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# Associations of Changes in Bone Turnover Markers with Change in Bone Mineral Density in Kidney Transplant Patients

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## Abstract

Background: Bone loss after kidney transplantation is highly variable. We investigated whether changes in bone turnover markers associate with bone loss during the first post-transplant year.

Methods: Bone mineral density was measured at 0 and 12 months, with biointact parathyroid hormone (PTH), bone specific alkaline phosphatase, intact pro-collagen type I N-terminal pro-peptide (PINP), and tartrate resistant acid phosphatase isoform 5b (TRAP5b) measured at 0, 3 and 12 months post-transplant (N=209). Paired transiliac bone biopsies were available in a subset (N=49). Between-group differences were evaluated by Student's *t* test, Wilcoxon signed-rank test, or Pearson's $X^2$ test.

Results: Changes in bone mineral density varied from –22 to +17%/year. Compared to patients with no change (±2.5%/year), patients who gained bone mineral density had higher levels of PTH (236 vs 136 pg/mL), bone specific alkaline phosphatase (31.7 vs 18.8 μg/L) and Intact PINP (121.9 vs 70.4 μg/L) at time of transplantation, a greater decrease in bone specific alkaline phosphatase (-40 vs -21%) and Intact PINP (-43 vs -13%) by 3 months, and lower levels of Intact PINP (36.3 vs 60.0 μg/L) at 12 months post-transplant. Patients who lost bone mineral density had a less marked decrease, or even increase, in Intact PINP (+22 vs -13%) and TRAP5b (-27 vs -43%) at 3 months, and higher Intact PINP (83.7 vs 60.0 μg/L) and TRAP5b (3.89 vs 3.16 U/L) at 12 months compared to patients with no change. If none of the biomarkers decreased by the least significant change at 3 months, an almost 2-fold (69 vs 36%) higher occurrence of bone loss was seen at 12 months post-transplant.

Conclusions: Bone loss after kidney transplantation was highly variable. Resolution of high bone turnover, as reflected by decreasing bone turnover markers, associated with bone mineral density gain, while increasing bone turnover markers associated with bone loss.

## Introduction

Fracture risk is increased in kidney transplant recipients,[1] particularly in the early post-transplant period.[2] Traditionally, substantial bone loss was expected following kidney transplantation,[3] but with the current steroid-sparing immunosuppressive protocols, the effect on the central skeleton seems overall neutral, with bone loss mainly at distal skeletal sites.[4] However, there is large inter-individual variability in bone mineral density changes post-transplant, with subsets of patients exhibiting bone mineral density loss, stability, or even gain during the first post-transplant year.[4,5]

Ongoing disturbances of mineral metabolism and consequent effects on skeletal remodeling contribute to bone loss after kidney transplantation. Ongoing hyperparathyroidism post-transplant associate with deterioration of cortical bone by high-resolution imaging,[6] which could explain the significant bone mineral density loss[4] and increased fracture risk[2] seen at the distal skeleton. Conversely, greater decreases in parathyroid hormone (PTH)-levels associate with bone mineral density gain during the first post-transplant year.[7] The normalization of skeletal remodeling brought on by the resolution of hyperparathyroidism is reflected by a reduction in circulating bone turnover markers.[4,8,9] These biomarkers are passively released from the bone during the process of skeletal remodeling and can be used as a non-invasive measure of overall skeletal bone turnover.[10]

In the realm of osteoporosis, bone turnover markers are used to assess treatment response and expected treatment benefits.[11,12] In a post-transplant setting, greater decreases in bone turnover markers associate with bone mineral density gain,[4] but it is unknown whether changes in these biomarkers early in the post-transplant course may be able to predict later changes in bone mineral density. This information could enable identification of patients at high risk of bone loss, who could benefit the most from early intervention.

To address this question, this study aimed to investigate how changes in bone turnover markers in the early post-transplant period would relate to later changes in bone mineral density in contemporary kidney transplant recipients.

## Methods

### *Cohort*

This observational cohort study included adult kidney transplant recipients participating in prospective, ongoing cohort-studies investigating skeletal health after kidney transplantation at the University Hospitals Leuven (ClinicalTrials.gov NCT00547040 and NCT01886950). Patients were recruited between October 2006 and September 2016. Relevant demographic data, comorbidities, medical therapy, and routine biochemistry were extracted from electronic patient files. The cohort was restricted to patients with bone densitometry at time of transplantation and at 12 months post-transplant, who also had study visits with blood sampling at time of transplantation, and 3- and 12-months post-transplant. Of 1343 patients prospectively enrolled at time of kidney transplantation, 333 patients had bone densitometry performed, and 235 of these had study visits at 3 and 12 months with blood samples available (**Supplemental Figure 1**). The only exclusion criterion was treatment with anti-resorptive therapy at any time-point during the first post-transplant year (n=26). Demographic data and markers of mineral metabolism were overall comparable between the selected patients and the overall cohort (**Supplemental Table 1**).

The study was approved by the local Research Ethical Committee (study IDs S52091 and S50111), and all patients provided written, informed consent for study participation. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the 'Declaration of Istanbul on Organ Trafficking and Transplant Tourism'.

***Immunosuppression***

Patients received a standard immunosuppressive regimen consisting of a calcineurin inhibitor, an antimetabolite (mycophenolate mofetil) and glucocorticoids (methylprednisolone). Glucocorticoids were discontinued at the discretion of the treating physician, based on immunological risk profile and the results of a protocolled kidney graft biopsy at 3 months post-transplant.

***Biochemical analyses***

Non-fasting blood samples were collected at time of admission for kidney transplantation and at study visits 3- and 12-months post-transplant. Samples were kept for <2 hours at 5°C before being centrifuged at 3000 rpm for 10 minutes and then aliquoted and processed or stored at -80°C until later analyses. Plasma albumin, hemoglobin, creatinine, total calcium, phosphate, total bicarbonate and total alkaline phosphatase were measured consecutively using standard laboratory techniques. Total alkaline phosphatase assays changed during the study period. Details of the conversions used are given in **Supplemental Methods**. Glomerular filtration rate (GFR) was estimated by the Chronic Kidney Disease Epidemiology Collaboration equation.[13] Biointact (1-84) PTH was measured by immunoradiometric assay (reference interval 3–40 ρg/mL).[14] 25-hydroxy vitamin D was measured by radioimmunoassay. Bone turnover markers were analyzed in batch after completion of the observational data collection. Bone-specific alkaline phosphatase, trimeric pro-collagen type I N-terminal pro-peptide (Intact PINP), and tartrate resistant acid phosphatase isoform 5b (TRAP5b) were measured using the IDS-iSYS instrument (ImmunoDiagnosticSystems, Boldon, UK). Values above the assay upper limit of quantification (Bone-specific alkaline phosphatase: 75 µg/L, Intact PINP: 230 µg/L, TRAP5b: 14 U/L) were determined after sample dilution. The assay-specific reference values are given in **Supplemental Methods**.

***Bone densitometry***

Bone densitometry was performed at the lumbar spine, proximal femur, and distal forearm by dual-energy x-ray absorptiometry (DXA)-scan (QDR-4500A or Discovery; Hologic, Marlborough, MA, USA), at time of transplantation and 1-year post-transplant (within ±1 mo). The Hologic Spine Phantom was scanned regularly to monitor scanner performance and stability. A single, certified operator, blinded to study details, analyzed all DXA scans. Coefficients of variation for repeat patient scans were 0.58% at lumbar spine, 0.56% at total hip, 1.40% at femoral neck, 0.98% at the 1/3 distal radius, and 1.10% at the ultradistal radius.

***Bone histomorphometry***

Transiliac bone biopsies were available for a subset of patients ($n$=49). A detailed report on these patients have been published previously.[9] Briefly, these samples were retrieved using a trephine with an internal diameter of 3.55 mm (Biopsybell 8G, Mirandola, Italy). Bone cores were fixed in 70% ethanol and embedded in a methylmethacrylate resin. 5-µm undecalcified sections were stained by the Goldner method, and an image analysis software (AxioVision version 4.51, Zeiss Microscopy, Zeiss, Germany) running a customized program was utilized to determine static parameters. An experienced bone pathologist semi-quantitatively assessed bone turnover (low, normal, high) and mineralization (normal, abnormal). All bone histomorphometric parameters are given in 2D using standardized nomenclature.[15]

***Statistical analyses***

Continuous variables are given as mean with standard deviation (±SD) if normally distributed or median with interquartile range [IQR] if skewed. Missing data were not imputed, and patients with missing data did not contribute to statistical analyses for the parameter in question. Dichotomous and categorical variables are given as number and proportion (%). Between-group differences were evaluated by Student's $t$ test, Wilcoxon signed-rank test, or Pearson's $X^2$ test, respectively. We

divided patients by whether or not they achieved a decrease in bone turnover marker levels greater than the least significant change of the biomarker at 3 months post-transplant. In stable hemodialysis patients, these are reported to be 23% for bone-specific alkaline phosphatase, 32% for Intact PINP, and 24% for TRAP5b.[16] A change in DXA bone mineral density >2.5% per year was considered a clinically relevant change.[17] Multivariable logistic regression including potential confounders (age, sex, body mass index, eGFR at 12 months, cumulative steroid dose at 12 months, and levels of PTH at time of transplantation) was used to investigate whether changes in bone turnover markers were independently associated with later changes in bone mineral density. All statistical analyses were performed using the statistical software solution STATA IC version 16.1 (StataCorp LP, College Station, TX, USA).

## Results

A total of 209 patients were included. Mean age was 53±12 years, 65% were men, and 15% had diabetes at time of transplantation. Cause of chronic kidney disease (CKD) was glomerulonephritis or vasculitis (25%), congenital disease (4%) or adult polycystic kidney disease (20%), chronic interstitial nephropathy (11%), diabetes mellitus type 1 or 2 (9%), hypertension or atherosclerosis (5%), other (4%), or unknown (21%). At time of transplantation, 144 (69%) patients had been treated with chronic intermittent hemodialysis for 32 [19, 52] months, and 55 (26%) had been treated with ambulatory peritoneal dialysis for 32 [20, 45] months. The remaining 10 patients (5%) were transplanted pre-emptively. A parathyroidectomy had been performed in 27 patients prior to transplantation.

Immunosuppression was maintained by a calcineurin inhibitor, with the majority of patients receiving tacrolimus (85%), in combination with mycophenolate mofetil and prednisone. Steroids were discontinued in 26% of patients between months 3 and 12. The median cumulative steroid dose, including any treatment given for acute rejection, was 1.40 [1.22, 1.71] g at 3 months and 2.37

[1.86, 2.75] g at 12 months. Six patients underwent a subtotal parathyroidectomy in the first post-transplant year, all between months 3 and 12 (median 172 days, range 112, 314). Five patients suffered a fragility fracture in the first post-transplant year; 2 of these were vertebral fractures and 3 were foot or ankle fractures.

***Changes in bone turnover markers***

Post-transplant biochemical measurements are shown in **Table 1**. Overall, median values of the bone turnover markers decreased by 3 months post-transplant. From 3 to 12 months, bone-specific alkaline phosphataselevels remained stable, Intact PINP decreased further, while TRAP5b increased slightly. Biointact PTH and total alkaline phosphatase levels decreased by 3 months, with no further changes from 3 to 12 months.

***Changes in bone mineral density***

At time of transplantation, the prevalence of osteoporosis (*T*-score ≤–2.5) was 18 to 37% at different skeletal sites (**Figure 1**). A T-score≤–2.5 at either spine or hip was seen in 30% of patients. Changes in BMD during the first post-transplant ranged from –22 to +17% (**Figure 2**). A significant decrease in bone mineral density was detected at the lumbar spine (–0.8%, [95% CI –1.5; –0.04], *p*=0.04), total hip (–1.2%, [–1.9; –0.4], *p*=0.003), femoral neck (–1.3%, [–2.0; –0.5], *p*=0.001), and ultradistal radius (–2.6%, [–3.6; –1.6], *p*<0.001), but not at the 1/3 distal radius (–0.5%, [–1.1; 0.1], *p*=0.11). A bone mineral density decrease of 2.5% or more from baseline was seen in 37% of patients at the lumbar spine, 39% at the total hip, 37% at the femoral neck, 23% at 1/3 the distal radius, and 53% at the ultradistal radius.

There was a direct correlation between eGFR at 12 months and BMD change at the total hip (Spearman's *rho* 0.16, *p* = 0.03) and femoral neck (*rho* 0.20, *p*=0.004), but not at the lumbar spine (*rho* 0.08, *p*=0.27), indicating higher prevalence of bone loss in patients with suboptimal kidney

graft function. There was a negative correlation between cumulative dose of steroids at 12 months and BMD change at the lumbar spine (*rho* –0.15, *p*=0.03), total hip (*rho* –0.19, *p*=0.009), but not at the femoral neck (*rho* –0.09, *p*=0.22) indicating a higher prevalence of bone loss with steroid exposure.

***Relationship between changes in biomarkers and bone density***

**Figure 3** shows trajectories of PTH and bone turnover markers in patients who lost, remained stable, or gained bone mineral density at the lumbar spine during the first post-transplant year, using a cutoff of 2.5%. Patients who gained bone mineral density had higher levels of PTH and bone turnover markers at time of transplantation and a greater decrease in PTH and biomarkers by 3 months compared to patients with a stable bone mineral density. For patients who lost bone mineral density, bone turnover markers decreased less markedly, or even increased slightly at 3 months, while at 12 months, all three biomarkers were significantly higher compared to patients who were stable. Other markers of mineral metabolism did not differ according to bone mineral density change (**Table 2**). Results were similar when considering bone mineral density changes at the proximal femur (**Supplemental Figure 2**). Trajectories of total alkaline phosphatase showed a pattern similar to, but less pronounced than, the bone turnover markers (**Supplemental Figure 3**).

***Association between early change in biomarkers and later bone loss***

To investigate the relationship between early changes in bone turnover markers and later bone loss, we dichotomized patients according to the decline in biomarkers at month 3 in "descenders", showing a decline greater than the least significant change for the biomarker and "non-descenders", showing a decline less than the least significant change or even an increase. The occurrence of bone mineral density loss was ~2-fold higher in non-descenders compared to descenders (**Figure 3**, **Supplemental Table 2**). If *none* of the biomarkers decreased at 3 months (*n*=55), 69% of patients

experienced bone loss at either spine or hip, while if *all* biomarkers decreased ($n$=53), this was true for 36% of patients (Pearson's $X^2$ $p$=0.001).

A decrease in bone turnover markers greater than the least significant change at 3 months remained independently associated with a higher prevalence of bone loss at 12 months after adjusting for age, sex, kidney function, cumulative steroid dose, and PTH levels at time of transplantation (**Table 3**).

***Bone biopsy findings***

Paired transiliac bone biopsies at time of and 12 months after kidney transplantation were available for 49 patients. At time of transplantation, static parameters indicated a higher skeletal remodeling rate in patients who later gained bone mineral density, while at 12 months post-transplant, these differences were no longer apparent. Patients who gained bone mineral density exhibited greater amounts of osteoid at time of transplantation, with significant decreases in these parameters at 12 months post-transplant (**Table 4**).

## Discussion

This study investigated the association between early changes in bone turnover markers and later changes in bone mineral density after kidney transplantation. Our main findings were as follows: Bone mineral density changes in the first post-transplant year were highly variable, with 30-40% of patients experiencing substantial bone loss, defined as a bone mineral density decrease by at least 2.5%. Decreasing levels of PTH and bone turnover markers by 3 months post-transplant associated with less pronounced bone mineral density loss, or even gain, while greater bone mineral density losses were seen if bone resorption markers remained high throughout the first post-transplant year.

Decreases in bone mineral density were seen at all skeletal sites in the first post-transplant year, except the 1/3 distal radius, with modest changes at spine and hip (~1%) and a more pronounced decrease at the ultradistal radius (~3%). Previous studies on the effect of kidney transplantation on skeletal health in patients receiving modern, steroid-sparing immunosuppressive therapy similarly

demonstrated a limited bone loss at the central skeleton.[6,18] However, the changes in bone mineral density post-transplant were highly variable, with subsets of patients losing, remaining stable, or even increasing in bone mineral density during the first post-transplant year. This pattern of variability, with gainers and losers of bone mineral density seems to be a consistent finding demonstrated in several other cohorts.[5,7]

Patients who gained bone mineral density during the first post-transplant year had higher levels of PTH and bone turnover markers at time of transplantation, with greater decreases in PTH and bone turnover markers at 3 months post-transplant. These findings indicate resolution of a high bone turnover state in bone mineral density gainers, which was confirmed in the subset of patients with available paired transiliac bone biopsies. Further, the histomorphometric analysis revealed that the amount of unmineralized bone (osteoid) was higher at time of transplantation and decreased at 1 year post-transplant in patients with bone mineral density gain. This supports the hypothesis that the gain in bone mineral density was caused by mineralization of preformed bone matrix, which accumulates in hyperparathyroid bone disease. This mechanism can be compared to what is seen after parathyroidectomy,[19] where rapid skeletal mineralization during the hungry bone syndrome can lead to impressive gains in bone mineral density.[20]

Conversely, patients who experienced bone mineral density loss during the first post-transplant year had lower levels of PTH at time of transplantation, with an increase in bone turnover markers during the first post-transplant year. At 12 months, the bone turnover markers were significantly higher in these patients. Thus, ongoing bone resorption signaled a greater prevalence of bone loss during the first post-transplant year.

Early (<3 months) changes in PTH and bone turnover markers associated with bone mineral density changes at 1 year post-transplant. This was not the case for other markers of mineral metabolism,

including phosphate, calcium, or 25-hydroxy vitamin D levels. Similar findings were seen in a post-hoc analysis of a small trial of denosumab after kidney transplantation.[21] The correlation was only demonstrated in the control group, not in the active treatment arm, which is surprising considering results from other trials of denosumab in patients receiving dialysis.[22] None of the other bone biomarkers measured associated with bone mineral density change[21]; however, these were all markers known to accumulate with reduced kidney function, making them less suitable in a CKD cohort.

In contrast to the bone turnover markers, PTH levels at 3 and 12 months were comparable across subgroups of bone mineral density change in the present study. Further, changes in bone turnover markers remained independent determinants of bone mineral density change after adjustment for demographic factors, which was not the case for PTH. In other words, significant differences in bone turnover markers were seen despite similar PTH levels, which could indicate either competing factors affecting bone turnover, or variability in the skeletal PTH responsiveness in kidney transplant recipients. The pathophysiology behind PTH hyporesponsiveness in CKD is unclear,[23] but the severity of hyperparathyroidism has been shown to be a main determinant, both in studies utilizing the gold standard calcemic response after PTH infusion[24] and in others using bone turnover markers as surrogate measures[25,26]. Thus, a desensitization of the skeleton may take place in severe hyperparathyroidism, and such adaptive changes could still be in effect post-transplant, reducing the diagnostic accuracy of PTH levels in the evaluation of bone turnover.[27] In effect, it may be more helpful to evaluate the skeletal response to PTH using the bone turnover markers, rather than relying on PTH levels alone. These biomarkers passively reflect the process of skeletal remodeling and convey information on the current status of bone turnover regardless of any underlying causes (glucocorticoids, inflammation, PTH levels, et cetera).[28]

Our findings indicate a usefulness of bone turnover markers in risk stratification post-transplant. Bone mineral density status is generally poor in kidney transplant recipients, with 20-30% of patients having *T*-scores in the osteoporotic range and 35-50% having *T*-scores in the osteopenic range at time of transplantation.[7,29,30] Considering that the risk of fractures is particularly high in the first post-transplant year,[2] timely intervention to minimize bone loss post-transplant could improve patient outcomes. Our findings indicate that an evaluation of bone turnover markers in the early post-transplant period could help identify patients at particularly high or low risk of bone mineral density loss, which in turn could enable an individualized approach to preventing further bone loss.

Strengths of this study include a substantial cohort of contemporary kidney transplant recipients with extended biochemical evaluation at several time points post-transplant. We measured bone biomarkers known to be largely unaffected by kidney function, which is of importance when applied in cohorts of patients with kidney dysfunction. A modern, steroid-sparing immunosuppressive protocol was utilized, and results should be generalizable to current day kidney transplant recipients. As limitations, we included patients with available study visits at 3 and 12 months after kidney transplantation, and the risk of selection bias should be considered. However, we found no marked differences in demography or mineral metabolism parameters in patients selected compared to the overall patient population. We excluded patients receiving anti-resorptive therapy, which would be expected to amplify the associations demonstrated. Bone biomarkers were measured in the non-fasting state and randomly with regards to the last dialysis session prior to kidney transplantation. However, the effect of fasting and dialysis on these biomarkers are limited,[31-33] and any variability caused should be random with respect to the associations studied. Our results could be exaggerated by regression to the mean, as patients with more severe hyperparathyroidism also had lower bone mineral density at time of transplantation. Movement away from extreme values could thus be expected for both of these parameters. We applied the

principle of least significant change to both bone mineral density and bone biomarker changes which should help overcome analytical and biological variability. Further, a sensitivity analysis excluding patients with osteoporosis at the lumbar spine at time of transplantation yielded identical results. We did not report on clinical outcomes (fractures), but bone mineral density as a surrogate marker of bone strength. Low bone mineral density has been shown to associate with risk of incident fractures in kidney transplant recipients.[29] Finally, the study cohort was exclusively White, with demographic data and mineral metabolism treatment targets reflective of Europe, and results may not be fully applicable to other population groups or regions of the world.[34]

In conclusion, bone mineral density changes after kidney transplantation were highly variable, and a subset of patients experienced substantial bone loss despite a steroid minimization protocol. Levels of bone turnover markers, and the changes in these markers, associated with bone mineral density changes. Our findings indicate that bone turnover markers may be useful in identifying patients with ongoing bone resorption who are at a high risk of bone loss in the first post-transplant year, which could enable an individualized approach to improving skeletal health after kidney transplantation.

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## Supplemental Material table of contents

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**Tables**

Table 1 Biochemistry and bone densitometry by timepoint in kidney transplant recipients

| | Missing | At transplant (*n* = 209) | At month 3 (*n* = 209) | At month 12 (*n* = 209) |
|---|---|---|---|---|
| *Medications* | | | | |
| Calcium containing phosphate binder or supplement | 0 | 138 (66) | 85 (41) | 78 (37) |
| Non-calcium containing phosphate binder | 0 | 93 (45) | 1 (0.5) | 0 (0) |
| Vitamin D supplement | 0 | 90 (43) | 43 (21) | 72 (34) |
| Active vitamin D | 0 | 93 (45) | 48 (23) | 51 (24) |
| Calcimimetic | 0 | 13 (6) | 0 (0) | 1 (0.5) |
| *Biochemistry* | | | | |
| eGFR (CKD-EPI), ml/min/1.73 m² | 7 | NA | 47 ± 17 | 53 ± 18 |
| Hemoglobin g/dL | 8 | 12.1 ± 1.5 | 11.4 ± 1.6 | 12.7 ± 1.7 |
| Albumin g/dL | 45 | 4.4 ± 0.4 | 4.4 ± 0.4 | 4.4 ± 0.4 |
| CRP, mg/L | 23 | 2.5 (1.1; 5.7) | 1.0 (0.8; 2.7) | 1.1 (0.6; 2.9) |
| Bicarbonate, mmol/L | 8 | 25 ± 3 | 22 ± 2 | 23 ± 3 |
| Total alkaline phosphatase, U/L | 20 | 89 (70; 121) | 73 (56; 96) | 76 (56; 98) |
| Biointact PTH, pg/ml | 17 | 141 [81; 254] | 46 [26; 75] | 43 [27; 78] |
| Phosphate, mg/dL | 11 | 4.7 ± 1.5 | 2.7 ± 0.6 | 3.1 ± 0.6 |
| Total calcium, mg/dL | 11 | 9.3 ± 0.8 | 9.6 ± 0.7 | 9.6 ± 0.6 |
| Magnesium, mg/dL | 164 | 2.3 ± 0.4 | 1.6 ± 0.3 | 1.7 ± 0.2 |
| 25-hydroxy vitamin D, ng/mL | 35 | 38 ± 17 | 30 ± 13 | 35 ± 16 |
| Bone-specific alkaline phosphatase, ug/L | 7 | 20.9 [14.9; 31.5] | 17.0 [11.2; 25.1] | 17.4 [11.5; 25.8] |
| Intact PINP, ug/L | 7 | 79.6 [51.7; 130.6] | 78.2 [47.7; 120.0] | 64.3 [32.0; 107.6] |
| TRAP5b, U/L | 7 | 5.11 [3.77; 7.06] | 3.14 [2.27; 4.13] | 3.27 [2.38; 4.83] |
| *Densitometry* | | | | |
| Lumbar spine *T*-score | 0 | -1.2 ± 1.5 | N/A | -1.3 ± 1.4 |
| Total hip *T*-score | 11 | -1.1 ± 1-1 | N/A | -1.2 ± 1.1 |
| Femoral neck *T*-score | 11 | -1.6 ± 1.0 | N/A | -1.7 ± 1.0 |
| 1/3 distal radius *T*-score | 118 | -1.3 ± 1.5 | N/A | -1.5 ± 1.7 |
| Ultradistal radius *T*-score | 118 | -1.9 ± 1.3 | N/A | -2.2 ± 1.3 |

Data are mean±SD, median [IQR], or *n* (%)

Abbr.: eGFR=estimated glomerular filtration rate, PINP=pro-collagen type I N-terminal pro-peptide, PTH=biointact parathyroid hormone (1-84), TRAP5b=tartrate resistant acid phosphatase isoform 5b

Table 2 Demographic and biomarkers in kidney transplant recipients by lumbar spine bone mineral density (BMD) change at 12 months post-transplant

| | *Missing* | BMD loss ($n$ = 78) | BMD stable ($n$ = 78) | BMD gain ($n$ = 53) |
|---|---|---|---|---|
| Age, yrs | 0 | 53 ± 13 | 54 ± 11 | 54 ± 11 |
| Sex, male | 0 | 44 (56) | 53 (68) | 38 (72) |
| Body mass index, kg/m² | 3 | 25 ± 5 | 25 ± 4 | 26 ± 5 |
| Diabetes mellitus, any type | 0 | 10 (13) | 13 (17) | 9 (17) |
| Dialysis vintage, mo | 0 | 32 [24; 50] | 29 [15; 42] | 37 [20; 54] |
| eGFR, 3 mo, ml/min/1.73m² | 0 | 47 ± 17 | 46 ± 16 | 50 ± 16 |
| eGFR, 12 mo, ml/min/1.73m² | 5 | 53 ± 18 | 52 ± 20 | 56 ± 14 |
| Cumulative steroids 3 mo, g | 1 | 1.41 [1.27; 1.72] | 1.36 [1.21; 1.72] | 1.43 [1.17; 1.70] |
| Cumulative steroids 12 mo, g | 1 | 2.48 [2.05; 2.81] ŧ | 2.23 [1.80; 2.52] | 2.34 [1.86; 2.75] |
| *Bone densitometry* | | | | |
| Lumbar spine BMD | 0 | 1.023 ± 0.169 | 0.962 ± 0.146 | 0.886 ± 0.169 |
| Lumbar spine T-score | 0 | -0.7 ± 1.5 | -1.3 ± 1.3 | -2.0 ± 1.5 |
| Total hip BMD | 9 | 0.858 ± 0.153 | 0.849 ± 0.133 | 0.810 ± 0.163 |
| Total hip T-score | 9 | -1.0 ± 1.1 | -1.1 ± 0.9 | -1.4 ± 1.2 |
| Femoral neck BMD | 9 | 0.708 ± 0.135 | 0.704 ± 0.112 | 0.663 ± 0.137 |
| Femoral neck T-score | 9 | -1.5 ± 1.1 | -1.6 ± 0.9 | -1.9 ± 1.1 |
| Phosphate, mg/dL | | | | |
| At transplantation | 2 | 4.8 ± 1.4 | 4.6 ± 1.7 | 4.6 ± 1.4 |
| At 3 mo | 6 | 2.6 ± 0.7 | 2.7 ± 0.6 | 2.8 ± 0.6 |
| At 12 mo | 5 | 3.1 ± 0.7 | 3.0 ± 0.6 | 3.1 ± 0.6 |
| % change at 3 mo | 6 | -38 ± 31 | -31 ± 31 | -30 ± 39 |
| % change at 12 mo | 6 | -30 ± 30 | -27 ± 29 | -25 ± 34 |
| Total calcium, mg/dL | | | | |
| At transplantation | 2 | 9.4 ± 0.7 | 9.2 ± 0.8 | 9.2 ± 0.8 |
| At 3 mo | 6 | 9.7 ± 0.7 | 9.6 ± 0.6 | 9.6 ± 0.7 |
| At 12 mo | 5 | 9.7 ± 0.7 | 9.6 ± 0.5 | 9.5 ± 0.5 |
| % change at 3 mo | 6 | 2.9 ± 8.4 | 5 ± 9 | 6 ± 11 |
| % change at 12 mo | 6 | 2.8 ± 8.6 | 5 ± 11 | 5 ± 11 |
| 25-hydroxy vitamin D, ng/mL | | | | |
| At transplantation | 1 | 37 ± 16 | 39 ± 17 | 38 ± 17 |
| At 12 mo | 14 | 38 ± 17 | 34 ± 16 | 34 ± 14 |
| Biointact PTH, pg/mL | | | | |
| At transplantation | 2 | 112 [57; 183] | 136 [82; 235] | 236 [115; 348] ŧ |
| At 3 mo | 6 | 41 [22; 76] | 48 [31; 79] | 50 [25; 70] |
| At 12 mo | 11 | 47 [27; 83] | 39 [26; 78] | 39 [29; 72] |
| % change at 3 mo | 6 | -56 [-75; -20] | -64 [-75; -47] | -79 [-87; -59] ŧ |
| % change at 12 mo | 13 | -59 [-75; 1.3] * | -68 [-82; -45] | -83 [-90; -62] ŧ |
| Total alkaline phosphatase, U/L | | | | |
| At transplantation | 12 | 82 [67; 107] | 90 [67; 116] | 108 [82; 139] |
| At 3 mo | 13 | 72 [52; 94] | 71 [55; 92] | 77 [60; 103] |
| At 12 mo | 18 | 82 [61; 108] | 76 [58; 96] | 63 [54; 90] |
| % change at 3 mo | 13 | -16 [-30; 5] | -20 [-33; 3] | -31 [-44; -5] |
| % change at 12 mo | 18 | -7 [-25; 20] | -17 [-34; 11] | -33 [-53; -24] |

| | | | | |
|---|---|---|---|---|
| Bone-specific alkaline phosphatase, µg/L | | | | |
| At transplantation | 0 | 17.4 [13.6; 23.8] | 18.8 [14.6; 28.6] | 31.7 [22.0; 54.2] ŧ |
| At 3 mo | 0 | 20.2 [12.3; 26.6] | 15.8 [11.0; 23.1] | 18.9 [12.1; 31.7] |
| At 12 mo | 7 | 20.6 [13.8; 29.2] | 16.0 [11.6; 23.9] | 15.2 [10.4; 22.5] |
| % change at 3 mo | 0 | -8 [-32; 36] | -21 [-48; 7] | -40 [-65; -6] * |
| % change at 12 mo | 7 | -1 [-26; 68] | -20 [-45; 28] | -55 [-72; -31] ŧ |
| Intact PINP, µg/L | | | | |
| At transplantation | 0 | 62.8 [44.6; 106.9] | 70.4 [53.5; 119.0] | 121.9 [88.1; 207.2] ŧ |
| At 3 mo | 0 | 78.1 [47.4; 125.2] | 73.7 [47.4; 110.2] | 89.8 [48.8; 122.6] |
| At 12 mo | 7 | 83.7 [47.3; 115.9] * | 60.0 [30.2; 96.0] | 36.3 [22.4; 77.0] * |
| % change at 3 mo | 0 | 22 [-29; 83] * | -13 [-43; 36] | -43 [-62; -10] ŧ |
| % change at 12 mo | 7 | 13 [-26; 75] ŧ | -27 [-65; 48] | -68 [-83; -42] ŧ |
| TRAP5b, U/L | | | | |
| At transplantation | 0 | 4.73 [3.17; 6.58] | 5.03 [3.73; 7.10] | 6.10 [4.08; 9.47] |
| At 3 mo | 0 | 3.37 [2.71; 4.52] ŧ | 2.94 [2.22; 3.67] | 3.00 [2.14; 4.25] |
| At 12 mo | 7 | 3.89 [2.93; 5.20] ŧ | 3.16 [2.17; 4.60] | 2.73 [1.76; 4.13] |
| % change at 3 mo | 0 | -27 [-48; 0.3] ŧ | -43 [-59; -19] | -52 [-66; -29] |
| % change at 12 mo | 7 | -13 [-42; 14] ŧ | -37 [-56; -17] | -54 [-67; -43] ŧ |

Data are mean ± SD or median [IQR], * P<0.05, ŧ P<0.01 and ŧ P<0.001 by Student's *t* test or Wilcoxon rank-sum test, compared to stable group

Abbr.: eGFR=estimated glomerular filtration rate, PINP=pro-collagen type I N-terminal pro-peptide, PTH=biointact parathyroid hormone (1-84), TRAP5b=tartrate resistant acid phosphatase isoform 5b

Table 3 Association between changes in biomarkers at 3 months and >2.5% decrease in bone mineral density at 12 months post-transplant

| | Lumbar spine (*n*=209) | | Total hip (*n* = 200) | | 1/3 distal radius (*n* = 124) | | Ultradistal radius (*n* = 124) | |
|---|---|---|---|---|---|---|---|---|
| ΔBiomarker at 3 mo | *OR* | *CI* | *OR* | *CI* | *OR* | *CI* | *OR* | *CI* |
| Biointact PTH decrease by 43% | 0.47 | [0.22; 1.00] | 0.51 | [0.23; 1.14] | 0.61 | [0.21; 1.80] | 0.92 | [0.37; 2.31] |
| Alkaline phosphatase decrease by 16% | 0.64 | [0.33; 1.23] | 0.64 | [0.33; 1.25] | 0.31 | [0.11; 0.87] | 0.49 | [0.22; 1.10] |
| Bone-specific alkaline phosphatase decrease by 23% | 0.39 | [0.21; 0.73] | 0.33 | [0.17; 0.64] | 0.44 | [0.17; 1.17] | 0.53 | [0.24; 1.16] |
| PINP decrease by 32% | 0.41 | [0.20; 0.82] | 0.44 | [0.21; 0.91] | 0.25 | [0.07; 0.86] | 0.36 | [0.15; 0.87] |
| TRAP5b decrease by 24% | 0.42 | [0.22; 0.82] | 0.53 | [0.27; 1.05] | 0.34 | [0.13; 0.89] | 0.44 | [0.18; 1.07] |

Multivariable logistic regression *odds ratios (OR)* with 95% confidence intervals (*CI)* after adjustment for age, sex, body mass index, PTH at time of transplantation, estimated glomerular filtration rate at 12 months post-transplant and cumulative steroid dose at 12 months post-transplant

Abbr.: eGFR=estimated glomerular filtration rate, PINP=pro-collagen type I N-terminal pro-peptide, PTH=biointact parathyroid hormone (1-84), TRAP5b=tartrate resistant acid phosphatase isoform 5b

Table 4 Bone histomorphometry by transiliac bone biopsy based on change in bone mineral density (BMD) at lumbar spine during the first year after kidney transplantation

| | *Missing* | Lumbar spine BMD loss (*n*= 15) | Lumbar spine BMD stable (n = 17) | Lumbar spine BMD gain (*n* = 17) |
|---|---|---|---|---|
| *Time of transplantation* | | | | |
| Bone turnover, L/N/H | 0 | 25/63/12%/ | 0/86/14% | 0/75/25% |
| ObPm/BPm, % | 0 | 1.6 [0.2; 4.9] | 1.4 [0.0; 3.1] | 7.7 [1.3; 11.5] ŧ |
| OcPm/BPm, % | 0 | 0.4 [0.0; 1.7] | 0.4 [0.0; 1.1] | 1.0 [0.4; 1.9] |
| EPm/BPm, % | 0 | 4.7 [1.7; 6.9] | 3.7 [2.2; 5.0] | 5.1 [4.0; 8.5] |
| OAr/BAr, % | 0 | 1.6 [0.7; 3.2] | 1.7 [1.2; 2.8] | 5.8 [2.1; 7.9] ŧ |
| OPm/BPm, % | 0 | 14.3 [7.6; 26.5] | 15.4 [12.4; 20.3] | 36.3 [26.2; 40.8] ŧ |
| *12 months post-transplant* | | | | |
| Bone turnover, L/N/H | 0 | 12%/88%/0% | 10%/90%/0% | 33%/58%/8% |
| BFR/TAr, um²/mm²/day | 9 | 210 [92; 312] | 126 [69; 221] | 427 [134; 493] |
| BFR/BS, um³/um²/year | 9 | 17 [9; 32] | 10 [5; 23] | 20 [7; 40] |
| Mlt, days | 9 | 44.3 [22.6; 68.7] | 36.0 [17.2; 72.8] | 22.6 [13.3; 52.7] |
| ObPm/BPm, % | 0 | 4.6 [0.9; 8.3] | 4.2 [0.0; 10.2] | 3.6 [1.3; 7.2] |
| OcPm/BPm, % | 0 | 0.7 [0.3; 1.5] | 0.4 [0.0; 0.7] | 0.6 [0.0; 1.2] |
| EPm/BPm, % | 0 | 3.5 [2.5; 5.5] | 2.7 [1.3; 3.4] | 2.7 [1.1; 4.1] |
| OAr/BAr, % | 0 | 3.4 [2.0; 5.8] | 3.0 [1.2; 5.9] | 2.0 [0.6; 5.3] |
| OPm/BPm, % | 0 | 23.1 [15.7; 38.2] | 25.2 [11.6; 46.7] | 17.1 [8.8; 31.8] |
| *Change at 12 months* | | | | |
| ΔObPm/BPm, % | 0 | 2.2 ± 4.6 | 3.6 ± 6.6 | -0.6 ± 6.6 |
| ΔOcPm/BPm, % | 0 | 0.1 ± 0.7 | -0.2 ± 1.0 | -0.6 ± 1.4 |
| ΔEPm/BPm, % | 0 | -1.3 ± 3.9 | -1.2 ± 3.1 | -3.0 ± 3.5 |
| ΔOAr/Bar, % | 0 | 2.2 ± 2.8 | 1.9 ± 3.8 | -1.7 ± 4.3* |
| ΔOPm/BPm, % | 0 | 9.0 ± 15.6 | 10.5 ± 22.8 | -9.1 ± 20.8* |

Mean ± SD or median [IQR], with P by Student's *t* test or Wilcoxon rank-sum test for significance compared to stable group, * p<0.05, ŧ p<0.01 and ŧ=p<0.001

Abbr.: BFR=bone formation rate, BPm=bone perimeter, Mlt=mineralization lag time, ObPm=osteoblast perimeter, OcPm=osteoclast perimeter, EPm=eroded perimeter, OAr=osteoid area, OPm=osteoid perimeter

## Figure legends

Figure 1: Prevalence of osteoporosis, defined as a dual-energy x-ray absorptiometry T-score <-2.5 at time of transplantation and at 12 months post-transplant

Figure 2: Changes in bone mineral density (BMD) from time of transplantation to 12 months post-transplant; number of patients and mean change in % from baseline given for each skeletal site

Figure 3: Trajectories of parathyroid hormone in times upper normal limit and bone turnover markers in patients who lost, remained stable, or gained bone mineral density (BMD) at the lumbar spine (LS) in the first post-transplant year; Medians with IQR, *marks P<0.05, ŧ P<0.01 and ŧ P<0.001 compared to stable group. PINP=pro-collagen type I N-terminal pro-peptide, TRAP5b=tartrate resistant acid phosphatase isoform 5b

Figure 4: Risk of lumbar spine and total hip bone mineral density (BMD) loss at 12 months by whether or not biomarkers decreased by the least significant change at 3 months post-transplant (D+: descender, non-D; non-descender), BALP=bone-specific alkaline phosphatase, PINP=intact pro-collagen type I N-terminal pro-peptide, PTH=biointact parthyroid hormone, TRAP5b=tartrate resistant acid phosphatase isoform 5b; * marks P<0.05, ŧ marks P<0.01 and ŧ marks P<0.001 by Pearson's $X^2$ test

Figure 1



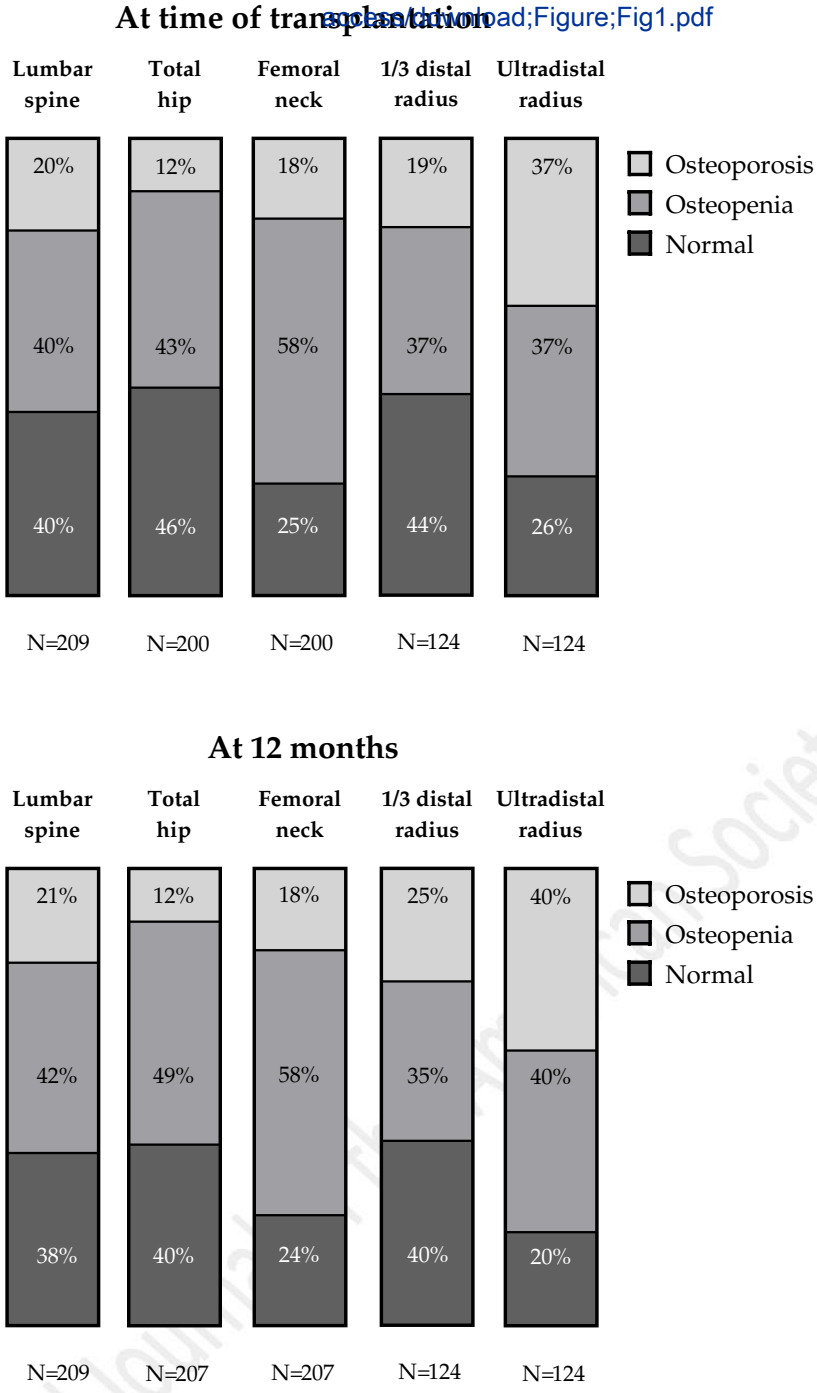


Figure 2



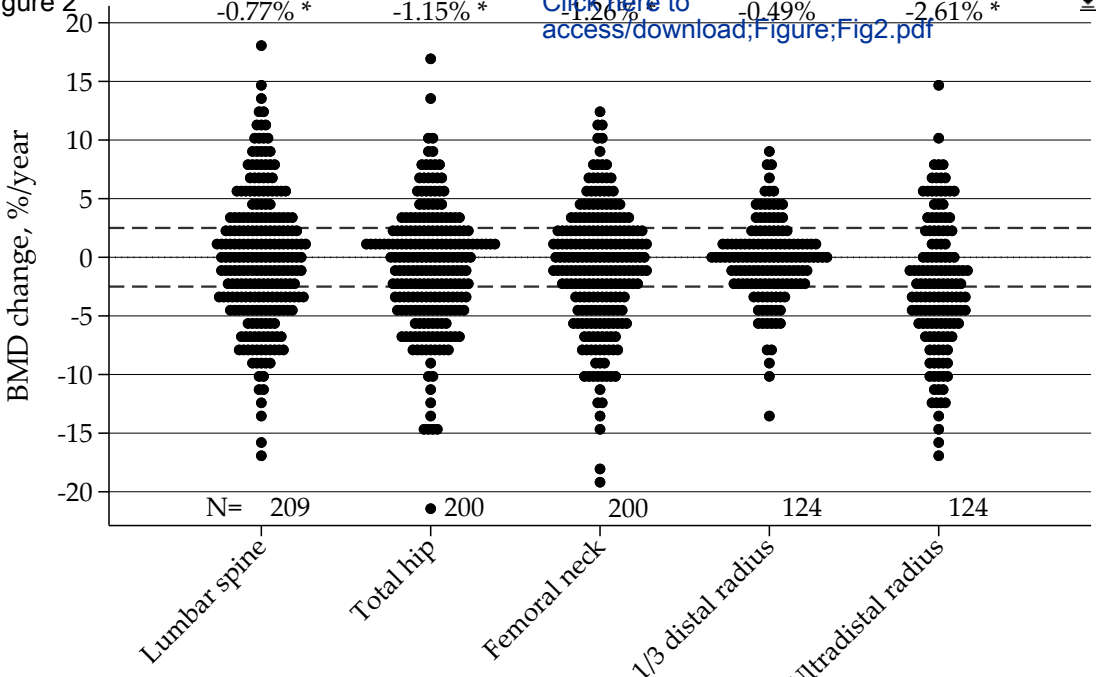


Figure 3



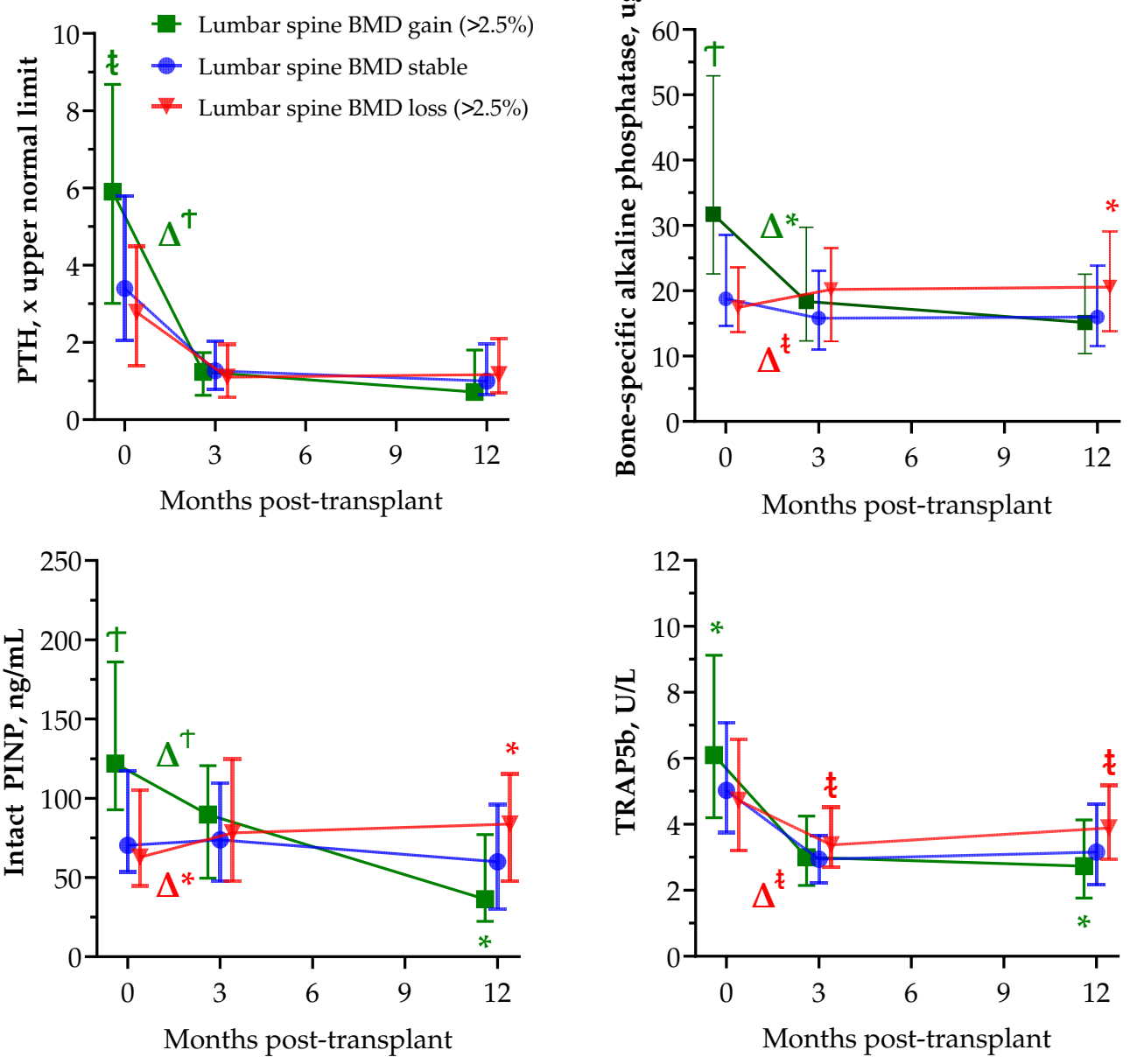
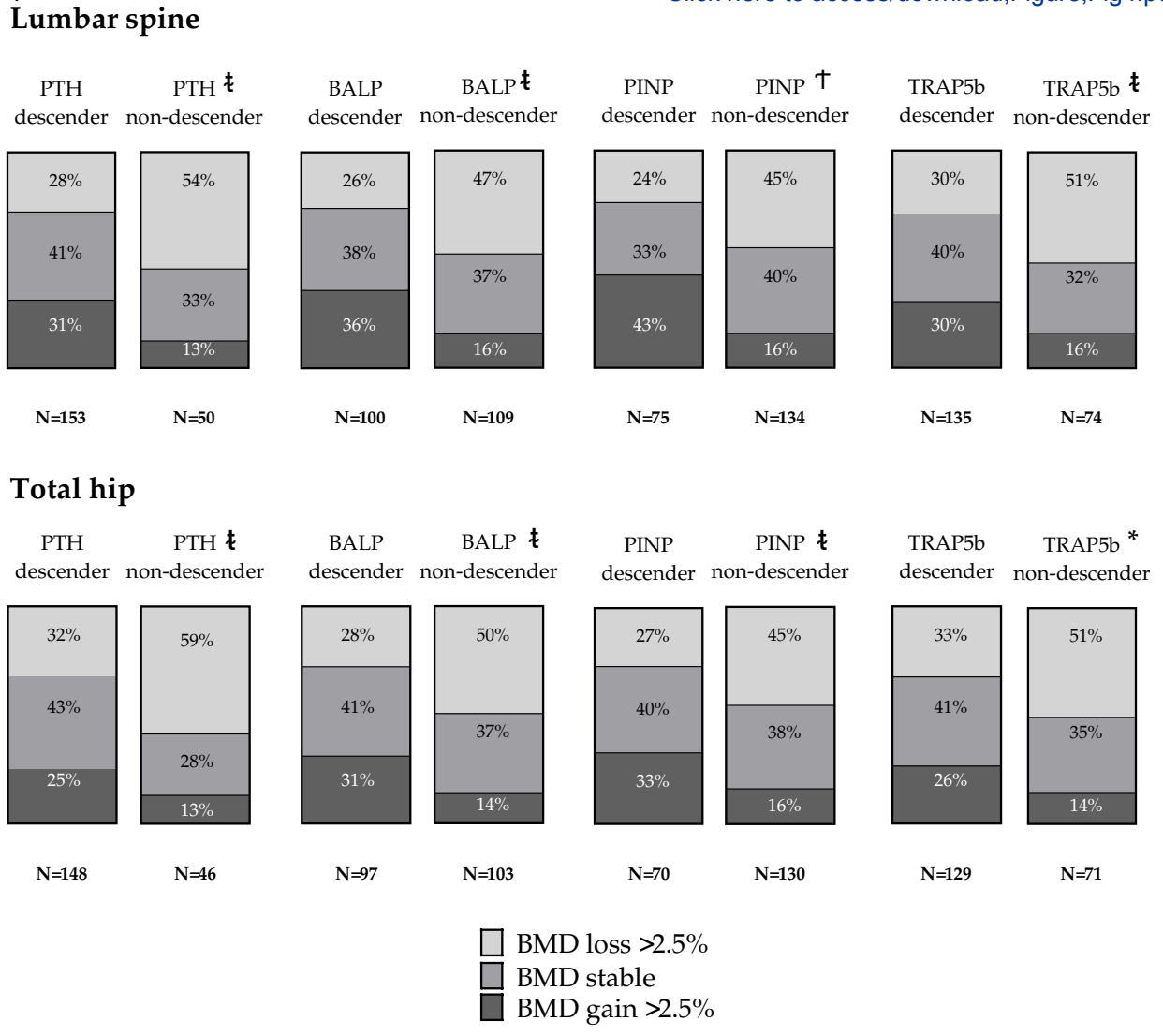


Figure 4





# Supplemental material

## *Table of contents*

***Supplemental Methods***

Until April 2012, the assay upper normal limits for total alkaline phosphatase were 240 U/L for men and 270 U/L for women, and conversion of data from this time-period was done by dividing values by 2.077 and 2.286, respectively. From April 2012 to October 2014, assay upper normal limits were 100 U/L for men and 113 U/L for women, and conversion was done by dividing by 0.869 and 0.952, respectively. From October 2014, assay upper normal limits were 105 U/L for men and 130 U/L for women.

The assay-specific reference values of the bone turnover markers utilized in this study were: Bone-specific alkaline phosphatase: 7.9–25.5 μg/L for men, 6.1–22.2 for pre-, and 7.1–23.9 μg/L for post-menopausal women; Intact PINP: 13–72 μg/L for men, 14–71 μg/L for pre-, and <83 μg/L for post-menopausal women; and TRAP5b: 1.4–6.1 U/L for men, 1.2–4.8 U/L for pre-, and 1.1–6.9 U/L for post-menopausal women.

Supplemental Table 1 Demographic data and biochemical markers of mineral metabolism in patients included versus the overall cohort of kidney transplant recipients

| | All patients (n=1343) | Missing DXA or study visit (n=1108) | Anti-resorptive therapy (n=26) | Included in this study (n=209) | Paired bone biopsies (n=49) |
|---|---|---|---|---|---|
| Sex, male | 793 (62) | 645 (62) | 13 (50) | 135 (65) | 38 (78) |
| Age, years | 55 ± 13 | 55 ± 13 | 60 ± 9* | 53 ± 12 | 55 ± 11 |
| Body mass index, kg/m² | 27 ± 5 | 28 ± 6 | 25 ± 4 | 25 ± 5 | 26 ± 5 |
| Diabetes, any type | 249 (19) | 212 (19) | 5 (19) | 32 (15) | 14 (29) |
| Receiving dialysis | 1178 (88) | 953 (86) | 26 (100) | 199 (95) | 46 (94) |
| Dialysis modality | | | | | |
| Hemodialysis | 949 (81) | 787 (83) | 18 (69) | 144 (72) | 30 (61) |
| Peritoneal dialysis | 229 (19) | 166 (17) | 8 (31) | 55 (28) | 16 (33) |
| *Biochemistry* | | | | | |
| Hemoglobin, g/dL | 11.8 ± 1.72 | 11.83 ± 1.75 | 11.82 ± 1.64 | 11.73 ± 1.56 | 12.0 ± 1.5 |
| Albumin, g/dL | 4.2± 0.6 | 4.2 ± 0.6 | 3.9 ± 0.6* | 4.2 ± 0.6 | 4.4 ± 0.3 |
| CRP, mg/L | 3.5 [1.5; 8.1] | 3.5 [1.5; 8.4] | 5.8 [2.5; 16.1]* | 2.8 [1.2; 6.5] | 5.7 [1.3; 5.8] |
| Bicarbonate, mmol/L | 24 ± 3 | 23 ± 3 | 23 ± 4 | 24 ± 4 | 24 ± 3 |
| Total calcium, mg/dL | 9.3 ± 0.9 | 9.2 ± 0.9 | 9.3 ± 0.7 | 9.3 ± 0.8 | 9.5 ± 0.7 |
| Phosphate, mg/dL | 4.5 ± 1.4 | 4.5 ± 1.4 | 4.5 ± 1.1 | 4.7 ± 1.6 | 4.7 ± 1.3 |
| Biointact PTH, pg/mL | 145 [72; 256] | 146 [70; 258] | 128 [60; 184] | 140 [81; 253] | 245 [150; 355] |
| Total alkaline phosphatase, U/L | 92 [71; 123] | 93 [72; 126] | 98 [80; 129] | 87 [69; 121] | 87 [67; 129] |

Mean ± SD, median [IQR], or n (%)

Abbr.: CRP=C-reactive protein, PTH=parathyroid hormone

Supplemental Table 2 Change in bone mineral density (ΔBMD) from baseline to 1 year in kidney transplant recipients, by whether or not a decrease in biomarkers greater than the least significant change was seen at 3 months post-transplant

| ΔBMD, %/year | Biointact PTH decrease by 43% | | | Bone-specific alkaline phosphatase decrease by 23% | | | Intact PINP decrease by 32% | | | TRAP5b decrease by 24% | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No | Yes | *P* | No | Yes | *P* | No | Yes | *P* | No | Yes | *P* |
| Lumbar spine, *(n=209)* | -2.71 (5.13) | 0.30 (5.16) | <0.001 | -2.23 (4.94) | 0.81 (5.44) | <0.001 | -2.01 (4.87) | 1.43 (5.61) | <0.001 | -2.31 (5.67) | 0.07 (5.06) | 0.004 |
| Total hip *(n=200)* | -3.90 (5.78) | -0.09 (5.00) | <0.001 | -2.36 (5.20) | 0.13 (5.23) | <0.001 | -2.09 (5.23) | 0.58 (5.17) | <0.001 | -2.58 (5.69) | -0.37 (5.00) | 0.003 |
| Femoral neck, *(n=200)* | -3.82 (5.33) | -0.27 (5.01) | <0.001 | -2.36 (5.54) | -0.09 (4.89) | 0.003 | -2.01 (5.44) | 0.12 (4.899 | 0.006 | -2.46 (5.16) | -0.60 (5.34) | 0.02 |
| 1/3 distal radius *(n=123)* | -1.32 (3.97) | -0.29 (3.20) | 0.15 | -0.90 (3.54) | -0.05 (3.33) | 0.21 | -1.06 (3.43) | 0.53 (3.30) | 0.02 | -1.73 (3.38) | 0.10 (3.35) | 0.004 |
| Ultradistal radius *(n=123)* | -4.21 (6.13) | -2.08 (5.17) | 0.06 | -3.35 (5.67) | -1.79 (5.68) | 0.14 | -3.47 (5.67) | -1.03 (5.48) | 0.02 | -4.97 (5.79) | -1.48 (5.34) | 0.001 |

Data are mean (SD), with P by Student's t test

Abbr.: PINP=pro-collagen type I N-terminal pro-peptide, PTH=parathyroid hormone, TRAP5b=tartrate resistant acid phosphatase isoform 5b

Supplemental Table 3 Sensitivity analysis of association between biomarkers and lumbar spine bone mineral density (BMD) loss excluding patients lumbar spine osteoporosis at time of kidney transplantation

| | Lumbar spine BMD loss (n=70) | Lumbar spine BMD stable (n=63) | Lumbar spine BMD gain (n=34) |
|---|---|---|---|
| Biointact PTH, pg/mL | | | |
| At transplantation | 111 (55; 189) | 113 (80; 220) | 219 (104; 326) |
| At 3 mo | 40 (21; 71) | 45 (29; 74) | 47 (20; 64) |
| At 12 mo | 45 (26; 80) | 37 (24; 78) | 33 (24; 62) |
| % change at 3 mo | -59 (-76; -20) | -62 (-75; -44) | -78 (-87; -61) |
| % change at 12 mo | -59 (-78; 6) | -66 (-81; -44) | -84 (-90; -54) |
| Bone-specific alkaline phosphatase, µg/L | | | |
| At transplantation | 17.3 (13.6; 23.4) | 18.2 (13.6; 26.8) | 27.0 (18.3; 41.5) |
| At 3 mo | 18.5 (12.0; 26.8) | 15.3 (10.5; 19.3) | 16.8 (12.6; 25.3) |
| At 12 mo | 20.4 (13.3; 27.0) | 14.6 (11.1; 23.9) | 12.4 (9.8; 19.7) |
| % change at 3 mo | -10 (-32; 36) | -19 (-47; 9) | -34 (-61; 3) |
| % change at 12 mo | -1 (-26; 68) | -17 (-44; 26) | -52 (-71; -20) |
| Intact PINP, µg/L | | | |
| At transplantation | 58.3 (43.4; 102.4) | 67.3 (51.5; 107.0) | 113.6 (70.4; 177.3) |
| At 3 mo | 76.5 (46.8; 123.9) | 71.4 (46.6; 114.3) | 92.1 (49.2; 118.4) |
| At 12 mo | 79.5 (44.7; 114.0) | 52.5 (29.3; 94.1) | 31.8 (18.5; 73.4) |
| % change at 3 mo | 27 (-24; 91) | -7 (-36; 37) | -39 (-58; -9) |
| % change at 12 mo | 18 (-26; 75) | -24 (-64; 54) | -70 (-84; -41) |
| TRAP5b, U/L | | | |
| At transplantation | 4.82 (3.17; 6.57) | 4.68 (3.68; 6.42) | 5.35 (3.83; 9.08) |
| At 3 mo | 3.39 (2.71; 4.58) | 2.60 (2.17; 3.67) | 3.00 (2.32; 4.19) |
| At 12 mo | 3.89 (2.75; 5.20) | 2.86 (2.11; 4.51) | 2.60 (1.56; 4.01) |
| % change at 3 mo | -27 (-47; 8) | -37 (-59; -14) | -46 (-66; -20) |
| % change at 12 mo | -12 (-43; 14) | -36 (-53; -13) | -53 (-64; -41) |

Data are mean ± SD or median [IQR]

Abbr.: PINP=pro-collagen type I N-terminal pro-peptide, PTH=biointact parathyroid hormone (1-84), TRAP5b=tartrate resistant acid phosphatase isoform 5b

Supplemental Table 4 Sensitivity analysis of association between biomarkers and lumbar spine bone mineral density (BMD) loss excluding patients with a parathyroidectomy before or after kidney transplantation

| | Lumbar spine BMD loss (n=56) | Lumbar spine BMD stable (n=71) | Lumbar spine BMD gain (n=43) |
|---|---|---|---|
| Biointact PTH, pg/mL | | | |
| At transplantation | 115 (87; 198) | 150 (90; 246) | 237 (128; 347) |
| At 3 mo | 41 (27; 70) | 48 (31; 81) | 49 (26; 65) |
| At 12 mo | 50 (34; 83) | 39 (26; 78) | 43 (30; 74) |
| % change at 3 mo | -61 (-78; -31) | -64 (-76; -50) | -79 (-87; -67) |
| % change at 12 mo | -60 (-78; -26) | -69 (-82; -50) | -82 (-89; -63) |
| Bone-specific alkaline phosphatase, µg/L | | | |
| At transplantation | 18.6 (14.0; 24.2) | 18.8 (15.0; 28.5) | 31.7 (22.6; 44.8) |
| At 3 mo | 20.6 (12.9; 26.3) | 16.0 (11.6; 23.1) | 18.9 (11.2; 28.0) |
| At 12 mo | 20.2 (13.7; 29.0) | 16.7 (11.9; 25.2) | 14.8 (9.7; 21.7) |
| % change at 3 mo | -10 (-33; 35) | -21 (-47; 1) | -43 (-66; -17) |
| % change at 12 mo | -5 (-26; 40) | -21 (-45; 28) | -53 (-72; -32) |
| Intact PINP, µg/L | | | |
| At transplantation | 71.9 (48.8; 104.3) | 71.7 (54.6; 117.4) | 123.6 (96.3; 186.0) |
| At 3 mo | 78.7 (58.1; 128.4) | 77.3 (50.9; 111.5) | 89.8 (49.6; 114.7) |
| At 12 mo | 79.6 (48.7; 111.1) | 60.3 (31.9; 96.0) | 39.4 (23.4; 77.0) |
| % change at 3 mo | 22 (-32; 81) | -11 (-41; 35) | -46 (-68; -11) |
| % change at 12 mo | 8 (-26; 62) | -30 (-66; 44) | -65 (-85; -38) |
| TRAP5b, U/L | | | |
| At transplantation | 5.04 (4.02; 6.71) | 5.11 (3.92; 7.13) | 6.44 (4.61; 9.13) |
| At 3 mo | 3.49 (2.84; 4.53) | 3.11 (2.25; 3.78) | 2.98 (2.15; 4.25) |
| At 12 mo | 3.86 (2.99; 4.89) | 3.21 (2.21; 4.51) | 2.79 (1.88; 4.10) |
| % change at 3 mo | -28 (-50; -10) | -45 (-59; -22) | -57 (-69; -34) |
| % change at 12 mo | -22 (-43; 5) | -40 (-56; -21) | -55 (-71; -42) |

Data are mean ± SD or median [IQR]

Abbr.: PINP=pro-collagen type I N-terminal pro-peptide, PTH=biointact parathyroid hormone (1-84), TRAP5b=tartrate resistant acid phosphatase isoform 5b

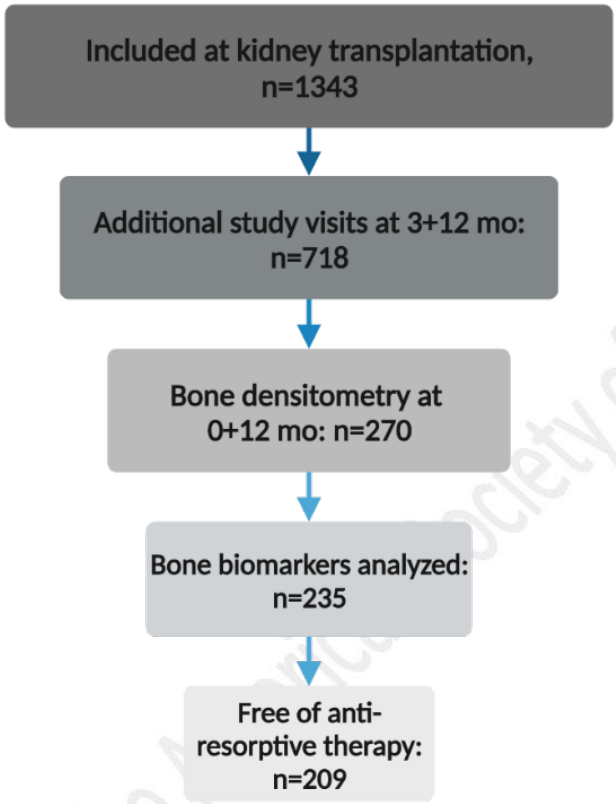
Supplemental Table 5 Sensitivity analysis of association between biomarkers and lumbar spine bone mineral density (BMD) loss excluding patients with normal LS BMD at time of kidney transplantation

| | Lumbar spine BMD loss (n=49) | Lumbar spine BMD neutral (n=46) | Lumbar spine BMD gain (n=31) |
|---|---|---|---|
| Biointact PTH, pg/mL | | | |
| At transplantation | 99 (37; 163) | 139 (73; 228) | 273 (128; 347) |
| At 3 mo | 40 (14; 57) | 46 (30; 75) | 52 (37; 75) |
| At 12 mo | 46 (19; 82) | 34 (24; 60) | 44 (31; 81) |
| % change at 3 mo | -56 (-75; -15) | -65 (-78; -48) | -80 (-87; -58) |
| % change at 12 mo | -48 (-76; 26) | -76 (-82; -50) | -81 (-89; -62) |
| Bone-specific alkaline phosphatase, µg/L | | | |
| At transplantation | 17.2 (13.4; 21.3) | 18.6 (15.3; 35.9) | 34.8 (22.6; 60.6) |
| At 3 mo | 15.9 (11.2; 23.6) | 15.9 (11.0; 24.5) | 20.5 (12.3; 34.4) |
| At 12 mo | 17.9 (12.1; 25.7) | 19.4 (11.7; 25.8) | 15.8 (11.1; 21.7) |
| % change at 3 mo | -8 (-35; 34) | -20 (-47; 4) | -42 (-69; -12) |
| % change at 12 mo | -5 (-30; 56) | -20 (-45; 29) | -56 (-72; -33) |
| Intact PINP, µg/L | | | |
| At transplantation | 56.2 (35.5; 106.3) | 84.2 (51.2; 126.1) | 123.6 (98.6; 360.4) |
| At 3 mo | 65.2 (42.8; 125.6) | 81.1 (46.0; 113.5) | 94.8 (51.4; 120.6) |
| At 12 mo | 82.1 (42.5; 111.1) | 64.9 (29.0; 97.6) | 45.7 (29.5; 79.2) |
| % change at 3 mo | 12 (-22; 73) | -16 (-41; 29) | -41 (-65; -10) |
| % change at 12 mo | 9 (-27; 75) | -27 (-67; 48) | -67 (-83; -38) |
| TRAP5b, U/L | | | |
| At transplantation | 4.57 (2.53; 6.36) | 5.03 (3.44; 7.95) | 6.44 (4.47; 10.21) |
| At 3 mo | 3.32 (2.51; 4.31) | 3.13 (2.25; 3.99) | 3.03 (1.98; 4.26) |
| At 12 mo | 3.56 (2.64; 5.33) | 3.24 (2.42; 4.91) | 2.76 (1.91; 4.22) |
| % change at 3 mo | -20 (-47; 18) | -38 (-58; -9) | -58 (-71; -33) |
| % change at 12 mo | -11 (-42; 7) | -34 (-57; -8) | -54 (-71; -46) |

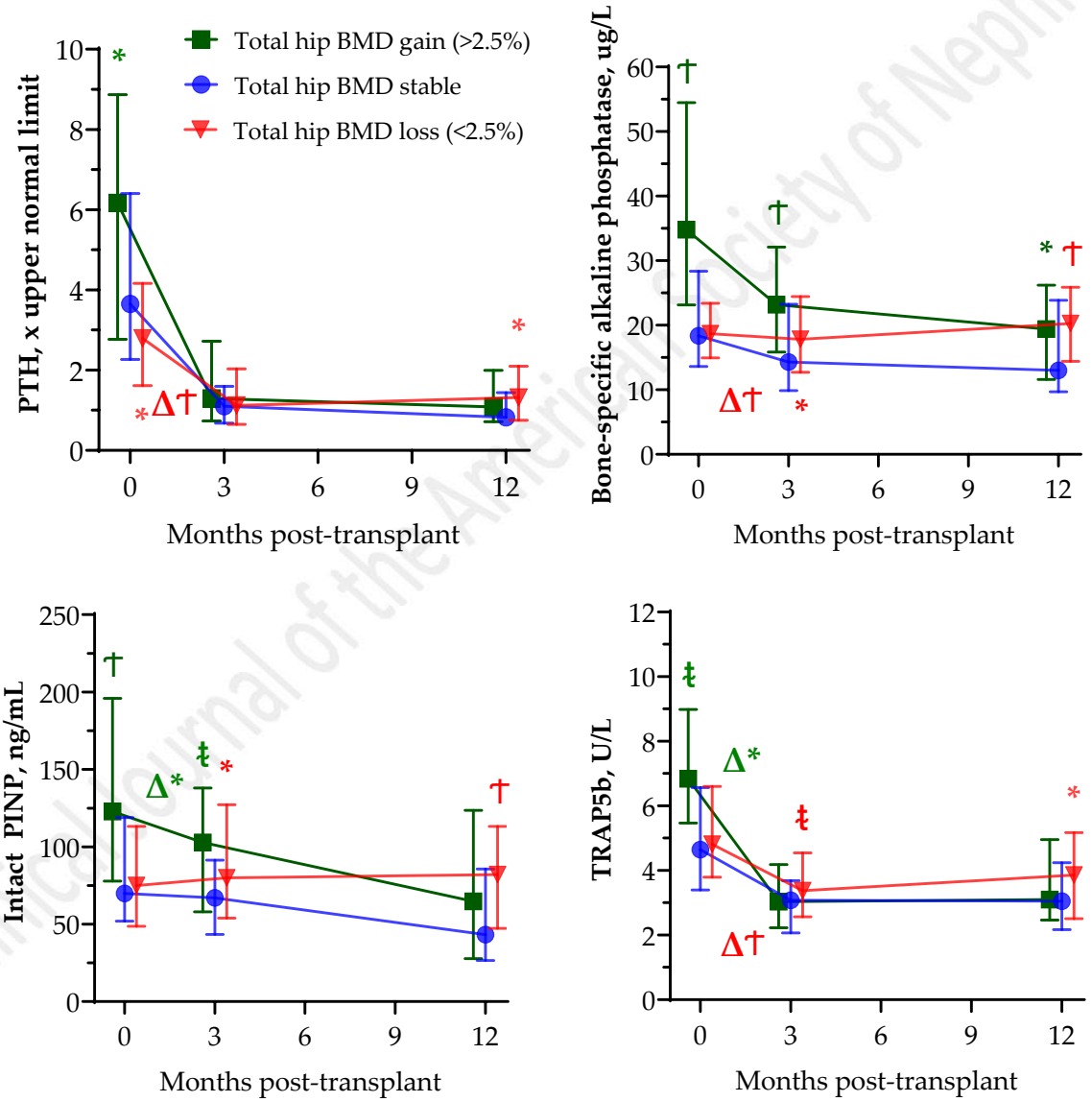
Data are mean ± SD or median [IQR]

Abbr.: PINP=pro-collagen type I N-terminal pro-peptide, PTH=biointact parathyroid hormone (1-84), TRAP5b=tartrate resistant acid phosphatase isoform 5b

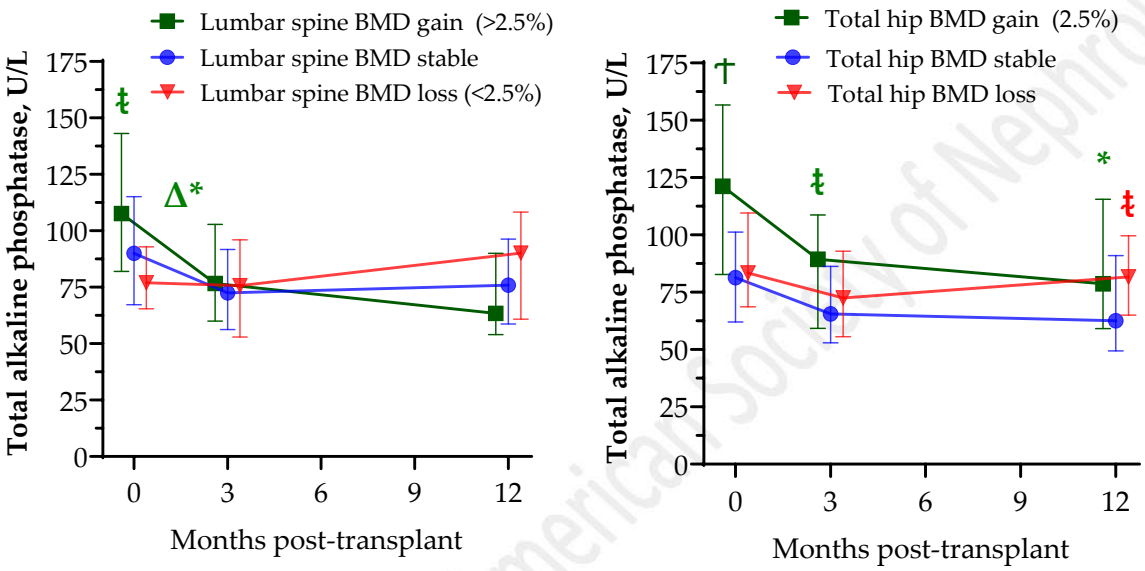
Supplemental **Figure** 1 Flow chart of selection of patients for this study.



Supplemental **Figure** 2 Trajectories of parathyroid hormone (PTH) and bone turnover markers (median with IQR) in patients who lost, remained stable, or gained total hip bone mineral density (BMD) by a 2.5% cutoff; * marks P<0.05, ŧ P<0.01 and ŧ P<0.001 by Wilcoxon rank-sum test compared to stable group. PINP=pro-collagen type I N-terminal pro-peptide, TRAP5b=tartrate resistant acid phosphatase isoform 5b



Supplemental **Figure** 3 Trajectories of total alkaline phosphatase in patients who lost, remained stable or gained bone mineral density (BMD) at the lumbar spine and total hip by a 2.5% cutoff; * marks P<0.05, ŧ P<0.01 and ϯ P<0.001 by Wilcoxon rank-sum test compared to stable group.



# Associations of changes in bone turnover markers with change in bone mineral density in kidney transplant patients

CJASN
Clinical Journal of the American Society of Nephrology

**Kidney transplant recipients**
**n = 209**

**Bone mineral density**
**0 and 12 months**
**post-transplant**

**PTH and**
**bone turnover markers**
**(BALP, intact PINP, TRAP5b)**
**0, 3, and 12 months**
**post-transplant**

**Paired transiliac bone**
**biopsies in a subset**
**n = 49**

**Compared to patients with no change in bone mineral density:**

**Patients who GAINED bone density**

| | |
|---|---|
| **At transplant** | **PTH** (236 *vs.* 136 pg/mL)<br>↑ **BALP** (31.7 *vs.* 18.8 µg/L)<br>↑ **PINP** (121.9 *vs.* 70.4 µg/L) |
| **3 m post-transplant** | Greater decrease in<br>↓ **BALP** (-40 *vs.* -21%)<br>↓ **PINP** (-43 *vs.* -13%) |
| **12 m post-transplant** | ↓ **PINP** (36.3 *vs.* 60 µg/L) |

**Patients who LOST bone density**

| | |
|---|---|
| **3 m post-transplant** | Lower decrease or increase<br>**TRAP5b** (-27 *vs.* -43%)<br>↑ **PINP** (+22 *vs.* -13%) |
| **12 m post-transplant** | ↑ **TRAP5b** (3.89 *vs.* 3.16 µg/L)<br>**PINP** (83.7 *vs.* 60 µg/L) |

**If none of the biomarkers decreased by the least significant change at 3 m, an almost 2-fold higher occurrence of bone loss was seen at 12 m post-transplant**

**Conclusions:** Bone loss after kidney transplantation was highly variable. Resolution of high bone turnover, as reflected by decreasing bone turnover markers, associated with bone mineral density gain, while ongoing bone resorption associated with bone loss.

Hanne Skou Jørgensen, Kathleen Claes, Dieter Smout et al. ***Associations of Changes in Bone Turnover Markers with Change in Bone Mineral Density in Kidney Transplant Patients***. CJASN doi: 10.2215/CJN.0000000000000368.
**Visual Abstract by José A. Moura-Neto, MD, FASN, FRCP**