Serum sclerostin: relation with mortality and impact of hemodiafiltration

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INTRODUCTION

Mortality in haemodialysis (HD) patients remains unacceptably high, despite various treatment-related refinements and improvements in our knowledge of the uraemic state [1–3]. A large proportion of deaths can be attributed to increased cardiovascular risk [4, 5]. However, this is only partly due to the high prevalence of traditional risk factors in this patient group, such as diabetes mellitus, hypertension and dyslipidaemia [6]. Non-traditional cardiovascular risk factors have also been shown to play a part. These include systemic (micro)inflammation, protein-energy wasting and chronic kidney disease-mineral bone disease (CKD-MBD) [7, 8], which has been specifically linked to extra-skeletal calcifications. The factors associated with the latter condition include calcium, phosphate, parathyroid hormone (PTH) and vitamin D. However, various other bone metabolism factors may also be involved, such as osteocalcin, (bone-specific) alkaline phosphatase (BALP), fibroblast growth factor 23 (FGF23) and sclerostin (Scl) [7, 9–11].

Scl, a 22 kDa-sized glycoprotein secreted by osteocytes, inhibits the canonical Wingless-type mouse mammary tumour virus integration site (Wnt) pathway by binding to lipoprotein receptor-related protein 5 and 6. Wnt signalling is involved in many different processes and performs a wide range of functions in various cardiovascular tissues. In healthy subjects, Scl inhibits osteoblast function and promotes the upregulation of osteoclasts,
resulting in decreased bone formation [12]. In CKD patients, serum Scl concentration (sScl) is inversely related to kidney function. It is especially high in subjects treated with HD [13]. These patients exhibit osteogenic changes in their vessel walls; however, the part played by sScl in this process is largely unknown. Studies on the association between sScl and mortality show conflicting results, ranging from inverse associations [14–16], via absence of an association [17, 18] to a positive relation [19].

The first of the present study’s three goals is to determine the association between sScl and mortality in end-stage kidney disease (ESKD) patients. As the molecular weight of sScl permits its removal by convective transport it is then possible to determine whether longitudinal data on sScl in conventional HD patients differ from those in individuals being treated by post-dilution online haemodialfiltration (HDF). If so, the third goal is to study the effect of the magnitude of the convection volume on sScl. Data from the CONVective TRANsfer STudy (CONTRAST) were used for this purpose.

MATERIALS AND METHODS

Patients and study design

The methods used were originally developed for the CONTRAST study (clinicaltrials.gov identifier NCT00205556) [20, 21]. In short, CONTRAST was a randomized controlled trial to evaluate the effect of post-dilution online HDF versus low-flux HD on all-cause mortality and on cardiovascular events. A total of 714 patients were enrolled in 29 dialysis facilities between 2004 and 2009 and followed until December 2010. ESKD patients ≥18 years of age were eligible for inclusion if they had been treated with HD two or three times per week for at least 2 months. The exclusion criteria were treatment with HDF or high-flux HD in the 6 months preceding randomization, a life expectancy of ≤3 months due to non-renal disease, severe non-compliance with dialysis treatment or participation in another clinical intervention trial. CONTRAST was approved by a central medical ethics review board. The study was conducted in accordance with the Declaration of Helsinki and with Good Clinical Practice guidelines. Written informed consent was obtained from all participants before enrolment.

Data collection

At baseline, data on demographics, medical history, biochemical values and treatment characteristics were collected. Material for laboratory measurements was drawn before dialysis. Routine samples were analysed using standard techniques, in local laboratories of the participating centres. The following formula was used to estimate the mean convection volume (substitution volume plus net ultrafiltration) delivered during the trial: mean convection volume delivered = (HDF treatments/total number of treatments) * mean convection volumes for the three treatments preceding the quarterly visit [20].

Serum samples

Seventeen of the 29 participating centres had the logistic capacity required to collect and store serum samples (at −80°C) from every patient at baseline and after 6, 12, 24 and 36 months. In these samples, sScl was measured—in a single run—at a central facility (Centre Hospitalier Universitaire de Lyon, Pierre Benite, France), using an enzyme-linked immunosorbent assay from Biomedica®, Vienna, Austria (reference value 19.0 pmol/L).

Follow-up

Follow-up was complete, as all patients who discontinued the randomized treatment (e.g. due to renal transplantation, a switch to peritoneal dialysis or moving to a non-participating centre) were still followed for cause-specific mortality. Thus, the data were analysed using an intention to treat approach. Adverse outcomes were continuously monitored. Cardiovascular death was classified into the following categories: myocardial infarction, aneurysm rupture, sudden death, terminal heart failure, haemorrhagic stroke or ischaemic stroke. An independent Endpoint Adjudication Committee reviewed the source documentation of all clinical outcomes [20, 21].

Statistical analysis

As sScl data were available for set intervals over a specific period of time, a time-varying analysis was performed. In that analysis, the last sScl in each time period was used to calculate the risk of mortality for that period. When an event (i.e. death) took place, the last value before that event was entered into the model. Thus, the time period between the determinant (sScl) and the outcome was as short as possible, which improved the accuracy of the risk estimates. The crude relation between sScl and mortality was not linear, i.e. above a certain threshold the relation between sScl and mortality did not vary (see crude results in Table 2). Accordingly, sScl was divided into four groups at each point in time, using the cut-off values applied to the sScl quartiles at baseline (group 1: <100 pmol/L; group 2: ≥100 and <139 pmol/L; group 3: ≥139 to <184 pmol/L; and group 4: ≥184 pmol/L). An interaction term between treatment modality and time-varying sScl, in relation to mortality, was derived. This was used to determine whether these dialysis modalities differed in terms of the effect of sScl on patient mortality. As there was no interaction (P ≥ 0.30), hazard ratios (HRs) were calculated in the pooled cohort (i.e. HD and HDF patients combined) for each sScl quartile versus the lowest quartile for mortality. This involved the use of Cox proportional hazards models, with sScl as a time-varying variable. The following confounders (derived from the published literature and based on physiological plausibility) were incorporated in the multivariable model: age, gender, diabetic status, history of cardiovascular disease, body mass index, dialysis vintage, residual kidney function, serum albumin and treatment modality. Since sScl can be a proxy for bone turnover, two additional models were fitted that incorporated the confounders mentioned above, plus BALP and PTH, were introduced [22]. A similar statistical approach was used to calculate HRs for cause-specific mortality (i.e. cardiovascular and non-cardiovascular mortality). The Cox model’s proportional hazard assumptions were checked using log minus log plots. It was found that none of the cases involved violated these assumptions.
Generalized linear mixed models (LMM) were used for the longitudinal analyses. These models used a random intercept alone or a random intercept plus a random slope, based on the lowest Aikake’s Information Criterion. As the intervals between laboratory measurements differed (i.e. either 6 months or 12 months), a continuous autoregressive covariance matrix was used in all LMMs. An LMM with a time/modality interaction term was fitted, to determine whether the rate of change per year differed for subjects treated with HD versus those treated with HDF. As this interaction term was positive (P = 0.004), LMMs stratified by treatment modality were used to calculate the rate of change over time for each dialysis modality.

Lastly, the influence of the magnitude of the convection volume on the change in sScl was investigated. For this purpose, all HD participants were considered as one a single category. The HDF participants were divided into three categories, based on tertiles of the convection volume reached (low-volume HDF <18.18 L/session; middle-volume HDF 18.18–21.95 L/session; high-volume HDF >21.95 L/session). The baseline sScl of these four categories differed, so the relative changes during 1 year of follow-up were investigated. All statistical analyses were performed with either SPSS 20.0 (IBM Inc., IL, USA) or RStudio (RStudio Inc, version 0.98.932).

Sensitivity analysis

A sensitivity analysis was performed to assess the robustness of the findings. In the CONTRAST study, as stated, all patients were followed until death or until the end of the study (i.e. intention to treat). As a sensitivity analysis, we ran crude and adjusted Cox regression models using an ‘on treatment’ approach, i.e. patients were censored the moment they discontinued the randomized treatment (e.g. due to renal transplantation, a switch to peritoneal dialysis or moving to a non-participating centre). This is particularly important as such events can substantially alter both the patient’s life expectancy and their sScl.

RESULTS

Baseline characteristics

Of the 714 patients included in CONTRAST, baseline sScl data were available for 396 subjects. No marked differences were observed in baseline characteristics between the original cohort and the current study group, nor between test subjects treated with either HD or HDF (Table 1). The study group had a mean (± standard deviation) age of 63.6 (± 13.9 years), 61.6% were male and 43.9% had a history of cardiovascular disease. The mean sPt/Vurea was 1.38 ± 0.21 per dialysis session. Their sScl values had a non-normal distribution, with a median of 139 pmol/L [interquartile range (IQR) 100–183 pmol/L], as shown in Figure 1.

Survival analysis

Median follow-up was 2.9 years (range 0.1–4.0 years). The results of the survival analysis are shown in Table 2. During the 4-year follow-up, 141 of the 396 subjects died. HRs were calculated for the pooled cohort, as there was no interaction (P ≥ 0.30) between treatment modality and the association between sScl and mortality. A crude analysis [HR 0.79 (95% confidence interval, CI, 0.49–1.25, P = 0.31)] revealed no difference in all-cause mortality risk for patients in the highest sScl quartile versus those in the lowest quartile. Following adjustment for confounders (model 1), however, the highest quartile was found to have a statistically significant reduction in mortality risk compared with patients in the lowest quartile [HR 0.51 (95% CI 0.31–0.86), P = 0.01]. Interestingly, the HRs for the quartiles in the adjusted models follow a linear trend (P for trend = 0.01). Adjustment for BALP or PTH (models 2 and 3, Table 2) produced no marked change in the HRs. The analyses of cause-specific mortality (i.e. cardiovascular death versus non-cardiovascular death) shown in Supplementary data, Table S1 yielded no significant results.

Impact of convective clearance on serum sclerostin

The longitudinal data on mean sScl (stratified by treatment modality) are shown in Figure 2. There was no difference in baseline sScl between the two treatment groups (P = 0.15). The change in sScl in patients treated with HD differed from that in patients treated with HDF (P for interaction = 0.004). A significant sScl change of −4.5 pmol/L/year (95% CI −8.0 to −0.9, P = 0.02) was observed in patients treated with HDF, while sScl remained stable in patients treated with HD (Δ +2.89 pmol/L/year, 95% CI −0.5 to 6.3, P = 0.09).

The baseline sScl in the HD group differed from the tertiles of convection volume in HDF patients [median (IQR) 143 (107–184), 121 (91–162), 140 (96–196) and 136 (90–266) pmol/L, respectively]. For this reason, Figure 3 shows the median relative changes in sScl for HD patients and HDF patients by tertiles of convection volume. In HD subjects, sScl increased by 5.3% in 1 year (IQR −9.1 to +21.7%). Conversely, in HDF patients, sScl fell over the course of a year by −1.7% (IQR −13.6 to +16.12%), −7.3% (IQR −24.3 to +6.8%) and −10.7% (IQR −25.1 to +2.2%), which respectively correspond to low, medium and high average convection volumes, respectively.

Sensitivity analyses

As shown in Supplementary data, Table S2, censoring patients the moment they discontinue the randomized treatment (‘on treatment’; e.g. due to renal transplantation) produced similar results to the analysis in which censoring took place at the end of the study or on the death of the subject (‘intention to treat’).

DISCUSSION

The first major conclusion that can be drawn from the results of the present study is that, in patients with ESKD, a high sScl is associated with a lower mortality risk. Although sScl has been associated with bone turnover [23], there was no marked change in the relation between sScl and clinical outcome after correction for BALP or PTH. The second conclusion is that sScl is influenced by dialysis modality, as sScl remained stable in HD patients but decreased over time in patients treated with HDF.
Finally, there is a linear, inverse association between convection volume and relative change in sScl over time.

Previous studies into the association between sScl and mortality in HD patients were based only on the baseline sScl. These studies reported heterogeneous results. Two European studies (n = 239 and n = 164) with follow-ups ranging from 2 to 4 years found no association between sScl and mortality [17, 18]. However, one single-centre Brazilian study in prevalent dialysis patients (n = 91) did report a positive relation [19].

At the other end of the spectrum, three European studies (n = 637, n = 100 and n = 207) in both incident and prevalent HD patients, with a similar follow-up, demonstrated results comparable to those obtained by the current study, i.e. a lower mortality risk for patients with higher sScl [14–16].

Differences in the assays used could account for some of the conflicting results obtained [24], but it seems unlikely that they account for the bulk of the reported inconsistencies. This is because the same assay was used in different studies that variously reported no association [18], a positive relation [19] and an inverse association [14]. The small Brazilian study that found a positive relation between sScl and mortality risk was based on a relatively young (mean age 42 years) and healthy population (15.4% had diabetes, versus >20% in the other studies) with a median dialysis vintage of 10 years versus <5 years in other studies or even incident patients [14]. Such differences may affect the relation between sScl and clinical outcome, which could account for some of the conflicting results obtained. Alternatively, the heterogeneous results could be due to differences in statistical adjustment, in residual kidney function or in the categorization of sScl.

Nevertheless, it is interesting to note that four studies investigating the potential causal relation between sScl and mortality (amounting to 1376 subjects from a total of 1870 subjects, including the present study, which investigated sScl as a time-varying variable) all report a lower mortality risk for patients with higher sScl [14–16].

Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Entire CONTRAST cohort (n = 714)</th>
<th>Study group (n = 396)</th>
<th>Investigated HD patients (n = 198)</th>
<th>Investigated HDF patients (n = 198)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.1 (13.7)</td>
<td>63.6 (13.9)</td>
<td>63.2 (13.8)</td>
<td>64.0 (14.0)</td>
</tr>
<tr>
<td>Sex (male gender)</td>
<td>445 (62.3%)</td>
<td>244 (61.6%)</td>
<td>126 (63.3%)</td>
<td>118 (59.6%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4 (4.8)</td>
<td>25.0 (4.8)</td>
<td>25.2 (4.7)</td>
<td>24.7 (4.9)</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes (yes)</td>
<td>170 (23.8%)</td>
<td>83 (21.0%)</td>
<td>39 (19.7%)</td>
<td>44 (22.2%)</td>
</tr>
<tr>
<td>Renal transplant</td>
<td>78 (10.9%)</td>
<td>40 (10.1%)</td>
<td>29 (14.6%)</td>
<td>11 (5.6%)</td>
</tr>
<tr>
<td>CVD (yes)</td>
<td>313 (43.8%)</td>
<td>174 (43.9%)</td>
<td>86 (43.4%)</td>
<td>88 (44.4%)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>148 (22)</td>
<td>148 (22)</td>
<td>149 (22)</td>
<td>148 (22)</td>
</tr>
<tr>
<td>Residual kidney function¹</td>
<td>376 (52.7%)</td>
<td>223 (56.3%)</td>
<td>108 (54.5%)</td>
<td>115 (58.1%)</td>
</tr>
<tr>
<td>Laboratory values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>11.8 (1.3)</td>
<td>11.9 (1.3)</td>
<td>11.8 (1.1)</td>
<td>11.9 (1.3)</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>5.08 (1.53)</td>
<td>5.20 (1.60)</td>
<td>5.10 (1.50)</td>
<td>5.20 (1.80)</td>
</tr>
<tr>
<td>Albumin⁰ (g/dL)</td>
<td>4.04 (0.38)</td>
<td>4.00 (0.40)</td>
<td>4.00 (0.42)</td>
<td>3.99 (0.38)</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>142 (37)</td>
<td>142 (39)</td>
<td>142 (42)</td>
<td>141 (35)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>9.7 (2.9)</td>
<td>9.8 (2.9)</td>
<td>10.1 (2.9)</td>
<td>9.5 (2.7)</td>
</tr>
<tr>
<td>Sclerostin (pmol/L)</td>
<td>N/A</td>
<td>139 (100–183)</td>
<td>143 (107–183)</td>
<td>125 (91–184)</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>381 (53.4%)</td>
<td>223 (56.3%)</td>
<td>116 (58.8%)</td>
<td>107 (54.0%)</td>
</tr>
<tr>
<td>Calcium antagonist</td>
<td>230 (32.2%)</td>
<td>130 (32.8%)</td>
<td>66 (33.3%)</td>
<td>64 (32.3%)</td>
</tr>
<tr>
<td>RAS inhibitor</td>
<td>351 (49.2%)</td>
<td>211 (53.3%)</td>
<td>105 (53.0%)</td>
<td>106 (55.3%)</td>
</tr>
<tr>
<td>Statin</td>
<td>369 (51.7%)</td>
<td>205 (51.8%)</td>
<td>95 (48.0%)</td>
<td>110 (55.6%)</td>
</tr>
<tr>
<td>Platelet aggregation inhibitor</td>
<td>240 (33.6%)</td>
<td>116 (29.3%)</td>
<td>60 (30.3%)</td>
<td>56 (28.3%)</td>
</tr>
<tr>
<td>Dialysis characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dialysis vintage (years)</td>
<td>2.00 (1.00–4.00)</td>
<td>1.83 (0.92–3.33)</td>
<td>2.0 (0.92–3.60)</td>
<td>1.67 (0.92–3.08)</td>
</tr>
<tr>
<td>spKt/V urea</td>
<td>1.40 (0.22)</td>
<td>1.38 (0.21)</td>
<td>1.37 (0.17)</td>
<td>1.40 (0.24)</td>
</tr>
</tbody>
</table>

Data are presented as mean (standard deviation), median (interquartile range) or number (percentage), as appropriate.

CONTRAST, CONvective TRAnsport STudy; BMI, body mass index; CVD, cardiovascular disease; SBP, systolic blood pressure; RAS, renin-angiotensin system; HD, haemodialysis; HDF, haemodiafiltration; N/A, not applicable.

¹Defined as diuresis ≥ 100 mL/24 h.

⁰Bromocresol green values.

FIGURE 1: Distribution of baseline serum sclerostin.
If there is indeed an inverse relation between sScl and mortality, then the question of causality must be addressed. Supplementary data, Table S1 shows that, if anything, the effect of sScl appears to be cardiovascular in nature. However, the small number of events involved \( (n = 44) \) resulted in very wide confidence intervals, so the results are rather inconclusive. If it is indeed a causal factor, sScl may prevent vascular calcification in ESKD patients. This is because a high sScl is related to the impaired proliferation of osteoblasts and increased apoptosis in healthy subjects, leading to reduced bone formation [25]. It is conceivable that these mechanisms may also apply to extra-skeletal calcifications. In CKD patients, a high sScl is related to extensive vascular calcification in coronary and abdominal vessels [26, 27]. Since sScl is considered to be an inhibitor of calcification in healthy subjects [25], a high sScl in CKD may represent a compensatory mechanism in individuals with a propensity for extra-skeletal calcifications (due to other uraemic derangements, such as abnormalities in phosphate and vitamin D metabolism) in this patient group [28]. This hypothesis is supported by the recent finding that the high sScl seen in CKD patients results not from reduced sScl excretion but rather from an increase in its production [29]. Alternatively, the picture may be much more complicated due to an altered balance between a variety of pro-calcifying (osteoprotegerin, calcium, phosphate) and anti-calcifying substances (fetuin A, matrix GLA protein), overall resulting in a dynamic and delicate pro-calcifying state, the overall result of which would be a dynamic and delicate pro-calcifying state. Lastly, increased intimal and medial calcifications may induce atherosclerotic plaque stabilization [30], which would reduce the risk of myocardial infarction and arterial emboli. Thus, a high sScl may protect patients against atherosclerotic cardiovascular events.

Our study found that, over time, sScl in HD (F) patients differed from that in HDF patients. Moreover, a graded, positive association was observed between the decline in sScl and convection volume. It is worth noting that several studies have reported an inverse relation between the magnitude of the convection volume and mortality risk, i.e. treatment with high-volume HDF is related to a reduced mortality risk [20, 31–33]. A beneficial survival effect of high-volume HDF and a concomitant reduction in sScl (high concentrations of which are related to improved survival) appears counterintuitive. However, perhaps it is simply the case that the assumed beneficial effects of high-volume HDF are overriding the assumed adverse effect of a lower sScl. Furthermore, the difference in mean sScl between quartile

### Table 2. HRs for 4-year mortality (intention to treat) stratified by time-varying sScl quartiles

<table>
<thead>
<tr>
<th></th>
<th>Crude&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Model 1&lt;sup&gt;1,2&lt;/sup&gt;</th>
<th>Model 2&lt;sup&gt;3,4&lt;/sup&gt;</th>
<th>Model 3&lt;sup&gt;5,6&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause mortality</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Q2 versus Q1</td>
<td>0.84 (0.54–1.33)</td>
<td>0.82 (0.51–1.32)</td>
<td>0.82 (0.51–1.33)</td>
<td>0.81 (0.50–1.32)</td>
</tr>
<tr>
<td>Q3 versus Q1</td>
<td>0.91 (0.58–1.44)</td>
<td>0.74 (0.44–1.23)</td>
<td>0.74 (0.45–1.23)</td>
<td>0.70 (0.42–1.18)</td>
</tr>
<tr>
<td>Q4 versus Q1</td>
<td>0.79 (0.49–1.25)</td>
<td>0.51 (0.31–0.86)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.51 (0.30–0.87)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.52 (0.31–0.89)&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Median (interquartile range) sScl of quartiles: Q1 80 (69–89), Q2 118 (111–124), Q3 157 (147–168), Q4 223 (198–335).

HR, hazard ratio; sScl, serum sclerostin; Q, quartile.

<sup>a</sup>Results are shown as HRs with 95% confidence intervals.

<sup>1</sup>Adjusted for age, sex, body mass index, diabetes, history of cardiovascular disease, dialysis vintage, serum albumin, residual kidney function and treatment modality.

<sup>2</sup>Adjusted as in model 1 plus bone-specific alkaline phosphatase.

<sup>3</sup>Adjusted as in model 1 plus parathyroid hormone.

<sup>4</sup>Adjusted as in model 1 plus parathyroid hormone.

<sup>5</sup>Indicates a significant difference in hazard at the level of \( P < 0.05 \).

FIGURE 2: Change in serum sclerostin (mean with standard error) over time, stratified by treatment modality. Solid line, haemodialysis; dashed line, haemodiafiltration.

FIGURE 3: Median relative change in serum sclerostin over 1 year of follow-up in categories of convection volume.
and quartile 4 (which shows a significant difference in mortality risk) is 201 pmol/L. It is likely that a decrease of 4.5 pmol/L/year (or even of 18 pmol over 4 years), as observed in the HDF group, has no effect. Thirdly, sScl may simply be an ‘innocent bystander’, a marker for CKD-MBD that plays no causal role in cardiovascular mortality. This possibility could also account for the conflicting results obtained by the above-mentioned studies regarding the association between sScl and mortality. If this is true, then the effect of HDF on sScl levels obviously has no effect on mortality. Alternatively, it is conceivable that HDF provides protection against cardiovascular events by restoring the mineral balance, as measured by reductions in phosphate and FGF23 [34, 35]. This, in turn, would affect the production of sScl.

One limitation of the present analysis is its observational nature. Furthermore, no other markers of bone metabolism (such as osteocalcin and FGF23) were available. Thirdly, the limited number of events involved prevented us from generating conclusive results with regard to cause-specific death. Lastly, we measured sScl with the Biomedica® assay. As different assays could (theoretically) bind different (degraded) sScl fragments, the choice of assay for measuring sScl concentrations could affect the measurement obtained. However, no data are currently available concerning the potential (degradation) fragments of Scl. Recent research has shown that, in healthy subjects, sScl values obtained using the Biomedica® assay can be up to 50% higher than those obtained with the TECO® assay [13, 36]. Therefore, the results of the present study (especially the new results on the influence of dialysis modality on sScl) should be confirmed in a cohort in which sScl is measured using other assays.

An important strength of the present study is the use of serial measurements for sScl in the survival analyses, which increases the robustness of the association between sScl and clinical outcome. Secondly, CONTRAST randomized patients to either HD or HDF, which made it possible to analyse the causal effect of dialysis modality on sScl. Thirdly, the assessment of all sScl measurements in a single run, at a central laboratory, eliminated inter-assay variability. Moreover, the present study was performed on a well-established ESKD cohort, using meticulous data collection procedures and with an endpoint adjudication committee to review all clinical events. Lastly, we performed a sensitivity analysis to increase the robustness of our findings.

Future research is urgently needed to determine whether clinical outcomes can be influenced by targeting sScl and the subsequent change in its concentration. This could involve antibodies to Scl, which are currently being tested in osteoporotic subjects [37]. Secondly, work is needed to identify the causal pathways of the above-mentioned relations. Logically, given the suggestion that sScl plays a part in vascular calcification, the next step should be to investigate any potential associations between sScl and various vascular parameters, such as carotid intima-media thickness, pulse wave velocity or left ventricular mass.

In conclusion, in ESKD patients, a high sScl is associated with a reduced mortality risk. Furthermore, sScl decreases in patients on HDF but remains unaltered in HD patients treated with HD. Lastly, the reduction in sScl in HDF patients is dependent on the magnitude of the convection volume. Given the clinical implications of these results, additional research is needed.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICT OF INTEREST STATEMENT

None declared.

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