Clinical Practice: Original Paper

nephron Clinical Practice

Nephron
DOI: 10.1159/000443845

Received: September 1, 2015
Accepted after revision: January 6, 2016
Published online: February 19, 2016

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

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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 ± 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 ± 12 vs. 64.7 ± 18 years), more frequently of the male gender (74 vs. 48%), had lower serum bone-specific alkaline phosphatases values (14 ± 9 vs. 20.4 ± 13 µg/l), were less frequently treated with alfacalcidol, displayed lower aortic calcification scores (9.5 ± 5 vs. 12.5 ± 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.

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-

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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 Wingless-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 upregulates OPG, which binds and inhibits receptor activator of nuclear factor κB-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, online-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 ± 0.22 ng/ml in men, 0.66 ± 0.22 in post-menopausal women and 0.59 ± 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 μg/l) and β-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-$(OH)_2$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 ultra-distal-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 ± 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 ≤0.05 were considered statistically significant.

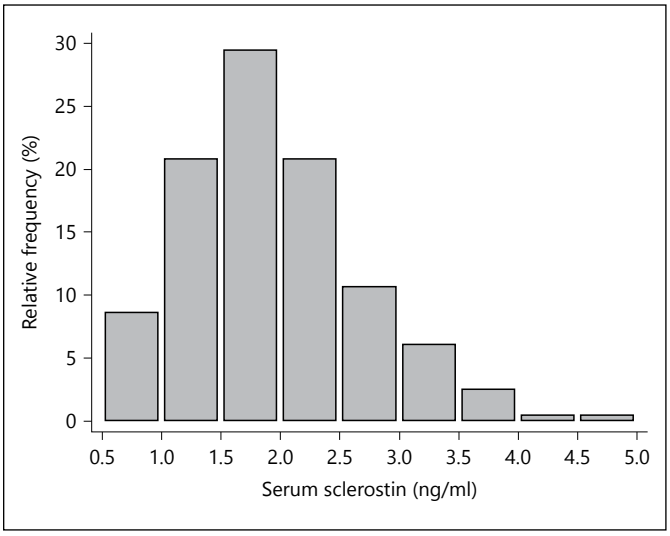

Fig. 1. Serum sclerostin levels distribution plot.

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 ± 14; 43% of female sex; 36.2% with diabetes; vintage 65.7 ± 83 months; thrice weekly session duration of 297 ± 75 min, post-dilutional online HDF in 27% of cases; and mean dialysate calcium concentration of 1.5 ± 0.17 mmol/l.

Baseline mean serum sclerostin was 1.9 ± 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 ± 12 vs. 64.7 ± 18 years, $p < 0.001$); more frequently of the male gender (74 vs. 48%, $p < 0.001$); had lower serum b-ALP values (14 ± 9 vs. 20.4 ± 13, $p < 0.05$); were less frequently treated with alfacalcidol (13 vs. 31%, $p < 0.05$); had lower aortic calcification scores (9.5 ± 5 vs. 12.5 ± 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, $1,25(OH)_2$-D (calcitriol) and FGF-23 levels were not significantly different between the 1st and 3rd sclerostine 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^2	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)$_2$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*
Kauppila 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*

* $p < 0.05$; ** $p < 0.001$ 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 retained 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

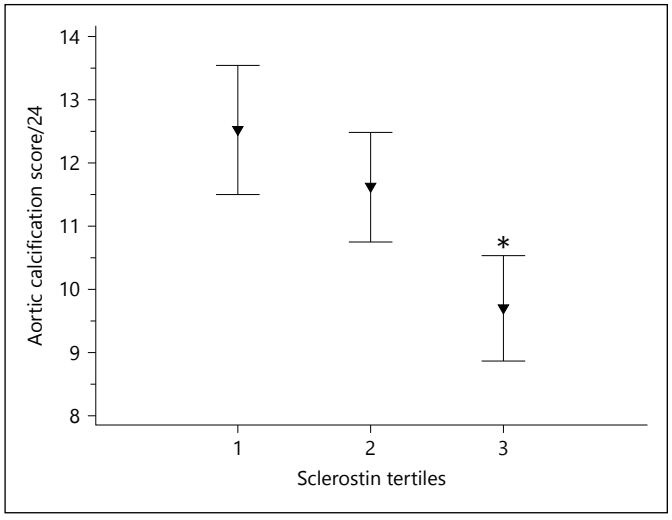

Fig. 2. Aortic calcification score according to the serum sclerostin levels tertiles. * $p < 0.05$.

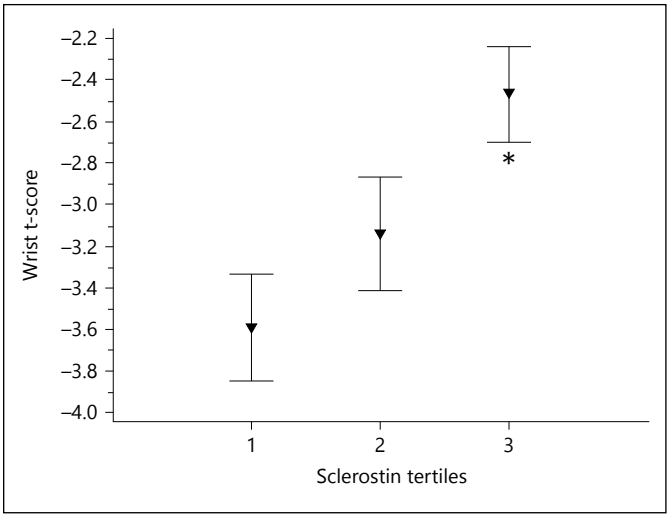

Fig. 3. Ultra distal radius (wrist) t-score according to serum sclerostin levels tertiles. * $p < 0.05$.

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

Kauppila 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^2	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)$_2$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

* $p < 0.05$; ** $p < 0.001$ 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/m²	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-$(OH)_2$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/cm²	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
Kauppila score (/24)	0.009	0.03	0.07	0.7	1.01	0.94–1.08

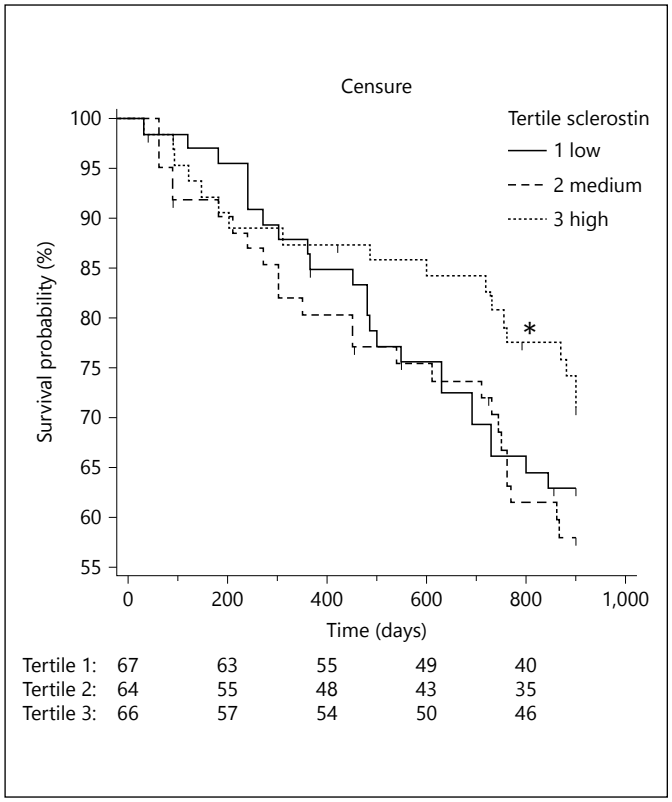

Fig. 4. Kaplan–Meier survival analysis according to baseline serum sclerostin levels tertiles. Tertile 3 vs. 2: HR 0.56, 95% CI 0.32–0.96, p = 0.04; tertile 3 vs. 1: HR 0.63, 95% CI 0.39–0.98, p = 0.04; tertile 2 vs. 1: ns. * p < 0.05.

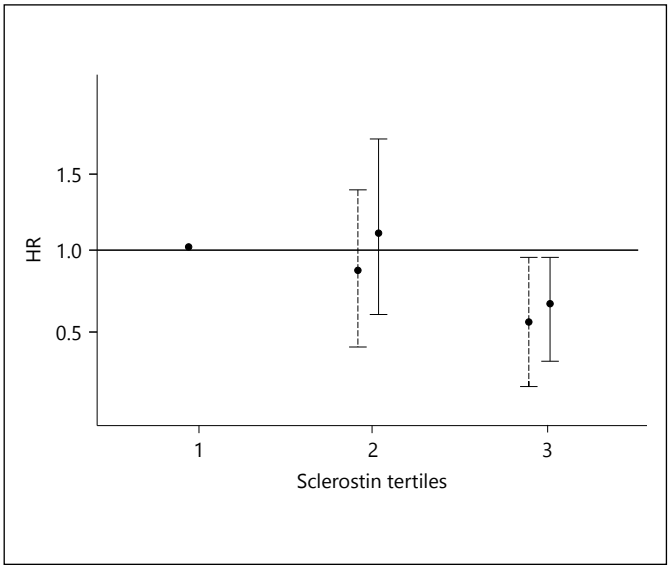

Fig. 5. HRs and 95% CI for mortality associated with sclerostin tertiles 2 and 3, relative to tertile 1, using unadjusted (–) and multivariable adjustment (—) analysis.

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© 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 Ca × P product and extraosseous calcification. Hence, Ca × P product in our study remained low (mean 3 $mmol^2/l$).

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.

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.

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