Improvement of Mineral and Bone Disorders After Renal Transplantation

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Background. Posttransplant mineral and bone diseases are causes of fractures, and their association with cardiovascular events is being studied. **Methods.** We analyzed the evolution of biochemical, histological, and imaging parameters pre– and 1 y post–renal transplantation in 69 patients and correlated mineral and bone findings with coronary calcifications. At inclusion and after 12 mo, clinical data and echocardiographic findings were recorded, and laboratory evaluations, radiography of the pelvis and hands, and bone biopsy were performed. Noncontrast cardiac computed tomography was performed during the second evaluation. **Results.** Serum levels of fibroblast growth factor 23 and sclerostin decreased in all patients, parathyroid hormone levels decreased in 89.8% of patients, bone alkaline phosphatase levels decreased in 68.1% of patients, and alpha-Klotho levels increased in 65.2% of patients. More than half of the patients presented with renal osteodystrophy at both biopsies, but histological findings improved: a significant transition from high to normal or low turnover and no significant differences in volume, mineralization defect, or cortical porosity at the 2 evaluations. Alpha-Klotho, sclerostin, and bone alkaline phosphatase shifts affect bone changes. Neither echocardiographic findings nor vascular calcification scores differed between the 2 points. Both the pretransplant period (dialysis vintage, sclerostin, and low bone volume at baseline) and the maintenance of abnormalities in the posttransplant period (high turnover posttransplant) were the most reliable predictors of the severity of the coronary calcification percentile. **Conclusions.** Renal transplantation improved bone and mineral abnormalities. The pretransplant period determines the severity of calcification.

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INTRODUCTION

With improved long-term outcomes in kidney transplantation,[1,2] cardiovascular (CV) disease and fractures have emerged as events that reduce both the quality of life and survival of renal transplant patients.[3,4] Posttransplant mineral and bone disease is considered to be one of the major causes of these outcomes. The evolution of bone disease after transplantation is not well defined, and studies are contradictory,[5-13] which may mirror the fact that many studies rely on only 1 bone biopsy. In this context, early biopsies in the posttransplant period can only reflect abnormalities in the pretransplant period.[5] Three recent double bone biopsy studies enrolling 36,[14] 31,[15] and 27 patients[16] showed that the remodeling pathology observed after renal transplantation was predominantly low-turnover disease; 2 of those studies showed no major changes in bone volume,[17] although in the Brazilian study by Marques et al,[15] the presence of low bone volume changed from 15% to 26%, and in all 3 studies, the presence of mineralization defects increased after transplantation. It should be noted

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A.C.F. and A.F. conceptualized and designed the study. A.C.F., M.M., C.S., P.C., I.A., D.N., F.C., R.R., R.S., B.C., and G.C. collected data and performed bone biopsies or laboratory evaluations. A.C.F., M.M., R.S., B.C., G.C., F.N., and A.F. analyzed the data. A.C.F. drafted the article. M.M., C.S., P.C., I.A., D.N., F.C., R.R., R.S., B.C., G.C., F.N., and A.F. revised the article. All authors approved the final version of the article and agreed with all aspects of the work.

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that in the study by Marques et al,[15] trabecular microarchitecture worsened, characterized by decreases in trabecular number and spacing. Although posttransplant mineral and bone disease improvement has a positive influence on the calcification progression rate,[4] to the best of our knowledge, no studies have explored the link between fibroblast growth factor 23 (FGF23) and sclerostin levels or metabolic bone disease and their role in vascular calcification in renal transplant recipients.

The aim of this study was to determine the prevalence, phenotype, and evolution of bone disease before and 1 y after renal transplantation and to correlate bone-associated biomarkers (FGF23, alpha-Klotho, sclerostin, parathyroid hormone [PTH], and bone alkaline phosphatase [BALP]) with bone histomorphometric parameters and CV disease.

MATERIALS AND METHODS

This was a prospective observational cohort study approved by the local ethics committees of the institutions and performed under the Strengthening the Reporting of Observational Studies in Epidemiology guidelines[18] of patients aged 18 to 66 y admitted to de novo renal transplant from November 2015 to February 2018 (*ClinicalTrials.gov*; ID: NCT02751099). The exclusion criteria were admission for double (liver-kidney and pancreas-kidney) transplantation, age outside the determined range, and major cognitive impairment. Written consent was obtained from all the participants. The patients were monitored for 12 mo.

This study was designed to perform evaluations at 2 time points: before engraftment (T0) and at the end of the first year (T1). At inclusion, demographic and medical past history were collected, transplant and donor data registered, as well as the evaluation of the last echocardiography. At both time points, routine laboratory analysis was performed using standard methods, and serum and plasma samples were stored at −80 °C for further analysis of BALP, FGF23, alpha-Klotho, and sclerostin. Intact PTH level was measured by immunochemiluminescence using a second-generation assay (Immulite 2000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Vitamin D (25(OH)D) levels were measured using a radioimmunoassay provided by immunodiagnostic systems (Boldon, United Kingdom). BALP was measured using an enzyme immunoassay with a monoclonal anti-BALP antibody (MIcroVue BAP). FGF23 levels were measured using a second-generation ELISA kit that detects epitopes within the carboxyl-terminal (C-Term) portion of FGF23 (Immunotopics, San Clement, CA). Alpha-Klotho was determined using a human soluble α-klotho assay kit, consisting of a solid-phase sandwich ELISA using 2 highly specific antibodies (Immuno-Biological Laboratories America, MN). Sclerostin levels were measured using a high-sensitivity enzyme immunoassay kit, which is a 96-well immune-capture ELISA (TECOmedical).

Horizontal manual puncture transiliac bone biopsies were obtained from the anterior iliac crest using a 7G trocar (Osteobell T) under general anesthesia (first biopsy) or with local anesthesia (second biopsy). In the case of the second bone biopsy, tetracycline hydrochloride, 500 mg, 12/12 h, 3 d, was given 1 mo and 1 wk before the biopsy. Biopsy specimens (4.5 mm × 1.0–1.5 cm) were fixed in 70% alcohol and dehydrated in 96% and 99.9% alcohol. The fragments were cleared with xylene and embedded in methyl methacrylate. Undecalcified 5-μm sections were stained with modified Masson-Goldner trichrome, toluidine blue, von Kossa, acid phosphatase, alkaline phosphatase, Perls, and solochrome azurine for static histomorphometric evaluation. Unstained 10-μm sections were prepared for fluorescent dynamic analysis in the second biopsies. Each sample was composed of 2 cortices and a cylinder of trabecular bone. One observer evaluated all biopsies: for cortical bone, cortical thickness and porosity, and for trabecular bone, volume (normal if bone/tissue volume ≥16%), bone remodeling (normal if osteoblast surface/bone surface [BS] 0.2%–3.5% and osteoclast surface/BS 0.1%–7.25%, plus bone formation rate/BS 18–38 $\mu m^3/\mu m^2/y$[19,20] in the biopsies with dynamic evaluation), and efficacy of mineralization (abnormal if osteoid thickness ≥12.5 μm plus no active osteoblasts in mineralization front or mineralization lag time >100 d in the biopsies with dynamic evaluation).[21,22] Based on our findings (median values and interquartile range), we considered an abnormal cortical porosity >10%. This decision was made because of the missing reference values in the literature. Mixed lesions were identified if both high remodeling and abnormal mineralization were present. Renal osteodystrophy (ROD) is defined as abnormal turnover or mineralization. Whenever static and dynamic evaluations provided different information, we reviewed the bone biopsy. Bone histomorphometry was analyzed using a semiautomatic technique with Osteomeasure software (Osteometrics, Atlanta, GA).

Echocardiography performed in M mode and 2 dimensions, to access both valve calcifications and left ventricular mass index (LVMI), calculated using the Devereux formula, indexed to body surface area, was performed at the time of the second bone biopsy and was compared with the one performed pretransplant. Female patients were considered to have left ventricular hypertrophy if LVMI was >95 g/m^2, whereas in male patients, LVMI was >115 g/m^2. Radiography of the pelvis and hands was performed to classify vascular calcifications using the Adragão score[23] at baseline and after 1 y. At the end of the study, patients who underwent a second bone biopsy underwent noncontrast cardiac computed tomography (CT) in a low-radiation exposure technique to quantify the coronary artery calcification score using the Agatston method,[24] with the exception of 3 patients who undertook prior angioplasty. This examination was performed only once, 1 y after the transplant.

Immunosuppression

Patients received induction immunosuppression (basiliximab or thymoglobulin, depending on the immunologic risk) and intravenous 500 mg of methylprednisolone intraoperatively and daily for 2 d, followed by maintenance of 20 mg of oral prednisolone (tapered throughout the year), mycophenolate mofetil (2 g/d with dose adjustments and reduction throughout the year), and tacrolimus (adjusted for levels of 8–12 ng/mL for 3 mo and 5–8 ng/mL thereafter). In 5 patients, low doses of everolimus were added to low doses of tacrolimus to minimize calcineurin inhibitor toxicity. Globally, patients are treated with steroid-based immunosuppression.

Statistical Analysis

The outcome variables of interest were bone histomorphometric parameters (turnover, mineralization, and volume) and extraosseous calcifications (Agatston percentiles score). The predictor variables were laboratory measurements of PTH, BALP, alpha-Klotho, FGF23, and sclerostin. Continuous variables are presented as medians (with interquartile ranges), and categorical variables are expressed as frequencies.

Evolution from baseline (bone biopsies, biochemical parameters, vascular calcification scores, or echocardiographic findings) was assessed using the Wilcoxon matched-pair signed-rank test or the McNemar test. Associations between demographic or laboratory data and bone biopsy data were obtained using the Mann-Whitney test, Fisher exact test, or Spearman correlation test. The different degrees of severity of coronary calcifications were evaluated using ordered logistic regression, and multivariate ordered logistic regression was performed to detect possible risk factors for coronary artery calcification. In this analysis, the outcome variable was the 3 levels of severity of coronary calcification percentiles, and we included plausible predictor variables that had a P value of ≤0.1 in the univariate model. The final model evaluated the relationship between high bone turnover and severity of coronary calcifications, adjusted for potential confounders that could theoretically interact with both turnover and calcifications (previous time on dialysis, estimated glomerular filtration rate by epidemiology collaboration equation, sclerostin baseline values, BALP 1 y after transplant, and bone volume at baseline).

All tests were performed using STATA version 13 software package, and statistical significance was set at $P < 0.05$.

RESULTS

During the recruitment phase, 151 patients underwent renal transplantation at our center: 84 were recruited, and

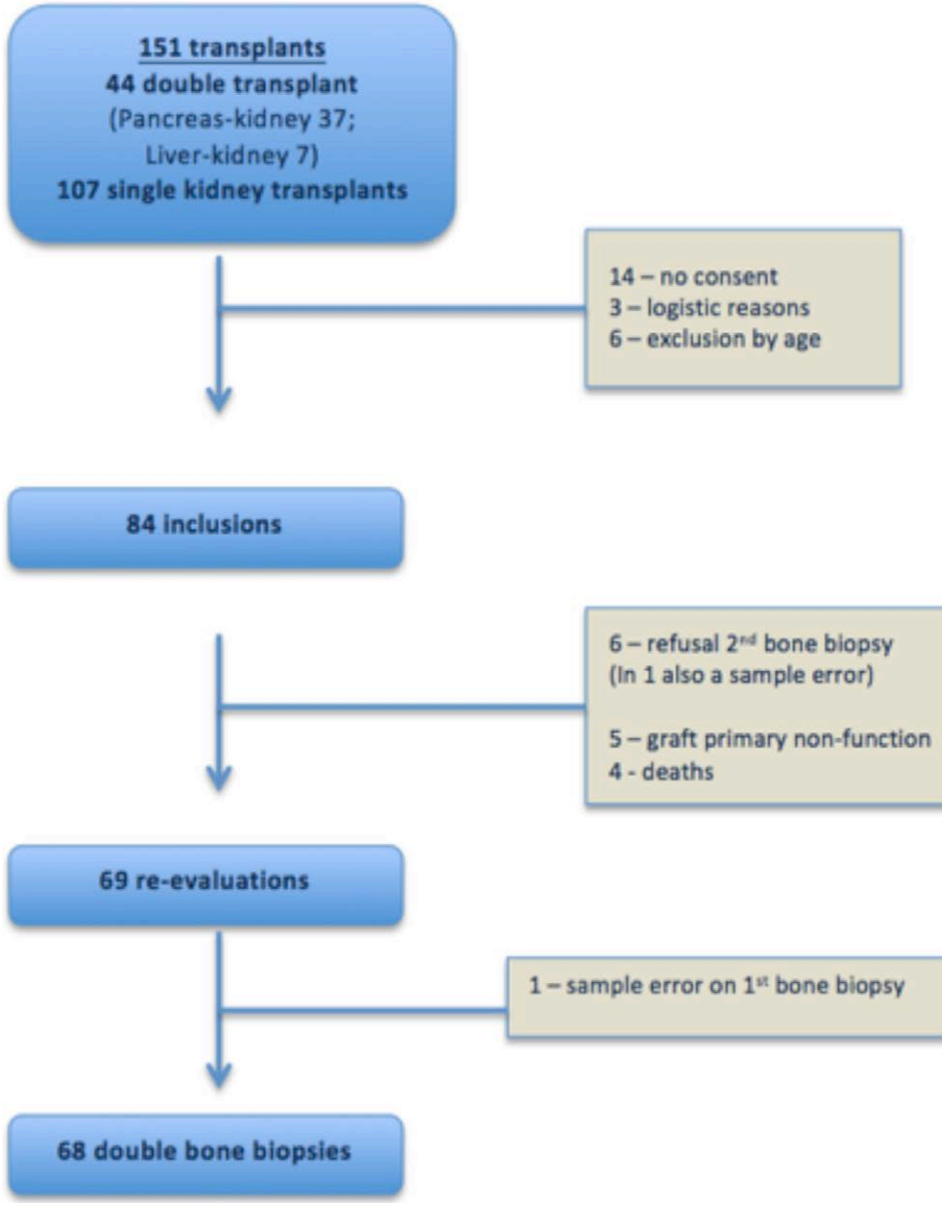

FIGURE 1. Flowchart of the study.

69 underwent a second evaluation, as shown in Figure 1. These patients were middle-aged, mostly Caucasian, and male, with a median dialysis vintage of almost 5 y. Six patients underwent parathyroidectomy before transplantation, presenting lower levels of PTH both at baseline and 1 y after the transplant but no other differences regarding other bone-related hormones or minerals or histomorphometric bone parameters, specifically bone formation rate/BS. None of the patients were prescribed antiosteoporotic drugs during the posttransplant period. Of the 16 patients who were on cinacalcet, 7 retained the drug after transplantation (Supplementary Data, **SDC,** http://links.lww.com/TP/C378). The demographic data are presented in Table 1.

TABLE 1.

Demographics and past history of the population

Demographic characterization (N = 69)	
Age (y), median (IQR)	53.0 (41.0–62.0)
Gender (M:F), n (%)	48 (69.6):21 (30.4)
Caucasian race, n (%)	53 (76.8)
BMI at transplant, kg/m^2, median (IQR)	24.5 (22.7–27.8)
PD (previous or current):HD, n (%)	9 (13.0):65 (94.2)
Dialysis vintage (mo), median (IQR)	55.0 (42.0–84.0)
Diabetes/PTD, n (%)	9 (13.0)/35 (50.7)
Hyperparathyroidism at transplant, n (%)[a]	50 (72.5)
Parathyroidectomy prior transplantation, n (%)	6 (8.7)
Cause of renal disease, n (%)	
Unknown	13 (18.8)
Hypertensive nephrosclerosis	11 (15.9)
ADPKD	11 (15.9)
Diabetic nephropathy (type 1 and 2)	6 (8.9)
Alport disease	2 (2.9)
Glomerulonephritis	
Chronic glomerulonephritis	5 (7.2)
IgA nephropathy/mensangial proliferation	6 (8.8)/1 (1.4)
HIVAN	1 (1.4)
FSGS	1 (1.4)
Membranous nephropathy	2 (2.9)
Lupus nephritis	1 (1.4)
Vasculitis	
Pauci-immune/Goodpasture	2 (2.9)/1 (1.4)
Lithiasis	3 (4.4)
CAKUT	3 (4.4)
Living kidney donor, %	10.1
Preemptive transplantation, %	0
GFR by CKD-EPI 1 y after transplant	53.0 (37.3–69.0) mL/min/1.73 m^2
Bone-related medication, n (%)	61 (88.4)
Phosphate binders	28 (40.6)
Cholecalciferol	21 (30.4)
Vitamin D analogs/calcitriol	44 (69.0)
Calcimimetics	22 (31.9)
Cumulative steroid dose (mg)	3580.0 (3257.5–4072.5)

[a]We assume a hyperparathyroidism diagnosis if patients were receiving vitamin D analogs or calcimimetics at the time of transplantation.

ADPKD, autosomal polycystic kidney disease; BMI, body mass index; CAKUT, congenital anomalies of the kidney and urinary tract; CKD-EPI, chronic kidney disease–epidemiology collaboration equation; FSGS, focal segmental glomerulosclerosis; GFR, glomerular filtration rate; HD, hemodialysis; HIVAN, HIV-associated nephropathy; IgA, immunoglobulin A; IQR, interquartile range; M:F, male:female; PD, peritoneal dialysis; PTD, posttransplant diabetes.

Metabolic Evaluation

Table 2 shows the differences between the pre- and 1-y follow-up after renal transplantation in 69 patients. In all patients, both sclerostin and FGF23 serum levels had decreased (sclerostin from 1.9 [1.3–2.7] ng/mL to 0.7 [0.5–1.0] ng/mL; FGF23 from 1806.5 [613.7–6281.6] pg/mL to 135.2 [101.1–168.6] pg/mL) with a median percentage reduction of 62.0% and 91.1%, respectively. PTH, BALP, and alpha-Klotho expressed some behavioral variability: 89.8% of patients had decreased PTH levels (from 475.0 [301.0–748.7] pg/mL to 135 [90.1–232.7] pg/mL), 68.1% had decreased BALP levels (from 33.8 [26.7–44.7] U/L to 23.0 [17.2–35.2] U/L), and 65.2% of patients had increased alpha-Klotho levels (from 571.0 [363.5–846.0] pg/mL to 945.2 [485.0–2044.2] pg/mL). Additional data regarding the metabolic evaluation are provided in the Supplementary Data (**SDC,** http://links.lww.com/TP/C378).

Histologic Evaluation

In the second bone biopsy, it was possible to analyze the dynamic parameters of the trabecular bone, as shown in Table 3. Overall, the histological findings improved, as patients with high bone turnover, low bone volume, abnormal mineralization, or bone porosity >10% decreased.

Cortical Bone

A nonsignificant trend toward lower cortical bone porosity was observed. The cortical thickness decreased significantly. The difference between cortical thicknesses at the 2 evaluations did not correlate with the cumulative steroid dose ($P = 0.269$).

Remodeling

Less than half of the patients had normal remodeling parameters in both biopsies (n = 33 versus n = 31; $P = 0.590$). We observed a large decrease in the number of patients with high-remodeling disease (20 versus 7 patients; $P < 0.001$) and a significant increase in patients with low-remodeling disease (15 versus 31 patients; $P = 0.002$), as demonstrated in Figure 2. Nevertheless, 33 (48.5%) maintained their original turnover, 29 (42.1%) decreased their turnover, and 6 (15.8%) increased their turnover, as shown in Figure 3.

Notably, increased bone remodeling occurred mostly in patients with low turnover at baseline ($P = 0.001$). Patients who had an increase in turnover had lower PTH levels at baseline (179.4 versus 481.1 pg/mL; $P = 0.032$), had higher calcium levels at baseline (10.3 versus 9.3 mg/dL), had a significant difference in the percentage of change of vitamin D (–95.1% versus 7.5%; $P = 0.016$), and had lower cumulative prednisolone dose (3150.0 versus 3755.0 mg; $P = 0.042$); 50% of those patients were under everolimus (versus 3.2% of patients who did not have an increased in bone turnover and were under everolimus; $P = 0.004$). Patients who had experienced decreases in bone remodeling categories had higher alpha-Klotho levels after 1 y of transplantation (1266.2 [619.0–2335.2] pg/mL versus 687.5 [453.3–1310.2] pg/mL; $P = 0.050$), greater increases in alpha-Klotho than at baseline (delta-Klotho of 798.4 [155.8–1908.2] pg/mL versus 14.2 [–375.7–967.7] pg/mL; $P = 0.036$), lower levels of sclerostin 1 y after transplantation (0.5 [0.3–0.9] ng/mL versus 0.8 [0.6–1.0] ng/mL;

TABLE 2.

Laboratory evaluation at baseline and 1 y after transplantation

Variable	Median (IQR) Baseline (N = 69)	12 mo (N = 69)	*P*
Hemoglobin (g/dL)	11.5 (10.9–12.6)	12.9 (12.2–14.3)	<0.0001
Glucose (mg/dL)	88.0 (79.0–102.0)	92.0 (81.0–103.0)	0.248
Urea (mg/dL)	**104.0 (66.0–138.0)**	**60.0 (44.0–78.0)**	**<0.001**
Creatinine (mg/dL)	**8.2 (5.7–10.6)**	**1.4 (1.1–1.8)**	**<0.001**
Uric acid (mg/dL)	**5.1 (3.5–7.0)**	**6.4 (5.6–7.1)**	**<0.001**
Alkaline phosphatase (U/L)	83.0 (61.0–103.0)	78.0 (57.0–119.0)	0.859
Albumin (g/dL)	4.2 (4.0–4.5)	4.3 (4.1–4.5)	0.509
Calcium (mg/dL)	**9.3 (8.7–9.6)**	**9.8 (9.3–10.4)**	**<0.0001**
Phosphorus (mg/dL)	**4.2 (3.3–5.1)**	**3.1 (2.8–3.5)**	**<0.0001**
Magnesium (mg/dL)	**2.3 (2.1–2.5)**	**1.7 (1.6–1.8)**	**<0.001**
Vitamin D (ng/mL)	20.2 (15.0–30.4)	22.5 (14.3–29.0)	0.881
iPTH (pg/mL)	**475.0 (301.0–748.7)**	**135.0 (90.1–232.7)**	**<0.001**
BALP (U/L)	**33.8 (26.7–44.7)**	**23.0 (17.2–35.2)**	**0.001**
FGF23 (RU/mL)	**1806.5 (613.7–6281.6)**	**135.2 (101.1–168.5)**	**<0.001**
Klotho (pg/mL)	**571.0 (363.5–846.0)**	**945.2 (485.0–2044.2)**	**<0.001**
Sclerostin (ng/mL)	**1.9 (1.3–2.7)**	**0.7 (0.5–1.0)**	**<0.001**

Statistical analysis was performed with the Wilcoxon matched-pair signed-rank test. Bold values when $P < 0.05$.
Normal range for PTH: 14.8 to 83.1 pg/mL; for vitamin D: 4.8 to 52.8 ng/mL; for FGF23: ≤180 RU/mL; Klotho normal value: 845 ± 330 pg/mL.
BALP is dependent on sex and age; the normal range in premenopausal women is 11.6 to 29.6 U/L, in postmenopausal women is 14.2 to 42.7 U/L, and in men is 15 to 41.3 U/L.
Sclerostin is dependent on sex and age; the normal values in premenopausal women are 0.45 ± 0.15 ng/mL, in postmenopausal women are 0.51 ± 0.14 ng/mL, and in men are 0.59 ± 0.13 ng/mL.
BALP, bone alkaline phosphatase; FGF23, fibroblast growth factor 23; iPTH, intact parathyroid hormone; IQR, interquartile range.

$P = 0.029$), and a higher percentage reduction in sclerostin (68.9% versus 59.4%; $P = 0.006$). These patients did not present with different estimated glomerular filtration rates.

Volume

We found that 12 of 22 patients with low volume at baseline normalized the volume (54.5%), as shown in Figure 4. Only 8 of 46 (17.5%) patients with normal bone volume had decreased bone volume. We notice that patients whose bone volume decreased after transplant were the ones with the highest body mass index at baseline (28.1 [26.4–29.4] kg/m^2 versus 24.2 [22.4–27.3] kg/m^2; $P = 0.007$), the highest levels at sclerostin at baseline (2.5 [2.2–4] ng/mL versus 1.7 [1.2–2.7] ng/mL; $P = 0.006$), and a significant decrease

TABLE 3.

Static and dynamic parameters of the bone biopsies

Histomorphometric bone parameters	Baseline (N = 68)	12 mo (N = 69)	*P*
Cortical bone			
Porosity (%)	7.4 (4.9–10.6)	5.9 (3.8–9.8)	0.094
Thickness (μm)	**737.9 (552.7– 973.9)**	**629.2 (403.5–849.2)**	**0.006**
Porosity >10%, n (%)	23 (33.3)	18 (26.1)	0.297
Trabecular bone			
Bone volume/tissue volume (%)	18.8 (14.3–24.3)	19.3 (15.8–24.8)	0.339
Osteoid surface/bone volume (%)	3.2 (1.7–4.9)	4.2 (2–5.8)	0.660
Osteoid thickness (μm)	**7.8 (6.7–10.3)**	**9.1 (6.8–12.6)**	**0.005**
Osteoid volume/bone volume (%)	3.2 (1.7–4.9)	4.2 (2.0–5.8)	0.261
Mineralized bone volume/tissue volume (%)	18.3 (13.7–23.0)	18.4 (14.7–23.6)	0.389
Osteoblast surface/bone surface (%)	**2.3 (0.7–5.5)**	**1.9 (1.1–3.2)**	**0.030**
Osteoclast surface/bone surface (%)	**1.3 (0.2–2.5)**	**0.4 (0–0.9)**	**<0.001**
Adjusted mineral apposition rate (μm/d)	–	0.3 (0.1–0.4)	–
Bone formation rate (μm^3/μm^2/d)	–	21.4 (4.7–32.2)	–
Mineralization lag time (d)	–	40.3 (25.5–85.0)	–
Low bone volume, n (%)	22 (32.3)	18 (26.5)	0.513
Normal bone turnover, n (%)	33 (48.5)	31(44.9)	0.590
Low-turnover bone disease, n (%)	**15 (22.1)**	**31 (44.9)**	**0.001**
Adynamic bone disease	7 (10.3)	10 (14.5)	0.405
Osteomalacia	1 (1.5)	3 (4.3)	0.157
High-turnover bone disease, n (%)	**20 (29.4)**	**7 (10.1)**	**<0.001**
Mixed renal osteodystrophy	2 (2.9)	0 (0)	0.157

Statistical analysis was performed using the Wilcoxon matched-pair signed-rank test or the McNemar test. Bold values when $P < 0.05$.

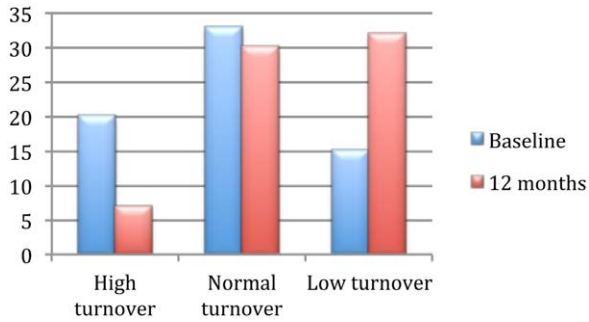

FIGURE 2. Differences in bone remodeling before and after transplantation (according to the number of patients).

in sclerostin values from baseline (–2.2 [–3.1 to –1.2] versus –1.1 [–1.6 to 0.7]; $P = 0.004$). No association with cumulative steroid dose was observed.

Mineralization

Very few patients had abnormal mineralization: there were 7 in the first bone biopsy (1 osteomalacia, 2 mixed ROD, 3 with normal volume and remodeling, and 1 with low volume and normal remodeling), and this number decreased to 5 in the second bone biopsy (3 osteomalacia, 2 with normal volume and remodeling), without statistical significance ($P = 0.479$). There were no differences in the mixed ROD ($P = 0.157$) or osteomalacia ($P = 0.157$) between the 2 points. Of the 7 patients classified as having abnormal mineralization, 5 (71.4%) had normalized mineralization, as shown in Figure 5; however, 3 additional patients (4.9%) moved from the normal group, leaving a total of 5 patients with this condition. The only factor associated with the reduced mineralization was the delta value of BALP: median was 7.3 (4.4–23.5) U/L higher at 12 mo versus –12.4 (–21.0 to 1.4) U/L in normal mineralization cases.

Imaging Exams Evaluation

The major echocardiographic findings did not differ from those at the baseline. Similarly, the vascular calcification score did not change, as shown in Table 4.

A CT scan was performed to quantify coronary artery calcification using the Agatston score. The percentiles of severity were homogeneous in the population: one third of the patients had mild coronary artery calcification (n = 22; 33.3%), one third had moderate coronary artery calcification (n = 24; 36.4%), and less than a third of the population had severe coronary artery calcification (n = 20; 30.3%).

Comparing the 3 levels of severity of the percentiles of coronary calcifications in univariate analysis, we observed that a longer time on dialysis; high sclerostin at baseline; high serum levels of calcium, BALP, and PTH 1 y after transplant; higher osteoid volume/bone volume; and higher cortical porosity 1 y after transplant were associated with calcification severity, as shown in Table 5. In the multivariate analysis, dialysis vintage ($P = 0.001$), baseline sclerostin levels ($P = 0.006$), baseline low bone volume ($P = 0.016$), and high bone turnover at 1 y after transplant ($P = 0.040$) were the main predictors of coronary calcification percentiles, as shown in Table 6.

DISCUSSION

Laboratory evaluation 1 y after transplant was as expected, considering the significant improvement in renal function: calcium and alpha-Klotho increased significantly,

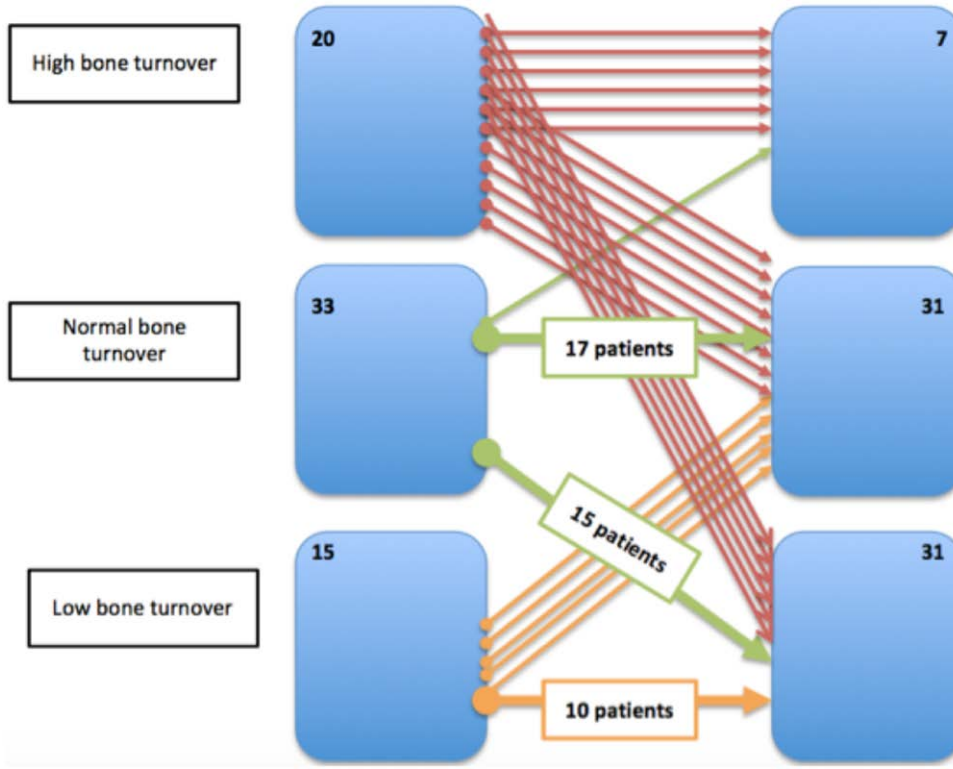

FIGURE 3. Changes in bone remodeling after transplantation.

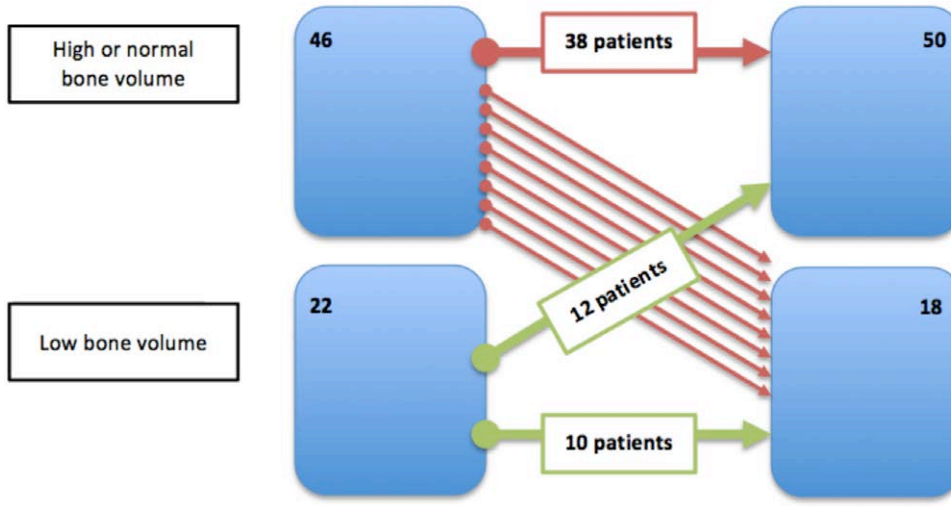

FIGURE 4. Changes in volume after transplantation.

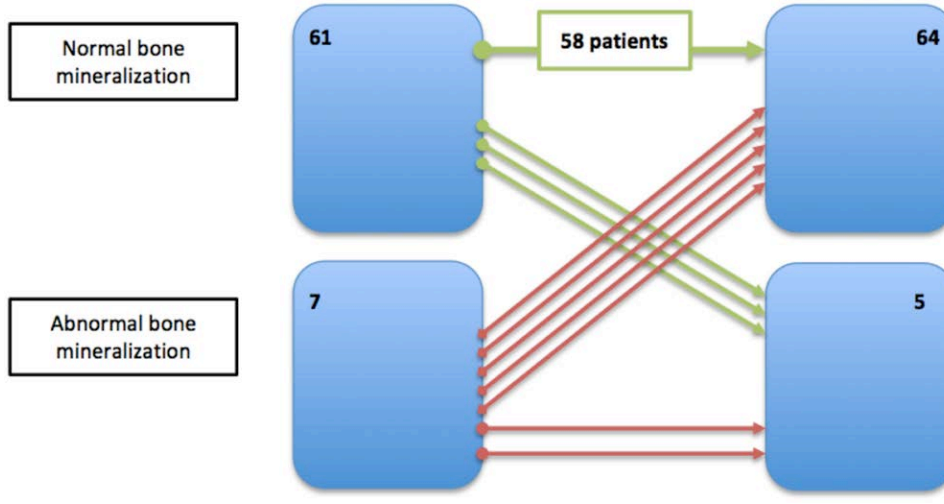

FIGURE 5. Changes in mineralization after transplantation.

TABLE 4.

Differences in imaging results comparing baseline to 1 y of follow-up

Imaging examinations	Baseline (N = 69)	12 mo (N = 69)	*P*
Echocardiographic findings			
Left ventricular mass index (g/m^2)	107.0 (91.5–140.5)	108.5 (98.0–138.0)	0.091
Interventricular septal thickness (mm)	11.0 (10.0–12.0)	11.0 (9.0–12.0)	0.492
Left ventricular hypertrophy, n (%)	29 (42)	26 (39.4)	1.000
Valve calcifications, n (%)	15 (21.7)	16 (23.5)	0.781
Left ventricular fractional shortening (%)	**40.0 (35.0–43.0)**	**43.0 (37.0–47.0)**	**0.015**
Vascular calcification score (Adragão score)			
Hands score	0 (0–2)	1 (0–2)	0.589
Pelvis score	0 (0–1)	0 (0–1)	0.873
Total score	1 (0–2)	1 (0–2)	0.196
Low score (0 and 1)/high score (≥2)	63.8%/36.2%	56.5%/43.5%	0.165

Statistical analysis was performed using the Wilcoxon matched-pair signed-rank or McNemar test. Bold values when $P < 0.05$.

TABLE 5.

Predictors for the percentile of Agatston coronary artery calcium score

	Agatston percentile ≤50% (n = 22)	Agatston percentile 51%–90% (n = 24)	Agatston percentile >90% (n = 20)	*P*
Agatston percentile	0 (0–0)	84 (74.0–87.5)	97.5 (94.0–99.0)	–
Age (y)	42.5 (33.0–50.0)	59.5 (49.5–63.0)	56.5 (41.5–60.5)	–
Male gender (%)	63.6	66.7	78.3	–
Caucasian race (%)	63.6	79.2	85.0	–
Valve calcifications	**9.1%**	**20.8%**	**36.8%**	**0.030**
Vascular calcifications	**0 (0–1)**	**2 (1–2.5)**	**1 (0.5–2)**	**0.021**
PD/only HD	27.3%/86.4%	4.2%/95.8%	10.0%/100%	0.053
Dialysis vintage (mo)	**51.5 (24.0–64.0)**	**48.5 (43.0–70.5)**	**79.5 (55.5–100.0)**	**0.003**
T0 PTH (pg/mL)	468.2 (308.0–671.3)	529.9 (290.1–774.4)	470.8 (273.0–956.2)	0.249
T0 calcium (mg/mL)	9.0 (8.7–9.5)	9.3 (8.6–9.6)	9.5 (9.0–10.1)	0.056
T0 phosphorus (mg/dL)	3.9 (3.2–4.9)	4.1 (3.3–5.0)	4.7 (3.5–5.2)	0.189
T0 magnesium (mg/dL)	2.2 (2.1–2.4)	2.1 (2.0–2.4)	2.3 (2.1–2.7)	0.199
T0 BALP (U/L)	32.1 (23.9–43.0)	34.3 (26.7–43.6)	36.4 (28.4–49.5)	0.399
T0 sclerostin (ng/mL)	**1.7 (1.2–2.2)**	**2.1 (1.3–2.9)**	**2.2 (1.7–2.9)**	**0.030**
T0 FGF23 (RU/mL)	1402.7 (463.9–6220.9)	1575.4 (599.4–3673.6)	4749.6 (778.6–8699.8)	0.151
T1 PTH (pg/mL)	**122.3 (84.9–179.3)**	**128.0 (88.3–181.0)**	**150.8 (113.4–256.4)**	**0.023**
T1 calcium (mg/mL)	**9.6 (9.2–9.9)**	**9.6 (9.3–10)**	**10.3 (9.6–10.8)**	**0.044**
T1 phosphorus (mg/dL)	3.1 (2.9–3.5)	3.1 (2.9–3.4)	2.8 (2.3–3.9)	0.681
T1 magnesium (mg/dL)	1.7 (1.6–1.8)	1.6 (1.4–1.8)	1.7 (1.6–1.9)	0.460
T1 BALP (U/L)	**18.2 (13.3–30.8)**	**26.4 (19.7–36.4)**	**27.6 (18.9–57.3)**	**0.008**
T1 sclerostin (ng/mL)	0.6 (0.4–0.8)	0.7 (0.5–0.9)	0.9 (0.5–1.2)	0.156
T1 FGF23 (RU/mL)	119.3 (88.5–143.2)	123.6 (96–164.1)	145.4 (119.9–196.1)	0.592
T0 BV/TV (%)	20.2 (14.1–26.0)	20.2 (16.3–25.1)	17.6 (13.3–21.5)	0.138
T1 porosity (%)	**4.4 (3.5–7.5)**	**5.3 (3.9–10.6)**	**7.3 (5.4–10.9)**	**0.017**
T1 BV/TV (%)	20.0 (15.0–24.8)	19.0 (17.1–23.4)	19.4 (14.3–26.3)	0.797
T1 OtV/BV	**4 (1.9–5.2)**	**3.9 (1.5–4.9)**	**5.3 (3.0–9.1)**	**0.021**
T1 high turnover	4.5%	4.2%	20.0%	0.069

Statistical analysis performed with ordered logistic regression. Bold values when $P < 0.05$.
BALP, bone alkaline phosphatase; BV/TV, bone volume/tissue volume; FGF23, fibroblast growth factor 23; HD, hemodialysis; OtV/BV, osteoid volume/bone volume; PD, peritoneal dialysis; PTH, parathyroid hormone; T0, at inclusion; T1, after 12 mo.

and phosphorus, magnesium, PTH, BALP, FGF23, and sclerostin decreased significantly. Despite these results, ROD was present in 68.1% of our patients 1 y after transplantation (adding remodeling and mineralization abnormalities); however, overall, we believe that the histological results improved. One important finding was that bone volume did not change pretransplant and posttransplant, and mineralization defects were not different between the 2 time points. Considering bone remodeling, a significant increase in low-turnover disease was observed (15–31 patients). In contrast, high bone turnover decreased, and hyperparathyroid bone disease was present in only 7 patients after transplantation. Neither echocardiographic findings nor vascular calcification scores differed between the 2 points. Nonetheless, 12 mo is a short period to observe major differences in the main echocardiographic findings. Dialysis vintage, sclerostin at baseline, low bone volume, and high bone turnover at 1 y after transplantation were the most reliable predictors of the percentile severity of coronary calcifications in this population.

TABLE 6.

Ordered logistic regression for independent associations with Agatston percentiles

	Agatston percentiles		
	OR	95% CI	*P*
Dialysis vintage (mo)	1.26[a]	1.09-1.45	0.001
T0 sclerostin (ng/mL)	2.61	1.38-4.92	0.006
T1 BALP (U/L)	1.38	1.04-1.90	0.050
GFR (mL/min/1.73 m^2)	0.95[a]	0.74-1.21	0.699
T0 BV/TV (%)	0.90	0.83-0.97	0.016
T1 high bone turnover	10.4	1.18-92.6	0.040

[a]For each 10-unit increase.
BALP, bone alkaline phosphatase; BV/TV, bone volume/tissue volume; CI, confidence interval; GFR, glomerular filtration rate; OR, odds ratio; T0, at inclusion; T1, after 12 mo.

We should recognize that this is a small, unicentric, and observational study; therefore, associations do not indicate a cause–effect relationship. Likewise, only the second bone biopsies had dynamic evaluations; the first biopsies could not benefit from tetracycline labeling, as patients were recruited on the day they were admitted to the hospital, meaning that turnover could be misclassified in the first bone biopsies.[20] Nevertheless, we did not find many discrepancies between quantitative and qualitative assessments of bone turnover in the second biopsies. We performed bone biopsies with a 7G trocar (4.5-mm inner diameter), but these were comparable with full 7.5-mm samples.[25] As we stored at –80 °C blood samples for non-routine analysis (BALP, FGF23, alpha-Klotho, and sclerostin), alpha-Klotho values can be inexact, as lower results can be obtained in stored versus fresh serum samples.[26,27] As this was a population listed for kidney transplantation,

meaning that these are the healthiest patients among patients with end-stage renal disease (ESRD), these results cannot be generalized to all ESRD patients. Finally, we classified 72.5% of the patients with secondary hyperparathyroidism based on the fact that those were receiving either vitamin D analogs, calcimimetics, or both at the time of transplantation. Despite this fact, the median value of PTH at baseline was 475.0 (301.0–748.7) pg/mL, which is consistent with most of the studies.

Overall, trabecular bone dynamics were similar to those published in recent literature,[14-16] although different reference ranges for turnover were applied (Salusky, composite parameters, and Malluche, respectively), as we still lack agreement in the turnover diagnostic cutoffs. Although a similar approach (double bone biopsy) was used in the aforementioned studies, we included an extraosseous calcification analysis, which has not been studied by other authors. In addition, the Belgian and Finnish studies included only Caucasian patients, a high number of males, and a high number of diabetic patients. Although our population characteristics were closer to those of the Brazilian cohort, they only addressed living-donor recipients and excluded those with low PTH levels and low bone turnover diagnosed by a bone biopsy before entry into the study.

We observed a significant reduction in the number of patients with high-turnover bone disease and a significant increase in the number of patients with low-turnover bone disease, with stable numbers of normal turnover. Six of our patients experienced an increase in bone remodeling, but this occurred mostly in patients with low turnover at baseline, which normalized their turnover. These patients presented with lower cumulative steroid doses, highlighting the importance of glucocorticoids in the activity of bone cells (inhibiting osteoblast function and promoting apoptosis).[28] Additionally, based on exploratory analysis, we observed that patients receiving everolimus also had increased bone turnover, which is in line with the belief that mammalian target of rapamycin inhibitors are sparing bone immunosuppressive agent.[5]

The reduction in bone turnover was associated with a greater increase in the levels of alpha-Klotho and higher levels of alpha-Klotho at 1 y after transplantation but not with PTH or BALP levels, as expected. In fact, PTH did not have an influence on trabecular bone dynamics. Although its median levels were above the normal level (135 pg/mL), its optimal range is unknown in renal transplanted patients.[5] In addition, changes in BALP were not associated with turnover deviations, but we noted that the development of abnormal mineralization posttransplantation occurred in patients with a greater increase in BALP levels than at baseline. A recent study showed that alkaline phosphatase levels could predict mineralization defects in a pediatric population.[29] In our population, BALP correlated well with nonmineralized bone (Supplementary Data, **SDC**, http://links.lww.com/TP/C378), and as this marker is produced by osteoblasts during bone formation,[30] inactivating pyrophosphate (which inhibits mineralization[31]) and osteopontin (which is a calcification inhibitor[32]), we can suspect some resistance to BALP actions at the bone after transplantation. In fact, after transplantation, osteoblast cells can become dysfunctional, have lower alkaline phosphatase expression,[33] and increase their own apoptosis, which contributes to bone disorders in these patients.[9] Although low bone turnover increased significantly, only 3 patients presented with osteomalacia, and only 10 patients presented with low turnover and low volume (adynamic bone disease). Even so, it is important to note that the presence of adynamic bone disease (defined as low turnover and low volume) did not increase with the transplant, which is a good result, as we recently published an association between adynamic bone disease and valve calcifications at baseline.[34] More important than having a low bone turnover after transplantation is having or maintaining a high bone turnover after the transplant, as this is associated with the severity of coronary calcifications. The introduction of vitamin D analogs (if calcium levels allow) or calcimimetics to halt bone turnover seems to be protective.[35]

Contrary to what is reported in the literature, we observed no loss of bone volume, even with a slight increase, as the number of patients with normal or high bone volume increased from 44 to 50 patients. It should be noted that we found no relationship between the cumulative steroid dose and bone volume or loss of bone volume. The decrease in bone volume after the transplantation was associated with the highest levels of sclerostin at baseline, which could be explained by the fact that sclerostin is an inhibitor of bone formation[36-38]; however, these results should be interpreted with caution, as decreased bone volume occurred in only 8 patients. Low bone volume and high sclerostin levels at baseline were correlated with calcification severity 1 y after the transplant, so it is of utmost importance to identify these patients to implement CV protective measures to safeguard them from an early CV event. We believe that it could be beneficial to perform a multicenter study to analyze the importance of performing bone densitometry some months after transplantation. For instance, it could rule out volume abnormalities in patients with a normal examination[39] and recognize patients who could benefit from more invasive studies (such as bone biopsies), especially those with higher calcification scores,[40] before starting antiresorptive or osteoformer therapies. These therapies could also be effective in those who do not benefit from the normalization of bone volume after transplantation.

Our transplant patients did not show progression in vascular calcification scores obtained by radiography of the hands and pelvis and did not show progression in valve calcifications. The fact that transplantation can slow the progression of calcifications has been suggested by other studies.[4] Because we did not have a control population, we cannot conclude that renal transplantation halts the evolution of calcifications. The Agatston percentiles correlated well with the vascular calcification score (and the presence of valve calcifications). We would expect to see more associations between bone-related hormones at baseline and Agatston percentile severity, as we suppose that coronary calcifications did not change significantly during a 12-mo period. FGF23 levels did not differ according to an increase in calcification percentile severity. Although increased expression of FGF23 in human calcified tissue has been shown,[41] Scialla et al[42] described that FGF23 does not have a role in inducing arterial calcification. The opposite was found with sclerostin, a soluble Wnt pathway antagonist and a negative regulator of bone metabolism.[37] Wnt signaling is involved in

vascular calcification, and increased sclerostin expression has been demonstrated during vascular smooth muscle cell calcification in an animal model.[38,43] Different studies have reached different conclusions regarding patients with chronic kidney disease. Some studies have demonstrated that high sclerostin levels are associated with better survival in hemodialysis patients,[44,45] suggesting a protective role through inhibition of vascular calcifications,[44,46] whereas other studies have found an association between high sclerostin levels and CV mortality in dialysis patients,[47] justified by the propensity for vascular calcifications via low-turnover bone disease,[37] leading investigators to speculate that a U-shaped dose effect could be the cause of these findings.[38] A recent study performed in patients with ESRD showed that sclerostin is associated with the degree of vascular calcifications.[48] Nevertheless, the role of sclerostin in CV health is very important to clarify, as a sclerostin antibody is being evaluated for osteoporosis treatment in postmenopausal women.[49] Patients with higher cortical porosity at 1 y after transplant also presented more severe percentiles of coronary calcification in univariate analysis but not in multivariate analysis, where only trabecular features were possible determinants of its severity (low bone volume and high bone turnover). This highlights the importance of trabecular bone in extraosseous calcifications in comparison with cortical bone, which is less metabolically active and probably has a more robust role in fracture prevention, which is relevant in these patients.[50]

CONCLUSIONS

Renal transplantation improves bone and mineral abnormalities. Not only the transplantation environment (ie, the presence of high bone turnover) but also the period on dialysis determines the severity of calcifications: dialysis vintage, sclerostin serum values, and bone volume at baseline were found to be predictors of severe calcifications. Preventing CV events through the timely identification of patients who would benefit from antiosteoporotic/bone remodeling control drugs should be considered in a prospective study.

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