# Sclerostin serum levels and vascular calcification progression in prevalent renal transplant recipients

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**Context:** Vascular calcification (VC) is prevalent and progressive in renal transplant recipients. Recent cross-sectional data suggest that activated Wnt signaling contributes to VC.

**Objective:** to investigate whether circulating levels of the Wnt antagonist sclerostin associate with progression of VC.

**Design:** post hoc analysis of the longitudinal observational Brussels Renal Transplant Cohort (BRTC) study.

**Setting:** Tertiary care academic hospital.

**Patients:** Coronary artery (CAC) and aortic calcification (AoC) were measured by multislice spiral CT in 268 prevalent renal transplant recipients (age 53 ± 13 years, 61% male) at baseline and re-measured in 189 patients after a median follow-up of 4.4 years. Baseline serum sclerostin levels were assessed on stored blood samples. Regression analysis was performed to identify determinants of baseline VC and progression.

**Main outcome measure:** progression of VC

**Results:** VC was present in up to 84% of participants at baseline. Almost half of the patients showed progression of VC, according to Hokanson criteria. The cross-sectional analysis at baseline demonstrated a direct association between sclerostin levels and VC score in univariate analysis, which became inverse after adjustment for age, gender and PTH level. Remarkably, a lower sclerostin level was identified as an independent determinant of a higher baseline AoC score in the final regression model. Moreover, baseline sclerostin levels showed an inverse association with VC progression, at least after adjustment for traditional risk factors.

**Conclusions:** serum sclerostin levels inversely associated with vascular calcification burden and progression in prevalent renal transplant recipients after adjustment for traditional risk factors. Our data corroborate previous findings in non-transplanted CKD patients and support the notion that sclerostin may be up-regulated in the vascular wall during the vascular calcification process as part of a local counter regulatory mechanism directed to suppress vascular calcification. Additional clinical and experimental data are required for confirmation.

Studies on the natural history of vascular calcification (VC) in RTRs are scarce and so far yielded discrepant findings (1, 2)(for review, see (3)). These discrepancies most probably reflect differences in case-mix and duration of follow-up, but may also relate to methodological issues. Indeed, computerized tomography (CT), being undoubtedly the most sensitive method to evaluate VC, is hampered by substantial interscan variability, especially in pa-

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Abbreviations:

tients with low calcium scores (4, 5). Without accounting for the relation between interscan variability and the CAC score, a bias may be introduced in the evaluation of changes in CAC (4). Recently, it has been recommended to use the transformed square root method of Hokanson for evaluating progression (6).

The pathogenesis of VC is complex and multifactorial, involving both traditional and nontraditional risk factors. The inextricable interdependence of vascular physiology, bone remodeling and mineral metabolism is increasingly recognized (7). Previous observational studies identified low 25(OH)VitD levels (1) and high PTH (8), calcium (9), and osteoprotegerin (9, 10) levels as predictors of VC progression in renal transplant recipients. Within this context, the canonical Wnt/β-catenin signaling pathway recently gained much attention (11), as an increasing body of evidence indicates that this pathway not only plays a crucial role in bone homeostasis (12), but is also involved in vascular (patho)biology (13–15). Wnt signaling is tightly regulated by secreted Wnt antagonists, with sclerostin and DKK1 being important representatives (16). Sclerostin is the gene product of *SOST*. *SOST* is expressed not only at sites of bone formation in bone and cartilage (17) but also in calcifying arterial tissue and valves (18–20). In a recent cross-sectional study including CKD patients not yet on dialysis, we observed higher serum sclerostin levels in patients with as compared to patients without vascular calcification. Remarkably, the association between vascular calcification and sclerostin, became inverse in the fully adjusted regression model (21). The latter observation suggests that SOST expression/secreted sclerostin may represent a counter-regulatory mechanism attenuating mineralization.

The present study aimed (a) to elucidate the natural history of coronary artery and aorta calcification in prevalent renal transplant recipients using robust criteria and (b) to confirm the hypothesis that high circulating sclerostin levels protect against progression of vascular calcification.

## Materials and Methods

This is a post hoc analysis of the longitudinal observational Brussels Renal Transplant Cohort (BRTC) study (1). In brief, the BRTC study was designed to elucidate the natural history and determinants of vascular calcification in prevalent renal transplant recipients (RTRs). All RTRs with a functional transplant for 1 year or longer and attending the outpatient clinic of the Cliniques universitaires Saint Luc (Brussels) for their annual or biannual in-depth control between February 3, 2004, to January 27, 2005 were invited to enter the study. Exclusion criteria were age younger than 18 years, residing abroad, or being a recipient of a multiorgan transplant. Three hundred nineteen patients were contacted, 300 of whom entered the study. The protocol was approved by the Ethics Committee of the Cliniques universitaires Saint Luc Medical School, and written informed consent was obtained from all patients.

### Vascular calcification

At inclusion, 281 patients underwent multislice spiral CT of the chest on a 16-slice scanner (Brillance; Philips Healthcare, Cleveland, OH, USA). The thoracic aorta and the 4 branches of the main coronary arteries were scored individually as previously described. Agatston scores of CAC and thoracic AoC were measured using a manufacturer algorithm (Heart Beat CS; Philips Healthcare) and expressed in HU. The Agatston scores were classified into 5 categories on the basis of their severity: 1 to 10 (minimal), 11 to 100 (mild), 101 to 400 (moderate), >400 (severe), and > 1000 (extreme). The Agatston score was measured again 3.5 or more years later in 189 patients.

### Demographics and biochemistry

At baseline, demographic, clinical, and medical history parameters, including history of a CV event (defined as myocardial, cerebrovascular, or lower-limb necrosis or revascularization or documented transient ischemic attack) were recorded by reviewing medical charts. Blood samples were obtained at inclusion to measure creatinine, cholesterol, triglycerides, glycemia, fibrinogen, homocysteine, 25-hydroxyvitamin D3 (25[OH]D3, calcidiol), 1,25-dihydroxyvitamin D3 (1,25[OH]2D3, calcitriol), intact parathyroid hormone (iPTH), and high-sensitivity C-reactive protein (CRP). Proteinuria was measured on a 24-hour urine collection. Glomerular filtration rate was estimated by the 4-variable Modification of Diet in Renal Disease (MDRD) Study equation at the time of inclusion. Induction and maintenance immunosuppressive drugs and all drugs prescribed at inclusion were recorded. Serum sclerostin was determined with an enzyme-linked immunosorbent assay (ELISA) (Tecomedical®, Sissach, Switzerland) in stored serum samples available in 269 participants at baseline.

### Statistical analysis

Results are presented as mean ± standard deviation, median (25th-75th percentile), or number (percentage), as appropriate. Variables presenting a right-skewed distribution were log-transformed. Univariate analysis was performed using t test, Wilcoxon sign-rank test, or $\chi 2$ test, as applicable. Linear and logistic regression analyses were performed to identify determinants of sclerostin baseline VC score and VC progression. VC scores were entered in the model as log(VC+1) to normalize VC score distribution and to avoid zero score exclusion. Significant progression/regression of VC was defined as a change ≥ 2.5 between the square root transformed values of baseline and follow-up calcium scores, because a change exceeding this magnitude most likely represents a real change in VC rather than interscan variability (4).

Demographic and other clinical/laboratory variables of interest were then evaluated in a univariable logistic regression model to determine their relationship to progression of VC. In addition, linear regression was performed with annualized absolute rate of change in vascular calcification as independent variable. The annualized absolute rate of change in vascular calcification (CAC or aorta calcification) was computed as the difference in Agatston score between the first and second scan di-

vided by the time between scans. Because of the need of logarithmic transformation, this analysis was restricted to patients with an annualized absolute rate of change $\geq 0$. A backward selection procedure was applied to identify candidate variables for the multivariable model. Candidate variables included, from the pool of historical risk factors and laboratory variables, those univariable predictors with $P \leq .2$. For the multivariable model, only those variables with a $P < .05$ were retained for the final variable selection.

All statistical analyses were performed using SAS, version 9.2, software. All tests were 2-tailed and $P < .05$ was considered significant

## Results

### Demographics

Patient demographics and relevant clinical and biochemical parameters are summarized in Table 1. Of the 268 patients included, 61.2% were men, 14.6% were diabetic. Mean age was 53 ± 13 years and mean eGFR was 51.5 ml/min/1.72m (2). A history of CV event was recorded in 31.7%. Mean time since transplantation was 7.8 ± 6.5 years.

### Determinants of serum sclerostin levels

The median sclerostin level was 0.84 [0.62–1.10] ng/ml. Patients with sclerostin level above the median were characterized by higher age, higher blood and pulse pressure, longer time since transplantation, higher total cholesterol, lower calcitriol level, and higher baseline vascular calcification score (Table 1). In univariate linear regression analysis, older age, male gender, diabetic state, lower eGFR and calcitriol levels were all associated with higher sclerostin levels. In multivariate regression analysis, higher age ($P < .0001$), male gender ($P = .002$), lower eGFR ($P = .002$), lower PTH ($P < .0001$) and lower calcitriol levels ($P < .05$) were identified as independent determinants of higher levels of circulating sclerostin. The same determinants but calcitriol were identified as determinants when sclerostin was assessed as dichotomous variable (above and below median, data not shown).

### Distribution of aortic and coronary artery calcification

Fifty-one (19%) individuals had no CAC at baseline. The severity of CAC was minimal in 18 patients (7%), mild in 43 patients (16%), moderate in 53 patients (20%), severe in 34 patients (13%), and extreme in 69 patients (26%). The distribution of AoC was comparable (weighted $\kappa$: 0.55): 44 individuals (16%) had no AoC at baseline. The severity of AoC was minimal in 26 patients (10%), mild in 34 patients (13%), moderate in 34 patients (13%), severe in 30 patients (11%), and extreme in 100 patients (37%). **Supplemental Table 1** compares the dis-

**Table 1.** Demographics and biochemistry in patients with sclerostin level below and above median at baseline

| | All | Below median | Above median | p-value |
|---|---|---|---|---|
| **Age (y)** | 53,0 (12,8) | 48,5 (13,6) | 57,5 (10,1) | <0,0001 |
| **Men (%)** | 61,2 | 56.0 | 66,4 | 0,08 |
| **BMI (kg/m$^2$)** | 26,36 (4,57) | 26,1 (4,95) | 26,61 (4,16) | 0.2 |
| **Diabetes (%)** | 14,6 | 10,5 | 18,7 | 0,2 |
| **Cardiovascular history (%)** | 31,7 | 28,4 | 35,1 | 0,2 |
| **History of smoking (%)** | 52,6 | 53,7 | 51,5 | 0,7 |
| **Current smoking (%)** | 14,18 | 20,2 | 8,2 | 0005 |
| **History of parathyroidectomy (%)** | 14,2 | 16,5 | 11,9 | 0,3 |
| **Systolic BP(mmHg)** | 136,1 (20,3) | 130,8 (18,3) | 141,5 (20,9) | <0,0001 |
| **Diastolic BP(mmHg)** | 82,3 (12,2) | 80,9 (10,7) | 83,7 (13,5) | 0.02 |
| **Pulse pressure (mmHg)** | 54,0 (16,4) | 50,0 (15,9) | 58,0 (15,9) | <0,0001 |
| **Statin (%)** | 38,8 | 35,1 | 42,5 | 0,2 |
| **Aspirin(%)** | 12,4 | 13,5 | 11,2 | 0,6 |
| **Calcium±VitD(%)** | 39,3 | 35,3 | 43,3 | 0,2 |
| **MDRD eGFR(ml/min/1,72m2)** | 51,5 (19,9) | 54,2 (20,3) | 48,7 (19,3) | 0.06 |
| **Dialysis vintage (yr)** | 2,4 (2,4) | 2,3 (2,3) | 2,4 (2,6) | 0.7 |
| **Creatinine (mg/dL)** | 1,63 (0,79) | 1,52 (0,64) | 1,73 (0,90) | 0.07 |
| **Living-donor Tx (%)** | 14,2 | 18,8 | 9,7 | 0,03 |
| **Time since Tx (yr)** | 7,82 (6,53) | 7,15 (6,45) | 8,49 (6,56) | 0.03 |
| **Glucose (mg/dL)** | 93 (86–105) | 92 (85–102) | 94 (86–107) | 0,21 |
| **hs-CRP (mg/dL)** | 1.61 (0.64–3.66) | 1,60 (0,60–3,26) | 1,69 (0,67–3,78) | 0,76 |
| **Hemoglobin (g/dL)** | 13,26 (1,61) | 13,06 (1,59) | 13,45 (1,62) | 0.09 |
| **Homocysteine ($\mu$Mol/liter)** | 15.4 (13.1–19.0) | 15,3 (12,6–18,2) | 15,8 (13,2–19,2) | 0,11 |
| **Proteinuria (g/24 h)** | 0,12 (0,07–0.27) | 0,13 (0,08–0,30) | 0,12 (0,07–0,25) | 0,99 |
| **Total Cholesterol (mg/dl)** | 199 (177–226) | 192 (171–218) | 207 (182–228) | 0,03 |
| **25(OH)D ($\mu$g/liter)** | 15 (11–22) | 14 (11–22) | 15 (10–21) | 0,72 |
| **1,25(OH)$_2$D (ng/liter)** | 31.0 (22.2–40.3) | 32,9 (25,4–42,3) | 28,9 (19,0–38,8) | 0005 |
| **Calcium (mg/dL)** | 9,52 (0,56) | 9,52 (0,55) | 9,52 (0,57) | 1.0 |
| **Phosphorus (mg/dL)** | 3,14 (0,75) | 3,06 (0,63) | 3,22 (0,86) | 0.30 |
| **Parathyroid Hormone (ng/liter)** | 41.2 (29.5–64.4) | 42,3 (30,1–66,0) | 41,0 (29,0–62,0) | 0,46 |
| **Osteoprotegerin (pg/mL)** | 11.9 (7.8–18.2) | 11,1 (8,1–16,1) | 12,5 (7,7–19,7) | 0,13 |
| **Sclerostin (ng/mL)** | 0,84 (0,62–1,09) | 0,62 (0,50–0,73) | 1,10 (0,96–1,30) | <0,0001 |
| **Baseline Coronary Artery Calcification** | 195 (7–1044) | 112 (0–979) | 254 (0–1335) | 0,01 |
| **Baseline Aorta Calcification** | 316 (9–2873) | 102 (1–1995) | 633 (60–3587) | 0,0004 |

Abbreviations: BMI: body mass index; BP: blood pressure; Tx: transplantation; VitD: vitamin D; eGFR: estimated Glomerular Filtration Rate; hs-CRP: high sensitive c-reactive protein;

tribution of patients in VC score categories at baseline and follow-up and illustrates the number of patients who actually shifted risk categories during the course of the study.

## Sclerostin and baseline aortic and coronary artery calcification

At baseline, median CAC score was 195 [7–1043]. In univariate linear regression, older age, male gender, diabetes, CV history, longer dialysis vintage, higher BMI, higher mean arterial blood pressure (BP), higher CRP, PTH, osteoprotegerin and sclerostin level and lower calcidiol levels were all associated with higher CAC score (**Table 2**). After adjustment for age, gender and PTH, sclerostin levels inversely associated with CAC score, although this lost significance. In the full model, older age, male gender, higher PTH, and longer dialysis vintage associated independently with higher baseline CAC score ($R^2$ 0.52, $P < .0001$).

At baseline, median AoC score was 316 [9–2873]. In univariate linear regression, older age, male gender, diabetes, CV history, longer dialysis vintage, higher BMI, higher CRP, PTH, osteoprotegerin and sclerostin level and lower calcidiol levels were all associated with higher AoC score (Table 2). In the full model, older age, male gender, CV history, longer dialysis vintage and lower sclerostin and calcitriol levels associated independently with baseline AoC score ($R^2$ 0.60, $P < .0001$).

## Sclerostin and vascular calcification progression

Paired scans were available in 189 patients. As described previously, patients with a repeat CT scan differed from those without a repeat CT scan, reflecting the expected selection bias due to death, resumption of dialysis or nonadherence as main causes of nonavailability of repeat CT (1). Median time between paired scans was 4.4 [4.2–4.6] years. The annualized absolute rate of change in CAC and AoC was 11 [0 to 57] mg and 6 [-1 to 68] mg, respectively. Progression of CAC was observed in 89 patients (47.1%), whereas significant regression was seen only in 4 subjects (2.1%). Progression of AoC was observed in 86 patients (45.5%), whereas significant regression was seen in 34 subjects (18.0%). Progression was most frequent in those with pre-existing VC (***Figure 1***). Among those with no calcification at baseline, progression was relatively infrequent (12% and 27% for CAC and aorta calcification, respectively). The absolute change in VC was small in those with little pre-existing VC but much greater in those with significant pre-existing VC (***Figure 2***).

Age (odds ratio (OR) [OR] per year 1.06, 95% confidence interval (CI) [CI] 1.03 to 1.09, $P < .0001$); gender (OR for males 2.1, 95% CI 1.1 to 3.7, $P = .02$); cardiovascular history (OR for absence 0.45, 95% CI 0.23 to 0.89 $P = .02$); baseline CAC score (log, OR 2.1, 95% CI 1.6 to 2.8, $P < .001$); serum phosphate (OR per mg 1.5, 95% CI 1.02 to 2.3, $P = .04$); serum PTH (log, OR 3.7, 95% CI 1.2 to 11.1, $P = .02$); serum sclerostin (log, OR 8.8, 95% CI 1.8 to 43.5, $P = .007$), serum calcidiol (log, OR 0.07, 95% CI 0.02 to 0.31, $P = .005$), pulse pressure (OR per mm Hg 1.02, 95% CI 1.002 to 1.04, $P = .03$), and use of statins (OR for patients free of statins 0.55, 95% CI 0.3 to 1.0, $P < .05$) were univariable predictors of CAC progression. In the final multivariable analysis, age (OR [OR] per year 1.04, 95% CI [CI] 1.006 to 1.08, $P = .02$); baseline CAC score (log, OR 1.8, 95% CI 1.3 to 2.6, $P = .0009$); serum phosphate (OR per mg/dl 2.03, 95% CI 1.22 to 3.37, $P = .006$); and serum calcidiol (log, OR per $\mu$g/L 0.07, 95% CI 0.01 to 0.37, $P = .002$) were independent predictors of CAC progression.

**Table 2.** Linear regression analysis with baseline Coronary Artery Calcification (log) and Aorta Calcification (log) as dependent variable

| | | Coronary Artery Calcification | | | | | | Aorta Calcification | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Univariate | | | Multivariate ($R^2$ = 0.57) | | | Univariate | | | Multivariate ($R^2$ = 0.60) | | |
| parameter | unit | β | t | p | β | t | p | β | t | p | β | t | p |
| **Age** | **yr** | 0.06 | 12.9 | <0.0001 | 0.05 | 12.0 | <0.0001 | 0.08 | 17.7 | <0.0001 | 0.08 | 13.5 | <0.0001 |
| **Dialysis vintage** | **yr** | 0.13 | 4.42 | <0.0001 | 0.1 | 4.64 | <0.0001 | 0.14 | 3.59 | <0.0001 | 0.1 | 3.85 | 0.0002 |
| **MAP** | **mm Hg** | 0.01 | 2 | <0.05 | | | | 0.01 | 1.63 | 0.1 | | | |
| **BMI** | **kg/m$^2$** | 0.05 | 2.4 | 0.02 | | | | 0.05 | 2.73 | 0.007 | | | |
| **Gender** | **M 0, F 1** | −0.72 | −4.73 | <0.0001 | −0.64 | −5.6 | <0.0001 | −0.41 | −2.3 | 0.02 | −0.32 | −2.3 | 0.008 |
| **Diabetes** | **No:0; Yes:1** | 0.81 | 3.79 | 0.0002 | | | | 0.46 | 3.6 | 0.0004 | | | |
| **CV history** | **No:0; Yes:1** | 1.05 | 6.86 | <0.0001 | | | | 1.4 | 8.29 | <0.0001 | 0.45 | 3.26 | 0.001 |
| **Smoking** | **No:0; Yes:1** | 0.16 | 0.73 | 0.5 | | | | 0.09 | 0.34 | 0.7 | | | |
| **Aspirin** | **No:0; Yes:1** | 0.3 | 1.27 | 0.21 | | | | 0.5 | 0.27 | 0.06 | | | |
| **Statin** | **No:0; Yes:1** | 0.74 | 4.82 | <0.0001 | 0.37 | 3.22 | 0.001 | 0.76 | 4.32 | <0.0001 | | | |
| **OPG (log)** | **log, pg/mL** | 0.99 | 2.86 | 0.005 | | | | 1.41 | 3.59 | <0.0001 | | | |
| **PTH (log)** | **log, ng/liter** | 0.97 | 3.7 | 0.003 | 0.39 | 2.0 | <0.05 | 0.96 | 3.2 | 0.002 | | | |
| **CRP (log)** | **log, mg/dL** | 0.32 | 2.2 | 0.03 | | | | 0.44 | 2.64 | 0.009 | | | |
| **Sclerostin** | **log, ng/mL** | 1.58 | 3.9 | 0.0001 | | | | 2.00 | 4.38 | <0.0001 | −0.78 | −2.2 | 0.03 |
| **Calcidiol** | **log, μg/liter** | −1.28 | −3.84 | 0.0002 | | | | −1.85 | −4.94 | <0.0001 | | | |
| **Calcitriol** | **log, ng/liter** | −0.58 | −1.72 | 0.09 | | | | −0.68 | −1.79 | 0.08 | −0.5 | −1.9 | 0.05 |

Other parameters with $P > 0.2$ in univariate analysis

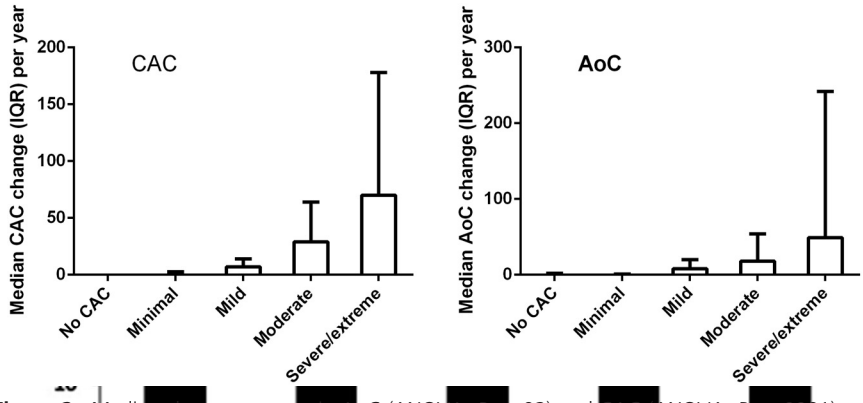


**Figure 2.** Median change per year in AoC (ANOVA, $P = .02$) and CAC (ANOVA, $P < .0001$) score, according to baseline severity.

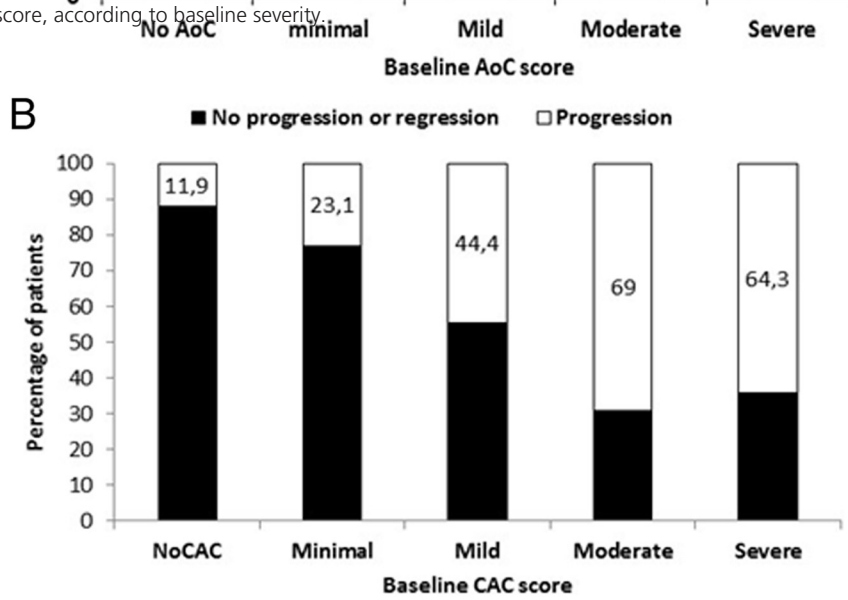


**Figure 1.** Progression of CAC according to baseline score (A: AoC, $P = .007$; B: CAC, $P < .0001$)

Age (OR [OR] per year 1.04, 95% CI [CI] 1.02 to 1.07, $P = .001$); baseline AoC score (log, OR 1.3, 95% CI 1.08 to 1.6, $P = .008$); and serum phosphate (OR per mg 1.7, 95% CI 1.13 to 2.59, $P = .01$) were univariable predictors of AoC progression. In the final multivariable analysis, age (OR [OR] per year 1.05, 95% CI [CI] 1.05 to 1.08, $P = .0005$) and serum phosphate (OR per mg 1.86, 95% CI 1.21 to 2.86, $P = .005$) were independent predictors of AoC progression.

We also performed linear regression with annualized absolute rate of change in VC as dependent variable. In univariate regression older age, male gender, diabetes, CV history, aspirin and statin use, higher pulse pressure, higher PTH, osteoprotegerin and sclerostin level, lower calcidiol level, longer dialysis vintage and higher baseline CAC score were all associated with faster progression of CAC (Table 3). After adjustment for age, gender, diabetes, CV history, pulse pressure, PTH, osteoprotegerin and calcidiol levels and dialysis vintage, sclerostin levels inversely associated with CAC progression rate ($R^2$=0.46). When baseline CAC score was entered in the model, only CV history ($P = .02$) and baseline CAC score ($P < .0001$) remained in the final model ($R^2$= 0.69, $P <$ .0001).

**Table 3.** Linear regression analysis with absolute annualized CAC change (log) as dependent variable

| | | Univariate | | | Multivariable Model1* ($R^2$ = 0.45) | | | Multivariable Model2* ($R^2$ = 0.69) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| parameter | unit | β | t | p | β | t | p | β | T | p |
| **Age** | **yr** | 0.04 | 7.67 | <0.0001 | 0.04 | 6.41 | <0.0001 | | | |
| **Dialysis vintage** | **yr** | 0.12 | 3.7 | 0.0003 | 0.08 | 3.24 | 0.002 | | | |
| **Pulse Pressure** | **Mm Hg** | 0.01 | 3.72 | 0.0003 | | | | | | |
| **Gender** | **M 0, F 1** | −0.47 | −3.46 | 0.0007 | −0.45 | −4.01 | <0.0001 | | | |
| **Diabetes** | **No:0; Yes:1** | 0.04 | 2 | <0.05 | | | | | | |
| **CV history** | **No:0; Yes:1** | 0.65 | 4.28 | <0.0001 | | | | 0.22 | 2.37 | 0.02 |
| **Aspirin** | **No:0; Yes:1** | 0.41 | 1.75 | 0.08 | | | | | | |
| **Statin** | **No:0; Yes:1** | 0.54 | 3.94 | 0.0001 | 0.30 | 2.55 | 0.01 | | | |
| **Phosphate** | **mg/dL** | 0.06 | 0.64 | 0.5 | | | | | | |
| **OPG (log)** | **log, pg/mL** | 0.47 | 1.73 | 0.08 | | | | | | |
| **PTH (log)** | **log, ng/liter** | 0.75 | 2.99 | 0.003 | | | | | | |
| **CRP (log)** | **log, mg/dL** | 0.47 | 1.73 | 0.09 | | | | | | |
| **Sclerostin (log)** | **log, ng/mL** | 1 | 2.84 | 0.005 | −0.94 | −2.68 | 0.008 | | | |
| **Calcidiol (log)** | **log, μg/liter** | −0.93 | −2.97 | 0.05 | −0.56 | −2.26 | 0.03 | | | |
| **Calcitriol (log)** | **log, ng/liter** | −0.03 | −0.09 | 0.9 | | | | | | |
| **Baseline CAC (log)** | **log, units** | 0.0005 | 8.2 | <0.0001 | | | | 0.59 | 17.8 | <0.0001 |

*Model 1: all parameters with $P < 0.2$ in univariate analysis, except baseline CAC

Model 2: all parameters with $P < 0.2$ in univariate analysis

In univariate linear regression older age, diabetes, CV history, aspirin and statin use, higher pulse pressure, higher sclerostin and lower calcidiol levels, and higher baseline AoC score were all associated with faster progression of AoC (Table 4). After adjustment for age, diabetes, CV history, aspirin and statin use, pulse pressure, phosphate and calcidiol levels, sclerostin levels inversely associated with AoC progression rate. When baseline aorta calcifcation score was entered in the model, only age ($P < .0001$), serum phosphate ($P < .0001$) and baseline AoC score ($P < .0001$) remained in the final model ($R^2 = 0.75$, $P < .0001$), with sclerostin falling out of the model in the last step ($P = .08$).

Collinearity was excluded in all regression analyses.

## Discussion

The main findings of the present prospective observational cohort study are (a) that vascular calcification is highly prevalent among renal transplant recipients and progressing in almost half and, (b) that circulating sclerostin levels inversely associate with vascular calcification burden and progression in renal transplant recipients after adjustment for traditional risk factors.

Vascular calcification is common among CKD patients. Reported prevalence rates range from 40% in patients with CKD stage 3, to 90% in patients with end stage renal disease (22). Variability between studies is substantial, which may be explained by case-mix, differences in arterial territories analyzed and in sensitivity of imaging techniques used. Studies exploring vascular calcification in renal transplant recipients reported prevalence rates between 61 and 75%. These prevalence rates, overall, are higher than observed in stage 3 CKD patients and lower than found in hemodialysis patients (3). Using a highly sensitive multislice spiral CT technique, we detected coronary and aortic calcifications in respectively 81 and 84% of our prevalent renal transplant recipients.

Studies investigating the natural history of coronary artery and especially aortic calcification in renal transplant recipients are limited. Applying the robust Hokanson criteria (4), we observed progression of CAC in 47% of patients. This percentage is at the higher end of the published spectrum (2, 10). Regression of calcification was observed in only 2%. Together with previous studies, this observation supports the notion that renal transplantation at best attenuates CAC progression (8, 23). Progression of Aoc was observed in 46%. This percentage is almost twice as high as reported by Meneghini et al in a cohort study including incident renal transplant recipients (9). Case-mix, shorter follow-up (1 year), and use of less sensitive imaging technique (lateral lumbar XR) probably explains the lower progression ratio in the latter study. Of interest and in accordance with literature data (9, 24), we demonstrate that prevalence, severity and natural history of vascular calcification in the aorta and coronary arteries closely correlate (data not shown), suggesting common pathophysiologic mechanisms.

It is well-recognized that vascular calcification is an actively regulated process similar to osteogenesis, and that bone-associated proteins may be involved. Wnt–$\beta$-catenin signaling recently emerged as important player not only in bone (16) but also in vascular (patho)biology (25). Overall, Wnt–$\beta$-catenin signaling promotes atherogenesis and vascular calcification (25–27).

The present study evaluated whether high circulating levels of the Wnt antagonist sclerostin protect against progression of vascular calcification. Data from the baseline

**Table 4.** Linear regression analysis with absolute annualized AoC change (log) as dependent variable

| | | Univariate | | | Multivariate Model1* ($R^2 = 0.68$) | | | Multivariate Model2* ($R^2 = 0.76$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| parameter | unit | $\beta$ | t | p | $\beta$ | t | p | $\beta$ | t | p |
| **Age** | **yr** | 0.06 | 12.0 | <0.0001 | 0.06 | 11.9 | <0.0001 | 0.02 | 4.83 | <0.0001 |
| **Dialysis vintage** | **yr** | 0.17 | 4 | 0.0001 | 0.08 | 2.9 | 0.005 | | | |
| **Pulse Pressure** | **Mm Hg** | 0.02 | 3.72 | 0.0003 | 0.009 | 2.9 | 0.005 | | | |
| **Gender** | **M 0, F 1** | −0.31 | −1.84 | 0.07 | −0.24 | −2.36 | 0.02 | | | |
| **Diabetes** | **No:0; Yes:1** | 0.6 | 2.42 | 0.02 | | | | | | |
| **CV history** | **No:0; Yes:1** | 0.43 | 2.18 | 0.03 | | | | | | |
| **Aspirin** | **No:0; Yes:1** | 0.62 | 2.42 | 0.02 | | | | | | |
| **Statin** | **No:0; Yes:1** | 0.68 | 4.3 | <0.0001 | 0.33 | 3.29 | 0.001 | | | |
| **Phosphate** | **mg/dL** | 0.2 | 1.88 | 0.06 | 0.22 | 3.57 | 0.0005 | 0.06 | 4.58 | <0.0001 |
| **OPG (log)** | **log, pg/mL** | 0.65 | 2 | <0.05 | | | | | | |
| **PTH (log)** | **log, ng/liter** | 0.54 | 1.72 | 0.09 | | | | | | |
| **CRP (log)** | **log, mg/dL** | 0.2 | 1.21 | 0.23 | | | | | | |
| **Sclerostin** | **log, ng/mL** | 1.61 | 3.9 | 0.0002 | −1.16 | −3.69 | 0.0003 | | | |
| **Calcidiol (log)** | **log, $\mu$g/liter** | −1.18 | −3.07 | 0.003 | | | | | | |
| **Calcitriol (log)** | **log, ng/liter** | −0.25 | −0.72 | 0.5 | | | | | | |
| **Baseline AoC (log)** | **log, units** | 0.56 | 17.2 | <0.0001 | | | | 0.43 | 10.1 | <0.0001 |

*Model 1: all parameters with $P < 0.2$ in univariate analysis, except baseline AoC

Model 2: all parameters with $P < 0.2$ in univariate analysis

cross-sectional analysis revealed a direct association between sclerostin levels and vascular calcification score in univariate analysis, which however became inverse after adjustment for age, gender and PTH, all determinants of circulating sclerostin levels and vascular calcification. Remarkably, a lower circulating sclerostin level was identified as independent determinant of a higher baseline AoC score in the final regression model, ie, after adjustment for traditional (older age, male gender, high BMI, presence of diabetes, hypertension) and nontraditional (inflammation, high PTH, low calcidiol, long dialysis vintage) risk factors. As such, these data confirm and extend our prior findings in CKD patients not yet on dialysis. In individuals with an annualized absolute rate of change of the calcification score equal or greater than zero, baseline sclerostin levels showed an inverse association with vascular calcification progression after adjustment for traditional risk factors (Table 3&4). Together with published experimental (18, 28) and clinical data (19, 21), our observations support the notion that sclerostin may be part of a local counter-regulatory mechanism directed to suppress progression of vascular calcification. Sclerostin produced in the vascular wall may spill over to the circulation and thereby contribute to circulating levels of the protein. In agreement with this line or reasoning, serum sclerostin levels were recently shown to inversely associate with mortality in prevalent hemodialysis patients, independent of traditional risk factors (29, 30).

Many of the risk factors that were related to baseline calcification remained predictive of vascular calcification progression, at least in univariate regression analysis. In the final multivariable regression model, only age, and serum phosphate were robustly identified as independent determinants of VC progression. Individuals without vascular calcification at baseline rarely (12%) showed progression. This finding confirms previous studies and indicate that baseline vascular calcification by far is the most important determinant of progression (31) (***figure 1&2***). It furthermore supports the thesis that vascular calcification might itself induce further inflammation and calcification in a positive feedback loop (32). The observation that factors being identified as independent determinants of baseline vascular calcification fail to demonstrate an association with progression may be explained by lack of statistical power. An alternative explanation is that baseline vascular calcification score reflects the lifetime burden, whereas progression is a more proximate measure, reflecting current disease activity. Interestingly, besides higher age and baseline calcification score, high serum phosphate levels independently associated with progression of VC. This observation is consistent with current notions of the biological roles of a disturbed phosphate metabolism in the pathogenesis of VC (33).

The present study has several strengths and limitations. Important strengths include the relatively large sample size with availability of follow-up scans in most participants, allowing the investigation of predictors of progression, and the availability of information on a broad panel of traditional and nontraditional risk factors of vascular calcification. We acknowledge that the association between baseline sclerostin levels and vascular calcification progression was rather weak. However, together with evolving experimental data and recent clinical observations in nontransplanted CKD patients, our findings may fuel further clinical and experimental research regarding the role of the Wnt/$\beta$-catenin pathway and its inhibitor sclerostin in vascular pathobiology. Such research is highly relevant, especially because antisclerostin antibodies are currently tested for the treatment of osteoporosis (34). The sample size, through relatively large, was insufficient to allow exploration of potential nonlinear relationships between sclerostin levels and vascular calcification burden and progression. Further limitations include missing data on bone healthy (bone mineral density (BMD), bone biomarkers, and histomorphometry) and menopausal state.

In conclusion, our data confirm that vascular calcification is prevalent among renal transplant recipients and progressive in almost half of the patients. Sclerostin levels inversely associated with vascular calcification burden and progression in prevalent renal transplant recipients after adjustment for traditional risk factors. These data are in line with previous findings in nontransplanted CKD patients and, in aggregate, support the thesis that Wnt signaling is important in vascular pathobiology.

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