

# High Serum Sclerostin Levels Are Associated with a Better Outcome in Haemodialysis Patients

Guillaume Jean<sup>a</sup> Charles Chazot<sup>a</sup> Eric Bresson<sup>c</sup> Eric Zaoui<sup>b</sup>  
Etienne Cavalier<sup>d</sup>

<sup>a</sup>Nephrocare Tassin-Charcot, Nephrology Haemodialysis, and <sup>b</sup>Novescia Rhône-Alpes, Laboratoire du Grand Vallon, Sainte Foy-les-Lyon, <sup>c</sup>Norimagerie, Infirmerie Protestante, Radiology, Caluire et Cuire, France; <sup>d</sup>Department of Clinical Chemistry, University of Liege, CHU Sart-Tilman, Liege, Belgium

## Key Words

Sclerostin · Haemodialysis · Survival rate · Bone mineral density · Alfacalcidol · Vascular calcification

## Abstract

**Background:** Sclerostin is an osteocyte hormone that decreases osteoblastogenesis. Sclerostin may play a key role in osteoporosis and also in vascular calcification (VC). In chronic kidney disease and haemodialysis (HD) patients, serum sclerostin levels are high. **Aim:** To assess the correlation of serum sclerostin levels with VC, bone mineral density (BMD), and survival rate in HD patients. **Methods:** A cross-sectional study was conducted in prevalent HD patients to correlate serum sclerostin tertiles with the Kauppila aortic calcification score, BMD scores and survival rate. **Results:** We studied 207 patients who had a mean serum sclerostin level of  $1.9 \pm 0.7$  ng/ml. Compared to patients in the 1st tertile of serum sclerostin levels (0.6–1.53 ng/ml), patients in the 3rd tertile (2.2–4.6 ng/ml) were significantly older ( $73.7 \pm 12$  vs.  $64.7 \pm 18$  years), more frequently of the male gender (74 vs. 48%), had lower serum bone-specific alkaline phosphatases values ( $14 \pm 9$  vs.  $20.4 \pm 13$  µg/l), were less frequently treated with alfacalcidol, displayed lower aortic calcification scores ( $9.5 \pm 5$  vs.  $12.5 \pm 7/24$ ) and had higher BMD scores. Furthermore,

patients of the 3rd tertile displayed a lower mortality rate compared to tertile 1 using multivariable adjusted Cox model (hazard ratio 0.5, 95% CI 0.25–0.93,  $p = 0.03$ ). The main factors associated with VC score were age, diabetes, cardiovascular disease, CRP level and Warfarin use. **Conclusion:** Our study of HD patients shows that higher serum sclerostin levels are associated with higher BMD, lower aortic calcification scores, and a better survival rate.

© 2016 S. Karger AG, Basel

## Introduction

Mineral and bone disorders are frequently observed in chronic kidney disease and haemodialysis (HD) patients, together with other physiological and cardiovascular abnormalities [1]. Deficiencies in calcitriol [2] and Klotho protein [3], together with phosphate retention [4], can lead to secondary hyperparathyroidism (SHPT). Sex hormone disorders, acidosis, metal accumulation, malnutrition, inflammation, diabetes and oxidative stress may also have consequences on bone metabolism [5, 6]. However, the physiological relationship between bone and cardiovascular disease is not well understood [7, 8]. Numerous bone proteins have been shown to be associated with pa-

tient outcome in CKD, such as osteoprotegerin (OPG) [9], fibroblast growth factor (FGF)-23 [10], bone-specific alkaline phosphatase (b-ALP) [11], and more recently sclerostin [12, 13].

Sclerostin is a 22 kDa glycoprotein product of the *SOST* gene in osteocytes that inhibits osteoblast and bone formation. The canonical Wntless-type mouse mammary tumour virus integration site (Wnt) pathway has a bone anabolic and anti-catabolic effect. Sclerostin acts as an inhibitor of the Wnt-coreceptor LRP5/6. In the deep mineralized bone, osteocytes can detect mechanical strain. When bone is subjected to mechanical forces, sclerostin is not secreted, and bone formation occurs. Therefore, sclerostin appears to play an important role in skeletal adaptation to mechanical forces [14]. Wnt signalling inhibits bone resorption and up-regulates OPG, which binds and inhibits receptor activator of nuclear factor  $\kappa$ B-ligand [15]. Serum sclerostin levels are increased in CKD and HD patients [16]. Whether this is due to decreased clearance or excess production has not yet been fully assessed. Cejka et al. [17] reported that in HD patients, serum levels of sclerostin are negatively correlated with parathyroid hormone levels, and positively correlated with bone mineral density (BMD) and bone volume [18]. In the animal model, Wnt signalling has been implicated in artery and valve calcification, and increased sclerostin expression has been demonstrated during vascular smooth muscle cell calcification [19]. In HD patients, it has been observed that sclerostin is locally produced in aortic valve tissue adjacent to areas of calcification [20].

Recently, Morena et al. [21] reported that in non-dialysis CKD patients, both high serum OPG and sclerostin levels are associated with vascular calcification; however, Claes et al. [22] reported discordant data. Viaene et al. [23] reported that high serum sclerostin levels are associated with better survival rate in HD patients. Thus, the role of sclerostin remains controversial.

The aim of our study, therefore, is to study the correlation of serum sclerostin levels with vascular calcification [23], BMD, and survival rate using conventional HD, on-line-post-dilution haemodiafiltration (HDF), and long dialysis sessions.

## Methods

Existing HD patients were enrolled in the study in July 2012, and were observed during 30 months. Exclusion criteria included the presence of monoclonal gammopathy and myeloma and other

malignancies, jaundice and specific bone diseases. The study was conducted in compliance with the Declaration of Helsinki and all patients gave their consent.

Patients were dialyzed thrice weekly (4–8 h) using polysulfone high-flux filters FX 60, 80, 100, 800 and 1,000 (Fresenius Medical Care®, Bad Homburg, Germany) in HD or online post-dilution HDF. Blood flow rate ranged from 220 to 400 ml/min, dialysate flow rate ranged from 350 to 800 ml/min. The standard dialysis calcium concentration was 1.5 mmol/l; however, 1.25 mmol/l was prescribed in cases where PTH levels were low (<100 pg/ml) and 1.75 mmol/l was recommended in cases where the PTH levels were high (>400 pg/ml).

The following patient information was recorded: medical history, including cardiovascular events and risk factors; treatments, including statins, warfarin, vitamin D, cinacalcet, and phosphate binders; and baseline results from standard laboratory tests.

Blood samples were obtained from nonfasting patients before a mid-week dialysis session. All laboratory parameters were measured in the same blood draw. Serum sclerostin levels were measured with ELISA (Sclerostin TECO® High sensitive, TECO Medical Sissach, Switzerland); reference values were  $0.83 \pm 0.22$  ng/ml in men,  $0.66 \pm 0.22$  in post-menopausal women and  $0.59 \pm 0.22$  in pre-menopausal women. Serum FGF-23 concentration was determined using the FGF-23 (C-Term) enzyme-linked immunosorbent assay kit (Immutopics, Inc., San Clemente, Calif., USA); both preceding assays were performed in the Clinical Chemistry Department, University of Liège (CHU Sart Tilman, Liège, Belgium). Intact PTH was measured using a second-generation assay (ElecSysG; Roche® Diagnostics, Meylan, France), reference values were 14–65 pg/ml. Measurements of b-ALP (Chemiluminescence, Beckman® Access, reference values 3.7–20  $\mu$ g/l) and  $\beta$ -Cross-Laps (CTX; Elecsys, Roche® Diagnostics, Meylan, France) were used as bone markers. 25-Hydroxyvitamin D (25-(OH)D) analyses were performed using Architect automat (Abbott® Laboratories, Abbott Park, Ill., USA). Calcitriol (1,25-(OH)<sub>2</sub>D) was measured after extraction, using chemiluminescence (LIAISON; DiaSorin® Inc., Stillwater, Minn., USA). Single-pool Kt/V was calculated using the second-generation logarithmic formula of Daugirdas. Daily protein intake was measured by calculating normalized protein nitrogen appearance. Common laboratory analyses were performed by the Grand Vallon Laboratory (NOVESCIA®, Lyon, France), and b-ALP and calcitriol were assayed by Biomnis® Laboratory, Lyon, France.

Standard radiological exams included BMD and a profile lumbar radiograph for aortic calcification assessment. BMD using DXA (HOLOGIC B QDR 4500C) and a regular radiological study were performed the same day. A t-score, for which the BMD of a young adult was used as a reference, and a Z-score, which was compared with the same-age population, were recorded at the ultradistal-radius of the forearm (with no vascular access), and at the femoral neck. BMD references curves used for women were the French cohorts (OFELY-ISOS-GENSET) for the spine, the National Health and Nutrition Examination Survey (NHANES III) for the hip and the OFELY for the forearm. For the men, American white curves were used with the NAHNES for the hip and the Kelly cohort for the other sites. The Kauppila score was calculated based on lateral aortic calcification scores, as described previously [24].

### Statistical Analysis

Results are reported as the means  $\pm$  SD. Due to the lack of recommendations for clinical thresholds of sclerostin, the patients were categorized into tertiles according to sclerostin levels. The serum sclerostin tertiles were compared using Student's *t* test or the Mann-Whitney *U* test, based on variable distributions. Fisher's exact test was used to compare proportions. Logistic regression was applied for factors associated with a serum sclerostin level higher than the median value. A regression correlation was applied when necessary. Kaplan-Meier and backward Cox proportional hazards models were used for the 30-month survival analysis. Variables that affected all-cause mortality in univariate analysis ( $p < 0.05$ ) were included in a multivariate Cox proportional hazards analysis by backward elimination at  $p < 0.05$ . The relative risk of death was expressed as a hazard ratio (HR). In each approach, data were censored at the time of transplantation, when the patient was transferred to another dialysis centre, when the patient was lost to follow-up or at the end of follow-up. All statistical analyses were performed using MedCalc software version 11.5.1.0 (MedCalc Software, Ostend, Belgium). Differences with  $p$  values  $\leq 0.05$  were considered statistically significant.

### Results

At the time of enrolment (July 2012), 227 patients were present in our centre. Of these, 20 patients were excluded due to active malignancy ( $n = 8$ ), myeloma ( $n = 2$ ), glucocorticoid-induced osteoporosis ( $n = 2$ ), recent parathyroidectomy ( $n = 2$ ) and actual hospitalization ( $n = 6$ ). The remaining 207 patients were included with the following characteristics: mean age of  $70.2 \pm 14$ ; 43% of female sex; 36.2% with diabetes; vintage  $65.7 \pm 83$  months; thrice weekly session duration of  $297 \pm 75$  min, post-dilutional online HDF in 27% of cases; and mean dialysate calcium concentration of  $1.5 \pm 0.17$  mmol/l.

Baseline mean serum sclerostin was  $1.9 \pm 0.7$  ng/ml; the distribution plot is displayed in figure 1. Baseline characteristics of patients in the 3 tertiles, according to the serum sclerostin levels, are displayed in table 1. Compared to patients in the 1st tertile (serum sclerostin: 0.6–1.53 ng/ml), patients in the 3rd tertile (2.24.6 ng/ml) were significantly older ( $73.7 \pm 12$  vs.  $64.7 \pm 18$  years,  $p < 0.001$ ); more frequently of the male gender (74 vs. 48%,  $p < 0.001$ ); had lower serum b-ALP values ( $14 \pm 9$  vs.  $20.4 \pm 13$ ,  $p < 0.05$ ); were less frequently treated with alfacalcidol (13 vs. 31%,  $p < 0.05$ ); had lower aortic calcification scores ( $9.5 \pm 5$  vs.  $12.5 \pm 7$ ,  $p < 0.05$ ; fig. 2); had lower Kauppila score  $>12/24$  (40 vs. 61%,  $p < 0.05$ ); and had higher BMD scores (hip and ultra-distal radius t-scores; fig. 3). By contrast, serum PTH, 25-(OH)D, 1,25(OH)<sub>2</sub>-D (calcitriol) and FGF-23 levels were not significantly different between the 1st and 3rd sclerostine

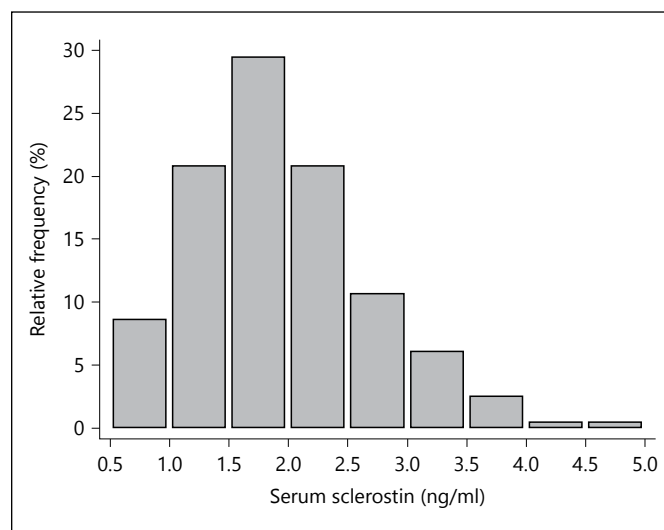


Fig. 1. Serum sclerostin levels distribution plot.

tertiles. Logistic regression of main factors associated with serum sclerostin tertiles are displayed in table 2. Factors significantly associated with aortic vascular calcification tertiles are displayed in table 3. Together with age, diabetes, cardiovascular disease, warfarin and central venous catheter use, and high serum level of CRP, lower serum sclerostin level is associated with a higher Kauppila score tertile.

Factors associated with hip t-score tertiles are displayed in table 4. Only lower dialysis vintage and higher serum sclerostin levels are associated with the higher BMD tertile.

The regression of Kauppila score vs. BMD score: hip t-score ( $r = 1.4$ ,  $p = 0.03$ ) and radius t-score ( $r = 0.2$ ,  $p = 0.009$ ), appeared weak even if significant.

On the 207 initial patients included, 66 (31.8%) died during the 30-month period. Only baseline age, dialysis vintage, cardiac disease, peripheral vascular disease, stroke and Kauppila calcification score were significantly ( $p < 0.05$ ) different between dead and alive patients.

Kaplan-Meier survival analysis (fig. 4) indicated that patients in the 3rd tertile had lower mortality rate after 30 months (tertile 3 vs. 2: HR 0.56, 95% CI 0.32–0.96,  $p = 0.04$ ; tertile 3 vs. 1: HR 0.63, 95% CI 0.39–0.98,  $p = 0.04$ ). This was confirmed using a multivariable adjusted Cox model (HR 0.5, 95% CI 0.25–0.93,  $p = 0.03$ ) for the 3rd tertile vs. the 1st tertile (table 5). HRs for all-causes mortality associated with tertiles 2 and 3 compared to tertile 1 using unadjusted and multivariable adjustment are displayed in figure 5.

**Table 1.** Baseline characteristics according to the serum sclerostin tertiles

Sclerostine tertiles, ng/ml	1st tertile 0.6–1.53 (n = 67)	2nd tertile 1.58–2.2 (n = 64)	3rd tertile 2.2–4.6 (n = 66)
Age, years	64.7±18	73±12	73.7±12**
Female gender, %	62	40	26**
Dialysis vintage, months	57.2±75	65.7±90	74.7±83
Diabetes, %	34.3	43.8	32
Body weight, kg	65.3±18	67.5±17	72.5±14
BMI, kg/m <sup>2</sup>	24.5±6	24.6±6	25.3±4
Peripheral vascular disease, %	19	25.4	19.7
Stroke, %	14.5	10.9	10.6
Cardiac disease, %	38	34	28
Parathyroidectomy, %	9	3	9
Dialysis session time, h:min	5:00±1:25	4:55±1:30	5:06±1:30
Dialysate calcium, mmol/l	1.53±0.17	1.48±0.17	1.51±0.19
On-line HDF, %	22	35	21
Central venous catheter, %	23	24	16
Kt/V	2.2±0.7	1.9±0.4	1.9±0.5
nPCR, g/kg/day	1.19±0.4	1.09±0.3	1.09±0.3
Sclerostine, ng/ml	1.1±0.2	1.87±0.16	2.8±0.6**
FGF-23, RU/ml	3,580±3,900	3,332±4,500	6,900±19,500
25-(OH)D, nmol/l	89±30	85.9±29	101±25
1,25-(OH) <sub>2</sub> D, pmol/l	88±45	82±50	83±51
Calcaemia, mmol/l	2.2±0.16	2.17±0.15	2.19±0.16
Phosphataemia, mmol/l	1.4±0.3	1.33±0.3	1.42±0.3
PTH, pg/ml	227±170	182±132	213±123
b-ALP, µg/l	20.4±13	20.2±15	14±9*
CTX, µg/l	1.6±0.8	1.6±0.9	1.5±0.9
CRP, mg/l	10.4±13	12.4±22	13±15
Albumin, g/l	35±4	34.3±4	35.2±4
Cholecalciferol, %, 100,000/week	88	93	81
Alfacalcidol, %, µg/week	31 (2.1±3)	20 (1.7±2)	13 (1.2±2)*
Oral calcium, %, g/day	21 (1.2±3)	33 (1.3±3)	28 (1.2±3)
Sevelamer, %, g/day	33 (3±5)	31 (3.1±4.6)	29 (3.5±6)
Cinacalcet, %, mg/day	13 (58±77)	10 (48±88)	6 (25±60)
Warfarin, %	21	20.3	21
Aortic calcification score (/24)	12.5±7	11.6±6	9.5±5*
Kaupila score >12/24, %	61	50	40*
Hip t-score	-2.7±1.1	-2.3±1.2	-2±1.5*
Ultra distal radius t-score	-3.6±1.7	-3.1±1.9	-2.4±1.7*

\* p &lt; 0.05; \*\* p &lt; 0.001 between the 1st and 3rd tertile.

## Discussion

Our results show that higher serum sclerostin levels are found mostly in aged male HD patients, and these levels are associated with higher BMD, lower b-ALP, less aortic calcification, and lower mortality.

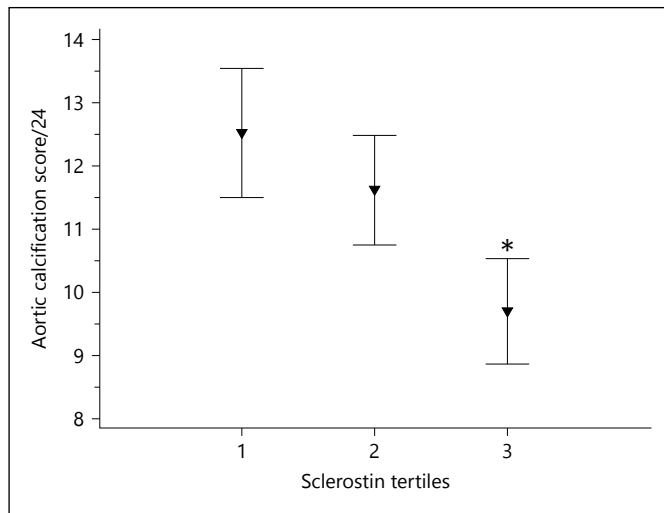
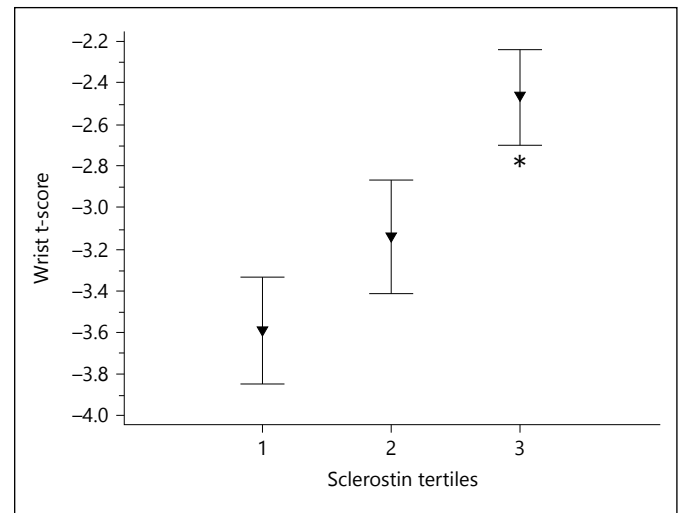
### *Sclerostin and CKD*

Sclerostin is a 22 kDa glycoprotein secreted by osteocytes, which is eliminated by the kidney, and may be re-

tained in CKD patients. Its precise metabolism is not known. In a cross-sectional study, Claes et al. [22] reported an increase in serum sclerostin when the glomerular filtration rate is decreased. Pelletier et al. [25] and Thambiah et al. [16] have published similar findings. However, the increasing mean age that parallels CKD stages could be a significant bias. The decrease of renal function with age, together with frequent immobility, has been hypothesized as a main cause of increased sclerostin levels observed in the elderly [25]. Recently, Cejka et al. [26] re-

**Table 2.** Logistic regression of factors associated with high serum sclerostin levels (>median value, 1.87 ng/ml)

Variable	OR	Coefficient	SE	p value	95% CI
Age, years	1.05	0.055	0.015	0.0002	1.026–11.089
Female gender, %	0.16	–1.79	0.4	<0.0001	0.075–0.362
Diabetes, %	1.24	0.21	0.39	0.58	0.569–2.711
Dialysis vintage, months	1.006	0.006	0.002	0.01	1.0015–11.011
Kauppila score (/24)	0.88	–0.12	0.03	0.0001	0.836–0.943
Hip t-score	1.73	0.55	0.15	0.0003	1.286–2.351

**Fig. 2.** Aortic calcification score according to the serum sclerostin levels tertiles. \*  $p < 0.05$ .**Fig. 3.** Ultra distal radius (wrist) t-score according to serum sclerostin levels tertiles. \*  $p < 0.05$ .

ported increased renal sclerostin elimination in CKD. Thus, increased sclerostin synthesis has been hypothesized to explain the higher serum sclerostin values in CKD patients.

#### *Sclerostin and Bone Mass*

We observed a clear relationship between BMD and serum sclerostin levels, as reported previously by Cejka et al. [18] in HD patients. However, Szulc et al. [27] found a similar relationship in male patients with normal renal function, and this association has been reported in post-menopausal women without CKD [28]. Moreover, individuals with genetically high bone mass have higher serum sclerostin levels than controls [29]. This could be in apparent contradiction with the known impact of sclerostin on post-menopausal osteoporosis [30]. Hence, in post-menopausal women, high serum sclerostin levels predict osteoporotic fractures more than other bone makers [31, 32]. Besides, Malluche et al. [33] reported

that baseline serum sclerostin level correlated with bone mass, but 1-year follow-up showed that high serum sclerostin predicts the loss of more bone mass. A possible explanation could be that serum sclerostin levels parallel osteocyte number and therefore bone mass.

Apart from serum sclerostin, we observed that only dialysis vintage is significantly, but negatively associated with BMD.

#### *Sclerostin and Bone Turnover*

In mice, it has been shown that PTH decreases osteocyte expression of sclerostin and gene SOST transcription [34]. In humans, there is a negative relationship between serum PTH and sclerostin levels [35]. The MINOS study of 710 males over 50 years reported a negative correlation between serum sclerostin values and bone turnover markers [27].

In this study, we observed that higher sclerostin levels were associated with lower serum bone-ALP levels.

**Table 3.** Baseline characteristics according to the aortic calcification score tertiles

Kaupila score (/24)	1st tertile 0–7	2nd tertile 8–16	3rd tertile 17–22
Age, years	63.8±17*	73.7±12	75.2±10
Female gender, %	40.8	44.4	40.3
Dialysis vintage, months	69.5±71	66.5±91	64±85
Diabetes, %	17*	36*	56*
Body weight, kg	66.4±17	69.5±16	70.4±17
BMI, kg/m <sup>2</sup>	23.5±5	25.2±5	25.3±5
Peripheral vascular disease, %	7	16	42*
Stroke, %	5.6	12.7	17.7
Cardiac disease, %	8.7	32.8	55.7*
Parathyroidectomy, %	7	6.3	6.4
Dialysis session time, h:min	5:00±1:28	5:05±1:26	4:39±1:21
Dialysate calcium, mmol/l	1.54±0.16	1.5±0.18	1.48±0.17
On-line HDF, %	25.4	28.6	25.8
Central venous catheter, %	12.7	17.5	37.1*
Kt/V	2.1±0.6	2±0.5	1.9±0.4
nPNA, g/kg/day	1.2±0.3	1.14±0.2	1.06±0.2
Sclerostine, ng/ml	2.15±0.7	2±0.7	1.8±0.6*
FGF-23, RU/ml	3,867±5,000	5,543±19,200	4,760±62,010
25-(OH)D, nmol/l	94±31	94.2±29	88±24
1,25-(OH) <sub>2</sub> D, pmol/l	88.4±49	85.6±48	81.1±39
Calcaemia, mmol/l	2.18±0.17	2.19±0.13	2.19±0.13
Phosphataemia, mmol/l	1.41±0.5	1.47±0.4	1.36±0.35
PTH, pg/ml	232±156	192±140	206±147
b-ALP, µg/l	18.7±14	16.5±11	18.5±14
CTX, µg/l	1.8±1*	1.6±0.8	1.45±0.7*
CRP, mg/l	10.2±19	8.6±11	18±23*
Albumin, g/l	36.2±4	34.7±3	33.6±4*
Cholecalciferol, %, 100,000/week	89.1	85.3	93.2
Alfacalcidol, %, µg/week	25.4 (2.2±3)	19 (1.3±2.2)	21.3 (1.5±3)
Oral calcium, %, g/day	28.2 (1.3±3)	31.2 (1.3±3)	20 (1.1±2)
Sevelamer, %, g/day	31.1 (3.1±5)	33.3 (3.2±5)	28 (3.3±4)
Cinacalcet, %, mg/day	12.7 (51±80)	6.3 (43±65)	16.1 (49±77)
Warfarin, %	8.7	12.7	42**
Aortic calcification score (/24)	2.7±2.8*	12.5±2.3	19.3±1.6*
Hip t-score	-2.1±1.2	-2.4±1.4	-2.5±1.4
Ultra distal radius t-score	-2.6±1.8	-3.1±2	-3.4±1.9

\* p &lt; 0.05; \*\* p &lt; 0.001 between the 1st and the 3rd tertile values.

This has also been reported by Viaene et al. [13] and Drechsler et al. [12] in HD patients, but not by Moysés et al. [36] using 2 different assays. Based on bone histology, Cejka et al. [17] reported that serum sclerostin and PTH levels were inversely related, and that low serum sclerostin levels were superior to high PTH levels in predicting high bone turnover. Bone resistance to PTH action [37] could explain the lacks of sclerostin inhibition in SHPT frequently observed in CKD and HD patients. In our study, we did not observe any association between serum sclerostin and PTH levels, as reported by

Morena et al. [21] in CKD non-dialyzed patients and Viaene et al. [13] and Moysés et al. [36] in HD patients. This discrepancy with other studies [12, 17, 22], is not understood.

The relationship between serum sclerostin and FGF-23 levels has not been well studied. Asamiya et al. [38] reported a positive association with low serum PTH level in HD patients. In animal models, PTH infusion decreases sclerostine expression, but increases FGF-23 secretion [39]. We failed to observe any relationship between these 3 hormones in our study.



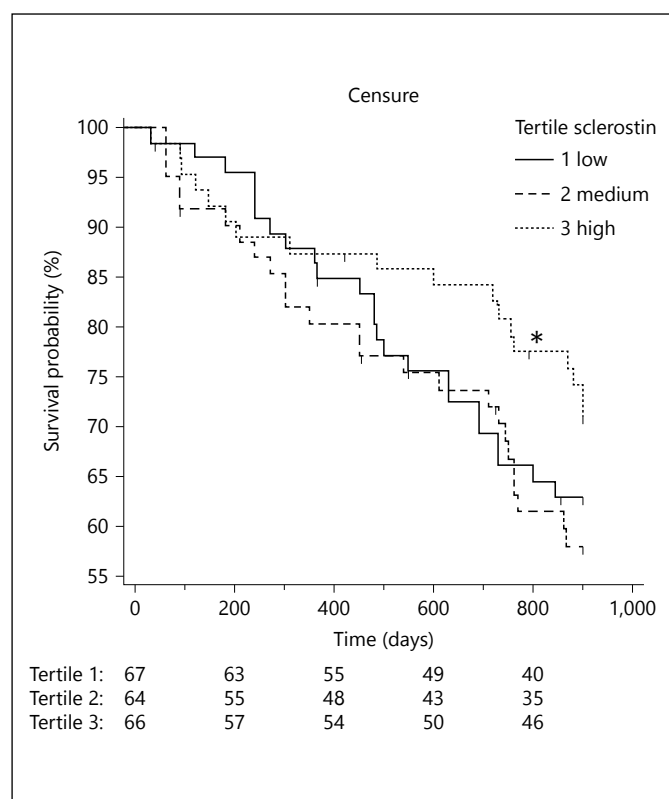
**Table 4.** Baseline characteristics according to the hip t-score tertiles

Hip t-score	1st tertile −5.6 to −3	2nd tertile −2.9 to −1.8	3rd tertile −1.7 to 2.3
Age, years	72.5±11	68.3±15	71.7±13
Female gender, %	33	47.4	49.2
Dialysis vintage, months	85±102	67±78	48.5±55*
Diabetes, %	38.6	36.8	38.1
Body weight, kg	67.4±16	67.1±17	72.5±16
BMI, kg/m <sup>2</sup>	24.4±5	24.9±5	26±5
Peripheral vascular disease, %	21.4	21.4	23.8
Stroke, %	15.7	10.5	7.8
Cardiac disease, %	30.4	29.1	41
Parathyroidectomy, %	7.1	7	4.7
Dialysis session time, h:min	5:01±1:24	4:58±1:31	4:53±1:25
Dialysate calcium, mmol/l	1.52±0.18	1.5±0.17	1.51±0.17
On-line HDF, %	22.9	29.8	27
Central venous catheter, %	24.3	22.8	20.6
Kt/V	2±0.6	2.1±0.6	1.9±0.5
nPNA, g/kg/day	1.1±0.2	1.2±0.2	1.12±0.2
Sclerostine, ng/ml	1.8±0.7	1.87±0.7	2.3±0.7*
FGF-23, RU/ml	2,960±2,900	4,572±6,600	6,000±19,900
25-(OH)D, nmol/l	94.8±29	89±30	95.3±24
1,25-(OH) <sub>2</sub> D, pmol/l	86.3±46	78±41	90.7±50
Calcaemia, mmol/l	2.19±0.13	2.19±0.13	2.2±0.13
Phosphataemia, mmol/l	1.31±0.3	1.48±0.3	1.39±0.3
PTH, pg/ml	186±113	210±152	219±155
b-ALP, µg/l	18.3±14	17.9±14	18.1±12
CTX, µg/l	1.57±0.8	1.55±0.7	1.76±0.9
CRP, mg/l	10.1±10	13.3±26	13.6±20
Albumin, g/l			
Cholecalciferol, %, 100,000/week	89.3	86.8	87.1
Alfacalcidol, %, µg/week	26.1 (2.2±3)	17.5 (1.2±3)	21.3 (1.7±3)
Oral calcium, %, g/day	20 (1.1±3)	28.3 (1.3±3)	34.2 (1.3±3)
Sevelamer, %, g/day	24.3 (2.9±5)	29.1 (3±5)	35.3 (3.3±4.6)
Cinacalcet, %, mg/day	14.3 (59±80)	7 (45±75)	11.1 (28±59)
Warfarin, %	24.3	14	25.4
Aortic calcification score (/24)	13.5±6	10.1±7	10.5±7
Hip t-score	−3.7±0.6	−2.2±0.3	−0.8±0.7**
BMD, g/cm <sup>2</sup>	0.5±0.1	0.66±0.1	0.8±0.2*
Ultra distal radius t-score	−3.9±2	−2.8±1.5	−2±1.7*

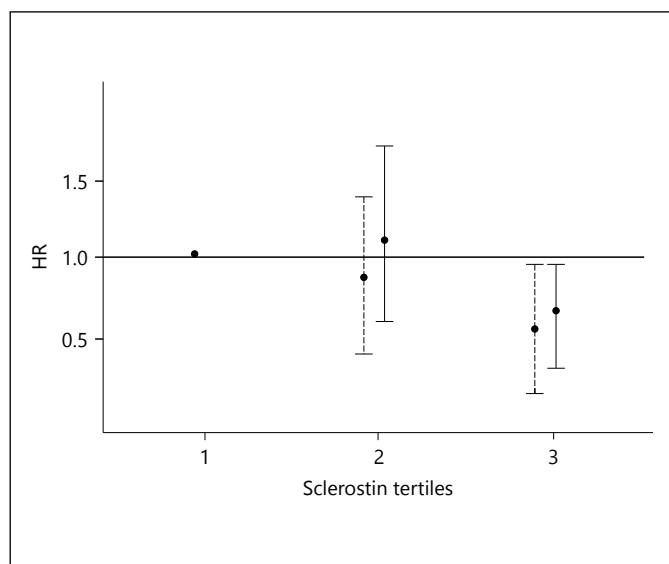
\*  $p < 0.05$ ; \*\*  $p < 0.001$  between the 1st and the 3rd tertile values. nPNA = Normalized protein nitrogen appearance.

**Table 5.** Survival analysis according to the multivariable adjusted Cox model

Covariate 1	b	SE	Wald	p value	Exp (b)	95% CI
Tertile sclerostine = 3	−0.7	0.33	4.5	0.03	0.5	0.25–0.93
Age, years	0.06	0.01	23	<0.0001	1.06	1.03–1.09
Dialysis vintage, months	0.006	0.001	18.5	<0.0001	1.006	1.003–1.008
Cardiac disease, %	0.33	0.3	1.1	0.28	1.38	0.76–2.5
Peripheral vascular disease, %	0.58	0.32	3.1	0.07	1.8	0.94–3.38
Stroke, %	−0.01	0.3	0.001	0.9	0.98	0.47–2.03
Kaupila score (/24)	0.009	0.03	0.07	0.7	1.01	0.94–1.08



**Fig. 4.** Kaplan–Meier survival analysis according to baseline serum sclerostin levels tertiles. Tertile 3 vs. 2: HR 0.56, 95% CI 0.32–0.96,  $p = 0.04$ ; tertile 3 vs. 1: HR 0.63, 95% CI 0.39–0.98,  $p = 0.04$ ; tertile 2 vs. 1: ns. \*  $p < 0.05$ .



**Fig. 5.** HRs and 95% CI for mortality associated with sclerostin tertiles 2 and 3, relative to tertile 1, using unadjusted (–) and multivariable adjustment (—) analysis.

### Sclerostin and Vascular Calcification

In our study, we observed that higher serum sclerostin levels are associated with less aortic calcification. Using the Kauppila score as we did, Claes et al. [22] reported a positive association between serum sclerostin levels and VC, but an inverse relationship using multivariate analysis. In HD patients, Brandenburg et al. [20] reported an association between high serum sclerostin levels and aortic valvular calcification, but not coronary artery calcification. Desjardins et al. [40] did not find any relationship between serum sclerostin levels and VC score (aortic valve and coronary arteries). Recently, Morena et al. [21] reported, in non-dialysis CKD patients, that both high OPG and sclerostin levels are associated with VC.

These apparent discrepancies could be due to different sclerostin assays used in the studies. However, Morena et al. [21] using the same TECO<sup>®</sup> assay reported results that were contradicting to ours. Another reason for this could be due to a difference in the nature of the studied population, CKD vs. HD patients.

Whether calcified cells in the arteries could be an important source of serum sclerostin production is not known. Besides, sclerostin should be a potent inhibitor of bone formation and mineralisation in the vascular wall. The relationship between osteoporosis and VC now is well recognized [7]. During calciphylaxis, there is increased expression of bone morphogenic protein-2 together with sclerostin in the cutaneous lesions [41].

In elderly patients, elevated serum sclerostin levels are not associated with bone-specific expression, and this elevation could be due to bone-independent vascular production [42]. We believe that sclerostin could be an important mediator between bone and vascular wall, at least in CKD and HD patients. Serum sclerostin levels could reflect bone mass sending feedback to stop further bone formation, and perhaps inhibit VC. In another model, high serum sclerostin levels may reflect adynamic bone disease with an inability to use both calcium and phosphates, leading to higher  $\text{Ca} \times \text{P}$  product and extraosseous calcification. Hence,  $\text{Ca} \times \text{P}$  product in our study remained low (mean  $3 \text{ mmol}^2/\text{l}$ ).

### Sclerostin and Survival

In non-CKD elderly men, the MONOS study failed to find any association between mortality and serum sclerostin levels [27]. In elderly women, Amrein et al. [35] also reported no relationship between serum sclerostin levels and patient outcome. Recently, Drechsler et al. [12] reported an association between high serum sclerostin levels and better survival rate in HD patients. The same association has been reported by Viaene et al. [13], but not



confirmed by Delanaye et al. [43]. Using the same sclerostin assay as Drechsler et al. [12], Viaene et al. [13] and Delanaye et al. [43], we confirmed the apparent protective effect of high serum sclerostin levels with better outcome in our studied population.

In contrast, Desjardins et al. [40] and Gonçalves et al. [44] reported an inverse relationship between serum sclerostin levels and survival rate. This discrepancy remains poorly explained and could be the result of using different assays in both CKD and dialysis patients [36]. However, our finding that higher sclerostin levels are associated with higher BMD, less aortic calcification and higher survival rate seems logical. This fits with the hypothesis that higher bone mass secretes more sclerostin, inhibiting further bone formation and vascular wall mineralisation.

### Limitations

Our study has some limitations due to the small number of patients. We used a simple radiological score to assess calcification, whereas Agatston score is considered to be the gold standard.

### References

- 1 Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group: KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). *Kidney Int Suppl* 2009;113:S1–S130.
- 2 Ishimura E, Nishizawa Y, Inaba M, Matsumoto N, Emoto M, Kawagishi T, Shoji S, Okuno S, Kim M, Miki T, Morii H: Serum levels of 1,25-dihydroxyvitamin D, 24,25-dihydroxyvitamin D, and 25-hydroxyvitamin D in nondialyzed patients with chronic renal failure. *Kidney Int* 1999;55:1019–1027.
- 3 Gutiérrez OM: Fibroblast growth factor 23, Klotho, and disordered mineral metabolism in chronic kidney disease: unraveling the intricate tapestry of events and implications for therapy. *J Ren Nutr* 2013;23:250–254.
- 4 Almaden Y, Canalejo A, Hernandez A, Ballasteros E, Garcia-Navarro S, Torres A, Rodriguez M: Direct effect of phosphorus on PTH secretion from whole rat parathyroid glands in vitro. *J Bone Miner Res* 1996;11:970–976.
- 5 Toussaint ND, Elder GJ, Kerr PG: A rational guide to reducing fracture risk in dialysis patients. *Semin Dial* 2010;23:43–54.
- 6 Andress DL: Adynamic bone in patients with chronic kidney disease. *Kidney Int* 2008;73:1345–1354.
- 7 Hofbauer LC, Brueck CC, Shanahan CM, Schoppet M, Dobnig H: Vascular calcification and osteoporosis – from clinical observation towards molecular understanding. *Osteoporos Int* 2007;18:251–259.
- 8 Gaudio A, Pennisi P, Bratengeier C, Torrisi V, Lindner B, Mangiafico RA, Pulvirenti I, Hawa G, Tringali G, Fiore CE: Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. *J Clin Endocrinol Metab* 2010;95:2248–2253.
- 9 Sigrist MK, Levin A, Er L, McIntyre CW: Elevated osteoprotegerin is associated with all-cause mortality in CKD stage 4 and 5 patients in addition to vascular calcification. *Nephrol Dial Transplant* 2009;24:3157–3162.
- 10 Jean G, Terrat JC, Vanel T, Hurot JM, Lorriaux C, Mayor B, Chazot C: High levels of serum fibroblast growth factor (FGF)-23 are associated with increased mortality in long haemodialysis patients. *Nephrol Dial Transplant* 2009;24:2792–2796.
- 11 Kovcsdy CP, Ureche V, Lu JL, Kalantar-Zadeh K: Outcome predictability of serum alkaline phosphatase in men with pre-dialysis CKD. *Nephrol Dial Transplant* 2010;25:3003–3011.
- 12 Drechsler C, Evenepoel P, Vervloet MG, Wanner C, Ketteler M, Marx N, Floege J, Dekker FW, Brandenburg VM; NECOSAD Study Group: High levels of circulating sclerostin are associated with better cardiovascular survival in incident dialysis patients: results from the NECOSAD study. *Nephrol Dial Transplant* 2015;30:288–293.
- 13 Viaene L, Behets GJ, Claes K, Meijers B, Blocki F, Brandenburg V, Evenepoel P, D'Haese PC: Sclerostin: another bone-related protein related to all-cause mortality in haemodialysis? *Nephrol Dial Transplant* 2013;28:3024–3030.
- 14 Robling AG, Niziolek PJ, Baldrige LA, Condon KW, Allen MR, Alam I, Mantila SM, Gluhak-Heinrich J, Bellido TM, Harris SE, Turner CH: Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. *J Biol Chem* 2008;283:5866–5875.
- 15 Ott SM: Sclerostin and Wnt signaling – the pathway to bone strength. *J Clin Endocrinol Metab* 2005;90:6741–6743.
- 16 Thambiah S, Roplekar R, Manghat P, Fogelman I, Fraser WD, Goldsmith D, Hampson G: Circulating sclerostin and Dickkopf-1 (DKK1) in predialysis chronic kidney disease (CKD): relationship with bone density and arterial stiffness. *Calcif Tissue Int* 2012;90:473–480.
- 17 Cejka D, Herberth J, Branscum AJ, Fardo DW, Monier-Faugere MC, Diarra D, Haas M, Malluche HH: Sclerostin and Dickkopf-1 in renal osteodystrophy. *Clin J Am Soc Nephrol* 2011;6:877–882.
- 18 Cejka D, Jäger-Lansky A, Kieweg H, Weber M, Bieglmayer C, Haider DG, Diarra D, Patsch JM, Kainberger F, Bohle B, Haas M: Sclerostin serum levels correlate positively with bone mineral density and microarchitecture in haemodialysis patients. *Nephrol Dial Transplant* 2012;27:226–230.

### Conclusion

Our study shows a direct association between high serum sclerostin levels and better survival rate in HD patients. The inverse association between serum sclerostin and aortic calcification may be the best explanation for this phenomenon. However, the mechanism for this association is poorly understood and remains controversial in the literature.

### Disclosure Statement

C. Chazot declares to have received a salary from Fresenius Medical Care France for this work.

### Statement of Ethics

This study did not require review/approval by the appropriate ethics committee.

- 19 Zhu D, Mackenzie NC, Millán JL, Farquharson C, MacRae VE: The appearance and modulation of osteocyte marker expression during calcification of vascular smooth muscle cells. *PLoS One* 2011;6:e19595.
- 20 Brandenburg VM, Kramann R, Koos R, Krüger T, Schurgers L, Mühlenbruch G, Hübner S, Gladziwa U, Drechsler C, Ketteler M: Relationship between sclerostin and cardiovascular calcification in hemodialysis patients: a cross-sectional study. *BMC Nephrol* 2013;14:219.
- 21 Morena M, Jaussent I, Dupuy AM, Bargnoux AS, Kuster N, Chenine L, Leray-Moragues H, Klouche K, Vernhet H, Canaud B, Cristol JP: Osteoprotegerin and sclerostin in chronic kidney disease prior to dialysis: potential partners in vascular calcifications. *Nephrol Dial Transplant* 2015;30:1345–1356.
- 22 Claes KJ, Viaene L, Heye S, Meijers B, d'Haese P, Evenepoel P: Sclerostin: another vascular calcification inhibitor? *J Clin Endocrinol Metab* 2013;98:3221–3228.
- 23 Loots GG, Kneissel M, Keller H, Baptist M, Chang J, Collette NM, Ovcharenko D, Plajzer-Frick I, Rubin EM: Genomic deletion of a long-range bone enhancer misregulates sclerostin in Van Buchem disease. *Genome Res* 2005;15:928–935.
- 24 Honkanen E, Kauppila L, Wikström B, Rensma PL, Krzesinski JM, Aasarod K, Verbeke F, Jensen PB, Mattelaer P, Volck B; CORD Study Group: Abdominal aortic calcification in dialysis patients: results of the cord study. *Nephrol Dial Transplant* 2008;23:4009–4015.
- 25 Pelletier S, Dubourg L, Carlier MC, Hadj-Aissa A, Fouque D: The relation between renal function and serum sclerostin in adult patients with CKD. *Clin J Am Soc Nephrol* 2013;8:819–823.
- 26 Cejka D, Marculescu R, Kozakowski N, Plischke M, Reiter T, Gessl A, Haas M: Renal elimination of sclerostin increases with declining kidney function. *J Clin Endocrinol Metab* 2014;99:248–255.
- 27 Szulc P, Boutroy S, Vilaythiou N, Schoppet M, Rauner M, Chapurlat R, Hamann C, Hofbauer LC: Correlates of bone microarchitectural parameters and serum sclerostin levels in men: the STRAMBO study. *J Bone Miner Res* 2013;28:1760–1770.
- 28 Garnero P, Sornay-Rendu E, Munoz F, Borel O, Chapurlat RD: Association of serum sclerostin with bone mineral density, bone turnover, steroid and parathyroid hormones, and fracture risk in postmenopausal women: the OFELY study. *Osteoporos Int* 2013;24:489–494.
- 29 Gregson CL, Poole KE, McCloskey EV, Duncan EL, Rittweger J, Fraser WD, Smith GD, Tobias JH: Elevated circulating sclerostin concentrations in individuals with high bone mass, with and without LRP5 mutations. *J Clin Endocrinol Metab* 2014;99:2897–2907.
- 30 Ardawi MS, Akhbar DH, Alshaikh A, Ahmed MM, Qari MH, Rouzi AA, Ali AY, Abdurafee AA, Saeda MY: Increased serum sclerostin and decreased serum IGF-1 are associated with vertebral fractures among postmenopausal women with type-2 diabetes. *Bone* 2013;56:355–362.
- 31 Ardawi MS, Rouzi AA, Al-Sibiani SA, Al-Senani NS, Qari MH, Mousa SA: High serum sclerostin predicts the occurrence of osteoporotic fractures in postmenopausal women: the center of excellence for osteoporosis research study. *J Bone Miner Res* 2012;27:2592–2602.
- 32 Arasu A, Cawthon PM, Lui LY, Do TP, Arora PS, Cauley JA, Ensrud KE, Cummings SR Study of Osteoporotic Fractures Research Group: Serum sclerostin and risk of hip fracture in older Caucasian women. *J Clin Endocrinol Metab* 2012;97:2027–2032.
- 33 Malluche HH, Davenport DL, Cantor T, Monier-Faugere MC: Bone mineral density and serum biochemical predictors of bone loss in patients with CKD on dialysis. *Clin J Am Soc Nephrol* 2014;9:1254–1262.
- 34 Bellido T, Ali AA, Gubrij I, Plotkin LI, Fu Q, O'Brien CA, Manolagas SC, Jilka RL: Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. *Endocrinology* 2005;146:4577–4583.
- 35 Amrein K, Dobnig H, Wagner D, Piswanger-Sölkner C, Pieber TR, Pilz S, Tomaschitz A, Dimai HP, Fahrleitner-Pammer A: Sclerostin in institutionalized elderly women: associations with quantitative bone ultrasound, bone turnover, fractures, and mortality. *J Am Geriatr Soc* 2014;62:1023–1029.
- 36 Moysés RM, Jamal SA, Gracioli FG, dos Reis LM, Elias RM: Can we compare serum sclerostin results obtained with different assays in hemodialysis patients? *Int Urol Nephrol* 2015;47:847–850.
- 37 Ritz E, Stefanski A, Rambauek M: The role of the parathyroid glands in the uremic syndrome. *Am J Kidney Dis* 1995;26:808–813.
- 38 Asamiya Y, Yajima A, Shimizu S, Otsubo S, Tsuchiya K, Nitta K: Associations between the levels of sclerostin, phosphate, and fibroblast growth factor-23 and treatment with vitamin D in hemodialysis patients with low intact PTH level. *Osteoporos Int* 2015;26:1017–1028.
- 39 Lavi-Moshayoff V, Wasserman G, Meir T, Silver J, Naveh-Many T: PTH increases FGF23 gene expression and mediates the high-FGF23 levels of experimental kidney failure: a bone parathyroid feedback loop. *Am J Physiol Renal Physiol* 2010;299:F882–F889.
- 40 Desjardins L, Liabeuf S, Oliveira RB, Louvet L, Kamel S, Lemke HD, Vanholder R, Choukroun G, Massy ZA; European Uremic Toxin (EUTox) Work Group: Uremic toxicity and sclerostin in chronic kidney disease patients. *Nephrol Ther* 2014;10:463–470.
- 41 Kramann R, Brandenburg VM, Schurgers LJ, Ketteler M, Westphal S, Leisten I, Bovi M, Jähnen-Dechent W, Knüchel R, Floege J, Schneider RK: Novel insights into osteogenesis and matrix remodelling associated with calcific uraemic arteriopathy. *Nephrol Dial Transplant* 2013;28:856–868.
- 42 Roforth MM, Fujita K, McGregor UI, Kirmani S, McCready LK, Peterson JM, Drake MT, Monroe DG, Khosla S: Effects of age on bone mRNA levels of sclerostin and other genes relevant to bone metabolism in humans. *Bone* 2014;59:1–6.
- 43 Delanaye P, Krzesinski JM, Warling X, Moonen M, Smelten N, Médart L, Bruyère O, Reginster JY, Pottel H, Cavalier E: Clinical and biological determinants of sclerostin plasma concentration in hemodialysis patients. *Nephron Clin Pract* 2014;128:127–134.
- 44 Gonçalves FL, Elias RM, Dos Reis LM, Gracioli FG, Zampieri FG, Oliveira RB, Jorgetti V, Moysés RM: Serum sclerostin is an independent predictor of mortality in hemodialysis patients. *BMC Nephrol* 2014;15:190.