol Dial Transplant 30(8):1345–1356. https://doi.org/10.1093/ndt/gfv081

12. Pelletier S, Confavreux CB, Haesebaert J, Guebre-Egziabher F, Bacchetta J, Carlier MC, Chardon L, Laville M, Chapurlat R, London GM, Lafage-Proust MH, Fouque D (2015) Serum sclerostin: the missing link in the bone-vessel cross-talk in hemodialysis patients? Osteoporos Int 26 (8):2165–2174. https://doi.org/10.1007/s00198-015-3127-9
13. Wang XR, Yuan L, Zhang JJ, Hao L, Wang DG (2017) Serum sclerostin values are associated with abdominal aortic calcification and predict cardiovascular events in patients with chronic kidney disease stages 3-5D. Nephrology 22(4):286–292. https://doi.org/10.1111/nep.12813
14. Yang CY, Chang ZF, Chau YP, Chen A, Yang WC, Yang AH, Lee OK (2015) Circulating Wnt/beta-catenin signalling inhibitors and uraemic vascular calcifications. Nephrol Dial Transplant 30(8):1356–1363. https://doi.org/10.1093/ndt/gfv043
15. Jean G, Chazot C, Bresson E, Zaoui E, Cavalier E (2016) High serum sclerostin levels are associated with a better outcome in haemodialysis patients. Nephron 132(3):181–190. https://doi.org/10.1159/000443845
16. Evenepoel P, Goffin E, Meijers B, Kanaan N, Bammens B, Coche E, Claes K, Jadoul M (2015) Sclerostin serum levels and vascular calcification progression in prevalent renal transplant recipients. J Clin Endocrinol Metab 100(12):4669–4676. https://doi.org/10.1210/jc.2015-3056
17. Payne RB, Little AJ, Williams RB, Milner JR (1973) Interpretation of serum calcium in patients with abnormal serum proteins. Br Med J 4(5893):643–646
18. Thomas IC, McClelland RL, Michos ED, Allison MA, Forbang NI, Longstreth WT Jr, Post WS, Wong ND, Budoff MJ, Criqui MH (2017) Density of calcium in the ascending thoracic aorta and risk of incident cardiovascular disease events. Atherosclerosis 265:190–196. https://doi.org/10.1016/j.atherosclerosis.2017.09.009
19. Craiem D, Chironi G, Casciaro ME, Graf S, Simon A (2014) Calcifications of the thoracic aorta on extended non-contrast-enhanced cardiac CT. PLoS ONE 9(10):e109584. https://doi.org/10.1371/journal.pone.0109584
20. Takasu J, Budoff MJ, O'Brien KD, Shavelle DM, Probstfield JL, Carr JJ, Katz R (2009) Relationship between coronary artery and descending thoracic aortic calcification as detected by computed tomography: the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 204(2):440–446. https://doi.org/10.1016/j.atherosclerosis.2008.09.041
21. Thomas IC, Thompson CA, Yang M, Allison MA, Forbang NI, Michos ED, McClelland RL, Budoff MJ, Criqui MH (2018) Thoracic aorta calcification and noncardiovascular disease-related mortality: the Multi-Ethnic Study of Atherosclerosis. Arterioscler Thromb Vasc Biol. https://doi.org/10.1161/ATVBAHA.118.310850
22. Ohya M, Otani H, Kimura K, Saika Y, Fujii R, Yukawa S, Shigematsu T (2010) Improved assessment of aortic calcification in Japanese patients undergoing maintenance hemodialysis. Int Med 49(19):2071–2075
23. Sullivan LM, Massaro JM, D'Agostino RB Sr (2004) Presentation of multivariate data for clinical use: the Framingham Study risk score functions. Stat Med 23(10):1631–1660. https://doi.org/10.1002/sim.1742
24. Kirkpantur A, Arici M, Altun B, Yilmaz MI, Cil B, Aki T, Bakkaloglu M, Turgan C (2008) Association of serum lipid profile and arteriovenous fistula thrombosis in maintenance hemodialysis patients. Blood Purif 26(4):322–332. https://doi.org/10.1159/000132388
25. Patsalas S, Eleftheriadis T, Spaia S, Theodoroglou H, Antoniadi G, Liakopoulos V, Passadakis P, Vayonas G, Vargemezis V (2007) Thirty-month follow-up of coronary artery calcification in hemodialysis patients: different roles for inflammation and abnormal calcium-phosphorous metabolism? Ren Fail 29(5):623–629. https://doi.org/10.1080/08860220701395010
26. Scialla JJ, Lau WL, Reilly MP, Isakova T, Yang HY, Crouthamel MH, Chavkin NW, Rahman M, Wahl P, Amaral AP, Hamano T, Master SR, Nessel L, Chai B, Xie D, Kallem RR, Chen J, Lash JP, Kusek JW, Budoff MJ, Giachelli CM, Wolf M, Chronic Renal Insufficiency Cohort Study Investigators (2013) Fibroblast growth factor 23 is not associated with and does not induce arterial calcification. Kidney Int 83(6):1159–1168. https://doi.org/10.1038/ki.2013.3
27. Jimbo R, Kawakami-Mori F, Mu S, Hirohama D, Majtan B, Shimizu Y, Yatomi Y, Fukumoto S, Fujita T, Shimosawa T (2014) Fibroblast growth factor 23 accelerates phosphate-induced vascular calcification in the absence of Klotho deficiency. Kidney Int 85(5):1103–1111. https://doi.org/10.1038/ki.2013.332
28. Desjardins L, Liabeuf S, Renard C, Lenglet A, Lemke HD, Choukroun G, Drueke TB, Massy ZA, European Uremic Toxin Work Group (2012) FGF23 is independently associated with vascular calcification but not bone mineral density in patients at various CKD stages. Osteoporos Int 23(7):2017–2025. https://doi.org/10.1007/s00198-011-1838-0
29. Roman-Garcia P, Carrillo-Lopez N, Fernandez-Martin JL, Naves-Diaz M, Ruiz-Torres MP, Cannata-Andia JB (2010) High phosphorus diet induces vascular calcification, a related decrease in bone mass and changes in the aortic gene expression. Bone 46(1):121–128. https://doi.org/10.1016/j.bone.2009.09.006
30. Patsalas S, Eleftheriadis T, Spaia S, Theodoroglou H, Panou E, Liakopoulos V, Antoniadi G, Passadakis P, Vayonas G, Kanakis E, Vargemezis V (2005) The value of computed tomography-derived coronary artery calcification score in coronary artery disease detection in asymptomatic hemodialysis patients. Ren Fail 27(6):683–688
31. Kirkpantur A, Balci M, Turkvatan A, Afsar B (2016) Serum sclerostin levels, arteriovenous fistula calcification and 2-years all-cause mortality in prevalent hemodialysis patients. Nefrologia 36(1):24–32. https://doi.org/10.1016/j.nefro.2015.07.006
32. Balci M, Kirkpantur A, Turkvatan A, Mandiroglu S, Ozturk E, Afsar B (2015) Sclerostin as a new key player in arteriovenous fistula calcification. Herz 40(2):289–297. https://doi.org/10.1007/s00059-013-3992-y
33. Zeng C, Guo C, Cai J, Tang C, Dong Z (2018) Serum sclerostin in vascular calcification and clinical outcome in chronic kidney disease. Diabetes Vasc Dis Res 15(2):99–105. https://doi.org/10.1177/1479164117742316
34. Wang X, Xiao Y, Mou Y, Zhao Y, Blankesteijn WM, Hall JL (2002) A role for the beta-catenin/T-cell factor signaling cascade in vascular remodeling. Circ Res 90(3):340–347
35. Wang X, Adhikari N, Li Q, Hall JL (2004) LDL receptor-related protein LRP6 regulates proliferation and survival through the Wnt cascade in vascular smooth muscle cells. Am J Physiol Heart Circ Physiol 287(6):H2376–H2383. https://doi.org/10.1152/ajpheart.01173.2003
36. Kanbay M, Solak Y, Siriopol D, Aslan G, Afsar B, Yazici D, Covic A (2016) Sclerostin, cardiovascular disease and mortality: a systematic review and meta-analysis. Int Urol Nephrol 48(12):2029–2042. https://doi.org/10.1007/s11255-016-1387-8
37. Pelletier S, Dubourg L, Carlier MC, Hadj-Aissa A, Fouque D (2013) The relation between renal function and serum sclerostin in adult patients with CKD. Clin J Am Soc Nephrol CJASN 8(5):819–823. https://doi.org/10.2215/cjn.07670712
38. Thambiah S, Roplekar R, Manghat P, Fogelman I, Fraser WD, Goldsmith D, Hampson G (2012) Circulating sclerostin and Dickkopf-1 (DKK1) in predialysis chronic kidney disease (CKD): relationship with bone density and arterial stiffness. Calcif Tissue Int 90(6):473–480. https://doi.org/10.1007/s00223-012-9595-4

39. Zhou H, Yang M, Li M, Cui L (2017) Radial artery sclerostin expression in chronic kidney disease stage 5 predialysis patients: a cross-sectional observational study. Int Urol Nephrol 49(8):1433–1437. https://doi.org/10.1007/s11255-017-1604-0
40. Cejka D, Marculescu R, Kozakowski N, Plischke M, Reiter T, Gessl A, Haas M (2014) Renal elimination of sclerostin increases with declining kidney function. J Clin Endocrinol Metab 99(1):248–255. https://doi.org/10.1210/jc.2013-2786
41. Confavreux CB (2011) Bone: from a reservoir of minerals to a regulator of energy metabolism. Kidney Int Suppl. https://doi.org/10.1038/ki.2011.25
42. Kogawa M, Wijenayaka AR, Ormsby RT, Thomas GP, Anderson PH, Bonewald LF, Findlay DM, Atkins GJ (2013) Sclerostin regulates release of bone mineral by osteocytes by induction of carbonic anhydrase 2. J Bone Miner Res 28(12):2436–2448. https://doi.org/10.1002/jbmr.2003
43. Brandenburg VM, D'Haese P, Deck A, Mekahli D, Meijers B, Neven E, Evenepoel P (2016) From skeletal to cardiovascular disease in 12 steps-the evolution of sclerostin as a major player in CKD-MBD. Pediatr Nephrol 31(2):195–206. https://doi.org/10.1007/s00467-015-3069-7
44. Baek K, Hwang HR, Park HJ, Kwon A, Qadir AS, Ko SH, Woo KM, Ryoo HM, Kim GS, Baek JH (2014) TNF-alpha upregulates sclerostin expression in obese mice fed a high-fat diet. J Cell Physiol 229(5):640–650. https://doi.org/10.1002/jcp.24487
45. Cejka D, Herberth J, Branscum AJ, Fardo DW, Monier-Faugere MC, Diarra D, Haas M, Malluche HH (2011) Sclerostin and Dickkopf-1 in renal osteodystrophy. Clin J Am Soc Nephrol CJASN 6(4):877–882. https://doi.org/10.2215/CJN.06550810
46. Drake MT, Khosla S (2017) Hormonal and systemic regulation of sclerostin. Bone 96:8–17. https://doi.org/10.1016/j.bone.2016.12.004