Low Bone Volume—A Risk Factor for Coronary Calcifications in Hemodialysis Patients

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Background and objectives: There is increasing evidence that altered bone metabolism is associated with cardiovascular calcifications in patients with stage 5 chronic kidney disease on hemodialysis (HD). This study was conducted to evaluate the association between bone volume, turnover, and coronary calcifications in HD patients.

Design, setting, participants, & measurements: In a cross-sectional study, bone biopsies and multislice computed tomography were performed in 38 HD patients. Bone volume/total volume, activation frequency, and bone formation rate/bone surface were determined by histomorphometry and coronary calcifications were quantified by Agatston scores.

Results: Prevalence of low bone turnover was 50% and of low bone volume was 16%. Among the studied traditional cardiovascular risk factors, only age was found to be associated with coronary calcifications. Lower bone volume was a significant risk factor for coronary calcifications during early years of HD, whereas this effect was not observed in patients with dialysis duration >6 yr. Histomorphometric parameters of bone turnover were not associated with coronary calcifications.

Conclusions: Low bone volume is associated with increased coronary calcifications in patients on HD.

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Cardiovascular disease and stroke account for 60 to 70% of all deaths in patients with chronic kidney disease (CKD) on maintenance hemodialysis (HD) (1,2). In a study utilizing electron beam computed tomography, dialysis patients presented with higher coronary calcification scores when compared with nondialysis patients (3). In HD patients, vascular calcifications were shown to be associated with cardiovascular morbidity and mortality (4). Conspicuously, however, two prospective trials on modification of traditional cardiovascular risk factors in CKD patients could not demonstrate significant differences in cardiovascular outcomes, including death between an intensive "multiple traditional risk factor intervention" group and the standard therapy group despite significantly improved traditional risk factor control in the former (5,6). Recent evidence shows that vascular calcifications in CKD can occur early, and that deposition of calcium in the vascular wall is a complex and tightly regulated process that is akin to bone mineralization (7,8). In addition, abnormalities in bone turnover and bone volume are associated with more vascular calcifications in uremic patients (9,10).

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Taking this evidence into consideration, the international initiative, Kidney Disease: Improving Global Outcomes, recommends the inclusion of evaluation for extraskeletal calcifications into a new classification for "chronic kidney disease-mineral and bone disorder" (11). Although an association between low bone turnover and increased vascular calcification (measured by a semiquantitative vascular calcification score) was reported previously (9), the relationship between histomorphometric parameters of bone formation and bone volume and quantitative determinations of coronary calcifications by multislice computed tomography (MSCT) while controlling for the traditional cardiovascular risk factors of hypertension, gender, age, cholesterol, and tobacco use has not been investigated in HD patients. The study presented here aims at providing this information.

Materials and Methods

Study Design

This is an extension study in a cohort of patients who participated in the Sevelamer Study Group randomized trial (12). Briefly, the Sevelamer Study Group study was a 54-wk randomized open-label study to compare the effects of sevelamer hydrochloride and calcium carbonate on bone histomorphometric parameters. This extension study investigates the interaction between histologically determined parameters of bone turnover/bone volume and coronary calcifications measured by MSCT in a cross-sectional study design. The Institutional Review Boards of all participating institutions approved the protocol. The study has been conducted in adherence to the Declaration of Helsinki, and all patients provided informed consent.

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Population

Thirty-eight patients provided informed consent for performing histomorphometric analysis of bone samples and MSCT for evaluation of coronary calcifications and were enrolled in this extension study. Inclusion criteria were ages 18 yr or older, dialysis duration of at least 3 mo, mental competence, and willingness to participate in the study. All patients were required to have stable serum phosphorus of ≤8.1 mg/dl because higher levels were considered indicative of medication noncompliance. The extension study called for providing consent to undergo evaluation for coronary calcifications by MSCT, which was performed on average 3.8 ± 1.9 mo after the bone biopsy. Exclusion criteria were failed kidney transplant during the past 6 mo; pregnancy; uncontrolled systemic illnesses or organic diseases with potential influence on bone metabolism such as diabetes mellitus, active or chronic liver disease, malabsorption, malignancy, and thyroid dysfunction; history of or present treatment with pharmacologic agents known to affect bone metabolism such as bisphosphonates, fluoride, calcitonin, glucocorticoids or other immunosuppressive agents, hormone replacement therapy, and selective estrogen receptor modulators; chronic alcoholism and/or drug addiction; and allergy to tetracycline compounds.

Hypertension was defined as blood pressure ≥140/90 mmHg and/or treatment with antihypertensive medications. Tobacco use was defined as inhalative smoking of ≥1 cigarette per day.

Bone Biopsies

Anterior iliac crest bone biopsies were done after tetracycline labeling under local anesthesia and conscious sedation. The labeling schedule consisted of a 2-d oral administration of tetracycline hydrochloride (250 mg twice daily) followed by a drug-free interval of 10 d, and subsequent oral administration of demeclocycline hydrochloride (300 mg twice daily) for 4 d. Bone biopsies were performed 3 to 4 d after completing the second label. Bone samples were obtained with the one-step electrical drill technique (Straumann Medical, Waldenburg, Switzerland). Bone samples were processed undecalcified and sections were stained with the modified Masson–Goldner trichrome stain, the aurin tricarboxylic acid stain, and solochrome azurin for assessment of stainable aluminum in bone (13). Unstained sections were prepared for phase contrast and fluorescence light microscopy. Histomorphometric analysis of bone was done at standardized sites in cancellous bone using the semiautomatic method (Osteoplan II, Kontron, Munich, Germany) at 200× magnification. Bone volume/total volume (BV/TV) was calculated for assessment of mineralized bone volume, whereas activation frequency (Ac.f.) and bone formation rate/bone surface (BFR/BS) were calculated for assessment of bone turnover. All bone samples were processed and analyzed without knowledge of the clinical data. BV/TV classification was based on three categories characterized by the distribution of our data (5th, 50th, and 95th percentile). The classification of "low," "normal," and "high" bone turnover was based on our normative database (14–16). The outcome group for low bone turnover was defined as Ac.f. <0.49 yr^{-1} and/or BFR/BS <1.8 $mm^3/cm^2/yr$. The outcome group for normal bone turnover was defined as Ac.f. 0.49 to 0.72 yr^{-1} and/or BFR/BS 1.8 to 3.9 $mm^3/cm^2/yr$. The outcome group for hig bone turnover was defined as Ac.f. >0.72 yr^{-1} and/or BFR/BS >3.9 $mm^3/cm^2/yr$.

Assessment of Coronary Calcifications

Vascular calcifications were assessed by a quantitative score using MSCT. MSCT scans were performed with the four-slice technique on the model Somatom Volume Zoom (Siemens AG, Erlangen, Germany). Slices of 2.5-mm thickness were acquired under the following conditions: 120 kVp, 130 mAs, and 0.5 gantry rotation time. All images were transferred to a workstation and analyzed with calcium scoring software (HeartView CT, Siemens AG, Erlangen, Germany). Quantification of coronary calcification was performed by calculating the Agatston (Agt.) score on the basis of the maximum x-ray attenuation coefficient (measured in Hounsfield units).

Biochemical Analyses

Blood was drawn at the time of the bone biopsy after an overnight fast. The following biochemical parameters were measured: serum calcium and phosphorus by an autoanalyzer (Hitachi 747, Globe Scientific Inc.), immunoreactive parathyroid hormoneby DPC IMMULITE PTH IRMA (Diagnostics Products Corporation, Los Angeles, California; normal range 16 to 87 pg/ml; intra- and interassay coefficients of variation <7 and <9%, respectively); 25-(OH)-vitamin D by LIAISON 25-OH Vitamin D assay (Diasorin, Saluggia, Italy; normal range 25 to 100 ng/ml; intra- and interassay coefficients of variation 4.1 and 7%, respectively); and total cholesterol was measured by the Synchron LX system (Beckman Coulter, Fullerton, California; desirable range <200 mg/dl; intra- and interassay coefficients of variation both <3%).

Statistical Analyses

Descriptive statistics are presented as means, medians, minimums, maximums, and SD stratified according to Agt. score groups of <100, 100 to 400, and >400. The variables Ac.f., BFR/BS, and HD duration had right-skewed distributions and were log transformed for analysis. Boxplots were used to characterize the distributions of BV/TV, log Ac.f., and log BFR/BS for each Agt. score group. Bivariate associations were assessed using the nonparametric Kruskal–Wallis test for continuous variables and Fisher's exact test for categorical variables. Multivariable associations were assessed using separate ordinal (proportional odds) logistic regression analyses to evaluate the effects of BV/TV, log Ac.f., and log BFR/BS on Agt. score, adjusted for measured demographic and biologic factors. All calculations were performed using the R statistical package (R Foundation for Statistical Computing, Vienna, Austria) and SAS version 9.1 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Demographic characteristics of the study population are presented in Table 1. There were no statistically significant differences between the coronary Agt. score groups except for age. The study included only nondiabetic patients.

BV/TV was low in 6 (16%), normal in 9 (24%), and high in 23 (60%) patients. Low bone turnover was diagnosed in 19 patients (50%), normal turnover in 4 patients (11%), and high turnover in 15 patients (39%) on the basis of Ac.f. and BFR/BS. There were no disagreements regarding classification of bone turnover between Ac.f. and BFR/BS. None of the biopsies showed positive staining for aluminum or iron. In unadjusted analyses, there were no statistically significant differences between the three Agt. score groups regarding the bone histomorphometric parameters BV/TV, Ac.f., and BFR/BS (Figure 1).

Ordinal logistic regression revealed that, among the studied traditional cardiovascular risk factors, age was the only variable that predicted Agt. score groups ($P = 0.02$). Our data showed that Agt. score groups were also predicted by BV/TV ($P = 0.02$); one unit (%) increase in BV/TV in patients with the same age and on HD for ≤2 yr decreases the odds of being in the high Agt. score group (Agt. >400) by 24% [odds ratio: 0.76; 95% confidence interval: 0.61 to 0.94]. In addition, the interaction term between BV/TV and HD duration was associated

Table 1. Demographic characteristics of the study population

Characteristic	Agatston Score			
	<100	100 to 400	>400	*P*
Age (yr)				0.03[a]
N	19	8	11	
mean (SD)	45.2 (15.2)	59.8 (14.9)	57.2 (15.1)	
median (min, max)	45 (21, 74)	59 (39, 76)	57 (37, 78)	
Cholesterol (g/L)				0.44[a]
N	19	8	11	
mean (SD)	1.70 (0.3)	1.6 (0.2)	1.5 (0.2)	
median (min, max)	1.7 (1.2, 2.4)	1.6 (1.2, 2.0)	1.5 (1.2, 2.0)	
Calcium (mg/dl)				0.35[a]
N	19	8	11	
mean (SD)	96 (6.6)	94 (6.2)	98 (5.7)	
median (min, max)	95.4 (87, 111)	93.6 (85, 104)	100 (87, 105)	
Phosphorus (mg/dl)				0.08[a]
N	19	8	11	
mean (SD)	5.4 (0.9)	4.6 (0.8)	5.0 (0.7)	
median (min, max)	5.5 (3.9, 7.2)	4.8 (3.2, 5.3)	5.0 (3.8, 6.3)	
iPTH[c] (pg/ml)				0.47[a]
N	19	8	11	
mean (SD)	620 (614)	293 (167)	570 (700)	
median (min, max)	353.4 (50, 2164)	259 (155, 679)	301 (100, 2572)	
25-(OH)-vitamin D (ng/ml)				0.78[a]
N	19	8	11	
mean (SD)	21.2 (7.8)	19.5 (6.2)	20.2 (10.0)	
median (min, max)	21.5 (7.8, 37.6)	17.5 (13.5, 27.8)	14.6 (11.0, 41.4)	
Dialysis duration (mo)				0.48[a]
N	19	8	11	
mean (SD)	73.1 (56.6)	44.9 (23)	87.2 (77.2)	
median (min, max)	48.3 (21, 206)	39.5 (19, 77)	45 (23, 242)	
Gender				0.27[b]
N	19	8	11	
male (*N*, %)	9 (47.3)	3 (37.5)	8 (72.7)	
female (*N*, %)	10 (52.6)	5 (62.5)	3 (27.3)	
Tobacco use				0.88[b]
N	19	8	11	
yes (*N*, %)	4 (21.0)	1 (12.5)	3 (27.3)	
no (*N*, %)	15 (79.0)	7 (87.5)	8 (72.7)	
Hypertension				0.63[b]
N	19	8	11	
yes (*N*, %)	11 (57.9)	4 (50.0)	8 (72.7)	
no (*N*, %)	8 (42.1)	4 (50.0)	3 (27.3)	

[a]*P* value computed from Kruskal–Wallis test.
[b]*P* value computed from Fisher's exact test.
[c]iPTH, immunoreactive parathyroid hormone.

with Agt. score groups ($P < 0.05$); the effect of BV/TV on the odds of being in a specific Agt. score group decreased with increasing HD duration (Figure 2) and was no longer statistically significant in patients on HD for >6 yr (odds ratio: 0.90; 95% confidence interval: 0.80 to 1.02).

To better study the interactions between BV/TV, age, and HD duration for predicting Agt. score groups, we divided these independent variables into three categories characterized by the distribution of our data (5th, 50th, and 95th percentiles). Classification according to these percentiles corresponded to BV/TV values of 15, 24, and 37%; ages of 30, 50, and 75 yr; and HD durations of 2, 4, and 17 yr. Because ordinal logistic regression revealed that the associations between bone volume (BV/TV) and coronary calcifications were no longer statistically

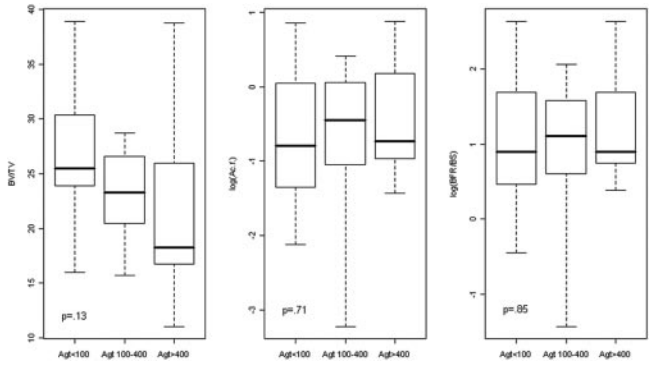

Figure 1. Distribution of values for bone volume/tissue volume (BV/TV), activation frequency (Ac.f.), and bone formation rate/bone surface (BFR/BS) among the Agatston (Agt.) score classes <100, 100 to 400, and >400. Box = median, 25 to 75%; T-bars = minimum and maximum values.

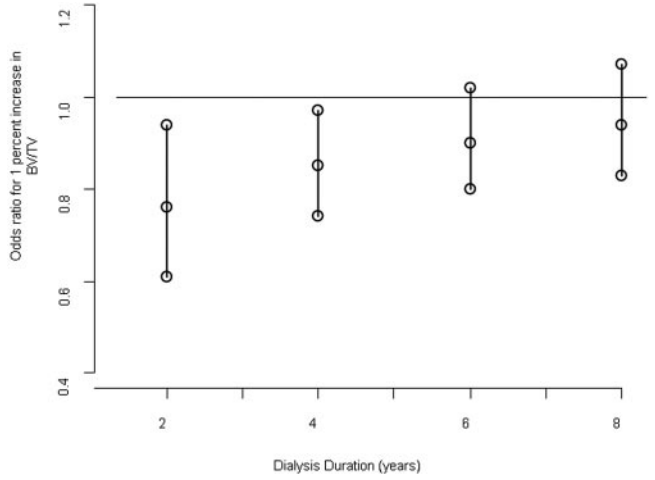

Figure 2. Effect of the interaction between BV/TV volume and hemodialysis (HD) duration on the odds of an Agt. score of >400. Odds ratio represents the change in the odds for an Agt. score >400 for each 1% increase in BV/TV. Vertical bars = 95% confidence interval.

significant after a HD duration of 6 yr, the sixth-year HD duration time point was also examined. Accordingly, Figure 3 depicts changes in the probabilities for the Agt. score group <100 (Figure 3, A through D) and for the Agt. score group >400 (Figure 3, E through H) for the three different BV/TV values (depicted on the *x*-axis) and three different ages (depicted by different lines) on the basis of the four different HD durations (Figure 3, A and E = 2 yr; Figure 3 B and F = 4 yr; Figure 3, C and G = 6 yr; and Figure 3, D and H = 17 yr). Irrespective of HD duration, increasing age carries a higher likelihood of having an Agt. score of >400 and accordingly a lower likelihood of having an Agt. score <100. This age effect was observed in each BV/TV class. However, the magnitude of this bone volume effect is conditional on HD duration; in the HD duration classes of 2 and 4 yr, lower BV/TV bears a higher likelihood of having an Agt. score >400 and, accordingly, lower likelihood of having

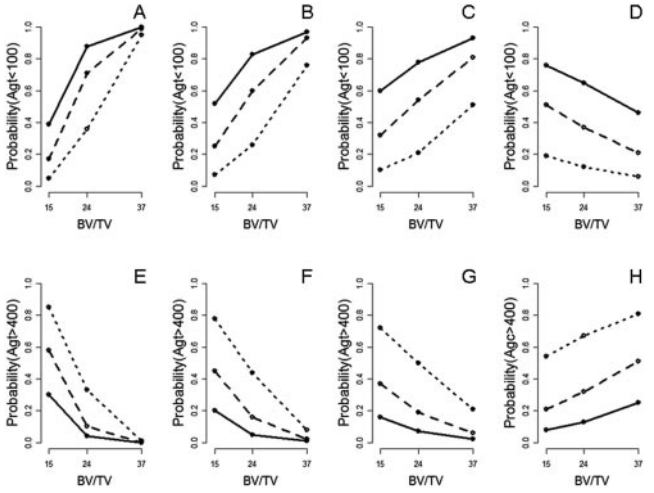

Figure 3. Changes in the probabilities for (A through D) the Agt. score group <100 and (E through H) the Agt. score group >400 for different BV/TV values and ages on the basis of four different HD durations (A and E = 2 yr, B and F = 4 yr, C and G = 6 yr, D and H = 17 yr). Solid line = 30-yr-old patient; long dashed line = 50-yr-old patient; short dashed line = 75-yr-old patient.

an Agt. score <100 irrespective of the age group (Figure 3). However, in long HD duration classes (17 yr), higher BV/TV is associated with lower likelihood of an Agt. score <100 and higher likelihood of an Agt. score >400 irrespective of age.

In the case of shorter HD duration (<6 yr), the incremental increase in the likelihood of having an Agt. score <100 is the largest for increasing BV/TV from the 5th to the 50th percentile for younger patients (30 yr), whereas older patients (75 yr) derive the highest benefit from increasing BV/TV from the 50th to the 95th percentile. It is of note that, after 2 yr of HD duration, a 30-yr-old patient with a low BV/TV has about the same probability of having an Agt. score <100 ($P = 0.39$) than a 75-yr-old patient with normal BV/TV ($P = 0.36$). Figure 3 also shows that the likelihood of having an Agt. score <100 is lower at the lowest BV/TV at the shortest HD duration than at normal or high BV/TV at the longest HD duration irrespective of age.

Ordinal logistic regression conducted with Ac.f. and BFR/BS revealed that only age was predictive of the Agt. score class of coronary arteries ($P = 0.03$). No specific interactions were found between variables of bone turnover and the studied traditional risk factors ($P > 0.05$).

Discussion

Our data confirm previous studies reporting on the effect of age on vascular calcifications in HD patients (17,18). We also confirm previous observations reporting lack of predictive value of the traditional cardiovascular risk factors of hypertension, smoking, gender, and cholesterol for cardiovascular outcomes in HD patients (5,6,18,19).

The novel aspect of our study is the investigation of the interaction between the bone volume and the bone turnover components of renal osteodystrophy (determined by histomorphometry) and coronary calcifications (determined by MSCT)

in prevalent HD patients. Low bone volume and bone loss are common findings in patients on renal replacement therapy (20,21). Our study demonstrates the importance of bone volume for predicting coronary calcifications. Specifically, it appears that lower bone volume is predictive of higher Agt. scores, reflecting higher risk of cardiovascular events (Agt. $\geq$100) irrespective of age. Although correlations between bone loss and progressive arterial calcifications were described in the general population, (22,23) there is little information available on their possible interaction in HD patients. Peripheral arterial calcifications assessed by plain radiographs and low bone volume were observed in an early study by Zuchelli and colleagues, but the authors did not examine a possible interrelation between bone volume and vascular calcifications and did not report on the contribution of traditional risk factors for cardiovascular calcifications (24). Similarly, assessment of bone mineral density by quantitative computed tomography in HD patients showed less coronary calcifications with higher vertebral bone mineral density (25). Although several serum markers of bone metabolism such as bone morphogenic proteins (26) and osteoprotegerin (27) were implicated in the pathogenesis of vascular calcifications, to our knowledge this is the first population-based study that describes the predictive value of histologically measured bone volume for coronary calcifications in HD patients.

An interesting finding in our study is that the effect of bone volume on Agt. scores is conditional on HD duration; if HD duration is >6 yr, the bone volume effect seems to be overridden by other risk and/or protective factors that determine coronary calcifications. In light of a 5-yr mortality rate of $\geq$75% for patients on dialysis (1), it appears that the bone volume effect is of great importance for most HD patients. The implications of lower bone volume for higher Agt. scores is further highlighted by our finding that 30-yr-old patients who exhibit low bone volume early in the course of HD have probabilities for low Agt. scores comparable to 75-yr-old patients with normal bone volume.

London and colleagues previously reported on the association between histologically diagnosed low bone turnover and arterial calcifications detected on plain radiographs and evaluated by semiquantitative calcifications scores in HD patients (9). Our study presented here did not find an association between bone turnover parameters and coronary calcifications measured by MSCT, although a relationship was demonstrated between histologically determined bone turnover and thoracic aorta and iliac artery calcifications in a previous publication (10). Discordance between predictors of aortic and coronary calcifications has been described in a series of publications from the large, population-based Multi-Ethnic Study of Atherosclerosis (MESA) (28–30). Accordingly, our findings supplement rather than contradict the findings of London and colleagues and highlight that studies aiming at predicting vascular calcifications have to take into account the site examined within the vascular tree, the morphology of the vascular tissue, and the localization of calcification within the vascular wall.

Limitations of our study are that patients were recruited from dialysis centers in Portugal and diabetic patients were not included. Accordingly, we cannot generalize our findings to other populations and draw inferences on a possible effect of diabetes mellitus on the observed interaction between bone volume and coronary calcifications. We are currently in the process of designing larger studies that focus on the contribution of renal osteodystrophy to coronary calcifications in different HD patient populations. On the basis of recent improvements in the sensitivity of MSCT scanners for detecting coronary calcifications, it is conceivable that the magnitude of the bone volume effect will be greater than observed in our presented here. More sensitive detection methods for coronary calcifications might also contribute to a more precise estimation of the contribution of bone turnover to coronary calcification.

In summary, our data implicate that low bone volume is a significant risk factor for coronary calcifications in HD patients, and that this risk is dependent on the patient's age and HD duration. Although statistical associations do not necessarily imply cause and effect relationships, our findings suggest that early prevention of bone loss in CKD patients might carry important implications for reducing coronary calcifications, especially because age and HD duration are not modifiable risk factors.

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Disclosures

None.

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