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Sclerostin: another bone-related protein related to all-cause mortality in haemodialysis?

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ORIGINAL ARTICLE

ABSTRACT

Background. Derangements in bone metabolism and vascular calcification (VC) substantially contribute to the accelerated cardiovascular morbidity and mortality in chronic kidney disease (CKD). The Wnt signalling pathway is increasingly recognized to play an important role in bone homeostasis and VC. Circulating levels of the Wnt inhibitor sclerostin are elevated in CKD patients. The present study investigated whether the circulating levels of sclerostin are associated with all-cause mortality in haemodialysis (HD) patients.

Methods. We performed a *post-hoc* survival analysis in 100 prevalent HD patients (68 ± 13 years, 40 male) recruited in 2006 who were prospectively followed for median 637 (8–1000, range) days. Parameters of mineral metabolism including bone-specific alkaline phosphatase (bsAP) and serum sclerostin were determined in spare blood samples collected at baseline.

Results. Serum concentrations of serum sclerostin amounted to 110 (82–151) [median (iqr)] pmol/L. Patients with sclerostin levels above median were characterized by older age, higher haemoglobin and creatinine level and lower bsAP concentration. During a median follow-up of 637 days, 31 patients died. Higher circulating sclerostin levels were associated with decreased mortality in prevalent HD patients: unadjusted hazard ratio (HR) 0.51 (0.24–1.06) (P = 0.06); HR adjusted for age and gender for serum sclerostin levels above versus below median was 0.33 (0.15–0.73) (P = 0.006). When bsAP was entered in the Cox regression analysis, it replaced sclerostin in the final model.

Conclusions. Our data show that high circulating sclerostin levels are associated with improved survival and suggest that a low bsAP activity may be in the causal pathway.

INTRODUCTION

Bone disease and vascular calcification (VC) are common in chronic kidney disease (CKD) and are important causes of mortality and morbidity [1]. Growing evidence suggests a molecular link between both entities, although the signalling pathways involved remain incompletely understood [2, 3].

The canonical Wingless-type mouse mammary tumour virus integration site (Wnt) pathway is increasingly recognized to play an important role in bone homeostasis. Efficient Wnt signalling relies on the presence of a transmembrane receptor complex, composed of the frizzled receptor and the low density lipoprotein receptor-related protein (LRP)-5 or LRP6 co-receptor. The effect of canonical Wnt signalling on bone is mediated by stimulation of stem cell and pre-osteoblast proliferation, induction of osteoblastogenesis, inhibition of osteoblast and osteocyte apoptosis and attenuation of osteoclastogenesis [4, 5]. Wnt activation thus confers dual anabolic and anti-catabolic benefits.

Soluble inhibitors of Wnt signalling, such as sclerostin and Dickkopf-related protein 1, bind to co-receptors LRP5/6 and inhibit their association with Wnts. The glycoprotein

sclerostin (22 kDa) is the secreted product of the SOST gene in osteocytes [4]. Ageing, diabetes, male gender, mechanical unloading of the skeleton, low parathyroid hormone (PTH) levels are all associated with high circulating sclerostin levels [6–9]. Sclerostin levels also increase along the progression of CKD. In haemodialysis (HD) patients, sclerostin levels correlate negatively with histomorphometric parameters of bone turnover, osteoblast number and function [10].

The arterial vasculature is the second most extensively calcified structure in the human body. Mounting evidence indicates that Wnt signalling is also involved in medial artery and aortic valve calcification [11–13]. Recently, increased expression of sclerostin was demonstrated during vascular smooth muscle cell calcification and confirmed by *ex vivo* analyses of a mouse model of medial calcification [13]. Meanwhile, expression of sclerostin has also been documented in calcified aortic valves in humans (unpublished data, V. Brandenburg). Although the role of sclerostin in calcifying vascular tissue remains to be determined, it may be speculated that as in the bone, sclerostin expression by newly embedded osteocyte-like cells in the vasculature at the onset of osteoid mineralization may serve as a negative feedback signal on osteoblasts [14]. Sclerostin produced either in the bone or in the vasculature may spill over to the circulation. Bone marrow plasma and peripheral serum sclerostin levels have been shown to strongly correlate [15]. Sclerostin may thus represent an important messenger in the cross talk between bone and the vasculature. Whether the circulating sclerostin levels are associated with mortality has so far not been investigated. The present study addresses this question in prevalent HD patients.

MATERIAL AND METHODS

Study design and patients

We performed a *post-hoc* survival analysis of patients included in a cross-sectional study in HD patients which assessed novel cardiovascular risk factors [16]. The study population consists of 100 patients, treated with maintenance HD for at least 3 months at the nephrology department of the University Hospital Gasthuisberg, Leuven, Belgium. Patients were enrolled between February and April 2006. All patients were ≥18 years of age and were treated three times for 4 h a week with HD or haemodiafiltration using synthetic polysulfone or polyamide dialysis membranes with a surface area of 1.4–1.8 m^2. Dialysis efficiency was targeted based on single pool K_t/V of urea nitrogen (spK_t/V) according to the National Kidney Foundation-Kidney Disease Outcomes Quality Initiative Guidelines. All patients were on low-dose native vitamin D (800 IU after every dialysis session) as part of a multivitamin preparation and 48 patients were on active vitamin D therapy. Six patients were treated with glucocorticoids. None of the patients received bisphosphonates or denosumab. The study was performed according to the Declaration of Helsinki and approved by the ethics committee of the University Hospital Leuven. Informed consent was obtained from all patients.

Clinical and biochemical parameters

Data on baseline demographics, presence of diabetes and cause of kidney disease were collected from medical files. Vascular history was defined as history of myocardial infarction, percutaneous coronary artery intervention, cardiac surgery, peripheral artery disease or cerebrovascular disease. Blood was sampled at the start and the end of a mid-week dialysis. Blood samples were collected and after centrifugation, serum was aliquoted and stored at −80°C until further analysis. Creatinine, urea nitrogen, haemoglobin, calcium, phosphate, C-reactive protein (CRP) and cholesterol were all measured using standard laboratory techniques. Albumin was measured using the bromcresol green method. Bone-specific alkaline phosphatase (bsAP) was measured using an electrophoretic method (ISOPAL, Analis) [17]. Serum concentrations of full-length (biointact) PTH were determined by an immunoradiometric assay, as described elsewhere [18]. Serum sclerostin was measured using enzyme-
linked immunosorbent assay (ELISA, Biomedica, Austria), performed according to the manufacturer's instructions. The intra and inter-assay coefficients of variation for sclerostin measurement were 4 and 7%, respectively. spK_t/V was calculated using the second-generation logarithmic formula of Daugirdas [19]. Residual renal function (RRF) was estimated from an interdialytic urine collection and calculated from the arithmetic mean of urea nitrogen and creatinine clearance. Anuria was defined as a urine output <100 mL/24 h and/or RRF <1 mL/min/1.72 m^2. As a measure of daily protein intake, normalized protein nitrogen appearance (nPNA) was calculated [20].

Endpoint evaluation

The primary endpoint was all-cause mortality. Mortality was prospectively recorded and coded, blinded from clinical and biochemical data. If information could not be obtained, the patient was assumed to be lost to follow-up starting from the date of the last actual visit. After review of available information, the cause of death was classified as either cardiovascular, infectious, malignancy or other. Cardiovascular deaths included fatal myocardial infarction, sudden death and death due to congestive heart failure. Cases of unobserved sudden death were considered cardiovascular death only when other potential causes could be excluded. Otherwise, they were classified as 'other cause of death'. When a patient was transplanted or transferred to another dialysis centre during the follow-up period, date of transplantation or transfer was recorded.

Statistical analysis

Continuous variables are expressed as mean [standard deviation (SD)] for normally distributed variables or median (interquartile range), otherwise. The Kaplan–Meier method was used to estimate cumulative incidence of the primary endpoint. Time to death was analyzed using Cox proportional hazard analysis. Variables that affected all-cause mortality ($P < 0.2$) in univariate analysis 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 transplantation, transfer to another dialysis centre, lost to follow-up or at the end of follow-up (1000 days). A P value of <0.05 was considered statistical significant. SAS 9.2 software was used for statistical analysis.

RESULTS

Study population characteristics

Population demographics and relevant biochemistry are shown in Table 1. Of the 100 HD patients, 60 were men, 34 were diabetic. Primary renal disease was diabetes (22%), glomerulonephritis/vasculitis (23%), interstitial nephritis (7%), cystic/hereditary/congenital (15%), miscellaneous (8%), vascular (2%) and unknown or missing (23%). The overall age was 68 ± 13 years, 77% of the patients were anuric. RRF amounted to 0.6 ± 1.3 mL/min/1.73 m^2.

Correlation of serum sclerostin with demographic and clinical parameters

Sclerostin levels were significantly higher in male than in female dialysis patients (126 versus 102 pmol/L, median; P = 0.004). Diabetic patients had significantly higher sclerostin levels when compared with non-diabetic HD patients (144.1 ± 74.8 versus 116.0 ± 55.0 pmol/L, P = 0.04). Patients with sclerostin levels above median were characterized by older age, higher haemoglobin and creatinine level and lower bsAP concentration. A (near-) significant correlation was observed with PTH (Spearman rank $r = -0.2$; P = 0.05), bsAP

Table1. Demographics

Variable	All	Sclerostin < median	Sclerostin > median	P value
Number of patients	100	50	50	/
Age	68 ± 13	65.0 ± 15.0	70.7 ± 10.5	0.03
Weight	65.4 ± 11.9	64.6 ± 13.9	65.9 ± 9.8	0.6
BMI	23.5 ± 4.0	23.4 ± 4.8	23.4 ± 3.2	0.9
Female (%)	40	47	35	0.2
Diabetes (Y) (%)	34	30	38	0.4
Active vitamin D (Y) (%)	47	51	45	0.5
Dialysis vintage (months)	39.9 (15.7–68.8)	45.1 (25.6–68.5)	36.0 (23.0–68.3)	0.9
Urea nitrogen (mg/dL)	119.6 ± 31.4	116.4 ± 32.0	124.2 ± 30.1	0.2
Creatinine (mg/dL)	7.4 ± 2.3	6.8 ± 2.5	8.0 ± 1.9	0.006
RRF (mL/min/1.73 m^2)	0.6 ± 1.3	0.9 ± 1.6	0.4 ± 0.8	0.09
nPNA (g/kg/day)	1.2 ± 0.4	1.2 ± 0.3	1.2 ± 0.3	0.2
spKt/V	1.6 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	0.3
Haemoglobin (g/dL)	11.7 ± 1.1	11.4 ± 1.1	11.9 ± 1.0	0.03
Bicarbonate (mEq/L)	23.9 ± 2.0	24.1 ± 2.0	23.7 ± 2.1	0.4
CRP (mg/L)	5.5 (2.6–9.4)	6.4 (2.8–11.7)	4.6 (2.0–7.6)	0.1
Albumin (g/L)	39.9 ± 3.5	39.6 ± 4.1	40.1 ± 2.9	0.5
Calcium (mg/dL)	9.5 ± 0.7	9.5 ± 0.8	9.5 ± 0.6	0.9
Phosphate (mg/dL)	4.6 ± 1.4	4.5 ± 1.6	4.8 ± 1.4	0.4
Cholesterol (mg/dL)	149.6 ± 34.0	143.4 ± 30.6	155.7 ± 36.8	0.08
PTH (ng/L)	61.9 (30.0–113.6)	70.7 (28.1–152.8)	56.4 (32.5–100.0)	0.3
Calcidiol (ng/mL)	14.8 (10.3–20.6)	14.4 (8.8–18.0)	17.8 (11.0–22.7)	0.06
Calcitriol (pg/mL)	14.0 (5.3–39.4)	10.0 (9.6–15.7)	11.2 (9.9–19.8)	0.2
Sclerostin (pmol/L)	110 (82–151)	81.7 (58.5–96.3)	150.6 (132.2–215.7)	<0.0001
bsAP (μg/L)	13.0 (9.7–21.6)	18.2 (10.1–26.1)	12.1 (9.1–14.4)	0.003

HD, haemodialysis; BMI, body mass index; Y, yes; N, no; RRF, residual renal function; nPNA normalized protein nitrogen appearance; spK_t/V, single pool K_t/V of urea nitrogen; CRP, C-reactive protein; PTH, parathyroid hormone; bsAP, bone-specific alkaline phosphate.

(Spearman rank $r = -0.34$; P = 0.0006) and carboxy-terminal collagen crosslinks (Spearman rank $r = -0.18$; P = 0.08). No interaction between the use of vitamin D and glucosteroids and sclerostin levels was found.

Sclerostin and all-cause mortality

Of the 100 HD patients, 31 patients died after a median follow-up of 637 (8–1000, range) days. As shown in the Kaplan–Meier curve (Figure 1), HD patients with serum sclerostin levels above median at the start of the observation period tended to have a better survival although significance was not reached (P = 0.06). After adjustment for age and gender, sclerostin levels above median were significantly associated with a better survival [HR 0.33 (0.15–0.73) (Table 2)]. In the full multivariate model, diabetes mellitus, shorter dialysis vintage, higher CRP and higher bsAP were associated with worse survival in prevalent dialysis patients. It is important to note that this association was independent of active vitamin D therapy, known to uncouple serum bsAP levels from bone turnover [21]. Data were similar when sclerostin was assessed as a continuous variable.

DISCUSSION

The major finding of the present *post-hoc* analysis was that after adjustment for age and gender, high circulating sclerostin levels were observed to be associated with better survival in prevalent HD patients.

In the last decade, the soluble Wnt signalling inhibitor sclerostin emerged as an important regulator of bone metabolism. More recently, sclerostin was also shown to be expressed in calcifying vasculature [13, 22]. The latter observation is not unexpected, given the similarities between bone formation and VC [23, 24]. It is hypothesized that the increased expression of Wnt inhibitors in calcifying vasculature may be a defensive response in order to limit further ossification [2]. Sclerostin produced in bone and vascular tissue may spill over into the circulation and exert systemic effects, although a direct proof for the latter is lacking. Sclerostin levels have been associated both with

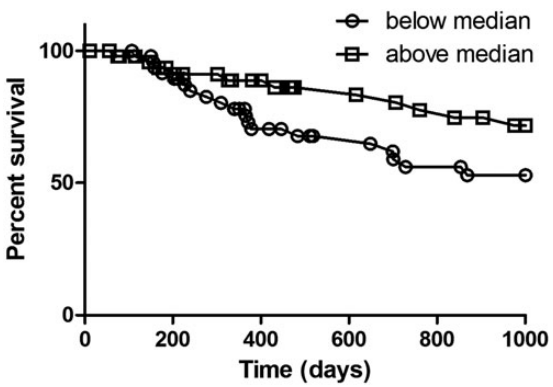

FIGURE 1: Kaplan–Meier curves of survival time of sclerostin above and below median. Log rank P = 0.06.

Table 2. Cox regression analysis of predictors of outcome in prevalent dialysis patients

	Unit of increase	HR	95%CI	P
Univariate[a]				
Age	1 year	1.05	1.01–1.09	0.02
Dialysis vintage	1 month	0.992	0.98–1.0	0.05
Hemoglobin	1 g/dL	0.78	0.55–1.10	0.16
Albumin	1 g/L	0.92	0.84–1.02	0.12
CRP	1 mg/L	1.03	1.01–1.05	0.002
Cholesterol	1 mg/dL	0.99	0.97–0.99	0.03
Diabetes mellitus	Y versus N	2.39	1.18–4.85	0.02
Vascular history	Y versus N	1.76	0.87–3.57	0.12
Sclerostin	Above versus below median	0.51	0.24–1.06	0.06
bsAP	1 µg/L	1.02	1.00–1.03	0.02
Multivariate models				
Age, gender, sclerostin				
Age	1 year	1.06	1.02–1.10	0.003
Gender	Female versus male	0.55	0.25–1.19	0.13
Sclerostin	Above versus below median	0.33	0.15–0.73	0.006
Full model				
Dialysis vintage	1 month	0.99	0.98–0.99	0.04
CRP	1 mg/L	1.03	1.01–1.05	0.006
bsAP	1 µg/L	1.02	1.00–1.04	0.02
Diabetes mellitus	Y versus N	2.82	1.27–6.23	0.01

CRP, C-reactive protein; bsAP, bone-specific alkaline phosphatase.
[a]Only parameters with P < 0.2 are shown; Relative risks of death for each variable are given as HR [95% confidence interval (CI)] and P value.

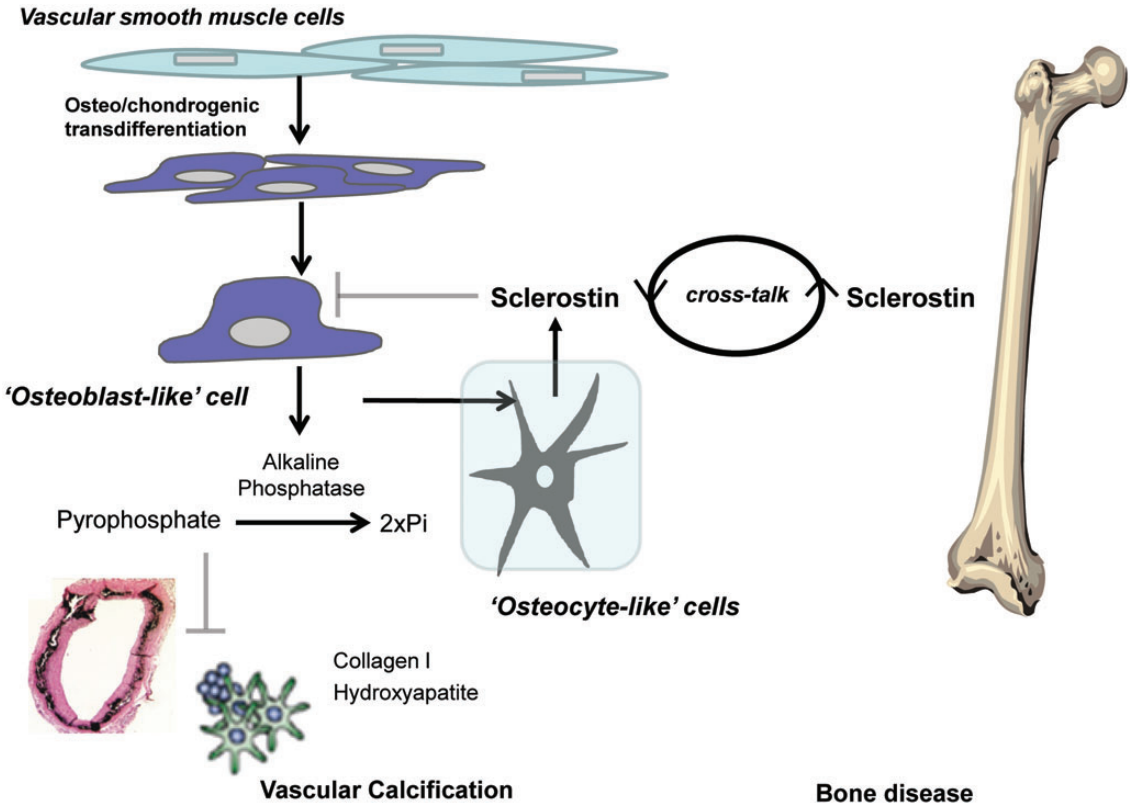

FIGURE 2: Vascular smooth muscles cells undergo osteo/chondrogenic transdifferentiation in a pro-calcifying environment. The resulting osteoblast-like cells induce alkaline phosphatases. These alkaline phosphatases catalyze the hydrolysis of PPi. In the late phase of VC, sclerostin is expressed. This can be interpreted as a defensive response that aims to block the Wnt pathway in order to reduce the mineralization in the vascular tissue. Sclerostin may spill over to the circulation and may reciprocally inhibit bone metabolism.

bone health (BMD, bone turnover) [10, 25–27] and with cardiovascular calcification burden [22, 28] (Claes K *et al.*, TH-PO 886, 45th American Society of Nephrology Congress, San Diego, USA, 2012). In addition, blocking sclerostin action with anti-sclerostin antibodies is a promising new therapeutic approach to osteo-anabolic therapy of osteoporosis [29]. As the arterial vasculature is the second most extensively calcified structure in the human body, the vascular contribution to circulating sclerostin levels is most probably substantial. The survival benefit in patients with high circulating sclerostin levels thus might be explained by an attenuated progression of VC.

Vascular calcification is very common in CKD patients, even in patients with early disease [30] and is associated with poor (cardiovascular) outcomes [31]. It is well established that the process of VC is controlled by a balance between procalcifying and anticalcifying regulatory proteins acting locally in the vessel wall and/or systemically in the circulation. Alkaline phosphatase is increasingly recognized as an inducer of VC in CKD [32]. Alkaline phosphate is upregulated under uremic conditions in vessels from rats, which leads to the hydrolysis and, therefore, inactivation of inorganic pyrophosphate (PPi), a potent inhibitor of hydroxyapatite crystal growth and a potential local and circulating inhibitor of VC [33]. This is referred to as the 'pyrophosphate hypothesis'. At least in animal models, VC can be induced by lowering PPi levels [33] and be prevented by administration of sodium pyrophosphate [34]. Sclerostin has been shown to be a chief suppressor of alkaline phosphatase activity [35, 36].

The upregulation of sclerostin (and other Wnt inhibitors) in calcifying vascular tissue most probably closes a feedback loop that aims to retard further mineralization [2, 13] (Figure 2). The observation that low bsAP levels replaced high sclerostin levels in the final regression model as an independent predictor of mortality supports this line of reasoning [37, 38] and corroborates previous clinical and experimental studies [32, 39, 40].

Taken together, sclerostin may serve as a master regulator of mineralization both in bone [41] and in vasculature [13] and thus, may represent an important effector in the bone–vascular axis [42]. Sclerostin spilling over from the vasculature may detrimentally affect bone and explain why VCs are inversely related to bone density and directly related to fractures [43, 44].

We acknowledge several limitations of our study. First of all, the sample size was small which confers the risk of a type 1 statistical error. Obviously, our data should be considered preliminary and need confirmation. Second, we lack data on bone histomorphometry and VC, which are of crucial importance to delineate the role of sclerostin in the cross talk between bone and vasculature. Whether sclerostin is simply a marker or a central mediator of (cardiovascular) morbidity and mortality in CKD needs to be elucidated by additional

experimental and clinical studies. This information is urgently required as blockade of sclerostin activity is currently explored as a novel treatment option for osteoporosis [29].

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CONFLICT OF INTEREST STATEMENT

None declared.

(See related article by Jean and Chazot. Sclerostin in CKD-MBD: one more paradoxical bone protein? *Nephrol Dial Transplant* 2013; 28: 2932–2935.)

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