Association of Serum Sclerostin Level, Coronary Artery Calcification, and Patient Outcomes in Maintenance Dialysis Patients

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Keywords

Sclerostin · Coronary artery calcification · Maintenance dialysis · Patient outcomes

Abstract

Objective: The objective of this study is to investigate the association between the serum sclerostin, the coronary artery calcification (CAC), and patient outcomes in maintenance dialysis patients. **Methods:** We performed a prospective cohort study of 65 maintenance dialysis patients in 2014, including 39 patients on peritoneal dialysis and 26 on hemodialysis, and followed up for 5 years. Parameters of mineral metabolism including bone-specific alkaline phosphatase, fibroblast growth factor 23, sclerostin, and other biochemical factors were determined at the baseline. Meanwhile, the CAC score was analyzed by cardiac computed tomography. **Results:** Serum sclerostin in hemodialysis patients was significantly higher than that in peritoneal dialysis patients (632.35 ± 369.18 vs. 228.85 ± 188.92 , $p < 0.001$). The patients with CAC were older, receiving hemodialysis, lower Kt/V, and had longer dialysis vintage, as well as higher levels of serum 25-(OH)-vit D and sclerostin. In multivariate logistic regression analysis, older age and lower Kt/V were risk factors for CAC. The area under the receiver operating characteristic curves for prediction of CAC by sclerostin was 0.74 (95% confidence interval 0.605–0.878, $p = 0.03$), and the cutoff value of sclerostin is 217.55 pg/mL with the sensitivity 0.829 and specificity 0.619. After 5 years of follow-up, 51 patients sur-

vived. The patients in the survival group had significantly lower age, sclerostin levels, and low CAC scores than the nonsurvival group. Old age (≥ 60 years, $p < 0.001$) and high CAC score (≥ 50 Agatston unit, $p = 0.031$) were significant risk factors for the patient survival. **Conclusions:** Sclerostin is significantly elevated in dialysis patients with CAC. But sclerostin is not a risk factor for CAC. After 5 years of follow-up, patients in the survival group are younger and have lower sclerostin levels and CAC scores. But sclerostin levels are not independent risk factors for high mortality in dialysis patients.

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Introduction

Chronic kidney disease-mineral bone disorder (CKD-MBD) is a major syndrome that occurs secondary to declining renal function and usually predicts poor outcomes [1, 2]. However, the exact mechanism of the relationship between the CKD-MBD and a high mortality is not yet fully elucidated. Meanwhile, the abnormal bone metabolism plays an important role in the pathogenesis of CKD-MBD [3, 4]. The various bone metabolic factors may be associated with the CKD-MBD, such as calcium, phosphate, parathyroid hormone (PTH), and vitamin D [5]. Recent studies have identified the new molecules including fibroblast growth factor 23 (FGF23), bone-specific alkaline phosphatase (BALP), and sclerostin may be in-

Fig. 1. Sclerostin: regulation, bone effect, and (hypothetical) link with vascular calcifications. Sclerostin inhibits the Wnt receptor (LRP5/6), inducing inhibition of differentiation and proliferation of the osteoblast precursors into mature osteoblasts. The link between sclerostin and vascular calcification is still uncertain. Wnt, Wingless-type integration site; LRP, lipoprotein receptor-related protein.

involved in the CKD-MBD and also become the risk makers for the cardiovascular outcomes [6, 7]. As a key aspect in the CKD-MBD, vascular calcification is an independent risk factor for the cardiovascular disease and is related to the adverse cardiovascular events [8, 9]. Among these emerging biomarkers, it is essential to find an indicator that could predict the presence and extent of vascular calcification. Sclerostin, an osteocyte-derived glycoprotein has attracted the researchers' attention [10, 11].

Sclerostin is the secreted product of the *SOST* gene in osteocytes. As a soluble inhibitor of Wingless-type mouse mammary tumor virus integration site (Wnt) signaling, sclerostin binds to the co-receptors low-density lipoprotein receptor-related protein-5 or lipoprotein receptor-related protein -6 and blocks their association with Wnts [12]. The effect of canonical Wnt signaling on bone is mediated by simulation of stem cells and pre-osteoblast proliferation, induction of osteoblastogenesis, and attenuation of osteoclastogenesis [12, 13]. Recently, the potential role of sclerostin in vascular calcification, in particular CKD patients, has been widely discussed in different studies [14–16]. However, the relationship between human serum sclerostin and vascular calcification is still unclear. The pathophysiological mechanism of sclerostin is shown in Figure 1. To date, some studies showed a positive correlation in vascular calcification [16–19]. On the other hand, a negative correlation was found between sclerostin and vascular calcification in 2 cross-sectional studies and a prospective observational cohort study [11, 20]. Claes et al.

[21] reported that the positive univariate correlation between aortic calcifications and sclerostin levels became inverse in multivariate analysis in predialysis patients. The previous studies also included vastly different population samples, such as hemodialysis patients, ESRD patients (predialysis, hemodialysis, and peritoneal dialysis), patients in CKD stage 3–4, and patients in CKD stage 1–5 not receiving dialysis. In the current study, we measured serum sclerostin and other CKD-MBD biomarkers, inflammation biomarkers, biochemical parameters, and degree of coronary artery calcification (CAC) score by cardiac computed tomography (CT) in ESRD patients receiving hemodialysis and peritoneal dialysis. Meanwhile, we also examined the relationship between these biomarkers and another cardiovascular outcomes-left ventricular mass index (LVMI) or patient survival after a 5-year follow-up period.

Materials and Methods

Study Design

A total of 65 maintenance dialysis patients from the Department of Nephrology at the First Affiliated Hospital of Nanjing Medical University were enrolled in this prospective cohort study between November 2014 and April 2015. Among these patients, 26 were undergoing hemodialysis, 39 were undergoing peritoneal dialysis. Inclusion criteria were aged 18 years or older and were able to provide consent. The patients were excluded if they had any acute inflammation, malignancy, acute myocardial infarction, pulmonary edema, and heart failure at the time of blood sample collection. The patients were followed up for 5 years. The study was

Table 1. Demographic and biochemical characteristics of maintenance dialysis patients

	Total (n = 65)	HD (n = 26)	PD (n = 39)
Male, n (%)	34 (52.3)	19 (73.1)	15 (38.5)
Age, years	48.38±13.18	54.62±12.45	44.23±12.09
Height, cm	165.12±8.56	166.85±8.06	163.97±8.81
Weight, kg	62.96±14.64	66.2±14.41	60.88±14.6
BMI, kg/m ²	22.86±4.24	23.55±4.58	22.42±4.01
Hemoglobin, g/L	106.11±16.78	106.04±16.15	106.15±17.40
Albumin, g/L	40.84±4.05	39.56±3.38	41.69±4.27
BALP, µg/L	20.56±10.11	17.96±9.58	22.42±4.01
Calcium, mmol/L	2.55±0.20	2.41±0.18	2.48±0.21
Phosphorus, mmol/L	1.68±0.60	1.73±0.59	1.65±0.60
Serum ferritin, ng/mL	282.08±257.01	200.73±242.86	336.31±254.75
25(OH)-vitamin D, ng/mL	33.84±27.41	59.42±25.72	16.79±9.17
Sclerostin, pg/mL	297.00 (156.85, 607.58)	632.35±369.18	228.85±188.92
LVMI, g/m ²	170.58±49.01	188.00±42.62	158.79±50.10
Dialysis vintage, months	24.00 (12.00, 60.00)	66.00 (24.00, 108.00)	18.00 (12.00, 30.00)
Alkaline phosphatase, U/L	74.90 (58.48, 112.23)	74.25 (58.83, 86.18)	75.80 (62.90, 115.10)
iPTH, pg/mL	275.00 (99.88, 571.58)	199.00 (50.83, 486.37)	277.20 (98.40, 497.10)
hsCRP, mg/L	1.37 (0.54, 4.01)	1.47 (0.48, 4.00)	1.19 (0.54, 4.05)
CAC score, AU	6.10 (0.00, 130.80)	91.80 (2.30, 665.15)	0.00 (0.00, 47.00)
FGF23, pg/mL	15,844.21 (2,875.01, 52,277.40)	24,907.51 (6,901.98, 53,736.12)	13,194.21 (1,916.72, 52,831.90)
Calcitriol use, n (%)	39 (60.0)	23 (88.5)	16 (41.0)
Ca-based phosphate binders use, n (%)	41 (63.1)	24 (92.3)	17 (43.6)
Ca-free phosphate binders use, n (%)	3 (4.6)	0	3 (7.7)
Calcimimetic use, n (%)	0	0	0

BALP, bone-specific alkaline phosphatase; LVMI, left ventricular mass index; iPTH, intact parathyroid hormone; hsCRP, hypersensitive C reactive protein; CAC score, coronary artery calcification score; FGF23, fibroblast growth factor 23; BMI, body mass index; Au, Agatston unit.

approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University. Written informed consent was obtained from each of the subjects.

Data on gender, age, body mass index, dialysis duration, primary disease, hypertension, and whether or not combined with diabetes mellitus were collected. During the follow-up period, blood biochemistry, intact PTH, sclerostin, FGF23, etc., imaging examinations such as echocardiogram and coronary artery CT scan were tested once a year.

Biochemical Measurement

Blood samples were collected from the participants in the morning after an overnight fast. Parameters of serum albumin, calcium, phosphorus, and alkaline phosphatase were measured by standard autoanalyzer techniques (Beckman AU5800). The hemoglobin level was analyzed with an automated hematology analyzer (Sysmex XT 4000i, GMI Inc.). The intact PTH (iPTH) level was measured using a UniCel DxI800 Access Immunoassay System (Beckman Coulter, Inc., Fullerton, CA, USA). Serum 25(OH)-D was measured by an electrochemiluminescence immunoassay (Modular Analytics E170, Roche Diagnostis, Mannheim, Germany). Bone alkaline phosphatase was determined using a solid-phase, monoclonal antibody immunoenzymetric assay (Ostase BAP, Immunodiagnostic Systems, Tyne & Wear, UK). Plasma FGF23 was measured using a human c-terminal ELISA kit (Immutopics, San Clemente, CA, USA). Serum sclerostin level was measured using ELISA (R&D

companies, America). LVMI was calculated according to the results of colored Doppler echocardiography.

Cardiac CT and CAC Score

Cardiac CT scans were performed using a 64-channel detector scanner (LightSpeed VCT; General Electric [GE] Healthcare, Milwaukee, WI, USA) in cine mode. Scans were ECG-gated, and a standard noncontrast protocol was used with a tube voltage of 100 kV, tube current of 200 mA, 350 ms rotation time, 2.5 mm slice thickness, and displayed field of 25 cm. Calcium deposits in the coronary arteries were identified by a radiologist with Level 2 competence and over 9 years of experience in interpretation of cardiac CT (JR). The data were processed and analyzed using an Advantage Workstation 4.4 (GE Healthcare). Smartscore 4.0 (GE Healthcare) was used to assess CAC scores. Calcified plaques were considered to be present if values crossed the standard threshold of 130 Hounsfield units. CAC scores were expressed in Agatston units (AUs) as described previously in detail. Total CAC score was calculated as the sum of the CAC scores in the left main artery, the left anterior descending artery, the left circumflex artery, and the right coronary artery.

Statistical Analysis

Normally distributed variables are expressed in mean ± standard error and non-normally distributed variables as median (25th and 75th percentiles), as appropriate. The correlation between the 2 variables was assessed by univariate regression. The comparison between

Table 2. Clinical and biochemical parameters according to values below and above median sclerostin

	Below median (<i>n</i> = 28)	Above median (<i>n</i> = 28)	<i>p</i> value
Male, <i>n</i> (%)	12 (42.9)	20 (71.4)	0.031
HD, <i>n</i> (%)	6 (21.4)	19 (67.9)	<0.001
Age, years	41.25±10.88	54.64±12.35	<0.001
Height, cm	164.43±9.82	167.11±7.67	0.340
Weight, kg	61.24±16.37	65.94±12.57	0.239
BMI, kg/m ²	22.37±4.37	23.50±3.74	0.307
Hemoglobin, g/L	106.08±15.88	104.96±14.44	0.692
Albumin, g/L	42.34±3.75	38.96±3.09	0.001
BALP, µg/L	20.05 (15.50, 30.40)	15.40 (11.70, 22.90)	0.068
Calcium, mmol/L	2.48±0.18	2.44±0.19	0.385
Phosphorus, mmol/L	1.64±0.50	1.76±0.72	0.472
Serum ferritin, ng/mL	222.50 (71.20, 405.10)	231.00 (89.70, 355.18)	0.961
25(OH)-vitamin D, ng/mL	16.37 (10.21, 37.43)	44.32±27.37	0.015
Kt/V	1.80 (1.46, 2.16)	1.34 (1.07, 1.72)	0.005
LVMI, g/m ²	158.00 (119.00, 203.00)	184.69±47.25	0.122
Dialysis vintage, months	24.0 (12.0, 60.0)	24.0 (12.0, 72.0)	0.232
Alkaline phosphatase, U/L	66.80 (55.43, 104.88)	74.80 (62.50, 91.40)	0.670
iPTH, pg/mL	310.00 (107.60, 691.80)	173.50 (36.40, 357.85)	0.023
hsCRP, mg/L	1.63 (0.37, 5.24)	1.98 (0.69, 4.22)	0.372
CAC score, Agatston unit	0 (0, 18.20)	81.65 (3.07, 636.35)	0.002
FGF23, pg/mL	11,507.85 (917.91, 30,062.27)	26,867.22 (7,698.13, 58,343.39)	0.091
Calcitriol use, <i>n</i> (%)	16 (57.1)	21 (75.0)	0.158
Ca-based phosphate binders use, <i>n</i> (%)	14 (50.0)	24 (85.7)	0.004
Ca-free phosphate binders use (%)	2 (7.1)	0	0.150
Calcimimetic use, <i>n</i> (%)	0	0	–

BALP, bone-specific alkaline phosphatase; LVMI, left ventricular mass index; iPTH, intact parathyroid hormone; hsCRP, hypersensitive C reactive protein; CAC score, coronary artery calcification score; FGF23, fibroblast growth factor 23; BMI, body mass index; Au, Agatston unit.

2 groups was made using either an independent *t* test or a Mann-Whitney U test for continuous variables. χ^2 test was used to compare the frequencies between groups. Univariate analysis was made using Pearson's correlation coefficients or Spearman's rank. To further analyze the relevant factors for calcification using the CAC score, we perform a multivariate analysis. A logistic regression model was used to screen the variables for CAC (no calcification: a CAC score of 0 and calcification: a CAC score of 1 or more). Independent risk factors associated with the patient survival were assessed using the Kaplan-Meier survival analysis and multivariate Cox regression model. Results are expressed as hazard ratios with 95% confidence intervals (95%). All analyses were performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corporation, Armonk, NY, USA). A probability (*p*) value <0.05 was considered statistically significant.

Results

Clinical and Biochemical Characteristics of the Maintenance Dialysis Patients

Our study population consisted of 65 maintenance dialysis patients undergoing hemodialysis or peritoneal di-

alysis. Demographics and biochemical characteristics of these patients are shown in Table 1. Of 65 participants included, 26 underwent hemodialysis and 39 received peritoneal dialysis. The causes of ESRD were chronic glomerulonephritis in 47 (72.3%) patients, diabetes in 6 (9.2%) patients, polycystic kidney disease in 6 (9.2%) patients, and the other disease also in 6 (9.2%) patients. The age, dialysis vintage, serum ferritin, 25 (OH)-vitamin D in hemodialysis patients were significantly higher than those in peritoneal dialysis patients. In contrast, serum albumin was obviously lower in hemodialysis patients than the patients undergoing the peritoneal dialysis.

Serum sclerostin in hemodialysis patients was significantly higher than that in the peritoneal dialysis patients (632.35 ± 369.18 vs. 228.85 ± 188.92 , $p < 0.001$). Similarly, compared with peritoneal dialysis patients, the hemodialysis patients had also significantly higher levels of CAC score ($91.8 [2.30, 665.15]$ vs. $0 [0, 47.00]$, $p < 0.001$) and LVMI (188.00 ± 42.62 vs. 158.79 ± 50.10 , $p = 0.026$).

Table 3. Demographics and biochemistry according to the presence or absence of CAC score

	Noncalcification 25 (39.70%)	Calcification 38 (60.30%)	<i>p</i> value
Male, <i>n</i> (%)	11 (44.0)	22 (57.9)	0.28
HD, <i>n</i> (%)	4 (16.0)	21 (55.3)	0.002
Age, years	39.76±10.30	53.61±11.72	<0.001
Height, cm	165.36±9.55	164.84±8.10	0.818
Weight, kg	62.03±15.31	62.78±13.97	0.842
BMI, kg/m ²	22.41±3.94	22.88±4.10	0.657
Hemoglobin, g/L	106.24±16.70	105.70±14.83	0.905
Albumin, g/L	41.70±4.53	39.91±3.11	0.126
BALP, µg/L	22.17±11.27	16.60 (12.90, 22.80)	0.461
Calcium, mmol/L	2.48±0.20	2.42 (2.35, 2.50)	0.536
Phosphorus, mmol/L	1.68±0.65	1.68±0.58	0.991
Serum ferritin, ng/mL	261.28±217.51	231.00 (84.07, 417.60)	0.855
25(OH)-vitamin D, ng/mL	15.87 (9.04, 29.64)	41.59±29.59	0.002
Kt/V	2.02±0.72	1.50±0.49	0.001
LVMI, g/m ²	168.00±52.43	172.32±47.28	0.747
Dialysis vintage, months	18.00 (9.60, 36.00)	33.00 (24.00, 75.00)	0.019
Alkaline phosphatase, U/L	65.20 (52.10, 106.60)	79.40 (65.75, 115.55)	0.061
iPTH, pg/mL	297.71±282.54	279.60 (134.05, 695.20)	0.286
hsCRP, mg/L	0.92 (0.43, 3.67)	1.55 (0.55, 4.09)	0.357
Sclerostin, pg/mL	187.40 (100.50, 327.20)	485.24±312.61	0.003
FGF23, pg/mL	24,823.29 (2,092.26, 47,813.16)	15,850.20 (3,933.29, 53,365.35)	0.673
Calcitriol use, <i>n</i> (%)	12 (48.0)	27 (71.1)	0.065
Ca-based phosphate binders use, <i>n</i> (%)	14 (56.0)	27 (71.1)	0.220
Ca-free phosphate binders use, <i>n</i> (%)	3 (12.0)	0	0.283
Calcimimetic use, <i>n</i> (%)	0	0	–

BALP, bone-specific alkaline phosphatase; LVMI, left ventricular mass index; iPTH, intact parathyroid hormone; hsCRP, hypersensitive C reactive protein; CAC score, coronary artery calcification score; FGF23, fibroblast growth factor 23; BMI, body mass index; Au, Agatston unit.

Table 2 shows the clinical and biochemical parameters in ESRD patients with sclerostin levels above and below the median. Compared with the patients with a serum sclerostin level below the median value of 297.0 pg/mL, those with a level above the median were characterized by male, older age, receiving hemodialysis, lower serum albumin level, lower Kt/V level, lower iPTH level, higher 25-(OH)-vitamin D level, higher CAC level, and higher Ca-based phosphate binders use.

Determinants of CAC in Maintenance Dialysis Patients

CAC was 60.3% patients. The prevalence of CAC was higher in hemodialysis patients than that in peritoneal dialysis. CAC was present in 84% of the hemodialysis patients and 44.7% of peritoneal patients. Table 3 compares the clinical and biochemical parameters between the patients with and without CAC. The patients with CAC were characterized by older age, longer dialysis duration, and lower Kt/V. The patients with CAC also had higher 25-(OH)-vitamin D, higher sclerostin, and had a higher

Table 4. Multivariate logistic regression model of risk factors for CAC score

Variables	Odds risk	95% CI	<i>p</i> value
Kt/V	0.087	0.016–0.484	0.005
Age, years	1.182	1.064–1.314	0.002

$R^2 = 0.592$, $\chi^2 = 31.455$, $p < 0.001$. CAC score, coronary artery calcium score; CI, confidence interval.

proportion of hemodialysis. However, levels of PTH, BALP, and FGF23 were not related to the CAC. In multivariate analysis, older age and high sclerostin level were correlated to the CAC.

In univariate logistic regression analysis, older age, lower Kt/V, and higher serum sclerostin level were associated with the presence of CAC. Multivariate logistic regression analysis revealed that older age and lower Kt/V were independent risk factors for CAC (Table 4). The

Fig. 2. ROC for prediction of CAC in maintained dialysis patients by sclerostin (AUC 0.74; $p = 0.03$). ROC, receiver operating characteristic curve; CAC, coronary artery calcification; AUC, area under the curve.

sclerostin level was not the independent factor related to the CAC ($p = 0.066$).

Receiver operating characteristic curves for prediction of CAC by sclerostin (area under the curve 0.74, 95% confidence interval 0.605–0.878, $p = 0.03$) are shown in Figure 2. The cutoff value of sclerostin was 217.55 pg/mL. The sensitivity was 0.829, and specificity was 0.619.

Risk Factors Influencing Survival of Maintenance Dialysis Patients

Over a follow-up period of 5 years, 51 (78.5%) of 65 patients were survived. The clinical and biochemical data of the patients in the survival or nonsurvival group are shown in Table 5. Kaplan-Meier survival analysis suggested that age <60 years ($p < 0.001$, log-rank test), CAC score <50 AU ($p = 0.010$, log-rank test), and serum sclerostin <400 pg/mL ($p = 0.017$, log-rank test) were associated with better survival (shown in Fig. 3). Cox regression analyses revealed that age (≥ 60 years, $p < 0.001$) and CAC score (≥ 50 AU, $p = 0.031$) were significant risk factors for patient survival (Table 6).

Discussion

Our study showed that high sclerostin level was related to CAC in maintenance dialysis patients. However, the sclerostin level was not the independent factor correlated to the CAC or patient survival. In the last decade, the Wnt signaling inhibitor, sclerostin, emerged as a key regulator

of the bone metabolism. Recently, sclerostin was also demonstrated to be expressed in calcifying vasculature [22]. Since there are some similarities between the mechanism of the bone metabolism and vascular calcification, more and more studies have focused on the relationship between bone metabolic markers and the vascular calcification [23]. As an important bone metabolic marker, sclerostin has been shown to be associated with the bone mineral density and bone turnover, though the nature of this association is not yet fully understood [7, 24, 25]. In addition, sclerostin antibody treatment could induce rapid and sustained increase in bone formation, bone mass, and bone strength in nonoperated bones in rats [26]. However, the clinical application of sclerostin antibodies as an effective treatment of osteoporosis is still in phase III trial [27]. Several clinical and biological variables have been described to be associated with the sclerostin concentration, such as age, CKD, PTH levels, and other bone biomarkers [28, 29].

Vascular calcification is common in CKD patients. Even in the early course of CKD when the serum phosphate level was in a normal range, vascular calcification occurred [9]. With CKD progression, the prevalence of vascular calcification is higher in ESRD patients. Furthermore, some studies reported some biological factors influence the relationship between vascular calcification and CKD-MBD [30, 31].

In the present study, we examined a number of new biological factors including BALP, FGF23, and sclerostin to determine which biomarker was associated with vascular calcification in ESRD patients. In line with some previous studies, we found that there was a positive association between the sclerostin levels and CAC [32, 33]. We could not demonstrate a correlation between higher levels of phosphorus, FGF23, BALP or iPTH, and CAC. Hernandez et al. [34] also found that in dialysis patients with severe hyperparathyroidism, PTH levels were not significantly correlated with CAC. After studying 89 epigastric artery biopsies from patients with end-stage renal disease, Qureshi et al. [17] also found that only sclerostin instead of FGF23 and BALP predicted vascular calcification among the circulating biomarkers of MBD. In contrast to these results, Claes et al. [21] studied 159 CKD patients with an average eGFR of 34 mL/min/1.72 m² and found that the association between sclerostin and aortic calcification switched from positive to negative when “CVD history” was introduced in the multivariate analysis [21]. Whether or not the “CVD history” should be included in the multivariate model is controversial because CVD is the outcome of vascular calcification rather than

Table 5. Clinical characteristics between survivors and nonsurvivors

	Nonsurvivors (n = 14)	Survivors (n = 51)	p value
Male (%)	9 (64.3)	25 (49.0)	0.375
HD (%)	7 (50.0)	19 (37.3)	0.539
Age, years	61.43±7.07	42.00 (37.00, 51.00)	<0.001
Height, cm	163.21±7.94	165.65±8.73	0.351
Weight, kg	62.86±11.76	62.99±15.40	0.978
BMI, kg/m ²	23.30±3.29	22.75±4.47	0.679
Dialysis vintage, months	24.00 (10.50, 63.00)	24.00 (12.00, 60.00)	0.619
Hemoglobin, g/L	111.50±19.77	104.63±15.77	0.177
Albumin, g/L	39.33±2.95	41.25±4.23	0.116
BALP, µg/L	15.15 (12.98, 29.27)	19.50 (13.20, 22.95)	0.865
Calcium, mmol/L	2.40±0.12	2.47±0.22	0.241
Phosphorus, mmol/L	1.66±0.70	1.69±0.57	0.842
Serum ferritin, ng/mL	177.95 (72.68, 719.85)	228.40 (85.20, 380.00)	0.848
25(OH)-vitamin D, ng/mL	37.64±29.31	22.44 (11.40, 46.84)	0.468
Kt/V	1.63±0.76	1.76±0.61	0.522
LVMI, g/m ²	180.64±59.46	168.17±46.62	0.454
Alkaline phosphatase, U/L	80.25 (68.45, 118.48)	74.50 (56.70, 102.50)	0.207
iPTH, pg/mL	163.85 (37.75, 549.18)	272.80 (107.60, 459.80)	0.430
hsCRP, mg/L	2.19 (0.58, 9.17)	1.24 (0.48, 3.75)	0.219
Sclerostin, pg/mL	564.04±354.64	257.25 (136.63, 478.10)	0.042
CAC score, Agatston unit	84.50 (11.40, 748.75)	1.30 (0.00, 91.80)	0.008
FGF23, pg/mL	3,637.13 (391.09, 64,826.56)	15,844.21 (4,982.98, 49,109.03)	0.297
Calcitriol use (%)	10 (71.4)	29 (56.9)	0.324
Ca-based phosphate binders use (%)	10 (71.4)	31 (60.8)	0.465
Ca-free phosphate binders use (%)	0	3 (5.9)	0.310
Calcimimetic use (%)	0	0	–

BALP, bone-specific alkaline phosphatase; LVMI, left ventricular mass index; iPTH, intact parathyroid hormone; hsCRP, hypersensitive C reactive protein; CAC score, coronary artery calcification score; FGF23, fibroblast growth factor 23 BMI, body mass index; Au, Agatston unit.

its cause. From a clinical point of view, the description of sclerostin as the cross talk between bone and vasculature is still an illusion. The expression of local sclerostin in blood vessels or bones was not related to circulating sclerostin levels, which may partly explain the inconsistency of the previous literatures. Qureshi et al. [17] found that vascular sclerostin mRNA and protein expressions did not differ between calcified and noncalcified vessels, suggesting that the vasculature is not a major contributor to circulating levels. Another study confirmed that the content of sclerostin in bone biopsies was increased despite a concomitant decrease of sclerostin in the serum after a successful kidney transplant [35]. These findings illustrate the limitations of the circulating sclerostin and the critical role of the bone biopsy to understand osteocyte biology in CKD-MBD [36]. Additional clinical and experimental studies are needed to clarify the mechanism of sclerostin in the vascular calcification.

In terms of patient survival, this study revealed that patients in the survival group were younger and had lower sclerostin levels and CAC scores. But after adjusting to correlation factors, older age and higher CAC scores were independent risk factors for high mortality. However, reports on the relationship between sclerostin and patient mortality were highly controversial. After 18 months of follow-up of 673 dialysis patients (HD and PD), the results of the Dutch Cooperative Dialysis Adequacy Study showed that the higher the sclerostin level, the lower the risk of cardiovascular death and all-cause mortality [15]. Contrary to this study, Gonçalves et al. [14] found that the high base level of serum sclerostin was associated with the worse survival in 91 hemodialysis patients. Kanbay et al. [37] examined 173 nondialyzed CKD patients and 47 control patients to show that higher sclerostin level was associated with fatal and nonfatal cardiovascular events. However, the relationship between the serum sclerostin level and all-cause mortality

Fig. 3. Kaplan-Meier patient survival curve for maintained dialysis patients with different risk factors. **a** Age. **b** Sclerostin level. **c** CAC score. CAC, coronary artery calcification.

Table 6. Cox proportional HR of patient survival (multivariate analysis)

Variable	HR	95% CI	<i>p</i> value
Age (≥60 years)	10.641	2.859–39.604	<0.001
CAC score (≥50 AU)	0.288	0.093–0.890	0.031

HR, hazard ratio; CI, confidence interval; CAC, coronary artery calcification; AU, Agatston unit.

disappeared after multivariable Cox regression adjustment. Consistent with the previous study, a meta-analysis also demonstrated that circulating sclerostin level was an independent risk factor of all-cause and cardiovascular mortality [38]. In this study, we also found that sclerostin increased significantly in non-survival patients, but its significance

disappeared after Cox regression analysis. The reason may be the small number of patients enrolled or the relatively short follow-up duration.

We acknowledge some limitations of our study. First, this is a single-center study, with a small sample size, which may confer the risk of a type 1 statistical error. Second, some baseline characteristics of PD patients at the time of enrollment were different from those of HD patients. Combining PD and HD patients to describe demographic and biochemical data might lead to results bias. Third, as a survival analysis, the 5-year follow-up period was relatively short, and more risk factors may not be found. Fourth, we lack the pathological data on the bone and vessel, which are crucial to delineate the role of sclerostin in the cross talk between bone and vasculature.

We conclude that hemodialysis patients with CAC had significantly higher levels of sclerostin. But sclerostin is

not a risk factor for CAC. In terms of patient outcomes, lower levels of sclerostin were in the survival patients. But sclerostin levels are not independent risk factors for high mortality in dialysis patients. Whether sclerostin is simply a marker or a central mediator of CAC and mortality in dialysis patients needs to be elucidated by additional studies.

Statement of Ethics

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Nanjing, Jiangsu, China) and all the patients signed a consent form prior to the study participation.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Research idea and study design: Yifei Ge and Changying Xing; data acquisition: Xueqiang Xu and Xiangbao Yu; data analysis/interpretation: Ningning Wang and Ming Zeng; statistical analysis: Buyun Wu; supervision or mentorship: Bo Zhang and Huijuan Mao.

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