Increased circulating sclerostin levels in end-stage renal disease predict biopsy-verified vascular medial calcification and coronary artery calcification

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Sclerostin, an osteocyte-derived inhibitor of bone formation, is linked to mineral bone disorder. In order to validate its potential as a predictor of vascular calcification, we explored associations of circulating sclerostin with measures of calcification in 89 epigastric artery biopsies from patients with end-stage renal disease. Significantly higher sclerostin levels were found in the serum of patients with epigastric and coronary artery calcification (calcification score 100 or more). In Spearman's rank correlations, sclerostin levels significantly associated with age, intact parathyroid hormone, bone-specific alkaline phosphatase, and percent calcification. Multivariable regression showed that age, male gender, and sclerostin each significantly associated with the presence of medial vascular calcification. Receiver operating characteristic curve analysis showed that sclerostin (AUC 0.68) predicted vascular calcification. Vascular sclerostin mRNA and protein expressions were low or absent, and did not differ between calcified and non-calcified vessels, suggesting that the vasculature is not a major contributor to circulating levels. Thus, high serum sclerostin levels associate with the extent of vascular calcification as evaluated both by coronary artery CT and scoring of epigastric artery calcification. Among circulating biomarkers of mineral bone disorder, only sclerostin predicted vascular calcification.

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Vascular calcification and bone disorders are features of the uremic phenotype entitled chronic kidney disease-mineral and bone disorder (CKD-MBD),[1] which begins early in the course of CKD and contributes to increased morbidity and mortality in this prematurely aged patient population.[2,3] Smoldering alterations in mineral homeostasis during worsening of kidney function promote vascular calcification, cardiovascular disease (CVD), bone disease, and poor outcome.[4] The mechanisms causing the CKD-MBD syndrome are not yet fully elucidated, but seem in part to be associated with the stimulation of osteocyte secretion. Although several biomarkers of CKD-MBD, such as calcium, phosphate, parathyroid hormone (iPTH),[5] fibroblast growth factor-23 (FGF23),[6] and bone-specific alkaline phosphatase (bALP)[7] predict outcome in end-stage renal disease (ESRD), there is a need of a biomarker that could predict the presence and extent of vascular calcification as well as outcome.

Among several emerging factors implicated in CKD-MBD, sclerostin, a osteocyte-derived glycoprotein has attracted recent interest.[8] Sclerostin acts as a soluble inhibitor of the Wnt signaling pathway and its physiological role is to reduce bone formation. The serum sclerostin levels increase with the progression of CKD[8,9] even though the renal elimination of sclerostin is reported to increase when renal function deteriorates,[10] whereas the levels decrease rapidly after renal transplantation (RTx).[11] These changes are likely explained by changes in the production of sclerostin in the bone,[12] and possibly other tissues, as renal function changes. This was supported by Fang *et al.*[13] who reported that increased osteocytic protein secretion, vascular calcification, and stimulated vascular osteoblastic transition occur already in early CKD. Although sclerostin is a soluble inhibitor of osteoblast function, positive correlations of serum sclerostin with bone mineral density have been reported in patients with Type 2 diabetes[14] and CKD patients.[15,16]

Mutations in the *SOST* gene, coding for sclerostin, associate with enhanced Wnt signaling, and lead to a phenotype characterized by marked increases in bone mineral density, bone volume, and bone formation.[17] Sclerostin antibodies are currently being evaluated in clinical trials as a treatment option for osteoporosis in postmenopausal women.[18] However, as sclerostin may also have extraskeletal effects,[19] the potential role of sclerostin in extraosseous calcification, in particular vascular calcification, needs to be further explored. Whereas circulating sclerostin was inversely related to the presence of calcified carotid artery plaques in patients with Type 2 diabetes,[14] a study in hemodialysis patients showed a positive correlation between sclerostin and the amount of aortic valve calcification.[20] On the other hand, a negative correlation was found between sclerostin and signs of vascular calcifications (Kauppila method) in a multivariate model including 164 hemodialysis patients.[21] Claes *et al.*[22] reported that the positive univariate correlation between aortic calcifications and sclerostin levels in predialysis patients became inverse in a multivariate model. Based on the effects by sclerostin on bone, one may speculate that increased levels of sclerostin counterregulate the progression of vascular calcification by inhibiting the mineralization process also in the vasculature. On the other hand, Fang *et al.*[8] recently showed that neutralization by a monoclonal antibody against the Dickkopf-related protein 1, which was similar to sclerostin is a Wnt inhibitor secreted mainly by osteocytes, leads to decreased levels of sclerostin, corrected osteodystrophy, and prevented CKD–induced vascular calcification in diabetic mice. However, in contrast to Dickkopf-related protein 1, sclerostin effects are tissue and context dependent even at times capable of stimulating Wnt signaling. As circulating Wnt inhibitors are involved in the pathogenesis of CKD-MBD, and as sclerostin serves as a regulator of the mineralization process in bone and potentially also in the vasculature, it could be hypothesized that elevated sclerostin levels predict poor outcome.

To date, clinical data on sclerostin as an outcome biomarker are inconsistent and elevated sclerostin have been reported to predict either higher mortality,[9,23] lower mortality,[24,25] or not being related to outcome.[21] These contradictory results imply that confounders need to be identified and accounted for. In the current study, we measured circulating sclerostin and other CKD-MBD biomarkers, inflammation biomarkers, body composition, and degree of coronary artery calcification (CAC) score by cardiac computed tomography (CT) in ESRD patients undergoing living donor RTx. Furthermore, the presence and extent of vascular calcification and protein mRNA expression of sclerostin in calcified and non-calcified vasculature were ascertained in vascular biopsies from the inferior epigastric artery. The extensive phenotyping allowed us to explore and validate the predictive role of the presence of vascular calcification with CAC score and several CKD-MBD biomarkers.

RESULTS

Demographics and clinical characteristics of the ESRD patients undergoing living donor RTx are shown in Table 1. Median S-creatinine did not differ significantly in dialyzed (784 μmol/l) versus non-dialyzed (664 μmol/l) patients. Similarly, median sclerostin level did not differ significantly in dialyzed (468 pg/ml) versus non-dialyzed (414 pg/ml) patients. Higher circulating sclerostin levels were found in patients with histological signs of moderate–extensive vascular calcification ($P=0.003$) (Figure 1). Nineteen patients with CAC score $\geqslant 100$ Agatston units (AU) by cardiac CT had significantly higher median levels of sclerostin compared with 46 patients with CAC score <100 AU (559 vs. 367 pg/ml; $P=0.001$) (Figure 1). No significant difference ($P=0.22$) in median sclerostin levels was found between diabetic (563 pg/ml) and non-diabetic (432 pg/ml) patients. In Spearman's rank (ρ) correlations, sclerostin concentrations were significantly associated with age ($\rho=0.38$; $P=0.002$), iPTH ($\rho=-0.33$; $P=0.002$), S-creatinine ($\rho=0.26$; $P=0.015$), high-density lipoprotein-cholesterol (HDL-cholesterol) ($\rho=-0.23$; $P=0.03$), lipoprotein(a) ($n=84$; $\rho=0.29$; $P=0.008$), skin advanced glycation end-product autofluorescence ($n=66$; $\rho=0.32$; $P=0.009$), calcium ($\rho=0.26$; $P=0.02$), bALP ($n=83$; $\rho=-0.37$; $P=0.0006$), total osteocalcin ($\rho=-0.24$; $P=0.03$), inactive (GLU) undercarboxylated osteocalcin ($\rho=-0.23$; $P=0.04$) and active (GLA) carboxylated osteocalcin ($\rho=-0.28$; $P=0.01$), tumor necrosis factor (TNF) ($\rho=0.24$; $P=0.03$), and 8-hydroxy-2′-deoxyguanosine ($\rho=0.23$; $P=0.04$). Sclerostin levels were not associated with gender, high-sensitivity C-reactive protein (hsCRP), interleukin-6, bone mineral density, phosphate, magnesium, troponin-T, 25(OH)D-vitamin, 1,25(OH)D-vitamin, cholesterol, triglycerides, pentosidine, or klotho. A multivariate regression model for determinants for plasma sclerostin showed that age ($\beta=0.41$; $P=0.007$), S-creatinine ($\beta=0.30$; $P=0.01$), and iPTH ($\beta=-0.25$; $P=0.05$) were associated with plasma sclerostin levels (Table 2). Eleven patients with pre-existing CVD had significantly higher median total CAC score (970 (0–3816) vs. 0 (0–455) AU; $P=0.001$) compared with 54 non-CVD patients.

Determinants of vascular calcification

Scoring by the pathologist. Moderate and extensive vascular calcification was present in 37 (42%) of 89 patients. As expected (Table 1), ESRD patients with vascular calcification were older ($P<0.001$), more often males ($P=0.04$), diabetics ($P=0.003$), and had higher body mass index ($P=0.002$). Moreover, patients with vascular calcification had significantly higher median total CAC score (133 vs. 0 AU; $P<0.001$), troponin T (23.5 vs. 14.0 μg/l; $P=0.04$), TNF (11.8 vs. 10.0 pg/ml; $P=0.03$), and lower median insulin growth factor-1 (IGF-1) (189 vs. 264 ng/ml; $P=0.03$) and HDL-cholesterol (1.2 vs. 1.5 mmol/l; $P=0.04$) levels. The association between magnitude of vascular calcification and total CAC scores by CT of the heart is depicted in Figure 2. In univariate analysis, sclerostin levels were significantly higher