

Sclerostin Levels Predict Cardiovascular Mortality in Long-Term Hemodialysis Patients: A Prospective Observational Cohort Study

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Summary

Sclerostin is a protein which is involved in bone metabolism and probably also in vessel wall function. This prospective observational cohort study evaluated the prognostic significance of sclerostin in hemodialysis (HD) patients. In total, 106 HD patients and 25 healthy controls participated in the study. HD patients were prospectively followed up for five years. Sclerostin was measured in serum using standard ELISA kits by Biomedica. Sclerostin concentrations in serum were higher in HD patients compared to the controls (89.2 ± 40.3 pmol/l vs. 32.8 ± 13.0 pmol/l, $p < 0.001$). Sclerostin levels were significant for cardiovascular mortality but not for overall mortality and mortality due to infection. A higher cardiovascular risk was connected to sclerostin concentrations above the median (> 84 pmol/l), HR (95 % CI): 2.577 (1.0002-10.207), $p = 0.04$. When sclerostin was evaluated together with residual diuresis in Kaplan-Meier analysis the worst prognosis due to cardiovascular events was observed in the group with high sclerostin and zero residual diuresis compared to all other patients ($p = 0.007$). In summary, serum sclerostin levels in HD patients were increased when compared to healthy subjects. High sclerostin levels were demonstrated as a risk factor for cardiovascular mortality. Further studies are required to clarify the pathophysiological mechanisms of sclerostin action in patients with renal failure before therapeutic measures can be established.

Key words

Cardiovascular • Hemodialysis • Mortality • Outcome • Sclerostin

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Introduction

Sclerostin is a product of the *SOST* gene located on chromosome 17q12-21 (Balemans *et al.* 2001, Brunkow *et al.* 2001). It is a glycoprotein with a molecular weight of 22 kDa. This protein is expressed in osteocytes buried in the bone mineralized matrix and influences bone metabolism (Poole *et al.* 2005). In addition, osteocytes develop a wide web with activated sensors capable of identifying markers in blood and secrete hormones including sclerostin and fibroblast growth factor 23 (FGF23) which influence distant organs (Dallas *et al.* 2013). It inhibits osteoblastogenesis and contributes to the development of osteoporosis. Sclerostin acts as an inhibitor of the Wnt/ β -catenin pathway, which is also important in blood vessels (van Bezooijen *et al.* 2007). Some of the sclerostin is secreted into general circulation where sclerostin can suppress the change of vascular smooth muscle cells into osteoblast-like cells (Ott 2015).

Sclerostin was first described in 2001 (Balemans *et al.* 2001, Brunkow *et al.* 2001) and till now (September

13, 2018) there are more than 1570 records for key words "sclerostin" or "SOST" in the medical database Medline, 235 of which are from 2017 and the first half of 2018. This profound interest and research is focused on the possible roles of sclerostin in a number of disease states. Decreased or lacking sclerostin was documented in sclerosteosis (Balemans *et al.* 2001, Brunkow *et al.* 2001) and van Buchem disease (Balemans *et al.* 2002), while its increase occurs with age (Mödder *et al.* 2011), physical inactivity (Amrein *et al.* 2012) and also in chronic kidney disease (Pelletier *et al.* 2013). Sclerostin is of significance in bone diseases mainly in osteoporosis where this molecule is a causal player (Cosman *et al.* 2016), but also in Paget disease, multiple myeloma or bone metastases (Terpos *et al.* 2012, Yavropoulou *et al.* 2012).

Special area of sclerostin significance are chronic kidney disease (CKD), mineral bone disease (MBD) and possibly vascular calcifications in patients with CKD (Cejka *et al.* 2011, Thambiah *et al.* 2012, Pelletier *et al.* 2015, Brandenburg *et al.* 2016). In CKD patients, serum sclerostin is elevated and may be produced ectopically in calcified vascular smooth muscle cells and calcified aortic valves (Zhu *et al.* 2011, Brandenburg *et al.* 2013). Whether this process is a sign of the final transformation of the vascular smooth muscle cells or a defensive mechanism is not well understood.

Sclerostin correlates negatively with parathormone (PTH) levels as documented in all the studies focused on sclerostin and PTH and is related to bone density. However, its relationship to the prognosis of CKD patients, mainly patients with end stage renal disease treated with long-term hemodialysis (HD), is ambiguous according to currently available studies (Zeng *et al.* 2018). Some researchers reported a positive correlation, whereas others suggested none or a negative correlation (Zeng *et al.* 2018).

The aim of the present study was to evaluate the prognostic significance of sclerostin in our cohort of HD patients during long-term follow-up.

Methods

Study design and subjects

This is a prospective observational cohort study performed in 2 dialysis centers in University hospitals in the Czech Republic. The study group consisted of 106 long-term HD patients (64 men and 42 women, mean age 61±14 years). In total, 111 patients were assessed for eligibility in this study. Four patients were excluded as

three were not in a stable clinical status at the beginning of the study, one patient refused to participate, and for another patient there was insufficient material available for the analysis of sclerostin. All of these factors lead to the 106 patients participating in the present study. The control group for the comparison of sclerostin concentrations consisted of 25 healthy subjects (16 men and 9 women, mean age 48±11 years). One part of the group of HD patients and controls has already been presented in our previous reports (Kalousová *et al.* 2014, Kalousova *et al.* 2015, Kalousová *et al.* 2017) The study was performed in adherence to the principles of the Helsinki Declaration and approved by the Ethical Committee. All participants gave informed consent prior to entering the study.

The causes of renal failure in our HD patients were as follows: diabetic nephropathy (26.4 %), hypertensive nephropathy (4.7 %), interstitial nephritis (20.75 %), glomerulonephritis (20.75 %), polycystic kidney disease (14.2 %) and multifactorial or unknown (13.2 %). Residual diuresis ranged from 0 to 2500 ml/24 hours. Of the patients, 53 were anuric, while the remaining 53 patients had median residual diuresis 1200 ml/24 hours. The majority of the patients had 4-hour dialysis session three times a week and polysulphone or modified cellulosic membranes were used at the beginning of the study. The duration of dialysis treatment was 26.5 months (median, range 1-359 months). 40.6 % of patients had diabetes, 58.5 % dyslipidemia and almost all patients had (94.3 %) hypertension. Cardiovascular disease was recorded in the personal history in 34 % (acute myocardial infarction in 15.1 %), cerebrovascular diseases in 3.8 % and peripheral vascular disease in 13.2 %. Smoking was recorded in 15.1 % of HD patients. The parameters of calcium phosphate metabolism of nearly the same cohort were presented in our previous study (Kalousova *et al.* 2015). The results of the routine baseline laboratory examinations are provided in Table 1.

The HD patients were treated with standard medication as recommended, which then changed during the follow up as appropriate (at the beginning of the study antihypertensive drugs in 59.4 %, diuretic drugs in 49.1 %, statins in 47.6 %, aspirin or other antiplatelet drugs in 17.6 %, iron, erythropoiesis stimulating agents (ESA), phosphate binders and vitamin D and its analogs.

The HD patients were prospectively followed up for five years or until death (April 2009 - April 2014). The causes of death were recorded and finally classified

Table 1. The results of the laboratory examinations of hemodialysis patients and healthy controls.

Parameter	HD patients	Controls	HD vs. controls – p value
Number of patients (men/women)	106 (64/42)	25 (16/9)	
Age (years)	61±14	48±11	<0.001
BMI (kg/m ²)	27.0±5.3	26.2±4.2	0.4, n.s.
Hemoglobin (g/l)	111.8±10.7	143.3±8.9	<0.001
Leukocytes (x10 ⁹ /l)	7.4±2.2	6.1±1.5	0.01
Creatinine (μmol/l)	778±218	91±12	<0.001
Urea (mmol/l)	23.0±6.7	5.6±1.8	<0.001
Calcium (mmol/l)	2.2±0.2	2.3±0.1	<0.001
Phosphate (mmol/l)	1.9±0.6	1.3±0.2	<0.001
Alkaline phosphatase (μkat/l)	1.5±0.9	0.8±0.2	<0.001
25-OH vitamin D ₃ (ng/ml)	18.0±15.6	48.7±18.3	<0.001
VDBP (μg/ml)	213±70.9	222±87.7	<0.001
Parathyroid hormone (pmol/l)	34.7±35.7	3.7±1.5	<0.001
Albumin (g/l)	40.8±3.5	47.3±3.2	<0.001
Cholesterol (mmol/l)	4.4±1.0	5.3±1.0	0.001
HDL cholesterol (mmol/l)	1.1±0.3	1.5±0.5	<0.001
LDL cholesterol (mmol/l)	2.4±0.9	3.0±0.8	0.001
Triacylglycerols (mmol/l)	2.1±1.1	1.7±0.9	0.1, n.s.
CRP (mg/l)	8.5±12.3	6.1±3.0	0.8, n.s.

Data are expressed as mean±SD (standard deviation). BMI, body mass index, CRP, C-reactive protein, HD, hemodialysis, HDL, high density lipoprotein, LDL, low density lipoprotein, VDBP, vitamin D binding protein, n.s., not significant.

based upon the patients' history as cardiovascular, infection, tumor or other. Almost half of the patients (52 patients, 49.1 %) died during the follow up. In 20 of those patients (i.e. 38.5 %) the causes of death were cardiovascular, in 18 patients (i.e. 34.6 %) they were caused by infection, in 4 patients (7.7 %) tumor and in 10 patients (20 %) the causes were other or unknown. 28 patients received a kidney transplant, 4 of which subsequently died during the follow-up.

Laboratory analyses

Blood samples in HD patients were collected after a long interdialysis interval. They were retrieved from the inserted dialysis needle prior to the administration of heparin, just before the beginning of the dialysis session. In the control group blood was taken after overnight fasting. The blood was standardly centrifuged for 10 min at 3000 rpm (rotations per minute) and routine biochemical parameters were measured in the fresh samples. The serum for special analyses was stored at -80 °C.

Vitamin D in the plasma was measured using high performance liquid chromatography. The vitamin D

binding protein was assessed using a standard enzyme linked immunosorbent assay (ELISA) kit (Quantikine, RD Systems, USA) according to the manufacturer's protocol. The parathyroid hormone (PTH) levels (second generation test) were determined using ECLIA (Electrochemiluminescence, Modular Roche, Germany). Details of the specific biochemical measurements are described in our previous study (Kalousova *et al.* 2015).

Sclerostin was measured in the serum using the ELISA kit (Biomedica, Austria) according to the manufacturer's protocol and all samples were analyzed in one laboratory. The results are expressed in pmol/l (1 pg/ml=0.044 pmol/l). Routine laboratory parameters were assessed using standard laboratory methods on automated analyzers.

Statistical analysis

Statistical softwares SPSS v.16 (SPSS Inc., Chicago, IL, USA, www.spss.com) and Wolfram Mathematica 11.2 were used for statistical analysis.

The results are shown as mean ± SD (standard deviation). The Mann-Whitney U-test was used for comparison between HD patients and controls.

Comparison of sclerostin between HD patients and healthy controls was further performed using the general linear model with adjustment for age after bootstrap correction of non-normality. Assessment of the relationship between parameters was performed using Kendall's tau correlation coefficient.

The significance of sclerostin for overall mortality, cardiovascular mortality and mortality due to infection were tested. Kaplan-Meier analysis was used to

construct real survival curves and hazard functions for hemodialysis patients based on sclerostin concentrations (below and above the median). The curves were compared using the log-rank test. The hazard ratios (relative risks of mortality, HR) of appropriate 95 % confidence intervals (CI) for each group of patients were calculated using the Cox proportional hazard model.

All results were considered statistically significant at $p < 0.05$.

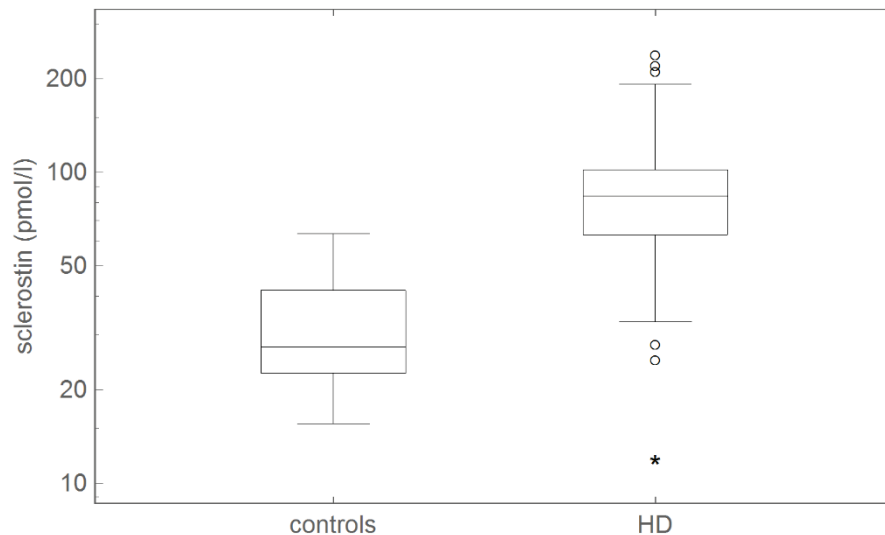


Fig. 1. Sclerostin in hemodialysis patients and healthy controls. HD vs. controls: 89.2 ± 40.3 pmol/l, 32.8 ± 13.0 pmol/l, $p < 0.001$, $p = 0.029$ after adjustment for age. HD – hemodialysis.

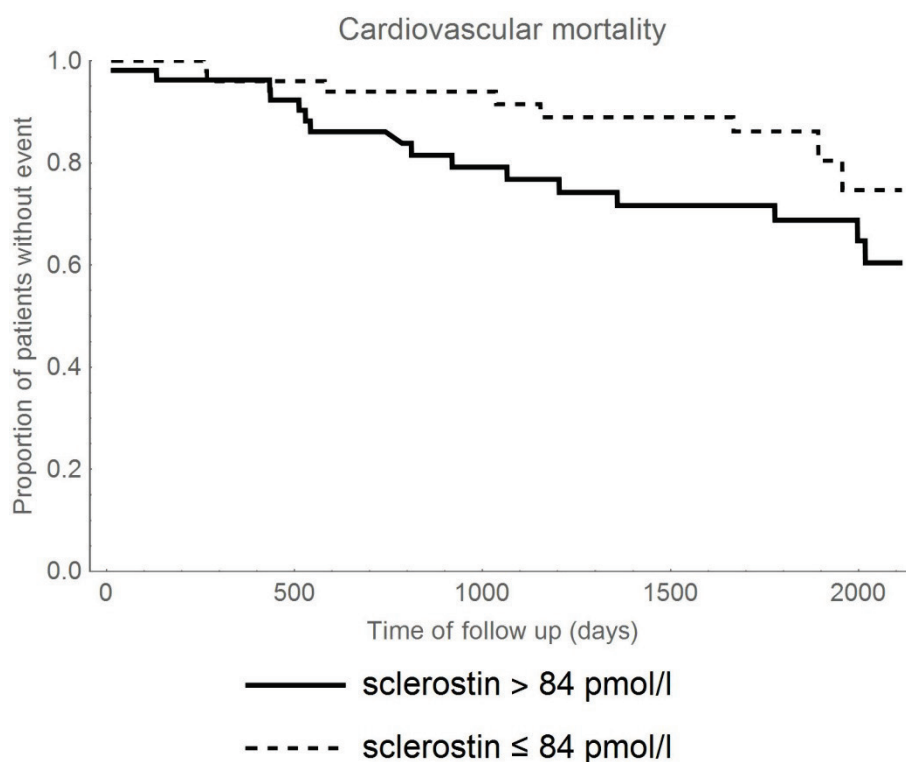


Fig. 2. Kaplan-Meier analysis showing cardiovascular mortality of hemodialysis patients based on sclerostin levels: sclerostin below (≤ 84 pmol/l) and above median (> 84 pmol/l). $p = 0.044$ (log-rank test)

Results

Sclerostin concentrations in serum are almost three times higher in the HD patients when compared to the controls (89.2 ± 40.3 pmol/l vs. 32.8 ± 13.0 pmol/l, $p < 0.001$, $p = 0.029$ after adjustment for age, Fig. 1) and were higher in HD men than in HD women (93.7 ± 38.6 pmol/l vs. 82.2 ± 42.4 pmol/l, $p = 0.019$). The results of other laboratory parameters are shown in Table 1.

In HD patients, sclerostin correlated with age ($\tau = 0.142$, $p = 0.034$), creatinine ($\tau = 0.224$, $p = 0.001$), and calcium ($\tau = 0.144$, $p = 0.030$), and was negatively correlated with cholesterol ($\tau = -0.131$, $p = 0.048$) and alkaline phosphatase ($\tau = -0.139$, $p = 0.035$). In the controls, no significant correlation between the basic laboratory parameters and age was observed. There was no difference in sclerostin levels between diabetics and non-diabetics, between patients with and without cardiovascular, peripheral vascular or cerebrovascular diseases in their personal history, and there was no relationship with hypertension. Additionally, there was no difference of serum sclerostin concentrations among various primary renal diagnoses. Sclerostin was higher in anuric patients (99.9 ± 43.2 pmol/l vs. 78.0 ± 34.3 pmol/l, $p = 0.004$) and correlated with residual diuresis ($\tau = -0.203$, $p = 0.005$ for all patients).

The Kaplan-Meier analysis did not find any

significance of sclerostin for overall mortality and mortality due to infection but sclerostin levels were significant for cardiovascular mortality ($p = 0.044$, Fig. 2). A higher cardiovascular risk was connected to sclerostin concentrations above the median (>84 pmol/l). This was also confirmed using the Cox proportional hazard model giving HR (95 % CI) 2.577 (1.0002-10.207), $p = 0.04$. When sclerostin was evaluated together with residual diuresis in Kaplan-Meier analysis: the worst prognosis due to cardiovascular events was observed in the group with high sclerostin and zero residual diuresis in comparison to all of the other patients ($p = 0.007$, Fig. 3). A similar result for sclerostin-anuria interaction also remained significant in the Cox model when sex, age, BMI and albumin were applied in the model, HR (95 % CI) 3.251 (1.094-9.663), $p = 0.034$. The same finding was also observed after further adjustment for comorbidities – diabetes mellitus, dyslipidemia and any pre-existing cardiac or vascular disease, HR (95 % CI) 7.864 (1.989-31.092), $p = 0.003$. Cox regression has not shown any significance for overall mortality and mortality due to infection.

In conclusion, sclerostin is elevated in HD patients and its higher concentrations (above the median concentrations in HD patients) are linked to higher cardiovascular mortality.

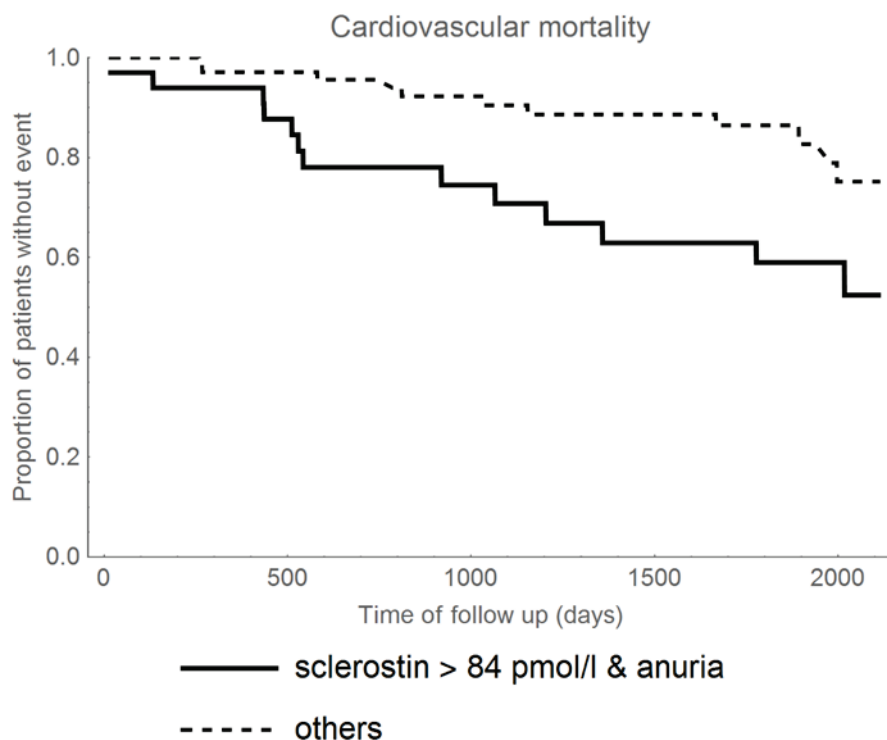


Fig. 3. Kaplan-Meier analysis showing cardiovascular mortality of hemodialysis patients based on sclerostin levels and residual diuresis: sclerostin below (≤ 84 pmol/l) and above median (>84 pmol/l) and residual diuresis 0 ml/24 hours (anuria) and >0 ml/24 hours. $p = 0.007$ (log-rank test)

Discussion

The present prospective observational cohort study shows that in long-term HD patients, the serum sclerostin levels are increased compared to healthy subjects. High levels of serum sclerostin were revealed as a risk factor for cardiovascular mortality.

In our study, serum sclerostin levels were three times higher in HD patients compared to subjects with normal kidney function, and were higher in males than in females similar to the results of another study by Sato *et al.* (Sato *et al.* 2018). Higher cardiovascular risk was connected to sclerostin concentrations above the median. Furthermore, the worst prognosis due to cardiovascular events was even more pronounced in the group of anuric HD patients with high sclerostin levels than in all other groups of HD patients.

In various studies conducted on HD patients, different methods of serum sclerostin levels measurement of HD patients were used, and the results also differ. Not only were the absolute concentrations for sclerostin levels discrepant, but the significance of sclerostin for patients' prognosis also showed conflicting results.

Firstly, in the clinical-laboratory studies currently available to date, sclerostin has been measured immunochemically using ELISA kits by three different manufacturers (mainly Biomedica, TECO Medical, and recently also R&D Systems) (Piec *et al.* 2016). Currently, a novel automated chemiluminescent sclerostin assay (LIAISON®, DiaSorin) has become available for research use (Drake *et al.* 2018). Several comparative studies have been performed and the results obtained from different assays differ (McNulty *et al.* 2011, Costa *et al.* 2014, Moysés *et al.* 2015, Mause *et al.* 2016, Piec *et al.* 2016), i.e. a standardization between the measurements of the serum sclerostin level in the HD population is required.

Secondly, the conflicting results evaluating a correlation between serum sclerostin levels and mortality may be linked to the varying lengths of the observational periods.

Finally, different age groups in heterogeneous HD patients, their comorbid conditions and the duration of dialysis treatment are further plausible confounding factors.

Some studies of the HD population showed that serum sclerostin levels were significantly associated with overall mortality (Gonçalves *et al.* 2014, Kanbay *et al.* 2014, Gong *et al.* 2018, Novo-Rodríguez *et al.* 2018),

whereas other studies demonstrated no association with overall mortality (Viaene *et al.* 2013, Jean *et al.* 2016, Drechsler *et al.* 2015, Lips *et al.* 2016, Delanaye *et al.* 2014, Nowak *et al.* 2015, Sato *et al.* 2018). The studies investigating sclerostin in cardiovascular disease (CVD) morbidity and mortality in HD populations are shown in Table 2.

Similarly to our study, sclerostin was demonstrated as an independent mortality predictor in 91 HD patients who were followed up with for 10 years. In that study, sclerostin was determined using the TECO Medical assay (Gonçalves *et al.* 2014). Additionally, an association of sclerostin levels (Biomedica assay) with overall mortality as well as cardiovascular events was found in non-dialyzed chronic kidney disease patients (Kanbay *et al.* 2014). A recent prospective observational study of serum sclerostin concentrations assessed by enzyme immunoassay (Biomedica assay) in 98 peritoneal dialysis (PD) patients during the 6-year study period suggested that high levels of serum sclerostin were significantly linked to new-onset cardiovascular events and cardiovascular mortality (Gong *et al.* 2018). A study by Novo-Rodríguez *et al.* (Novo-Rodríguez *et al.* 2018) fully supports our results. These authors concluded that high sclerostin levels (measured using the Biomedica kit) are related to mortality due to cardiovascular causes (follow up 2007-2014), but no association was found between sclerostin and infectious (non-cardiovascular) causes of mortality in their cohort of 130 hemodialysis patients (75 patients with diabetes mellitus). An increase of serum sclerostin by 10 pmol/l resulted in a 31% increase in cardiovascular mortality. According to the authors, the clinical implication of these findings are based on the possible use of serum sclerostin as a new biomarker for cardiovascular mortality risk in order to establish preventive strategies. This is also in perfect agreement with our conclusion.

However, Viaene *et al.* (2013) assessed sclerostin using the Biomedica kit in 100 prevalent dialysis patients who were followed up with for 637 days, a much shorter period compared to our study and have shown that high circulating sclerostin levels are associated with improved survival. In addition, a cross-sectional study of prevalent HD patients by Jean *et al.* (Jean *et al.* 2016) showed that higher serum sclerostin levels were linked to higher bone mineral density, lower Kupilla aortic calcification scores and a better survival rate. In the NECOSAD study performed with 673 HD and PD patients (4-years follow up) where sclerostin was

Table 2. Studies investigating sclerostin in CVD morbidity and mortality in HD

Authors	Method (ELISA)	Study population	Study type	Study duration	Study outcomes
<i>Kalousová et al. 2019</i>	Biomedica	HD (n=106)	Prospective observational	5 years	High sclerostin levels were significant for CVD mortality
<i>Gonçalves et al. 2014</i>	TECO Medical	HD (n=91)	Prospective observational	10 years	High sclerostin was associated with CVD in prevalent HD population
<i>Kanbay et al. 2014</i>	Biomedica	CKD (3-5) (n=173)	Prospective observational	26 months	High sclerostin levels were associated with CVD in non-dialyzed CKD population
<i>Viaene et al. 2013</i>	Biomedica	HD (n=100)	Post-hoc survival analysis in a cross sectional study	637 days	High sclerostin levels were associated with improved survival in prevalent HD patients
<i>Drechsler et al. 2016</i>	TECO Medical High sensitive	HD and PD (n=637) 90 % prevalence of HD patients (NECOSAD study)	Observational prospective follow-up cohort study	18 months and 4 years	High serum sclerostin levels were associated with lower short-term CVD and overall mortality in HD patients
<i>Lips et al. 2016</i>	Biomedica	HD (n=198) and HDF (n=198) (CONTRAST study)	Prospective observational	2.9 years	High sclerostin levels were associated with lower mortality risk
<i>Delanaye et al. 2014</i>	TECO Medical	HD (n=164)	Prospective observational	2 years	No association between baseline sclerostin concentrations and mortality
<i>Nowak et al. 2015</i>	TECO Medical	HD (n=239)	Multicentre prospective observational	1461 days	No association between sclerostin and all-cause mortality
<i>Jean et al. 2016</i>	TECO Medical High sensitive	HD (n=207)	Cross-sectional prospective observational	30 months	Higher serum sclerostin levels were associated with better survival
<i>Sato et al. 2018</i>	Biomedica	HD (n=389)	Prospective observational cohort study	72 months	Serum sclerostin was not an independent predictor in maintenance HD patients
<i>Kanbay et al. 2016</i>	Metanalysis	HD (n=1788) 9 studies			Serum sclerostin was not associated with overall and CVD mortality

CKD chronic kidney disease, CVD cardiovascular disease, ELISA enzyme linked immunosorbent assay, HD hemodialysis, PD peritoneal dialysis.

measured using the HS EIA kit TECO Medical, high levels of serum sclerostin were associated with lower cardiovascular mortality (mainly short-term mortality) in dialysis patients (Drechsler *et al.* 2015). However, statistically significant results were obtained only when HD and PD patients were evaluated together, while in separate categories of HD or PD patients, the trend was not significant. In a recent study of 396 HD patients with a median follow up of 2.9 years (CONTRAST study) (Biomedica assay) high serum sclerostin was associated with a lower mortality risk (Lips *et al.* 2016). Another study with 164 HD patients followed up for 2 years found no difference in the baseline concentrations of sclerostin (TECO Medical kit) in the survivors and non-survivors (Delanaye *et al.* 2014). Similarly, no significance of sclerostin for the mortality of HD patients (239 HD patients, 1461 days follow up, measurement of sclerostin using the TECO Medical kit) was described by Nowak *et al.* (2015). A study by Sato *et al.* in 2018 found no association between serum sclerostin (assessed by Biomedica assay) and (total) mortality in a cohort of 389 Japanese hemodialysis patients with 42 months of follow-up (Sato *et al.* 2018). A recent quantitative meta-analysis of 9 studies of CKD patients (1788 patients included in total) revealed no relationship between serum sclerostin concentrations and either overall or cardiovascular mortality (Kanbay *et al.* 2016).

Similarly, studies focusing on sclerostin in vascular calcifications in end-stage renal disease do not yield the same results. For example, Yang *et al.* reported an inverse correlation between sclerostin and aortic calcification scoring (calculated from plain X ray films of both posterior-anterior and lateral views) and future cardiovascular events (Yang *et al.* 2015). Similarly, other studies (Delanaye *et al.* 2014, Evenepoel *et al.* 2015, Lee *et al.* 2016, Sato *et al.* 2018) have shown the connections between elevated sclerostin levels and a lower prevalence and severity of vascular calcifications. Most probably, sclerostin may restrain the progression of vessel calcification by a similar mechanism of antagonising bone formation.

However, in the study by Qureshi *et al.* (2015) the increased sclerostin levels were described as a positive predictor of vascular calcifications. High serum sclerostin was associated with coronary artery calcification evaluated by CT as well as with biopsy verified epigastric artery calcification (Qureshi *et al.* 2015). The cross-sectional study by Morena *et al.* in 2015 found that bone turnover inhibitors, sclerostin and

osteoprotegerin, specifically their high serum levels, were independently correlated with coronary artery calcifications with potential additive impact in 241 non-dialyzed chronic kidney disease (non-HD CKD) patients, in the context of low to normal PTH levels (Morena *et al.* 2015). These findings further emphasize the conclusion that verified optimal values for PTH, osteoprotegerin and sclerostin should be established in the HD and non-HD CKD population with the goal of preventing vascular calcifications. Paradoxically the positive association between high sclerostin levels and vascular calcifications might be due to the inhibitory effect of sclerostin, counterbalancing other mechanisms involved in vessel calcifications.

Sclerostin levels behave differently in acute settings like critical illness. For example, in the study by Koch *et al.* sclerostin levels were significantly increased in critically ill patients, but were not a predictor of mortality (Koch *et al.* 2017). Sclerostin serum levels were not different in terms of the gender or age of critically ill patients. Sclerostin concentrations were associated with disease severity and independent on the presence of sepsis. Pre-existing kidney, or liver impairment, abnormalities of bone metabolism, but not cardiovascular disease were major influencing factors on the circulating sclerostin concentrations in the critically ill patients (Koch *et al.* 2017).

Interestingly, a recent study by Cejka *et al.* (2014) concluded that the increasing levels of sclerostin in patients with a progressive decline of kidney function occur not because of the renal sclerostin retention, but because of the increased production of sclerostin by osteocytes (Cejka *et al.* 2014).

Given the knowledge that inactivating mutations of SOST, the gene for sclerostin, leads to formation of strong dense bones, there are emerging novel treatment strategies against sclerostin which stimulate bone formation and decrease bone resorption. Trials using romosozumab have now reached phase I and II (McClung *et al.* 2014, Padhi *et al.* 2014). Romosozumab is a humanized monoclonal antibody that targets sclerostin. A clinical trial (phase III) on the treatment of osteoporosis using romosozumab was performed on 7180 postmenopausal women without renal failure. The trial demonstrated decreased risk of vertebral fracture after 12 months in the group treated with romosozumab, while adverse events including cardiovascular ones, were comparable between the treated and non-treated groups (Cosman *et al.* 2016). These promising results in treating

osteoporosis with anti-sclerostin antibody might be of value as an aid in developing strategies beneficiary for both bone health and vascular calcifications in patients with kidney disease, as well as possibly improving the survival in HD population.

Treatment of HD patients with hemodiafiltration (CONTRAST study) lead to a decrease of serum sclerostin levels, and this decline was dependent on the magnitude of convection volume (Lips *et al.* 2016). However, the possible removal of sclerostin during a single dialysis procedure with a high-flux membrane does not explain the decrease in sclerostin levels in the long-term. Additionally, the secondary analysis of the CONTRAST study also demonstrated an association between high sclerostin levels and a lower mortality risk. A study by Carlson *et al.* found that the clearance of sclerostin during hemodialysis occurs at a lower rate but intradialytic plasma levels of substrates involved in bone metabolism including sclerostin remains largely unchanged (Carlson *et al.* 2017). The role of sclerostin as the mediator between bone and vasculature in chronic disease – mineral bone disorder (CKD-MBD) remains a fascinating analytical and clinical area for future further investigations.

Our present study had some limitations. Firstly, the study was observational and some unknown confounding factors might not have been considered when interpreting the results. However, we performed a profound statistical analysis, which was identified and adjusted for relevant variables. Secondly, the study

evaluated only a moderate sample of the HD population of two hemodialysis centres, although the proportion of the deceased HD patients due to infectious or cardiovascular causes still allowed us to perform the mortality analysis.

We can summarize that sclerostin seems to be a potentially significant biomarker in patients with chronic kidney disease treated with long-term hemodialysis. High levels of serum sclerostin were revealed as a risk factor for cardiovascular mortality in long-term HD patients. However, for the proper usage of sclerostin in clinical practice we would need comparable laboratory methods, randomized controlled trials, a better understanding of bone turnover as well as of detailed mechanisms of vascular and extraosseal calcifications. Based on this novel information we could discuss the possible therapeutic measures to influence sclerostin and explore the significance of mortality risk in those patients with chronic kidney disease treated by hemodialysis.

Conflict of Interest

The authors declare that they have no conflict of interest.

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