ORIGINAL ARTICLE

Bone densitometry versus bone histomorphometry in renal transplanted patients: a cross-sectional study

Ana Carina Ferreira[1,2], Marco Mendes[1], Cecília Silva[1], Patrícia Cotovio[1], Inês Aires[1,2], David Navarro[1], Fernando Caeiro[1], Rute Salvador[3], Bruna Correia[3], Guadalupe Cabral[3], Fernando Nolasco[1,2] & Aníbal Ferreira[1,2]

1 Nephrology Department, Hospital Curry Cabral, CHULC, Lisbon, Portugal
2 Nova Medical School, Lisbon, Portugal
3 Tissue Repair and Inflammation Lab, CEDOC, Lisbon, Portugal

Correspondence
Ana Carina Ferreira, Rua da Beneficência n°8, 1050-099 Lisbon, Portugal.
Tel.: 003517924229;
fax: 003517921214;
e-mail: carina.ferreira@fcm.unl.pt

ClinicalTrials.gov ID NCT02751099.

SUMMARY

Bone loss leads to increase risk of fractures in renal transplantation. The aim of this study was to analyse the relationship between bone densitometry (DXA) findings, bone histomorphometry and bone-related molecules 1-year after renal transplantation. We performed a cross-sectional study of *de novo* renal transplanted patients that agreed to perform a bone biopsy and a DXA examination 1 year after transplantation. All patients underwent a laboratory evaluation, bone biopsy, DXA examination and cardiac CT 1 year after transplantation. 67 patients were included, 16 had a normal examination, and 18 patients were classified as having osteoporosis by DXA. Correlations between bone mineral density and *T*-scores of total femur and femoral neck were the ones that best correlated with bone volume assessed by a bone biopsy. The sensitivity of DXA for osteoporosis diagnosis was 47.0%, and the specificity was 81.2%. The positive predictive value was 50.0%, and the negative predictive value (NPV) was 80.0%. DXA parameters also correlated with klotho and sclerostin serum levels. In this population, a normal examination excluded the presence of osteoporosis, helping in identifying patients that would not benefit from therapy. Overall, densitometry in total femur and femoral neck correlated well with bone volume measured by bone biopsy.

Transplant International 2021; 34: 1065–1073

Key words
bone biopsy, bone densitometry, renal transplantation

Received: 16 February 2021; Revision requested: 23 March 2021; Accepted: 14 April 2021;
Published online: 19 May 2021

Introduction

Improved long-term survival in renal transplant patients has led to cardiovascular (CV) disease and fractures emerging as important complications in those patients [1]. Disturbed mineral metabolism has been investigated as a probable cause for fractures in renal transplanted patients, as, contrary to chronic kidney disease (CKD), hypophosphatemia, hypercalcemia and hypomagnesaemia are frequent metabolic complications in the early post-kidney transplant period [2].

It is assumed that accelerated bone mineral density loss occurs within the first 6–12 months of transplantation, especially in trabecular bone, and that bone loss occurs in 11–56% of the renal transplanted recipients, due to altered mineral metabolism. Additionally,

© 2021 Steunstichting ESOT. Published by John Wiley & Sons Ltd
doi:10.1111/tri.13888

younger age, longer dialysis vintage and low BMI (<23 kg/m^2) seem to be risk factors for this event [3]. Other risk factors for bone loss in transplantation are immunosuppressive agents, such as high steroid doses. Bone loss, with osteopenia or osteoporosis, reflects the disproportion between bone formation and bone resorption post-transplantation, which increases the risk of fractures [4]. Diagnosis of osteoporosis in the renal transplant setting is challenging, because in some, if not in the majority of transplanted patients, low volume is accompanied by other mineral bone disorders. Apart from bone loss, abnormal bone quality, in terms of mineralization, cortical porosity and trabecular bone architecture seem to increase the risk of fractures. Nevertheless, the importance of impaired bone quality in the postrenal transplantation fracture risk is not well defined [5]. It is postulated that fracture risk is fourfold higher in transplanted patients than the general population [6] and 34% higher than in dialysis patients in the first 3–6 months post-transplantation, and slowly decreases 1% each month thereafter [3]. It is estimated that 10–25% of renal transplanted patients will fracture over their follow-up [6,7]. Nevertheless, a recent meta-analysis failed to get the same assumptions [8]. The most relevant risk factors for fracture are diabetes and pancreas–kidney transplantation, BMI <23 kg/m^2, white race, age, female gender, immunosuppression (glucocorticoid dose and duration), abnormal PTH and probably, hypophosphatemia [2–4,9].

Although we assume bone loss and disturbed mineral metabolism early post-transplantation, the phenotype of bone disease after transplantation is not well defined, as data on bone histomorphometry is contradictory [6,10–17], and double bone biopsy studies are needed to determine and quantify the loss of bone volume after transplantation. Most studies assess bone loss after kidney transplantation using dual-energy X-ray absorptiometry (DXA) to measure bone mineral density.

The aim of this study was to analyse the relationship between densitometry findings, bone biopsy data obtained 1 year after transplantation and levels of bone-related molecules [phosphorus (Pi), calcium (Ca), magnesium (Mg), parathyroid hormone (PTH), bone alkaline phosphatase (BALP), calcitonin, vitamin D (vitD), alpha-klotho, fibroblast grow factor (FGF) 23, sclerostin] in renal transplanted patients.

Methods

We performed a cross-sectional study in a sample of *de novo* renal transplanted patients, aged 18–66 years old, who underwent to a bone biopsy 1-year post-transplantation (*ClinicalTrials.gov* ID NCT02751099). Exclusion criteria consisted of admission for double transplantation (pancreas–kidney and liver–kidney transplantation), age outside the determined range or major cognitive dysfunction.

To perform this study, we selected patients included in a bone disease assessment study that agreed to perform a bone biopsy 1-year after transplantation. We asked those patients to perform an additional examination – DXA, and we explained to the potential participants the aims of this analysis. All patients had the opportunity to ask questions. Written consent was obtained from all participants prior to entering the protocol. Consent to take part in the study was recorded in each patient's notes and in the study records. The institutional local Ethic Committee approved this study.

At inclusion, demographic, comorbid and therapeutic data at baseline and transplant and donor data were registered. All admitted patients performed laboratory, a noncontrast cardiac CT to quantify coronary artery calcification score using the Agatston method [18] and a bone densitometry within a week pre or after the bone biopsy. DXA parameters included the evaluation of mineral bone density, *Z*-scores and *T*-scores of lumbar spine, femoral neck and total femur. Osteoporosis was defined by a *T*-score ≤2.5 in lumbar spine or femoral neck or total femur.

Laboratory evaluation used standard methods for hemogram, creatinine, urea, ionogram, uric acid, liver function, Ca, (corrected for hypoalbuminemia), Pi, Mg and calcitonin. Intact PTH was measured by immunochemiluminescence using a second-generation assay (Immulite 2000; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Vitamin D [25(OH)D] was measured with RIA provided by IDS (Boldon, UK). Also, on both occasions, blood samples were stored at −80 °C for further analysis of BALP, FGF23, and its cofactor alpha-Klotho and sclerostin. BALP was measured using an enzyme immunoassay (EIA) utilizing a monoclonal anti-BALP antibody (MIcroVue BAP). FGF23 was measured using a 2nd generation enzyme-linked immunosorbent assay (ELISA) kit, which detected epitopes within the carboxyl-terminal (C-Term) portion of FGF23 (Immunotopics, San Clement, CA, USA). Alpha-Klotho was determined using a human soluble α-klotho assay kit, consisting of a solid phase sandwich ELISA using 2 kinds of highly specific antibodies (IBL America, Minneapolis, MN, USA). Sclerostin was measured using a high sensitivity EIA kit, which is a 96-well immune-capture ELISA

Transplant International 2021; 34: 1065–1073
© 2021 Steunstichting ESOT. Published by John Wiley & Sons Ltd

(TECOmedical). All measurements were performed according to the manufacturers' instructions.

Bone biopsies by manual puncture, using a 7G trocar (Osteobell T®), with local anaesthesia, were obtained from the anterior iliac crest. Tetracycline hydrochloride, 500 mg, 12/12 h, 3 days was given one month and one week before the biopsy. Biopsy specimens of around 4.5 mm × 1.0–1.5 cm were fixed, dehydrated, cleared with xylene and embedded in methyl-methacrylate. Decalcified 5-µm sections were stained with our routine staining: modified Masson–Goldner trichrome, Toluidine Blue, von Kossa, acid phosphatase, alkaline phosphatase, Perls and solochrome azurine for static histomorphometric parameters evaluation. Unstained 10-µm sections were prepared for fluorescent dynamic analysis. Cortical bone was characterized by its cortical thickness and cortical porosity; trabecular bone characterized by bone remodelling and degree of cellular activation, efficacy of mineralization, bone formation rate and mineral deposition rate. Evaluation of possible metal deposits was performed. Bone histomorphometry was analysed using a semiautomatic technique in the Osteomeasure software (Osteometrics, Atlanta, GA, USA).

Cortical porosity superior to 10% was considered abnormal. Trabecular bone volume was considered low if bone volume/tissue volume – TV/BV < 16%; bone remodelling was considered low if osteoblast surface <0.2% and/or osteoclast surface <0.1%; and was considered high if osteoblast surface >3.5% and/or osteoclast surface >7.25% plus bone formation rate and mineral apposition rate analysis; mineralization defects were obtained if osteoid thickness ≥12.5% and mineralization lag time >100 days [19].

Immunosuppression

Patients received induction immunosuppression (basiliximab or thymoglobulin, depending on the immunological risk) and intravenous 500 mg of methylprednisolone intraoperative and daily for two days, followed by maintenance of 20 mg of oral prednisolone (tapered through the year), mycophenolate mofetil (2 g daily with dose adjustments and dose reduction through the year) and tacrolimus (adjusted for levels of 8–12 ng/ml for 3 months and 5–8 ng/ml thereafter).

Statistical analysis

Continuous variables were expressed as median (interquartile range) and categorical variables as frequencies. Correlations between DXA and bone biopsy volume measurements were obtained by Mann–Whitney test or pairwise correlation test. Associations between histomorphometric and DXA data and serum levels of bone-related variables or demographic data were accessed by Mann–Whitney test, Fisher exact test, Kruskal–Wallis rank test or Spearman correlation test, depending on the variable. Multivariate analysis was performed using linear regression models.

All tests were performed using STATA version 13 software package, and a $P < 0.05$ was considered significant.

Results

From the 69 patients included in the bone disease assessment study running in our unit, 67 patients agreed in performing a DXA examination. Those 67 patients were mostly Caucasian and male, middle-aged (53 years), with a median dialysis vintage of 55 months. Six patients had been submitted to a parathyroidectomy prior to transplant. Of the 21 women, 14 were in menopause. The median body mass index was 24.6 (22.0–27.8) kg/m^2. The median prednisolone cumulative dose was 3580.0 (3257.5–4072.5) mg, but the median cumulative steroid dose (prednisolone + methylprednisolone) was 5697.5 (5207.0–7310.0) mg. Five patients were treated with low doses of everolimus and low doses of tacrolimus, in an attempt to minimize calcineurin inhibitors toxicity. No patient had been prescribed anti-osteoporotic drugs during the post-transplant period. Cholecalciferol supplementation was implemented in 24 patients (35.8%), paricalcitol in seven patients (10.4%) and cinacalcet treatment in seven patients (10.4%). Characterization of the population can be found in Table 1.

Metabolic evaluation and Histologic evaluation

One year after transplantation, laboratory values were in the expected and normal range, as shown in Table 2. The only parameter above the normal was PTH, but the optimal range in renal transplanted patients is unknown [6]. Vitamin D levels were similar irrespective of supplementation.

Renal osteodystrophy (ROD) was present in 47 (70%) of our patients: low turnover bone disease in 32 patients (10 with low volume); high turnover bone disease presented in seven patients (3 with low volume); osteomalacia in three patients, with no cases of mixed ROD. We also had two cases of abnormal

© 2021 Steunstichting ESOT. Published by John Wiley & Sons Ltd

Table 1. Demographic and past history of the population.

Demographic characterization	
Age at transplantation (years)	53.0 (42.0–62.0)
Gender (M:F)	46 (68.7%):21 (31.3%)
Caucasian race, *n* (%)	53 (79.1%)
PD (previous or current):HD, *n* (%)	9 (13.4%):63 (94.0%)
Dialysis vintage (months)	55 (41.0–84.0)
Hypertension at transplantation, *n* (%)	57 (85.0%)
Diabetes|PTDM, *n* (%)	9 (13.4%)|11 (15.9%)
Hyperparathyroidism at transplant, *n* (%)	48 (71.6%), median value 529.9 pg/ml
Parathyroidectomy prior to transplant (*n*, %)	6 (9.0%)
HIV, HBV, HCV (*n*, %)	2 (2.9%):0:3 (4.5%)
Aetiology of renal disease	
Unknown	13 (19.4%)
ADPKD	11 (16.4%)
Hypertensive nephrosclerosis	9 (13.4%)
Diabetic nephropathy (type 1 and 2)	6 (8.9%)
Alport disease	2 (3.0%)
Glomerulonephritis	
Chronic glomerulonephritis	5 (7.5%)
IgA nephropathy|mesangial proliferation	6 (8.9%)|1 (1.54%)
HIVAN	1 (1.54%)
FSGS	1 (1.54%)
Membranous nephropathy	2 (3.0%)
Lupus nephritis	1 (1.5%)
Vasculitis	
Pauci-immune|goodpasture	2 (3.0%)|1 (1.5%)
Lithiasis	3 (4.5%)
CAKUT	3 (4.5%)

Table 2. Laboratory evaluation 1 year after transplantation.

Laboratorial evaluation 1-year after transplantation	
Haemoglobin (g/dl)	12.9 (12.2–14.3)
Platelets ($\times$1000/μl)	228 (176–256)
Glucose (mg/dl)	92.0 (81.0–103.0)
Urea (mg/dl)	60.0 (44.0–78.0)
Creatinine (mg/dl)	1.4 (1.1–1.8)
Glomerular filtration rate (ml/min/1.73 m^2)	52.4 (35.9–68.7)
Uric acid (mg/dl)	6.4 (5.6–7.1)
Sodium (mEq/l)	141 (140–142)
Potassium (mEq/l)	4.5 (4.2–4.9)
Chloride (mEq/l)	106 (105–108)
Alkaline phosphatase (U/l)	78.0 (57.0–119.0)
Albumin (g/dl)	4.3 (4.1–4.5)
Total cholesterol (mg/dl)	181.0 (159.0–212.0)
Calcium (mg/dl)	9.8 (9.3–10.4)
Phosphorus (mg/dl)	3.1 (2.8–3.5)
Magnesium (mg/dl)	1.7 (1.6–1.8)
Calcitonin (ng/dl)	2.0 (2.0–4.9)
Vitamin D (ng/ml)	22.5 (14.3–29.0)
iPTH (pg/ml)	135.0 (90.1–232.7)
Bone alkaline phosphatase (U/l)	23.0 (17.2–35.2)
FGF23 (RU/ml)	135.2 (101.1–168.5)
Klotho (pg/ml)	945.2 (485.0–2044.2)
Sclerostin (ng/ml)	0.7 (0.49–0.96)

Transplant International 2021; 34: 1065–1073
© 2021 Steunstichting ESOT. Published by John Wiley & Sons Ltd

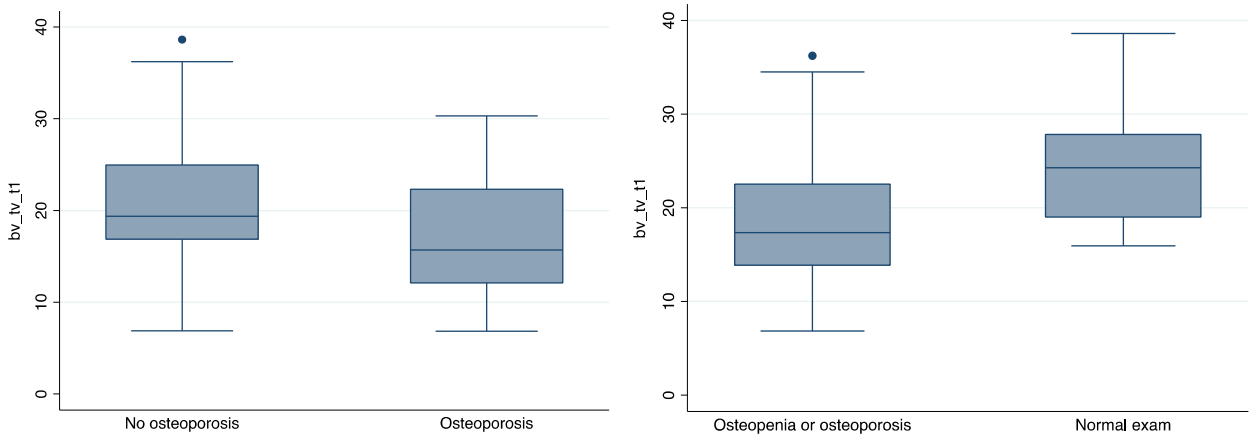

Figure 1 Differences of bone volume at the bone biopsy in the presence of osteoporosis or in a presence of a normal examination by DXA.

mineralization, with normal turnover and volume and six cases of osteoporosis, as no other abnormality was observed, aside from low bone volume. We also found that 26.8% of our patients (n = 18) had abnormal cortical bone porosity.

A large number of patients, specifically 48 (71.6%), presented with normal bone volume. The remaining 19 patients with low volume had adynamic bone disease (n = 10), hyperparathyroid bone disease (n = 3) or osteoporosis (n = 6). We could not find associations between bone volume data measured in bone fragments and serum bone-related parameters, renal function, age, gender, menopause status, BMI or therapeutic drugs. Nevertheless, we found a trend of association between Caucasian race and low bone volume, compared to other races. It is worth saying we found no significant difference between bone volume in T0 and in T1.

Imaging evaluation

Bone densitometry was performed in 67 patients (Table 3) and revealed that 18 patients (26.9%) had osteoporosis, 33 patients (49.0%) had osteopenia, and 16 patients had a DXA examination normal. Patients with osteoporosis were Caucasian (P = 0.02), had a lower BMI (P = 0.0005) and had a higher percentile of coronary calcifications (P = 0.04) comparing to the patients without this diagnosis obtained by DXA.

Overall, bone volume measured in the bone biopsies correlated well with densitometry findings. Correlations with total femur mineral bone density (P < 0.001), its Z-scores (P = 0.001) and its T-scores (P = 0.005), with lumbar spine mineral bone density (P = 0.007), its Z-scores (P = 0.007) and its T-scores (P = 0.006), and with femoral neck mineral bone density (P = 0.003), and its z-scores (P = 0.004) had statistically significance. Also, we observed a difference in bone volume obtained by a bone biopsy when classifying patients by presence or absence of osteoporosis via DXA (osteoporosis – BV/TV 17.3% vs. no osteoporosis BV/TV 21.0%, P = 0.054) or when classifying patients by presence of a normal DXA examination (normal examination – BV/TV 24.3% vs. osteoporosis or osteopenia – BV/TV 18.6%, P = 0.003; Fig. 1). The DXA parameters that correlated better with the bone volume measured in the bone biopsies were the mineral bone density and T-scores of total femur (both with ROC curve of 92.0%) and of femoral neck (both with ROC curve of 93.0%).

In what concern to the diagnosis of osteoporosis obtained by DXA (in 18 patients) and comparing it with histomorphometric measurements (Table 4), 9 out of these 18 patients effectively had low volume (BV/TV <16.0%) at the bone biopsy. The sensitivity of the DXA examination to detect low bone volume (or osteoporosis) when comparing to the gold standard (a bone biopsy) was of 47.0% and the specificity of the test was 81.2%. The positive predictive value was 50.0%, and the negative predictive value (NPV) was 80.0%.

Sixteen patients had a normal DXA exam, and all of them had a BV/TV ≥16.0% in the bone biopsy. This reinforces the previous results about specificity and NPV of the DXA examination. Still, 4 out of these 16 patients had abnormal mineralization (although normal volume), and 9 had low bone turnover (although normal volume), these numbers included two patients with osteomalacia.

We found correlations between the results of DXA imaging and both alpha-klotho and sclerostin serum levels. The group of patients with higher alpha-klotho levels also had higher values of bone mineral density

© 2021 Steunstichting ESOT. Published by John Wiley & Sons Ltd

Table 3. Bone densitometry performed 12 months after transplantation.

Bone densitometry	
Lumbar spine	
Bone mineral density	1.1 (1.0–1.2)
T-score	−1.1 (−1.9 to −0.1)
Z-score	−0.8 (−1.8 to 0.4)
Femoral neck	
Bone mineral density	0.9 (0.8–1.0)
T-score	−1.4 (−2.2 to −0.6)
Z-score	−0.7 (−1.5 to 0)
Total femur	
Bone mineral density	0.9 (0.8–1.0)
T-score	−1 (−2.1 to −0.4)
Z-score	−1 (−1.45 to 0.2)
FRAX risk	
Osteoporotic fracture|hip fracture	3.5% (2.2–6.2)|0.8% (0.2–2.7)

(0.89 vs. 0.78, $P = 0.007$), and significantly different *T*-scores in femoral neck (−1.1 vs. −1.9, $P = 0.009$), with no significant differences in its *Z*-scores. These associations were maintained after adjusting for age and for renal function (Table 5). Patients with a normal DXA examination were mostly in the group presenting the highest levels of klotho ($P = 0.03$). Nevertheless, after adjusting for age, this association was lost.

Looking at sclerostin, people with higher levels of sclerostin, had higher bone mineral density (1.02 vs. 1.12, $P = 0.02$), *T*-scores (−1.65 vs. −0.7, $P = 0.002$) and *Z*-scores (−1.3 vs. 0, $P = 0.005$) at spine, and higher bone mineral density (0.86 vs. 0.97, $P = 0.03$), *T*-scores (−1.5 vs. −0.7, $P = 0.02$) and higher *Z*-scores (−1.2 vs. −0.25, $P = 0.007$) at total femur. All those association were maintained, after adjusting for age and for renal function (Table 6).

Overall, we found no correlation between the values obtained by DXA and coronary calcifications scores or percentiles obtained by cardiac CT or with bone-related therapy, namely vitamin D supplementation or cinacalcet prescription or even with renal function.

Table 4. Osteoporosis detected by a bone biopsy and by DXA scan.

	Volume BV/TV		
T-score	<16%	≥ 16%	Total
≤2.5	9	9	18
>2.5	10	39	49
Total	19	48	67

Table 5. Multivariate analysis for associations between mineral bone density of femoral neck and klotho levels.

	Femoral neck – bone mineral density		
	Coefficient	IC 95%	*P*-value
Klotho	0.09	0.006 to 0.17	0.03
Age	−0.001	−0.004 to 0.002	0.5
GFR	0.0004	−0.001 to 0.002	0.6

Table 6. Multivariate analysis for associations between mineral bone density of lumbar spine or total femur and sclerostin.

	Coefficient	IC 95%	*P*-value
Lumbar spine – bone mineral density			
Sclerostin	0.13	0.02–0.24	0.02
Age	−0.0008	−0.001 to 0.003	0.7
GFR	0.001	−0.001 to 0.003	0.4
Total femur – bone mineral density			
Sclerostin	0.11	0.01–0.24	0.02
Age	−0.0008	−0.007 to 0.001	0.2
GFR	0.001	−0.002 to 0.002	0.9

Discussion

In this study, we found that low bone volume, obtained by a bone biopsy, was present in 28.4% (19 patients) of our population. Of these 19 patients, only 6 had true osteoporosis, as the other 13 patients presented either with remodelling or mineralization abnormalities. We also found that in this population, DXA examination had a good specificity and a good NPV. The examination was useful in ruling out true low bone volume and

© 2021 Steunstichting ESOT. Published by John Wiley & Sons Ltd

is useful in selecting patients that would not need osteoporosis treatment.

We must recognize that this is an observational, cross-sectional, unicentric study, with a small sample of transplanted patients. Also, we know that a bone biopsy is a snapshot of a given moment, and, like any biopsy, can have sampling errors.

The World Health Organization (WHO) defines osteoporosis as 'a systemic skeletal disease characterized by low bone mass and microarchitecture deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture' [20,21], and in clinical practice, the diagnosis is based upon DXA measurements, as *T*-score 2.5 standard deviation or more below the young adult mean bone mineral density [22]. As the most important factors for osteoporosis are advanced age and glucocorticoids therapy, it is not difficult to imagine that our transplanted patients are at risk of the disease. Age is the most relevant factor, and for any kind of *T*-score on DXA, the risk of fracture will rise in aged persons [22]. In our patients, we found no relation between bone volume assessed by histomorphometry or by DXA and age. We found a trend towards lower bone volume in Caucasian race. Also, in our patients, cumulative steroid doses did not correlate with bone volume. Steroids reduce bone formation mediated by direct inhibition of osteoblasts function, impair osteoblastogenesis, increasing osteoblasts and osteocytes apoptosis (the latter inducing osteolysis), and increase osteoclastogenesis [23], by raising the expression of the receptor activator of nuclear factor –kB ligand (RANKL) [6,9]. In addition, glucocorticoids reduce muscle mass leading to a higher risk of falls [9], decrease secretion of oestrogen and androgen, and activate vitamin D catabolism [24], reducing the absorption of calcium from the gastrointestinal tract and renal tubular cells, resulting in a negative calcium balance [25], leading to maintenance or development of secondary hyperparathyroidism [23]. Finally, glucocorticoids up-regulates Wnt antagonists [6], aggravating bone loss. We can suppose that as steroid doses were similar in all patients, and as the sample size is small, the differences in bone volume were not relevant.

Nevertheless, we must recognize that isolated changes in bone volume (with normal turnover or mineralization) can be present in renal transplanted patients, but in a minority of patients, as we have shown. In line with what we already know, DXA did not give any information on bone turnover and mineralization, for which we need a bone biopsy. Other limitations of this examination is that it cannot differentiate cortical and trabecular bone; cannot assess microarchitecture, and finally, DXA measures areal BMD and not volumetric BMD. Still, recently, in 2017, the update on CKD-MBD KDIGO guidelines reviewed the utility of performing DXA image tests in CKD patients, based on the new data [26–29], and recommends bone mineral density testing in CKD patients for fracture risk assessment, if the results will impact on treatment decisions [30]. Although its limitations, DXA remains the standard method for predicting fracture risk, comparing to peripheral quantitative computed tomography (HR-pQCT) [31] or even with trabecular bone score [32]. The screening for fracture risk is possible through the Fracture Risk Assessment Tool (FRAX), a tool developed by WHO in 2008. Unfortunately, this tool is not validated for patients younger than 40 years of age and is not validated for kidney transplant recipients. Nevertheless, in 2014, Naylor and co-workers showed a positive prediction of fracture risk with FRAX in kidney transplant recipients [33]. We could not investigate this tool, as only one patient fractured, during the course of the 12 months in which we followed these patients. A recent study from Belgium of 502 patients transplanted between 2006 and 2013 showed a fracture incidence of 14.2 fractures per 1000 person-years, with a median time to first fracture of 17 months [34].

One interesting finding was that imaging from total femur and hip were the sites that best correlated with bone volume assessed by bone biopsy, compared to lumbar spine. But in a recent consensus, hip and lumbar spine were named as the best for the evaluation of bone mineral density in CKD patients [35].

Curious was the fact that klotho and sclerostin did correlate with DXA findings, but not with bone volume measured by histomorphometry. The Wnt/β-catenin pathway is important for bone formation: once activated, bone is formed; once inhibited, bone formation is halted. Genetic mutation on the Wnt/β-catenin pathway leads to premature coronary disease and severe osteoporosis, providing evidence of the importance of the Wnt signalling in the bone-vessels axis [36]. Klotho acts as an antagonist of Wnt/β-catenin pathway activation through interactions with extracellular activators of the pathway [37], and sclerostin is a direct inhibitor of the pathway [38,39]. We can suppose that patients with the highest mineral bone density have increased levels of those hormones as a feedback loop.

© 2021 Steunstichting ESOT. Published by John Wiley & Sons Ltd

With this study, we show that if a patient has a normal DXA examination, we can assume that the patient most probably is not eligible for osteoporosis treatment, as the examination had shown to have high specificity. If classified as having osteoporosis, prior to any directed treatment, we must confirm the diagnosis with a bone biopsy, with the advantage that it will also allow to exclude low or high bone turnover or even osteomalacia. Bone biopsy is the only method that quantifies and evaluates bone mineralization, making it possible to distinguish osteoporosis from hyperabsorption and osteoporosis from deficient bone formation. However, this is an expensive, invasive and one-shot procedure and is performed in only a few centres worldwide [2,3,40], due to the expertise needed. The future could combine newer imaging techniques with newer laboratory biomarkers towards a virtual bone biopsy [41].

Conclusions

Post-transplant mineral and bone disease are present in a very expressive number of transplanted patients, and fracture risk is high in this population. DXA bone mineral density and its *T*-scores of total femur and femoral neck correlated well with bone volume assessed by a bone biopsy. Performing a DXA examination can allow identifying patients that will not benefit from antiresorptives or osteoformers therapy.

Authorship

ACF and AF: concept and design the study. ACF, MM, CS, PC, IA, DN, FC, RS, BC and GC: collected data or perform bone biopsies or performed laboratory evaluations. ACF, MM, RS, BC, GC, FN and AF: analysed the data. ACF: drafted the article. MM, CS, PC, IA, DN, FC, RS, BC, GC, FN and AF: revised the paper; all authors approved the final version of the paper and agree with all aspects of the work.

Funding

This study was made possible with a grant of the Portuguese Society of Nephrology and the Portuguese Society of Transplantation/Astellas.

Conflict of interest

Authors do not have any conflict of interest relating with this manuscript. The results presented in this paper have not been published previously in whole or part, except in abstract format. The data that support the findings of this study are available upon reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Acknowledgements

We thank CHULC, the institution where the study was conducted. We thank the Portuguese Society of Nephrology and the Portuguese Society of Transplantation/Astellas for awarding us a research grant. We would like to thank Sandra Silva for help with administrative work and Dr Isabel Mesquita for help in samples handling. We also thank the surgeons Ana Pena and Sofia Carrelha and all the operating room staff for being available to help us with the bone biopsies performed in the operating room. We thank the Radiology Department of Hospital Santo António dos Capuchos for performing the bone densitometry in our patients.

Ethical approval

This study has been reviewed by the appropriate ethics committee and has therefore been performed in accordance with the ethical standards laid down in an appropriate version of the 2000 Declaration of Helsinki as well as the Declaration of Istanbul 2008. As stated clearly in the text, all persons gave their informed consent prior to their inclusion in the study.

REFERENCES

1. Seeherunvong W, Wolf M. Tertiary excess of fibroblast growth factor 23 and hypophosphatemia following kidney transplantation. *Pediatr Transplant* 2011; **15**: 37.
2. Kalantar-Zadeh K, Molnar MZ, Kovesdy CP, Mucsi I, Bunnapradist S. Management of mineral and bone disorder after kidney transplantation. *Curr Opin Nephrol Hypertens* 2012; **21**: 389.
3. Alshayeb HM, Josephson MA, Sprague SM. CKD-mineral and bone disorder management in kidney transplant recipients. *Am J Kidney Dis* 2013; **61**: 310.
4. Bellorin-Font E, Rojas E, Carlini RG, Suniaga O, Weisinger JR. Bone remodeling after renal transplantation. *Kidney Int Suppl* 2003; **63**: S125.
5. Drueke TB, Evenepoel P. The bone after kidney transplantation. *Clin J Am Soc Nephrol* 2019; **14**: 795.
6. Evenepoel P. Recovery versus persistence of disordered mineral metabolism in kidney transplant recipients. *Semin Nephrol* 2013; **33**: 191.

© 2021 Steunstichting ESOT. Published by John Wiley & Sons Ltd

7. Naylor KL, Li AH, Lam NN, Hodsman AB, Jamal SA, Garg AX. Fracture risk in kidney transplant recipients: a systematic review. *Transplantation* 2013; **95**: 1461.
8. Sidibé A, Auguste D, Desbiens L-C, *et al*. Fracture risk in dialysis and kidney transplanted patients: a systematic review. *JBMR Plus* 2019; **3**: 45.
9. Buckley L, Humphrey MB. Glucocorticoid-induced osteoporosis. *N Engl J Med* 2018; **379**: 2547.
10. Martins L, Queiros J, Ferreira A, *et al*. Renal osteodystrophy: histologic evaluation after renal transplantation. *Transplant Proc* 2000; **32**: 2599.
11. Carlini RG, Rojas E, Weisinger JR, *et al*. Bone disease in patients with long-term renal transplantation and normal renal function. *Am J Kidney Dis* 2000; **36**: 160.
12. Montalban C, De Francisco ALM, Mariñoso ML, *et al*. Bone disease in long-term adult kidney transplant patients with normal renal function. *Kidney Int Suppl* 2003; **63**: S129.
13. Rojas E, Carlini RG, Clesca P, *et al*. The pathogenesis of osteodystrophy after renal transplantation as detected by early alterations in bone remodeling. *Kidney Int* 2003; **63**: 1915.
14. Cruz EA, Lugon JR, Jorgetti V, Draibe SA, Carvalho AB. Histologic evolution of bone disease 6 months after successful kidney transplantation. *Am J Kidney Dis* 2004; **44**: 747.
15. Abdallah KA, Jorgetti V, Pereira RC, *et al*. Improvement of adynamic bone disease after renal transplantation. *Braz J Med Biol Res* 2006; **39**: 31.
16. Lehmann G, Ott U, Stein G, Steiner T, Wolf G. Renal osteodystrophy after successful renal transplantation: a histomorphometric analysis in 57 patients. *Transplant Proc* 2007; **39**: 3153.
17. Borchhardt K, Sulzbacher I, Benesch T, Fodinger M, Sunder-Plassmann G, Haas M. Low-turnover bone disease in hypercalcemic hyperparathyroidism after kidney transplantation. *Am J Transplant* 2007; **7**: 2515.
18. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990; **15**: 827.
19. Dempster DW, Compston JE, Drezner MK, *et al*. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR histomorphometry nomenclature committee. *J Bone Miner Res* 2013; **28**: 2.
20. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. *Am J Med* 1993; **94**: 646.
21. Lippuner K. The future of osteoporosis treatment – a research update. *Swiss Med Wkly* 2012; **142**: w13624.
22. Sambrook P, Cooper C. Osteoporosis. *The Lancet* 2006; **367**: 2010.
23. Canalis E, Mazziotti G, Giustina A, Bilezikian JP. Glucocorticoid-induced osteoporosis: pathophysiology and therapy. *Osteoporos Int* 2007; **18**: 1319.
24. Cianciolo G, Galassi A, Capelli I, Angelini ML, La Manna G, Cozzolino M. Vitamin D in kidney transplant recipients: mechanisms and therapy. *Am J Nephrol* 2016; **43**: 397.
25. Malluche HH, Monier-Faugere MC, Herberth J. Bone disease after renal transplantation. *Nat Rev Nephrol* 2010; **6**: 32.
26. Naylor KL, Garg AX, Zou G, *et al*. Comparison of fracture risk prediction among individuals with reduced and normal kidney function. *Clin J Am Soc Nephrol* 2015; **10**: 646.
27. Yenchek RH, Ix JH, Shlipak MG, *et al*. Bone mineral density and fracture risk in older individuals with CKD. *Clin J Am Soc Nephrol* 2012; **7**: 1130.
28. Iimori S, Mori Y, Akita W, *et al*. Diagnostic usefulness of bone mineral density and biochemical markers of bone turnover in predicting fracture in CKD stage 5D patients – a single-center cohort study. *Nephrol Dial Transplant* 2012; **27**: 345.
29. West SL, Lok CE, Langsetmo L, *et al*. Bone mineral density predicts fractures in chronic kidney disease. *J Bone Miner Res* 2015; **30**: 913.
30. Ketteler M, Block GA, Evenepoel P, *et al*. Executive summary of the 2017 KDIGO chronic kidney disease-mineral and bone disorder (CKD-MBD) guideline update: what's changed and why it matters. *Kidney Int* 2017; **92**: 26.
31. Bouquegneau A, Salam S, Delanaye P, Eastell R, Khwaja A. Bone disease after kidney transplantation. *Clin J Am Soc Nephrol* 2016; **11**: 1282.
32. Silva BC, Leslie WD, Resch H, *et al*. Trabecular bone score: a noninvasive analytical method based upon the DXA image. *J Bone Miner Res* 2014; **29**: 518.
33. Naylor KL, Leslie WD, Hodsman AB, Rush DN, Garg AX. FRAX predicts fracture risk in kidney transplant recipients. *Transplantation* 2014; **97**: 940.
34. Evenepoel P, Claes K, Meijers B, *et al*. Bone mineral density, bone turnover markers, and incident fractures in de novo kidney transplant recipients. *Kidney Int* 2019; **95**: 1461.
35. Evenepoel P, Cunningham J, Ferrari S, *et al*. European Consensus Statement on the diagnosis and management of osteoporosis in chronic kidney disease stages G4–G5D. *Nephrol Dial Transplant* 2021; **36**: 42.
36. Brandenburg VM, D'Haese P, Deck A, *et al*. From skeletal to cardiovascular disease in 12 steps-the evolution of sclerostin as a major player in CKD-MBD. *Pediatr Nephrol* 2016; **31**: 195.
37. Liu H, Fergusson MM, Castilho RM, *et al*. Augmented Wnt signaling in a mammalian model of accelerated aging. *Science* 2007; **317**: 803.
38. Drechsler C, Evenepoel P, Vervloet MG, *et al*. High levels of circulating sclerostin are associated with better cardiovascular survival in incident dialysis patients: results from the NECOSAD study. *Nephrol Dial Transplant* 2015; **30**: 288.
39. Viaene L, Behets GJ, Claes K, *et al*. Sclerostin: another bone-related protein related to all-cause mortality in haemodialysis? *Nephrol Dial Transplant* 2013; **28**: 3024.
40. Wesseling-Perry K, Bacchetta J. CKD-MBD after kidney transplantation. *Pediatr Nephrol* 2011; **26**: 2143.
41. Diamond T, Elder GJ. Is there a practical role for bone biopsy in chronic kidney disease? *Nephrology* 2017; **22**(Suppl 2): 22.

© 2021 Steunstichting ESOT. Published by John Wiley & Sons Ltd