Bone histomorphometry in de novo renal transplant recipients indicates a further decline in bone resorption 1 year posttransplantation

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ABSTRACT

Renal transplantation is believed to have a major impact on bone health. The present prospective observational bone biopsy study aimed to define the natural history of bone histomorphometry parameters in contemporaneous de novo renal transplant recipients. Paired bone biopsies were performed at the time of transplantation and at one year post-transplantation in an unselected cohort of 36 patients referred for deceased kidney replacement. Parameters of mineral metabolism and circulating bone turnover markers were monitored as well. Static parameters of bone formation and especially bone resorption being already low-normal in the majority of patients at the time of renal transplantation, further declined during the first post-transplant year. However, inter-individual variation was substantial, and significance was reached only for bone resorption parameters. Bone mineralization and trabecular bone volume were within the normal range at the time of transplantation (83.3% and 91.7% of graft recipients, respectively) and showed little change one year post-transplantation. Changes in osteoclast number were paralleled by changes in circulating tartrate-resistant acid phosphatase 5b levels. Finally, cumulative glucocorticoid dose, but not the post-transplantation parathyroid hormone level, associated with trabecular bone loss. Thus, the impact of renal transplantation on bone histomorphometry is limited with only bone resorption, being already low at the time of transplantation, showing a further decline.

INTRODUCTION

For patients with end stage renal disease, kidney transplantation undoubtedly is the best treatment option. Worldwide, the numbers of transplanted kidneys rise steadily. In the US alone, more than 17500 kidneys are transplanted annually. The development of novel immunosuppressive therapies has led to a tremendous increase in the 1-year survival rates of renal allografts [1]. Accordingly, improving the long-term survival and quality of life of renal transplant recipients has become a major focus of post-transplantation patient care and includes prevention of cardiovascular complications, diabetes mellitus, cancer, and fractures. According to estimates, approximately 7-10 % of all renal transplant recipients will suffer one or more fractures over their lifetime [2-7]. The overall fracture risk after renal

transplantation is several-fold higher than in healthy individuals [5;6], and is 30% higher during the first 3 years after transplantation as compared to patients on dialysis [8]. Both impaired bone quantity and quality may account for the increased fracture risk in renal transplant recipients.

Bone biopsies are the gold standard to evaluate bone health. Bone biopsy studies in renal transplant recipients, however, are scanty and often hampered by cross-sectional design, heterogeneity and small sample size [9].

Bone metabolism in transplant recipients depends on pre-existing damage to the bone acquired during the period of renal insufficiency, and damage caused by immunosuppression and modulating factors independent of renal transplantation (gender, gonadal function, diabetes, acid-base balance).
Obviously, the bone histomorphometric pattern in patients with end stage renal disease, and by extension in renal transplant candidates, has shown important changes over the last two decades with adynamic bone disease nowadays being the most prevalent bone disease[10-13]. Also with regard to immunosuppressive therapy in renal transplant recipients, practices have changed substantially, with steroid minimization protocols rapidly gaining popularity. The impact of these evolutions on the phenotype of post-transplant bone disease so far has not been investigated. Accurate diagnosis of bone turnover abnormalities is of paramount importance since the therapeutic approaches to the various abnormalities are distinctly different.

The aim of the present prospective observational cohort study was to define the natural history of renal bone disease in contemporaneous *de novo* renal transplant recipients.

RESULTS

Patient characteristics:

Demographics and details on immunosuppressive and mineral metabolism therapy are summarised in ***table 1***. Mean age of the patients was 54.2± 12.2 year; 80.6% were males. Mean BMI was 24.9±3.7 kg/m^2; 28%, 8.3% and 8.3% of patients had a history of diabetes mellitus, parathyroidectomy and low impact fracture, respectively. Primary renal diagnosis was diabetic nephropathy in 13.9%, glomerulonephritis or vasculitis in 16.7%, interstitial nephritis in 5.6%, hypertensive or large vessel disease in 11.1%, cystic, or hereditary, or congenital disease in 25%, and missing diagnosis in 27.8% of the patients. In almost 40% of the renal transplant patients, therapy with glucocorticoids was halted between M3 and M12. Acute allograft rejection occurred in 3 patients (8.3%). All rejection episodes (3 in 3 patients) were successfully treated with corticosteroid pulse therapy. The cumulative steroid dosage was 945±421 and 1680±673mg at M3 and M12, respectively.

Laboratory parameters:

Table 2 shows the time course of laboratory parameters of mineral metabolism after renal transplantation. In agreement with literature data, PTH, FGF23, and sclerostin levels markedly decreased, while calcitriol levels increased during the first 3 months following transplantation. An increase of serum calcium levels and a drop of serum phosphorus levels accompanied these changes. Between M3 and M12, PTH and calcitriol levels showed changes in the same direction, but at a much slower rate, while FGF23 levels stabilized and sclerostin levels showed a discrete rebound (***figure 1***). Hyperparathyroidism at M12, defined by a PTH level above 1.5 times upper normal limit, with and without hypercalcemia, defined by a total calcium level above 10.3 mg/dL, was observed in 13.9% and 27.8%, of the patients respectively. Circulating levels of bsAP remained stable, while levels of TRAP5b showed a significant decline after transplantation. Serum IL-6 levels significantly declined following transplantation.

Apart from gender distribution, demographics and routine biochemical parameters did not differ between the study population and patients transplanted

within the same period but not included in the study (n=310) (***supplementary table 2***).

Bone histomorphometry

Bone biopsies performed at the time of transplantation showed low, normal and high turnover in respectively 44.4, 52.8 and 2.8% of the patients, as determined using the surrogate markers. Defective mineralization and low bone volume were observed in 16.7 and 8.3% of patients, respectively. Twelve months after transplantation, histomorphometric parameters of bone volume and mineralization were almost unaltered, while static parameters of bone turnover, at least numerically, substantially declined (***Table 3 and figure 2***). Significance, however, was only reached for parameters of bone resorption (E.Pm/B.Pm, Oc.Pm/T.Pm)

Bone density

Paired DXA scans were available in a subset of patients only (lumbar spine: n=22; hip: n=22; radius: n=10). At none of the sites studied, bone mineral density showed significant changes following transplantation (***Table 3***).

Correlation analysis

Circulating levels of PTH, sclerostin, FGF23, OPG, and sRANKL at M12 did not correlate with bone changes during the first post-transplant year, whatever histomorphometric parameter was examined. Changes in trabecular bone volume, expressed as percentage of total bone volume (delta [Δ] B.Ar/T.Ar) correlated with ΔBMD at the total hip only (R=0.54, p=0.01, n=22). Cumulative steroid exposure at M12 significantly correlated with ΔB.Ar/T.Ar (**figure 3**) and ΔBMD, at least at the total hip and femoral neck. ΔOc.Pm/E.Pm correlated significantly with ΔTRAP5B levels (R=0.34, p=0.04). ΔTRAP5B correlated significantly with ΔbsAP (R=0.69, p<0.0001).

DISCUSSION

The main findings of this 1 year-prospective observational bone biopsy study in *de novo* renal transplant recipients may be summarized as follows: *first*, bone formation and especially resorption, being already low-normal in the majority of renal transplant recipients at the time of transplantation, showed a further decline during the first post-transplant year; *second*, bone mineralization and (trabecular) bone volume were within the normal range in renal transplant recipients at the time of transplantation and showed little change during the first post-transplant year; *third*, changes in osteoclast number were paralleled by circulating TRAP5b levels and *fourth*, cumulative glucocorticoid dose associated with trabecular bone loss.

In agreement with data from recent large bone biopsy surveys in patients with end stage renal disease [12-15], the vast majority of patients enrolled in the present study showed low or normal bone turnover at the time of transplantation. Of note, the prevalence of normal bone turnover in our study cohort was indeed higher than previously reported by Malluche *et al.* [12] and Sprague *et al*.[13]. Both case mix (gender, dialysis vintage, age,…) and differences in diagnostic criteria may account for this apparent discrepancy. PTH levels were on average 4.5 times the upper normal limit, similar to what is observed in DOPPS and well within KDIGO recommendations[16]. Altogether, these data indicate that PTH hyporesponsiveness may be a bigger issue than currently acknowledged[17].

Repeat bone biopsies 1 year after transplantation showed a decline of bone turnover and especially bone resorption parameters. Circulating levels of TRAP5b, a biomarker of osteoclast number/activity, changed accordingly. This observation confirms and extends data from previous longitudinal bone biopsy studies in *de novo* renal transplant recipients [18-21]. However, different from most of these studies, bone turnover of patients enrolled in the present study was normal or even low at the time of transplantation. In aggregate, it may be concluded from these studies that renal transplantation suppresses bone activity, whatever the bone turnover is at the time of engraftment. Glucocorticoids and mineral metabolism disturbances have been suggested to be implicated in the

pathogenesis of low bone turnover in renal transplant recipients [9;20;22]. A negative correlation has indeed been reported between bone turnover and cumulative doses of glucocorticoid, both early[20] and late[9] after transplantation. The mechanisms by which glucocorticoids may affect bone metabolism are multiple. Experimental studies indicate that glucocorticoids promote osteoblast and osteocyte apoptosis and inhibit osteoblastogenesis, resulting in defective bone formation. Glucorticoid excess also directly reduces osteoclast production but, in contrast with the increase in osteoblast apoptosis, the lifespan of osteoclasts is prolonged [23]. In the present study, however, we failed to confirm cumulative dose of glucocorticoid as a significant determinant of bone formation (change) in renal transplant recipients. This might be explained by the fact that both parameters of bone formation rate and glucocorticoid exposure were rather low and homogeneous among participants, limiting the statistical power to find correlations.

Besides glucocorticoids, post-transplant mineral metabolism disturbances may modulate bone turnover. PTH is a key regulator of bone turnover. The primary target of PTH is the osteoblast. PTH increases the number, activity and lifespan of osteoblasts and also protects osteoblasts against steroid induced apoptosis[24]. Opposite to Rojas *et al.*, we failed to observe a correlation between PTH levels and parameters of bone formation. This may due either to lack of power related to homogeneity of the study population with most patients having PTH levels within KDIGO target or to the presence of conditions disturbing or overriding classical PTH signalling in the bone. These include glucocorticoid therapy (discussed earlier), ongoing PTH hyporesponsiveness (related to residual renal dysfunction) and hypophosphatemia.

Hypophosphatemia has been associated with osteoblast dysfunction. In the study by Rojas *et al.*, patients showing post-transplant osteoblast apoptosis had significantly lower serum phosphorus levels than those without evident apoptosis. In the present study, serum phosphate levels did not correlate with indices of bone turnover. Similar results were obtained using either single year 1 phosphorus level or the time-averaged-concentration (Tx up to M12) (data not shown).

Remarkably, bone turnover in renal transplant recipients presenting with hypercalcemic hyperparathyroidism at month 12 was either low or normal. This observation confirms that hypercalcemic hyperparathyroidism, being a common finding in *de novo* renal transplant recipients, not necessarily implies high bone turnover disease[25]. The pathophysiological mechanism underlying post-transplant hypercalcemia thus remains to be elucidated. Contrary to primary hyperparathyroidism, persistent hypercalcemic hyperparathyroidism in renal transplant recipients often goes along with low calciuria. This presentation mimics the phenotype of familial hypocalciuric hypercalcemia, an inherited disease caused by a heterozygous inactivating mutation of calcium-sensing receptor (CaSR) gene. Additional studies are required to evaluate the CaSR expression and functionality in renal transplant recipients.

Bone mineralization at the time of transplantation was normal in most patients and showed little change after transplantation. The low prevalence of mineralization defects in our transplant population contrasts with literature data reporting delayed mineralization in up to 88% of renal transplant recipients [9;19]. Differences in diagnostic criteria and case-mix, mainly with regard to mineral metabolism control, may account for these discrepant findings. No clinical or biochemical factors were found to predict delayed mineralization, neither in the present, nor in previous bone biopsy studies. More specifically, 25(OH)D, 1.25$(OH)_2$D, phosphate levels and FGF23 did not differ between patients with and without mineralization defect.

Trabecular bone volume, expressed as percentage of total tissue volume (B.Ar/T.Ar), was within the normal range in more than 90 % of patients at the time of transplantation and numerically decreased only minimally over the first post-transplant year. Cumulative glucocorticoid exposure was the only parameter correlating with trabecular bone loss. This observation confirms and extends previous data in renal transplant recipients, indicating that also in the setting of renal transplantation glucocorticoids are a predominant factor causing trabecular bone loss [9;26].

DXA scans, performed in a subgroup of patients, equally failed to show significant changes. This finding conflicts with older studies demonstrating significant bone mineral density loss often exceeding 5% during the first year after transplantation, but agrees with more recent cohort studies reporting no or only minimal losses. Steroid minimisation most probably accounts to a large extent for this favourable trend. In agreement with previous studies[27], we observed a significant correlation between areal BMD and B.Ar/T.Ar, however not at all sites and not consistently across time points. Correlation coefficients did not differ between sites rich in cortical vs trabecular bone. Changes in areal BMD also were concordant with changes in B.Ar/T.Ar.

An important strength of the present study is the longitudinal design with availability of repeat bone biopsies in a substantial number of patients. While previous studies often selected patients with low bone mineral density or disturbed mineral metabolism [19;25;28], no selection criteria besides being an adult renal transplant candidate were applied in the present study. Our study has also some limitations. *First*, only static histomophometric parameters were determined in the present study since double tetracyclin labelling was not possible at the time of transplantation due to the unpredictable timing of the deceased kidney transplant procedure. *Second*, our study, although being among the largest in its kind, is hampered by small sample size, limiting statistical power. Extrapolation moreover warrants caution, as findings might be different in patients with different ethnic and renal disease background or subjected to a different immunosuppressive regimen. Collaborative efforts, similar to what has been achieved in the setting of end stage renal disease, are needed to tackle the power issue and to allow the identification of robust clinical and biochemical determinants of bone histomorphometry (changes) in renal transplant recipients.

In conclusion, our data indicate the impact of renal transplantation on renal bone disease is limited in contemporaneous transplant recipients. Bone activity, being already in the low-normal range in most patients at the time of transplantation tends to further decline. Bone loss is limited, with glucocorticoid exposure remaining a dominant determinant.

METHODS

Patients and study protocol:

This is an interim analysis on patients enrolled in an ongoing prospective observational study that aims to unravel the natural history of CKD-MBD after transplantation (NCT01886950). All patients referred for a single kidney transplant at the University Hospitals Leuven between October 2010 and August 2013, were eligible for inclusion. The study protocol included a bone biopsy at the time of transplantation and 12 months later. Patients with an adequate bone biopsy both at the time of transplantation and at month 12 and no history of exposure to anti-resorptive agents were included in the present interim analysis (n=36). Demographics and data on immunosuppressive and mineral metabolism therapy were retrieved from electronic files.

Immunosuppressive and mineral metabolism therapy:

At the time of transplantation, triple immunosuppressive therapy was initiated with glucorticoids, a calcineurin inhibitor (tacrolimus or cyclosporine) and an antimetabolite (mycophenolate mofetil). Intravenous methylprednisolone was administered at the dose of 500 mg on the day of transplantation and 40 mg at the first postoperative day. Subsequently, methylprednisolone was started at the dose of 16 mg orally and tapered to 8 mg orally during the second month, and 4mg orally from around the third month on, as decided by the treating physician. Clinical parameters and findings on protocol renal transplant biopsy at month 3, determined whether glucocorticoids were halted or continued. Cyclosporine and tacrolimus dosing was concentration controlled according to standard protocols. Mycophenolate mofetil dosage was adjusted in case of intolerance.

Mineral metabolism therapy was stopped at the time of transplantation and resumed posttransplant if deemed necessary by the treating physician.

Biochemical analyses:

Blood samples were collected immediately before transplantation [pre] (random, nonfasting) and at month 3 (M3) and 12 (M12) post-transplantation [post] (fasting). Samples were stored for <2 h at 5°C until centrifugation. Upon arrival at the laboratory, the blood samples were centrifuged at 3000 rpm for 10 min,

aliquoted, and stored at –80°C until analysis. Twenty-four hour urine samples were collected at M3 and M12, shaken, aliquoted, and stored at –80°C until analysis. Creatinine, hemoglobin, total and ionized calcium, phosphate, and total alkaline phosphatase, were all measured using standard laboratory techniques. Serum 1,25(OH)$_2$D (calcitriol) and 25(OH)D (calcidiol) levels were measured using a radioimmunoassay [29;30]. Serum concentrations of full-length (biointact) PTH were determined by an immunoradiometric assay (IRMA), as described elsewhere [31]. Albumin was measured using the bromocresol green method. Serum sclerostin (TECOmedical, Sissach, Switzerland), biointact fibroblast growth factor 23 (FGF23) (Immutopics, San Clemente, CA, USA), interleukin-6 (IL-6) (IBL, Hamburg, Germany), osteoprotegerin (OPG), bone specific alkaline phosphatase (bsAP) and tartrate-resistant acid phosphatase 5b (TRAP5b) (Quidel, Ohio USA) were measured using ELISA kits according to the manufacturer's instructions. Inter- and intra-assay variation for all assays was < 10%, and recovery levels between 84 – 106% according to the manufacturers. The eGFR was calculated using the CKD-EPI equation. Time average concentrations of phosphate during the first year after transplantation were calculated according to the trapezoidal rule.

Bone biopsy and bone histomorphometry:

A transiliac bone biopsy was performed at the time of transplantation (general anaesthesia) and at M12 (local anaesthesia ± light sedation) using a needle with an internal diameter of 4.5 mm (Osteobell, Biopsybell), at a site 2 cm posterior and 2 cm inferior to the anterior iliac spine. Since the timing of deceased donor kidney transplantation is unpredictable, bone biopsies at the time of transplantation were performed without prior double tetracycline labelling. At M12, patients received double tetracycline labelling. The labelling schedule consisted of two 3-days oral tetracycline (2 x 500 mg/day) administration sessions separated by an 11-days tetracycline free interval. Bone biopsies at M12 were performed 4 days after the last intake of tetracycline.

The method for quantitative histomorphometry of bone has been described elsewhere [32]. Briefly, biopsy specimens were fixed in 70% ethanol and embedded in a methylmethacrylate resin. Undecalcified 5-µm thick sections were stained by

the method of Goldner for quantitative histology to determine static bone parameters. Ten-µm thick sections were mounted unstained in 100% glycerol for fluorescence microscopy and visualization of tetracycline labels to determine dynamic bone parameters. All results are reported as measurements in two dimensions using nomenclature established by the American Society for Bone and Mineral Research[33]. Bone analysis was performed in the Laboratory of Pathophysiology of the University of Antwerp, Belgium, using an image analysis program (AxioVision v 4.51, Zeiss, Germany) running a custom program. Key parameters that were assessed included perimeter of active osteoblasts on osteoid perimeter (Ob.Pm/O.Pm) (%), perimeter of active osteoclasts on eroded perimeter (Oc.Pm/E.Pm) (%), eroded perimeter on bone perimeter (E.Pm/B.Pm) (%), bone area on tissue area (B.Ar/T.Ar) (%), osteoid area on bone area (O.Ar/B.Ar) (%) and osteoid width (µm). Fibrosis was scored as present or absent. Osteoid seams less than 2 µm in width were not included in primary measurements of osteoid width or area.

In bone biopsies obtained at the time of transplantation, i.e. without tetracycline labelling, we used the bone area to total tissue area (B.Ar/T.Ar), osteoid area to bone area (O.Ar/B.Ar), and the ratio of osteoblast-covered perimeter to total bone perimeter (Ob.Pm/B.Pm) as surrogate markers for bone volume, mineralization, and turnover, respectively (***supplementary table 1***). The cut-off values were determined on biopsies obtained at M12 after double tetracycline labels.

Bone densitometry:

Measures of bone mineral density (BMD) at the lumbar spine, hip and radius by dual X-ray absorptiometry (DXA, Synarc Imaging, Newark, CA) were collected within 2 weeks after engraftment and at M12. Due to logistic obstacles, DXA scans were available in an unselected subset of patients only (n=22).

Statistical analysis:

Results were expressed as mean±SD or median (interquartile range), as appropriate. The Wilcoxon signed-rank test was used to compare median values at time of transplantation and M12. Spearman's correlation was used to examine

the relationship between bone histomorphometric parameters and clinical and biochemical parameters.

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FIGURE LEGENDS:

Figure 1: Time course of laboratory parameters of mineral metabolism after successful renal transplantation.

Figure 2: Changes in TMV following successful renal transplantation.

Figure 3: Correlation between change B.Ar/T.Ar and glucocorticoid exposure during the first post-transplant year

Table 1: Demographics, clinical characteristics and therapy

Age (yrs)	54.2 ± 12.2		
Gender M/F (%)	80.6/19.4		
Length (cm)	175.0 ± 8.5		
Weight (kg)	76.5 ± 13.5		
BMI (kg/m^2)	24.9 ± 3.7		
Dialysis vintage (Yrs)	2.34 (1.42-3.34)		
Renal diagnosis (%)			
Diabetic nephropathy	13.9		
Glomerulonephritis/vasculitis	16.7		
Interstitial nephritis	5.6		
Hypertensive/large vessel disease	11.1		
Cystic/hereditary/congenital diseases	25.0		
Miscellaneous	0.0		
Etiology unknown or missing	27.8		
Diabetes Mellitus (%)	28		
Parathyroidectomy (%)	8.3		
Prior low impact fracture (%)	8.3		
Immunosuppression	**Tx**	**M3**	**M12**
Steroids (%)	-	88.8	61.1
CNI (%)	-	88.9	97.2
Antimetabolite (%)	-	88.9	86.1
MP mg/d	-	3.5 ± 1.3	2.2 ± 2.0
Cumulative MP dose (g)	-	945 ± 421	1680 ± 673
Mineral Metabolism	**Tx**	**M3**	**M12**
Phosphate binder (%)	83	-	-
Ca (%)	83	22.2	19.4
Nutritional Vitamin D (%)	55.2	25.0	44.4
Active Vitamin D (%)	55.2	5.5	11.1
Cinacalcet (%)	13.8	0	0
Bicarbonate supplement (%)	31.0	41.7	33.3

Abbreviations: CNI: Calcineurin inhibitor; MP: Methyl Prednisolone; Ca: calcium, either as calcium containing phosphate binder or calcium supplement

Table 2: Biochemistry

	Tx	M3	M12	ANOVA	Paired t-test (Tx vs M12)
Creatinine (mg/dL)	8.06 ± 2.71	1.85 ± 0.68	1.61 ± 0.60	<0.0001	<0.0001
eGFR (ml/min 1.73m^2)	-	44.7 ± 17.2	50.9 ± 16.0	-	0.0008
Bicarbonate (mmol/L)	25.4 ± 3.25	22.3 ± 2.8	23.7 ± 3.3	0.0009	0.01
Albumin (g/dL)	44.4 ± 5.1	44.9 ± 3.9	42.4 ± 7.7	0.30	0.08
Ca (mg/dL)	9.1 ± 0.8	9.7 ± 0.8	9.7 ± 0.8	0.002	0.0007
tCa > 10.3 mg/dL (%)	0	17.1	19.4	0.03	-
iCa (mmol/L)	-	1.29 ± 0.12	1.25 ± 0.1	-	0.10
iCa > 1.27 mmol/L (%)	-	48.6	30.6	0.1	-
Phos (mg/dL)	4.41 ± 1.22	2.61 ± 0.64	3.13 ± 0.78	<0.0001	<0.0001
Phosphate <2.3 mg/dL (%)	0	34.3	8.3	<0.0001	
Magnesium (mg/dL)	2.21 ± 0.35	1.59 ± 0.32	1.67 ± 0.25	<0.0001	<0.0001
biPTH (ng/L) [Normal: 4-40]	234.8 (140.2-340.0)	72.1 (49.5-116.2)	52.3 (32.0-79.7)	<0.0001	<0.0001
biPTH x UNL	5.9	1.8	1.3	-	-
PTH low/adequate/high (%)	14.7/64.7/20.6	-	-	-	-
25(OH)D	39.9 (33.4-50.1)	27.7 (22.3-36.2)	34.2 (25.7-45.7)	0.002	0.004
25(OH)D$_3$< 30 ng/L	44.4	52.9	13.9	0.002	-
1.25(OH)$_2$D	37.8 (27.3-40.9)	54.1 (49.2-71.0)	61.6 (52.6-75.6)	<0.0001	<0.0001
FGF23 (ng/l) [Normal: <50]	1591.1 (475.4-4665.4)	94.4 (68.7-125.0)	101.3 (88.4-128.7)	<0.0001	<0.0001
Sclerostin (ng/L)	1.72 (0.88-2.39)	0.54 (0.47-0.65)	0.68 (0.53-0.84)	<0.0001	<0.0001
tAP (U/L)	73.5 (62.0-134.7)	78.1 (63.0-98.7)	82.4 (69.3-92.4)	0.01	0.004
BsAP (ng/ml) [2.9-22.4]	34.7 (21.2-44.6)	26.9 (22.2-39.4)	31.5 (22.4-36.8)	0.40	0.20
Osteocalcin (ng/ml) [11.0-55.9]	80.0 (30.9-127.0)	18.4 (12.7-28.1)	16.3 (11.1-26.2)	<0.0001	<0.0001
NTX (nmol/l) [5.4-24.2]	172.9 (102.8-297.9)	24.5 (21.9-46.7)	25.3 (18.7-45.6)	<0.0001	<0.0001
Trap5b (U/L) [0.49-5.31]	4.7 (3.0-7.0)	3.26 (2.17-4.79)	3.01 (2.04-4.77)	0.01	0.0003
IL-6	4.46 (2.79-5.12)	2.84 (1.59-3.98)	2.34 (1.21-4.67)	0.01	0.0009

24h urinary Ca excretion	-	81.3 (45.7-102.9)	104.7 (68.6-148.0)	-	0.07
24h fractional Ca excretion	-	0.89 (0.58-1.37)	1.19 (0.81-1.54)	-	0.30
24h urinary phosphate excretion	-	683.8 (611.8-916.5)	862.1 (684.0-1075.7)	-	0.01
24h fractional phosphate excretion	-	34.5 (27.3-43.2)	32.2 (25.3-37.9)	-	0.04

Abbreviations: eGFR: estimated glomerular filtration rate; tCA: total calcium; iCa: ionized calcium; biPTH: bio-intact PTH; UNL: upper normal level; tAP: total alkaline phosphatase; BsAP: bone specific alkaline phosphatase; NTX: N-terminal telopeptide; TRAP5b: tartrate resistant acid phosphatase; IL-6: interleukin-6; TAC: time averaged concentration since transplantation

Table 3: bone histomorphometry at time of transplantation and at 1 year (M12).

	TX	M12	p-value (signed rank), (n)*
B.Ar/T.Ar (%)	22.6 (17.9 - 27.8)	19.4 (17.2 – 26.8)	0.3
O.Ar/B.Ar (%)	2.1 (1.1 – 3.9)	2.0 (1.1 – 4.3)	0.7
O.Pm/B.Pm (%)	20.3 (11.8 – 28.9)	15.0 (9.8 – 25.0)	0.4
O.Wi (µm)	7.9 (7.1 – 10.2)	9.1 (6.1 – 12.4)	0.5
Ob.Pm/O.Pm (%)	8.7 (0.0 – 13.4)	4.4 (0.0 – 19.1)	1.0
Ob.Pm/T.Pm (%)	1.9 (0.0 – 4.0)	0.6 (0.0 -3.7)	0.6
E.Pm/B.Pm (%)	4.7 (3.3 – 8.0)	2.1 (0.1- 4.3)	0.003
Oc.Pm/E.Pm (%)	15.0 (0.0 – 24.0)	0.0 (0.0 - 25.0)	0.3
Oc.Pm/Tt.Pm (%)	0.9 (0.0 – 1.5)	0.0 (0.0 - 0.6)	0.001
Tb.Wi	145.5 (126.3 – 169.5)	135.8 (117.0 - 172.9)	0.4
TB.N	2.0 (1.7 – 2.3)	1.9 (1.5 - 2.4)	0.7
T (low/nl/high) (%)	44.4/52.8/2.8	63.9/36.1/0.0	NS
M (delayed/nl) (%)	16.7/83.3	22.2/77.8	NS
V (low/nl) (%)	8.3/91.7	11.1/88.9	NS
Fibrosis, yes present (%)	11.0	3.0	NS
R13 BMD	0.76 (0.69 – 0.81)	0.72 (0.68-0.81)	0.3 (n=10)
R13 T-score	-0.104 (-0.241- -0.010)	-0.194 (-0.259- -0.010)	1.0 (n=10)
UDR BMD	0.423 (0.392 – 0.481)	0.416 (0.383 – 0.446)	0.5 (n=10)
UDR T-score	-1.703(-2.527 - -0.997)	-2.121 (-2.746 - -1.406)	0.5 (n=10)
LS BMD	0.952 (0.878 – 1.094)	0.945 (0.848 – 1.114)	0.5 (n=22)
LS T-score	-1.353 (-2.159 - -0.195)	-1.544 (-2.408 - -0.234)	0.5 (n=22)
FN BMD	0.689 (0.601 – 0.762)	0.675 (0.632 – 0.779)	0.5 (n=22)
FN T-score	-1.756 (-2.324 - -1.237)	-1.799 (-2.175 - -1.111)	0.2 (n=22)
TH BMD	0.854 (0.777 – 0.899)	0.838 (0.778 – 0.901)	0.8 (n=22)
TH T-score	-1.185 (-1.695 - -0.686)	-1.294 (-1.689 - -0.545)	0.8 (n=22)

*, n=36, unless otherwise specified

Abbreviations: B.Ar: bone area; T.Ar: tissue area; O.Ar: Osteoid area; O.Pm: osteoid perimeter; B.Pm: bone perimeter; O.Wi: osteoid width; Ob.Pm: osteoblast perimeter; E.Pm: eroded perimeter; Oc.PM: osteoclast perimeter; Tt.Pm: total perimeter: Tb.Wi: trabecular width; TB.N: trabecular number; T: turnover; M: mineralization; V: volume; BMD: bone mineral density; R: radius; UDR: ultradistal radius region of interest; LS: lumbar spine; FN: femoral neck; TH: total hip

Supplementary Table 1: TMV classification of bone biopsies based on static parameters

		Parameter
Turnover	Low	Ob.Pm/B.Pm < 1.5%
	Normal	1% < Ob.Pm/B.Pm < 7.4%
	High	Ob.Pm/B.Pm >7.4%
Mineralization	Normal	O.Ar/B.Ar ≤4.9%
	Abnormal	O.Ar/B.Ar >4.9%
Volume	Low	B.Ar/T.Ar ≤14.6%
	Normal	B.Ar/T.Ar >14.6%

Abbreviations: Ob.Pm: osteoblast perimeter; B.Pm: bone perimeter; O.Ar: Osteoid area; B.Ar: bone area; T.Ar: tissue area

Supplementary Table 2: Demographics and routine biochemical parameters in study cohort vs patients transplanted within the same period but not included in the study

	All (n=310)	**Study cohort (n=36)**	**p-value**
Age (yrs)	54.0 ± 13.8	54.2 ± 12.2	0.80
Gender M/F (%)	61.0/39.0	80.6/19.4	0.02
Length (cm)	171.2 ± 9.6	175.0 ± 8.5	0.01
Weight (kg)	72.7 ± 15.5	76.5 ± 13.5	0.20
BMI (kg/L^2)	24.5 ± 4.6	24.9 ± 3.7	0.70
Dialysis vintage (Yrs)	3.02 (1.43-4.44)	2.34 (1.42-3.34)	0.40
Renal diagnosis (%)			0.40
Diabetic nephropathy	10.3	13.9	
Glomerulonephritis/vasculitis	26.5	16.7	
Interstitial nephritis	7.1	5.6	
Hypertensive/large vessel disease	5.2	11.1	
Cystic/heriditary/congenital diseases	21.3	25.0	
Miscellaneous	6.1	0.0	
Etiology unknown or missing	23.6	27.8	
Diabetes Mellitus (%)	22	28	0.40
tCa (mg/dL)	9.3 0.7	9.1 0.9	0.50
Phosphate (mg/dL)	4.46 ± 1.44	4.41 ± 1.22	0.90
biPTH (ng/L) [4-40]	168.0 (86.1-290.9)	234.8 (140.2-340.0)	0.20
$25(OH)D_3$	39.4 (28.8-52.7)	39.9 (33.4-50.1)	0.60
$1.25(OH)_2D_3$	32.1 (23.7-45.0)	37.8 (27.3-40.9)	0.70
tAP (U/L)	94.1 (71.4-131.4)	73.5 (62.0-134.7)	0.50

Abbreviations: BMI: body mass index; tCA: total calcium; biPTH: biointact PTH, tAP: total alkaline phosphatase

FIGURES
Figure 1:

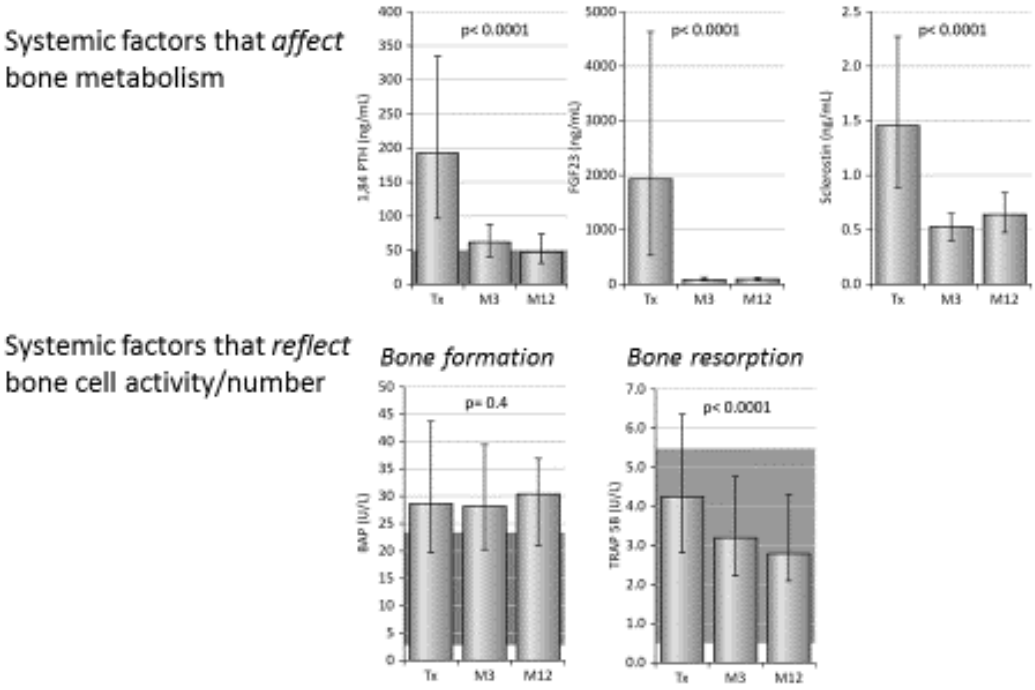


Figure 2:

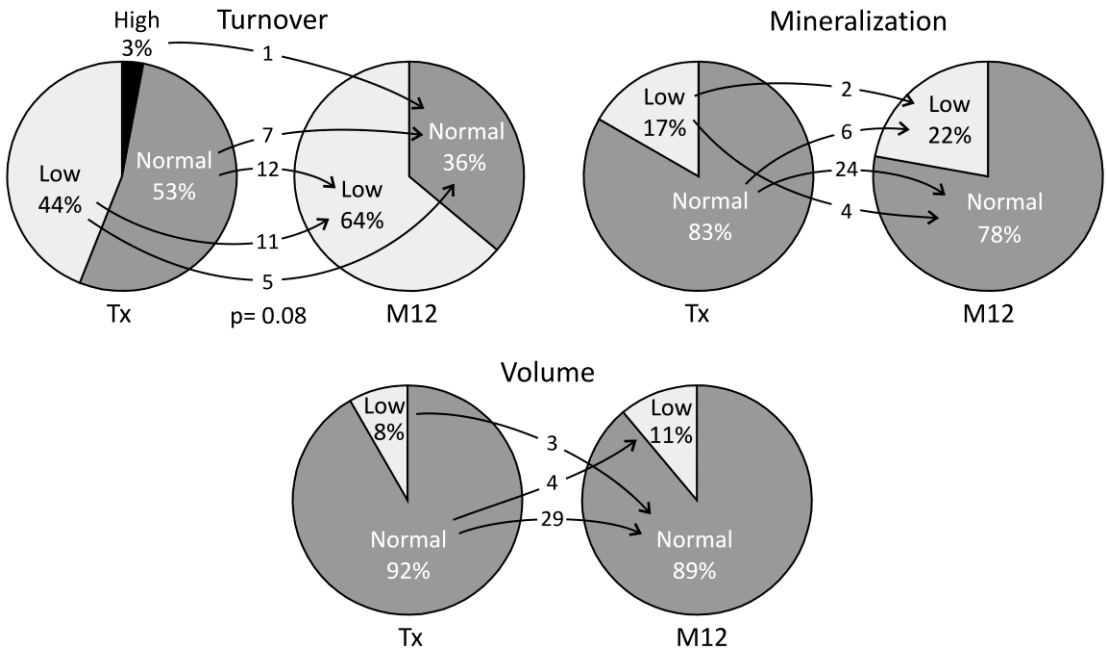
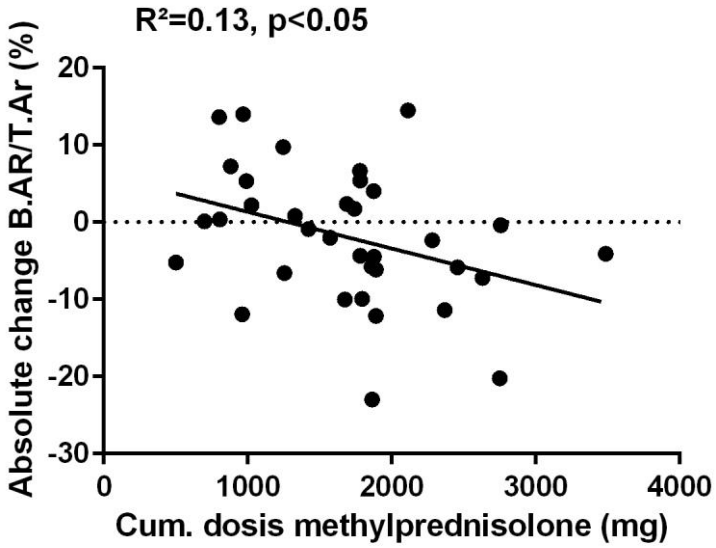


Figure 3:



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