High levels of circulating sclerostin are associated with better cardiovascular survival in incident dialysis patients: results from the NECOSAD study

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ABSTRACT

Background. Sclerostin is a Wnt pathway antagonist regulating osteoblast activity and bone turnover, and it plays a role in cardiovascular calcification processes. Previous findings indicate that sclerostin regulation is disturbed in chronic kidney disease (CKD). The aim of this study was to assess the association of circulating sclerostin levels with mortality in dialysis patients.

Methods. From a prospective cohort study of incident dialysis patients in the Netherlands, all patients with measured circulating sclerostin at 3 months after the start of dialysis (baseline) were included in the present analysis: n = 673, age 63 ± 14 years, mean serum sclerostin (ELISA) 1.24 ± 0.57 ng/mL. By Cox regression analyses, we assessed the association of sclerostin levels with cardiovascular and non-cardiovascular mortality both in the short (18 months) and long term (4-year follow-up).

Results. Serum sclerostin levels in the entire cohort correlated with intact parathyroid hormone levels (r = −0.25, P < 0.001), age (r = 0.16, P < 0.001) and serum alkaline phosphatase (r = −0.13, P = 0.001). After adjustment for various clinical and biochemical parameters, patients in the highest sclerostin tertile had a significantly lower risk of cardiovascular death [hazard ratio 0.29, 95% confidence interval (CI) 0.13–0.62] and for all-cause mortality (0.39, 95% CI 0.22–0.68) within 18 months compared with patients of the lowest tertile. The association of sclerostin levels with outcome was less pronounced for long-term cardiovascular mortality and absent for non-cardiovascular mortality.

Conclusions. High levels of serum sclerostin are associated with lower short-term cardiovascular mortality in dialysis patients. The exact mechanisms of this association, e.g. how sclerostin influences or reflects uraemic vascular calcification, need to be investigated in further studies.

Keywords: CKD-MBD, end-stage renal disease, mortality, renal osteodystrophy, sclerostin

INTRODUCTION

Disturbances in mineral and bone metabolism as well as cardiovascular disease in uraemia constitute an integral part of the chronic kidney disease-mineral and bone disorders (CKD-MBD) syndrome [1]. CKD-MBD is closely associated with increased morbidity and mortality in patients with advanced CKD or end-stage renal disease (ESRD). Numerous cohort studies revealed an association between deranged circulating bone biomarkers with adverse clinical outcome in patients with ESRD. For example, high (bone-specific) alkaline phosphatase (AP) levels [2, 3], high phosphate levels and high calcium levels [4], increased fibroblast growth factor-23 [5] or very low and very high parathyroid hormone (PTH) levels [6] have all been associated with increased mortality. These laboratory abnormalities reflect a disturbed bone and mineral metabolism. However, besides reflecting bone disease, virtually all of these biomarkers have been implicated in what is called accelerated calcifying uraemic arteriosclerosis underlining the importance of the so-called bone-vascular axis in...
Sclerostin and outcomes in dialysis patients

Materials and Methods

Study design

NECOSAD is an observational prospective follow-up study in ESRD patients in the Netherlands. NECOSAD enrolled incident dialysis patients [both haemodialysis (HD) and peritoneal dialysis (PD)] in 38 participating dialysis centres since 1997. Study visits took place at the start of dialysis, at 3 months, 6 months and subsequently at 6-month intervals until the date of loss to follow-up (death, kidney transplantation or transfer to a non-participating dialysis centre) or the end of the follow-up at 1 January 2009. Demographic and clinical data as well as blood samples were obtained at the start of long-term dialysis treatment, and at subsequent study visits.

For the present analysis, the 3-month visit after the start of dialysis treatment was defined as baseline. All laboratory data and clinical conditions of the patients as described in the present analyses refer to this time point.

Patients

Patients with ESRD who were at least 18 years old and started long-term dialysis therapy, either HD or PD, for the first time were invited to participate in NECOSAD.

In the present analysis, all patients in whom the amount of collected blood was sufficient to measure sclerostin at 3 months after initiation of dialysis (the baseline of our study) were included. The medical ethical committees of the participating centres approved the study, and all patients gave their written informed consent before inclusion.

Data collection

The NECOSAD database recorded patients’ data upon demographics and clinical conditions including age, sex, ethnicity, smoking habits, primary kidney disease and comorbidity. Primary kidney diseases and causes of death were classified according to the coding system of the European Renal Association—European Dialysis and Transplant Association (ERA-EDTA). Diagnoses of comorbid conditions were reported by the patients’ nephrologists and used to calculate the comorbidity score according to Khan [17]. Residual renal function expressed as glomerular filtration rate (GFR) was calculated as the mean of creatinine and urea clearance, corrected for body surface area (mL/min/1.73 m²).

Biochemical measures including cholesterol, haemoglobin, total serum calcium, phosphorus, intact parathyroid hormone (iPTH) total AP and albumin were measured by standard laboratory techniques in different centres at 3 months after the start of dialysis. Additionally, sclerostin was measured centrally at the RWTH Aachen University Hospital, Germany. The blood samples for sclerostin were also collected at 3 months after initiation of dialysis therapy. Blood was drawn immediately prior to the dialysis session and centrifuged according to standard techniques and stored at −80°C until their analysis.

Sclerostin measurement.

Sclerostin was assessed by the TECO® Sclerostin EIA Kit, which is a 96-well immuno-capture ELISA. All measurements were done within one batch by personnel blinded to the study results. Serum samples were incubated with a biotinylated polyclonal antibody as well as with a horseradish peroxidase-labelled secondary monoclonal antibody that specifically recognizes human sclerostin. Sclerostin molecules are captured on the plate through the binding of streptavidin to the biotinylated primary antibody. After overnight incubation, the unbound material was washed away. After this washing step, the tetramethyl benzidine substrate, which reacts with the horseradish peroxidase, was added to the well and colour was formed. After 15 min of incubation, the reaction was stopped with HCl and the plate was read using a plate reader at 450 nm. The amount of colour generated is directly proportional to the amount of sclerostin in the sample. According to the instruction manual for human sclerostin HS EIA kit provided by TECOmedical (TECOmedical AG, Sissach, Switzerland), the lower limit of detection is 0.008 ng/mL and the upper limit of quantification is 3.34 ng/mL. The intra- and inter-assay precision as determined by assaying 20 replicates of 4 serum samples in 10 different assays was quantified as below 4.3% for within-run coefficient of variation and below 4.9% for between-run coefficient of variation. Each kit used for NECOSAD sclerostin measurements was accompanied by six internal quality controls revealing a coefficient of variation = 3.9% for low sclerostin controls [0.51 (0.38–0.63)] and a coefficient of variation = 3.7% for high sclerostin controls [1.41 (1.05–1.76)].

Definition of end points

Cardiovascular mortality was defined as death due to the following causes: myocardial ischaemia and infarction, hyperkalaemia, hypokalaemia, cardiac arrest, (hypertensive) cardiac...
failure, fluid overload, cerebrovascular accident, haemorrhage from ruptured vascular aneurysm, mesenteric infarction and cause of death uncertain/unknown. All other causes of death were designated as non-cardiovascular mortality.

Statistical analyses

Mean values with standard deviations (SD) were calculated for continuous variables, and median values with interquartile range as appropriate. Categorical variables were expressed as proportions.

We performed correlation analyses to examine associations between sclerostin, PTH and total AP. Survival analyses were performed to assess associations of sclerostin levels with all-cause mortality, cardiovascular (CV) mortality and death from non-cardiovascular causes. Due to the lack of recommendations for clinical thresholds of sclerostin, the patients were categorized into tertiles according to sclerostin levels.

Cumulative mortality curves were calculated using Kaplan–Meier analysis for all-cause mortality. This method is known to profoundly overestimate the cumulative mortality when analysing competing end points [18]. Analysing separately CV mortality and non-CV mortality is a clear example of competing end points. For that reason, we calculated the cumulative mortality curves for CV mortality and non-CV mortality using competing risk analysis, taking into account that patients dying from CV causes are no longer at risk of dying from non-CV causes, and vice versa [19].

By Cox regression analyses, we calculated hazard ratios (HRs) with 95% confidence intervals (95% CIs) for subsequent short-term (18 months) and longer-term (4 years) periods, according to sclerostin levels at baseline. The lowest category of sclerostin (≤0.95 ng/mL) was thereby used as the reference group. All analyses were adjusted for potential confounders including age, sex, residual GFR, blood pressure, levels of serum albumin, haemoglobin, calcium, iPTH and AP.

A two-sided P-value <0.05 was considered statistically significant. Analyses were performed using SPSS version 21.0.

RESULTS

Patients

A total of 2021 patients with ESRD who started long-term dialysis and still participated in NECOSAD at 3 months after the initiation of dialysis therapy (the baseline of the present work) were included. Of those, sclerostin was measured in 673 patients (=study population), in whom the amount of collected blood was sufficient for the measurement of sclerostin. These patients were included in the present analyses and N = 59 (8.8%) patients were peritoneal dialysis patients.

In the study population, the mean (±SD) age was 63 ± 14 years and 57% of the patients were male. The mean serum sclerostin concentration at baseline was 1.24 ± 0.57 ng/mL. Compared with patients without CKD and without overt cardiovascular disease, this dialysis-associated sclerostin level is ~2-fold higher than previously described by our group with the same sclerostin assay [14] (n = 57; 17 males, 40 females; mean age 48 ± 20 years: 0.58 ± 0.26 ng/mL). Storage time and time of inclusion into the NECOSAD follow-up period did not influence the mean sclerostin levels, since no statistically significant difference between samples was detectable which were obtained within the first 4 years and the last 4 years. After stratifying the samples according to the median of storage time, the sclerostin concentration was 1.25 ± 0.56 and 1.23 ± 0.58 ng/mL in older samples and younger samples, respectively.

The baseline patient characteristics are summarized in Table 1. Patients with low sclerostin concentrations were younger and had a lower blood pressure. Furthermore, the percentage of female patients was higher. Low sclerostin concentrations were associated with lower haemoglobin, albumin and cholesterol levels, higher PTH and AP levels and a higher residual GFR. Dialysis modality and body mass index were comparable across sclerostin concentrations.

Association of sclerostin with demographic and clinical characteristics and parameters of bone and mineral metabolism

The serum concentration of sclerostin was significantly higher in males (1.31 ± 0.55 ng/mL) when compared with females (1.14 ± 0.57 ng/mL) (P < 0.01). In contrast, there were no significant differences between haemodialysis and...
peritoneal dialysis patients (1.24 ± 0.56 versus 1.26 ± 0.64 ng/mL). Furthermore, sclerostin levels were comparable between diabetic and non-diabetic dialysis patients (1.26 ± 0.52 versus 1.23 ± 0.58 ng/mL).

By correlation analyses, sclerostin was positively related to age (r = 0.16, P < 0.001) and inversely to residual GFR (r = −0.18; P < 0.001). Regarding parameters of bone and mineral metabolism, sclerostin levels were inversely associated with iPTH (r = −0.25, P < 0.001) and AP (r = −0.13, P = 0.001). Furthermore, sclerostin levels showed a positive association with total serum calcium (r = 0.12, P = 0.001) as well as a weak association with phosphate (r = 0.09, P = 0.02). Additionally, we divided the patients into groups according to the median of PTH and AP. The patients with both low PTH (<13 pmol/L) and low AP (<65 IU/L) concentrations showed significantly higher sclerostin levels (1.34 ± 0.50 ng/mL) when compared with the patients with both high PTH and high AP concentrations (1.11 ± 0.54 ng/mL, P < 0.001).

### Sclerostin status and all-cause mortality

We investigated short- and long-term mortality according to sclerostin concentrations. The respective follow-up intervals were 18 months and 4 years following the measurements of sclerostin at 3 months after initiation of dialysis. Patients of the highest sclerostin tertile revealed a significant cardiovascular survival benefit as compared with patients of the lowest tertile (Table 2). In detail, the age- and sex-adjusted HRs for the 18-month and 4-year follow-up periods were 0.42 (95% CI: 0.25–0.71) and 0.62 (95% CI: 0.43–0.89), respectively. The association largely persisted after adjustment for parameters of bone mineral metabolism and other confounders (HR at 18 months: 0.39, 95% CI: 0.22–0.68; HR at 4 years: 0.60, 95% CI: 0.35–0.98). The results of the Kaplan–Meier analyses are shown in Figure 1.

### Sclerostin status and cardiovascular mortality

Cardiovascular mortality was significantly lower in patients with high sclerostin concentrations (Table 2). Kaplan–Meier analyses showed a better cardiovascular survival for patients with higher sclerostin levels (Figure 2). Compared with patients of the lowest sclerostin tertile, the age- and sex-adjusted HRs for patients of the highest sclerostin tertile were 1.03 (95% CI: 0.72–1.47) and 1.04 (95% CI: 0.71–1.56) for the 18-month follow-up and 1.03 (95% CI: 0.62–1.73) and 1.05 (95% CI: 0.62–1.77) for the 4-year follow-up. After adjustments for confounders including parameters of bone mineral metabolism, these HRs were stable with 0.29 (95% CI, 0.13–0.62) and 0.59 (95% CI, 0.35–0.98) for the 18 months and the 4-year follow-up, respectively. Of note, the patients of the intermediate sclerostin tertile also had a statistically significant cardiovascular survival benefit when compared with those of the lowest tertile. The fully adjusted HRs for patients of the middle when compared with the lowest sclerostin tertile were 0.40 (95% CI, 0.20–0.82) in the short term and 0.53 (95% CI, 0.31–0.89) in the longer term, respectively.

We also analysed the association between sclerostin tertiles and cardiovascular mortality after separating PD and HD patients. Both sub-group analyses showed the same association between sclerostin concentration tertiles and cardiovascular outcome (higher levels of baseline sclerostin are associated with better outcome). However, only the pooled analyses (n = 673) revealed the above-mentioned statistically significant results in terms of survival benefit with higher baseline sclerostin levels.

### Sclerostin status and non-cardiovascular mortality

Sclerostin did not significantly associate with non-cardiovascular mortality (Table 2). There was a tendency for a lower risk of non-cardiovascular mortality in patients of the

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**Table 2. HRs with 95% CI for all-cause, cardiovascular and non-cardiovascular mortality according to sclerostin tertiles**

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<th>18 months</th>
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<tr>
<td>Second tertile</td>
<td>0.63 (0.39–1.03)</td>
<td>0.72 (0.50–1.04)</td>
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<tr>
<td>Third tertile</td>
<td>0.42 (0.25–0.71)</td>
<td>0.62 (0.43–0.89)</td>
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<tr>
<td>Second tertile</td>
<td>0.62 (0.37–1.03)</td>
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<td>Third tertile</td>
<td>0.39 (0.22–0.68)</td>
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<td><strong>Cardiovascular mortality</strong></td>
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<td>Second tertile</td>
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<td>1.02 (0.59–1.77)</td>
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<tr>
<td>Third tertile</td>
<td>0.58 (0.25–1.39)</td>
<td>0.64 (0.35–1.16)</td>
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Adjusted 1: adjustments for age and sex.  
Adjusted 2: additional adjustments for GFR, blood pressure, levels of serum albumin, haemoglobin, calcium, PTH and AP.

**FIGURE 1**: Kaplan–Meier analyses showing all-cause mortality according to baseline tertiles of sclerostin concentration.
highest sclerostin tertile. In addition, no benefit was seen for patients of the middle sclerostin tertile when compared with the patients of the lowest tertile.

**DISCUSSION**

The major finding of our study is that high or intermediate levels of circulating sclerostin were strongly associated with lower risk factor for future all-cause and cardiovascular mortality in incident dialysis patients, particularly in the short term. Cardiovascular mortality was 70% lower in patients of the highest tertile of sclerostin within 18 months when compared with patients of the lowest tertile. Adjustments for age, sex, residual GFR, blood pressure, levels of serum albumin, haemoglobin, calcium, iPTH and AP had a minor impact only, supporting the independent nature of sclerostin on this difference in mortality.

Sclerostin, the protein product of the *SOST* gene, is an osteocyte product that functions as a soluble antagonist of the Wnt/β-catenin signalling and profoundly influences bone metabolism [8, 9]. Blocking sclerostin in humans via antibody application is regarded a promising tool to enhance physiological mineralization processes in bone via osteo-anabolic properties, which stabilizes bone mineral density in postmenopausal mineralization processes via osteo-anabolic properties [14–16]. These human data are experimentally supported by *in vitro* data also showing sclerostin expression in vascular smooth muscle cells in a pro-calcific milieu [23]. Notably, high levels of circulating sclerostin correlated positively with the extent of valvular calcification as assessed by computed tomography quantification [14,15]. Within the complex spectrum of CKD-MBD dysregulation of sclerostin signalling is an early event [24].

With the present data from the NECOSAD, we substantially expand previous study findings about the association of circulating sclerostin and survival in ESRD patients [25]. Viaene *et al.* [25] measured sclerostin levels in 100 prevalent dialysis patients from a single centre who were followed for a median of 637 days. They included at baseline prevalent haemodialysis patients who already survived on average 40 months (16–69 months) on dialysis, in contrast to the incident ESRD patients from NECOSAD. Viaene *et al.* [25] dichotomized their cohort according to the median level of sclerostin and detected a significant survival benefit for patients above the median after adjusting for age and gender (HR 0.33, 95% CI: 0.15–0.73, *P* = 0.006). However, within a fully adjusted model including bone-specific alkaline phosphates the association between survival and sclerostin lost statistical significance [25]. In contrast, our NECOSAD results still indicate an independent association with sclerostin and outcome even after adjustments for AP. The reasons for these discrepancies between the two cohorts remain speculative. Power issues, case-mix and differences between sclerostin assays may be involved. Cohort characteristics as well as sclerostin assay characteristics may also contribute to the fact that the present NECOSAD cohort could not reproduce differences between males and females as previously described [25]. However, the key message is comparable in both studies, i.e. the higher the circulating sclerostin—the better the outcome. This association is particularly striking because the patients within the highest sclerostin tertile in NECOSAD were older and revealed a higher comorbidity index.

Additional studies are required to elucidate pathophysiological mechanisms underlying this association. Because the physiological role of bone sclerostin is down-regulation of mineralization, we might speculate that the function of sclerostin in the vasculature is comparable [26]. It should be of note that in a recent bone biopsy study [27] increased circulating sclerostin levels in the elderly were not accompanied by an increase in sclerostin mRNA levels in bone fuelling the hypothesis that non-skeletal sources, including the vasculature, may be of relevance. Looking at the convincing efficacy of anti-sclerostin antibodies in increasing skeletal mineralization, it appears mandatory to investigate what kind of effects such sclerostin blocking effects have in the vasculature [20]. A recent prospective randomized study in 261 postmenopausal women receiving romosozumab (a humanized monoclonal anti-sclerostin antibody) over 12 months did not report remarkable cardiovascular side effects. However, the exact baseline cardiovascular disease burden in the study cohort is unknown [20].

Potential limitations of this study need to be acknowledged. The important question remains unanswered if the association between sclerostin and mortality reflects causality. We cannot assess a link between sclerostin concentration, vascular calcification status and mortality, because the cardiovascular status (e.g. calcification) was not systematically assessed. Moreover, our study lacks data upon renal osteodystrophy, so the triangular relationship between bone metabolism and sclerostin levels as well as outcome remains speculative. Additional limitations of our study are the missing data on phosphate binder, active vitamin D or calcimimetic treatment and if such CKD-MBD...
therapy influences sclerostin levels. We also acknowledge that our data solely rely on single spot measurements of sclerostin.

In conclusion, high levels of circulating sclerostin at 3 months after dialysis start were strongly associated with short-term cardiovascular survival over 18 months in dialysis patients from NECOSAD. This association remained strong and significant even after adjustments for a wide range of potential confounders including CKD-MBD-related factors. With all necessary limitations, the present data may suggest a protective role of sclerostin in CKD-MBD vascular disease. However, currently sclerostin does not qualify yet as biomarker for cardiovascular risk assessment in dialysis patients. Further studies need to assess if sclerostin measurements add benefit to patient care if incorporated to clinical routine regarding parallel monitoring of bone and vascular disease. Moreover, it is undetermined if therapeutic modifications of sclerostin translate into the corresponding alterations in outcome.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the results presented in this paper have not been published previously as a full-text manuscript.

REFERENCES


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