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Serial changes in bone histomorphometry following kidney transplantation

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Abstract

Background: Over the past decade, the management of chronic kidney disease - mineral and bone disorder has changed substantially, altering the pattern of bone disease in chronic kidney disease. We aimed to evaluate the natural history of kidney bone disease in contemporary kidney transplant recipients and dialysis patients.

Design, settings, participants, and measurement: Sixty-one dialysis patients referred to kidney transplantation participated in this prospective cohort study during November 2009 and December 2010. We performed baseline bone biopsies while the patients were on dialysis and repeated the procedure in 56 patients two years after kidney transplantation or two years after baseline if transplantation was not performed. Measurements of mineral metabolism and bone turnover as well as dual energy X-ray absorptiometry scans were obtained concurrently.

Results: 37 of 56 participants received a kidney transplant, of which 27 underwent successful repeat bone biopsy. The proportion of patients with high bone turnover declined from 63% at baseline to 19% two years after kidney transplantation, while the proportion of those with low bone turnover increased from 26% to 52%. Of 19 participants remaining on dialysis after two years, 13 underwent successful repeat biopsy. The proportion of patients remaining on dialysis with high bone turnover decreased from 69% to 31%, and low bone turnover increased from 8% to 38%. Abnormal bone mineralization increased in transplant recipients from 33% to 44%, but decreased in patients remaining on dialysis from 46% to 15%. Trabecular bone volume showed little change after transplantation, but low bone volume increased in patients remaining on dialysis. Bone mineral density did not correlate with histomorphometric findings.

Conclusions: Bone turnover decreased over time both in patients remaining on dialysis and in kidney transplant recipients. Bone mineral density and bone biomarkers were not associated with bone metabolism changes detected in bone biopsy.

INTRODUCTION

Despite successful kidney transplantation metabolic bone disorder persists in a substantial number of kidney transplant recipients (1). The bone damage in transplant recipients is due to pre-existing bone disease associated with kidney disease, and especially bone loss is further aggravated by immunosuppressive therapy (2). Also other factors independent of kidney transplantation (*e.g.* sex, diabetes, dialysis duration, altered vitamin D metabolism, and hypogonadism) impair post-transplant bone health. The fracture risk in kidney transplant patients is markedly increased and the overall risk is 4-fold higher than in healthy individuals (3) and 30% higher than in dialysis patients during the first three years post-transplantation (4). Approximately 10% of kidney transplant recipients experience a fracture during their lifetime. Besides fractures, bone disease in patients with kidney disease may cause osteonecrosis or bone pain and aggravate cardiovascular disease, thus contributing to poor allograft and patient outcomes (5, 6).

Changes in clinical treatment practice of chronic kidney disease - mineral and bone disorder over the last decade have considerably altered the pattern of bone disease in patients with kidney disease. Low turnover bone disease is currently the most common bone histomorphometric pattern in dialysis patients (7-11). With regard to immunosuppressive therapy, there has been growing interest in steroid-sparing regimens in recent years. It is plausible that these alterations have an influence on post-transplant bone patterns.

Transiliac crest bone biopsy is considered the gold standard in assessment of bone metabolism. Although some biochemical markers of bone turnover are able to discriminate bone turnover, they are not sufficiently accurate to guide treatment decisions (8). The prevalence and histologic pattern of contemporary post-transplant bone disease are not well characterized; to date, only a few bone biopsy studies have been conducted on kidney transplant recipients.

The improvement of bone mineral metabolism after kidney transplantation was anticipated, and thus the primary outcome of this prospective, observational cohort study was a decrease of bone turnover after kidney transplantation. The other aim of this study was to characterize the evolution of bone disease in wait-listed dialysis patients.

METHODS

Participants and study protocol

One hundred and twenty-three dialysis patients were screened and seventy-eight patients enrolled in this study upon receiving their informed consent to participate. The study protocol was approved by the Research Ethics Board of the Division of Medicine, Helsinki University Central Hospital (approval no. 446/13/03/01/08) and conducted in accordance with the Declaration of Helsinki. All of the screened patients came from the Hospital District of Helsinki and Uusimaa, had been on maintenance dialysis at least one month and were planned or wait-listed for kidney transplantation from deceased donor. During the study patients were treated and followed up in our institution. Enrollment and baseline studies were performed between November 2009 and December 2010 and follow-up studies were done between January 2012 and May 2014.

Besides maintenance dialysis and planned kidney transplantation, inclusion criteria comprised age ≥ 18 years, willingness and mental competence to sign informed consent. Exclusion criteria were severe illnesses that might hinder kidney transplantation *(e.g*. malignancy or active infection) and pregnancy.

In this prospective longitudinal study iliac crest bone biopsy, dual-energy X-ray absorptiometry (DXA) scans, and blood draws for measurement of plasma biochemical markers were performed at baseline and repeated after two years in patients who continued on dialysis. In those patients who received a kidney transplant the second biopsy was taken two years after kidney transplantation. Blood samples were collected within one month and DXA scans within three months of bone

biopsy. Demographic characteristics along with data on immunosuppressive and mineral metabolism therapy were obtained from electronic patient records.

The clinical and research activities reported here are consistent with the Principles of the Declaration of Istanbul as outlined in the `Declaration of Istanbul on Organ Trafficking and Transplant Tourism´. The baseline results have been published elsewhere (12).

Immunosuppressive and mineral metabolism therapy

The treating physician prescribed the medication for mineral metabolism disorder according to KDOQI and KDIGO recommendations. At the time of transplantation mineral metabolism therapy was stopped and continued if considered necessary by the treating physician.

At baseline, dialysate calcium concentration was 1.25 mmol/l (2.5 mEq/l) in hemodialysis patients and 1.25-1.35 (2.5-2.7 mEq/l) in peritoneal dialysis patients.

All patients were treated with triple immunosuppressive therapy consisting of mycophenolate mofetil, calcineurin inhibitor (cyclosporine or tacrolimus) and methylprednisolone. Intravenous methylprednisolone was given 250 mg during transplantation and then 1mg/kg orally from the day after transplantation. Three weeks after transplantation methylprednisolone was tapered to a dose of 12mg/day. At around the third and the sixth month methylprednisolone dose was tapered to 8 and 6-4 mg, respectively. In the patients treated with tacrolimus, the dose of intravenous methylprednisolone was 125 mg and oral methylprednisolone was tapered to 8 and 4 mg, respectively, at around one and six months after transplantation. Cyclosporine and tacrolimus dose were altered according to through levels and mycophenolate mofetil dosage were adjusted in case of side effects. According to the protocol in our clinic at the time of the study, methylprednisolone was discontinued one year after engraftment, except in patients with a history of rejection**.**

Bone biopsy and histomorphometric analysis

Patients received labeling with tetracycline (3x 500 mg/day) over two separate 2-day periods. The interlabel time was 10 days. After local anesthesia, bone samples were obtained by using vertical technique and 8G – 11G needle (T-Lok, Angiotech, Reading, PA, USA) 5-14 days after the second labeling.

The method for quantitative histomorphometry has been described elsewhere (12). In brief, samples were fixed in 70% ethanol and embedded in polymethylmetacrylate. For the determination of static and dynamic parameters, we used stained and unstained 5 µm sections, respectively. Histomorphometric analyses were performed at standardized sites in cancellous bone by either of two experienced histomorphometrists (ISB or HK) at x200 magnification using a semiautomatic image analyzer (BioquantOsteoII, Bioquant Image Analysis Corporation, Nashville, TN, USA). The histomorphometry readers were blinded to the source of the samples.

The parameters assessed were mineralized and unmineralized tissue and bone volume. Osteoid surfaces, measured osteoid volume, osteoid thickness, and eroded surfaces were identified. Osteoblast- and osteoclast-covered trabecular surfaces were measured, as were trabecular thickness, trabecular number, and trabecular separation. Dynamic indices were defined using fluorescence microscopy. Mineralizing surfaces were measured and the mineral apposition rate determined. All results are reported based on established nomenclature (13).

Bone turnover was assessed by the bone formation rate per bone surface (BFR/BS) and activation frequency (Ac.f.). Reference values for normal Ac.f. are 0.49-0.72/year and for BFR/BS 18-38 μm^3 /μm^2/year (14). In the absence of labeling, the assessment of bone turnover was made using osteoblastic and osteoclastic surfaces and the reference values were applied as Z-scores based on Rehman *et al.* (15). Abnormal mineralization was identified when osteoid surface/bone surface in lamellar bone was more than 2 SD over the mean (14) and mineralization lag time in lamellar bone

was above 100 days (13). The normal range of cancellous bone volume/tissue volume (BV/TV) was 16.8-22.9% (15).

Bone densitometry

DXA scan obtained with Lunar Prodigy scanner (GE Healthcare, Little Chalfont, UK) was used for the measurements of bone mineral density at the lumbar spine and femoral neck. The coefficients of variation for DXA measurements were lumbar spine 1% and femoral neck 1.5%. The bone mineral density values were given in g/cm², and individual patient´s results were expressed as T-scores.

Biochemical analyses and other data

Routine methods were used to analyze plasma inorganic phosphate and ionized calcium. Until October 2012 25-hydroxyvitamin D was analyzed using in-house high performance liquid chromatography (HPLC) assay and therafter using electrochemiluminescence (ECLIA) assay (Elecsys 2010 analyzer, Roche Diagnostics GmbH, Mannheim, Germany). HPCL as well as ECLIA measurements include both D2 and D3 metabolites. 1,25-dihydroxyvitamin D was analyzed using chemiluminescence 1,25 dihydroxyvitamin D assay (CLIA) and Liaison XL analyzer (DiaSorin S.p.A. , Saluggia, Italy). Intact parathyroid hormone (PTH) levels were studied using a chemiluminescence immunoassay (Roche Elecsys 2010 Analyzer). Levels of bone-specific alkaline phosphatase (BsAP) were measured by spectrophotometric assay (IDS-iSYS Ostase® BAP) (Immunodiagnostic Systems Ltd., London, UK) and osteocalcin by electrochemiluminescence immunoassay (N-MID® Osteocalcin, Roche Diagnostics GmbH, Mannheim, Germany). The estimated glomerular filtration rate (GFR) was calculated using the CKD-EPI equation.

Statistical analysis

We used Mann-Whitney U-test and Chi-Square test for continuous and categorical variables, respectively, to compare differences in parameters between study groups. Kendall's tau correlation coefficient was applied to determine correlations between continuous variables. To compare differences in categorical and continuous variables at baseline and study-end, we used McNemar's test and Wilcoxon signed-rank test, respectively. For characteristics that could not regress after transplantation (*e.g.* history of fracture), p-values were not calculated. For statistical analysis, we divided bone biopsy findings into three subgroups according to bone turnover (low, normal, and high) and determined mineralization and bone volume (TMV classification) (14). For comparisons between TMV groups, we used Kruskal-Wallis test. We performed all analyses with SPSS for Windows (version 24, SPSS, Chicago, IL, USA), and all values are presented as median and interquartile range (25-75 percentiles).

RESULTS

Figure 1 presents the flowchart of the study cohort. At baseline 17 of 78 consented patients were excluded. Two patients died and three withdrew their consent after baseline, thus 56 patients were included for the follow-up study. After two years, 37 patients had received a kidney transplant and 29 of them (78%) consented to a second biopsy. Thirteen patients of 19 patients remaining on dialysis (68%) complied for re-biopsy. Bone biopsy sample quality was adequate for histomorphometric analysis in 27 kidney transplant recipients and in all 13 patients remaining on dialysis.

Characteristics of kidney transplant recipients

Demographic characteristics and details of immunosuppressive and mineral metabolism therapy of kidney transplant recipients with or without representative repeat bone biopsies at baseline and follow-up are displayed in Table 1. The patients without repeat bone biopsies were older and had more coronary artery disease. At the time of the second biopsy, the median age of kidney transplant patients with repeated bone biopsy (interquartile range) was 50 (43-62) years; 22 patients (81%) were men and 11 (41%) had diabetes.The median dialysis duration was 15 (7-29) months before the first biopsy, which was taken the median of nine (5-22) months before

transplantation. The median vintage of dialysis treatment was 28 (18-45) months. The median time between first and second biopsies was 36 (30-47) months. The median time for the second biopsy after kidney transplantation was 25 (23-26) months.

At baseline the median daily dosages for calciumcarbonate, alphacalcidol, paricalcitol and cinacalcet were 1500 (1000-2000) mg, 0.4 (0.25-0.5) µg, 2.1µg and 30 mg, respectively. After two years the median daily dosages for calciumcarbonate, alphacalcidol, paricalcitol and cinacalcet were 500 (500-1250) mg, 0.4 (0.2-1.0) µg, 0µg and 75 (40-90) mg, respectively. Two patients experienced acute rejection after engraftment and were treated successfully with corticosteroid pulse therapy. The cumulative dose of methylprednisolone per patient was 2009 (1464-2600) mg and 2176 (1464-2854) mg over the first and the second year, respectively. The median dose of cyclosporine was 175 (125-200) mg/day and tacrolimus 3.1 (3.0-4.4) mg/day. Twenty-six patients used mycophenolate daily with the median dose 1000 (500-1000) mg.

Characteristics of patients who continued on dialysis

Demographic characteristics, details and median doses of mineral metabolism drugs of patients remaining on dialysis patients with or without representative repeat bone biopsies at baseline and follow-up are displayed in Supplemental Tables 1 and 2. The median age of dialysis patients was 55 (interquartile range) (48-67) years at the time of the second biopsy and the median duration of dialysis was 58 (39-114) months. Previous parathyroidectomy was done to six (22%) kidney transplant recipients and to six (46%) patients who remained on dialysis, but the difference did not reach statistical significance.

Bone histomorphometry:

Bone histomorphometry of kidney transplant recipients

Bone biopsy findings at baseline and two years after kidney transplantation are shown in Figure 2.

In accordance with TMV classification, the proportion of patients with low bone turnover increased from 26% to 52%, while the proportion of those with high bone turnover decreased from 63% to 19% two years after kidney transplantation. The proportion of patients with normal bone turnover was 11% at baseline and 29% after kidney transplantation. Abnormal mineralization and low bone volume increased from 33% to 44% and from 33% to 37% of patients, respectively. However, bone volume normalized in six patients after kidney transplantation, but *de novo* bone loss was detected in seven patients (26%). We did not identify any factors associated with new bone loss. We did not recognize any differences in bone histomorphometric parameters between patients in different treatment modalities prior to transplant.

Bone histomorphometric parameters in kidney transplant recipients are shown in Table 2. At baseline osteoid thickness in kidney transplants recipients without repeat biopsies was significantly lower compared to transplant recipients with repeat biopsies. After transplantation bone formation rate/bone surface, osteoblastic and osteoclastic surface/bone surface significantly decreased and osteoid thickness increased in patients with repeat biopsies.

Bone histomorphometry of patients who continued on dialysis

Repeat bone biopsy findings and bone histomorphometric parameters of patients remaining on dialysis are presented in Figure 3 and Supplemental Table 4. The proportion of high bone turnover in patients remaining on dialysis decreased from 69% to 31%, and low bone turnover increased from 8% to 38%. Abnormal bone mineralization decreased in patients remaining on dialysis from 46% to 15%.

Biochemical findings and correlations

Table 3 and Supplemental Table 3 present key laboratory values at baseline and follow-up for kidney transplant recipients and patients remaining on dialysis with repeat bone biopsies.

Plasma ionized calcium levels increased, while levels of inorganic phosphate, PTH, and osteocalcin significantly decreased. At baseline, 19 transplant candidates (70%) presented with vitamin D deficiency (25-hydroxyvitamin D < 20 ng/L), but after kidney transplantation both calcidiol and calcitriol levels increased significantly. Eleven kidney transplant recipients had hyperparathyroidism (PTH level higher than 1.5-fold the upper normal limit) and nine were hypercalcemic (ionized calcium > 5.2 mg/dl/pH 7.4). Five (of 11) kidney transplant recipients presented with hypercalcemic hyperparathyroidism, and one of these patients had high bone turnover. High bone turnover was found in one hypercalcemic kidney transplant recipient and five hypercalcemic patients had low bone turnover.

No statistically significant differences emerged in bone metabolic markers between turnover groups in kidney transplant recipients. PTH levels were significantly lower (P=0.03) in patients with previous parathyroidectomy. In transplanted patients, PTH levels correlated with osteoclastic surface/bone surface and osteoid thickness (R=0.416, P=0.004 and R=0.431, P=0.003, respectively). Osteocalcin levels correlated with osteoblastic and osteoclastic surface/bone surface (R=0.418, P=0.005 and 0.484, P=0.001, respectively), osteoid surface/bone surface (R=0.333, P=0.02) as well as with osteoid thickness (R=0.391, P=0.008). BsAP levels correlated with osteoid thickness and osteoclastic surface/bone surface (R=0.323, P=0.03 and R=0.305, P=0.04, respectively).

Bone mineral density and bone volume

Due to logistical hurdles repeat DXA scans were available in a subset of patients only. At baseline and follow-up 26 (96%) and 22 (81%) of biopsied kidney transplant recipients, respectively, had DXA imaging. Osteoporosis (T-score < -2.5) in lumbar spine and femoral neck was found in three (12%) and five (19%) of kidney transplant recipients at baseline, respectively. After transplantation, none of the patients had osteoporosis in lumbar spine, but six patients (27%) had osteoporotic femoral neck T-scores. Femoral neck T-score was significantly lower (P=0.03) after kidney

transplantation (Table 2).

Repeat DXA scans were available for 11 biopsied patients who continued on dialysis (85%). Femoral neck T-score significantly decreased (P=0.04) after two years on dialysis (Supplemental Table 4).

Bone loss detected in bone biopsy was not associated with age, sex, BMI, dialysis duration, or cumulative dose of glucocorticoid in either group. The distribution of low bone volume in bone biopsy did not differ between turnover groups at baseline or follow-up.

We could not demonstrate statistically significant associations between BV/TV and either T-scores or bone mineral density ($g/cm^{2)}$ in lumbar spine or femoral neck in either group.

DISCUSSION

Our results confirm previous observations of a decline in bone formation and mineralization in the late post-transplant period (16-23). Although bone loss showed little change overall, new bone loss was detected in one-quarter of the patients after kidney transplantation. Transplant recipients experienced more fractures than patients who remained on dialysis, but the difference was not statistically significant. Five kidney transplant recipients presented with hypercalcemic hyperparathyroidism, but only one of them had high bone turnover. Bone formation and mineralization declined also in dialysis patients, and the proportion of patients with bone loss doubled in two years. We could not demonstrate correlations between bone metabolic markers or bone mineral density and bone histomorphometric findings in either group.

In contrast to recent large bone biopsy studies of dialysis patients (7, 8, 24), low bone turnover was less frequent in our cohort despite the detected decrease in bone formation. In agreement with previous bone biopsy studies in kidney transplant recipients, bone formation declined after transplantation; nevertheless, 48% of our patients had normal or high turnover (2, 25, 26). Earlier studies are, however, poorly comparable in terms of patient characteristics, immunosuppressive regimen, and timing of biopsy sampling after

engraftment. Three bone biopsy studies in contemporary kidney transplant recipients were identified. The proportion of patients with high bone turnover in the present study confirms the findings of Neves *et al.*, despite notable differences in case mix (20), but differs substantially from the studies of Evenepoel *et al.* and Carvalho *et al.* (21, 27). Sex distribution, dialysis duration, and immunosuppressive therapy were comparable to those in Evenepoel *et al.* (21), but our patients were slightly younger and had a greater proportion of diabetes. Despite the history of parathyroidectomy in almost one-third of the patients included, 80% of the transplant candidates had normal or high turnover bone disease. The pattern of pre-existing bone disease, differences in study cohort as well as original kidney disease, and timing of bone biopsy probably account for these disparate findings. Also dialysis modalities (with 48% of biopsied patients on peritoneal dialysis at baseline) in addition to variations in dialysate calcium concentration, dialyzers, and peritoneal fluids might explain the overt differences between these studies. The decline in bone formation in dialysis patients suggests oversuppression of secondary hyperparathyroidism with medication. Similar to previous observations (19, 21), transplanted patients with hypercalcemic hyperparathyroidism presented also with low or normal bone turnover. This observation implies, as already suggested by Borchhardt *et al.* (19), that hypercalcemic hyperparathyroidism may result from increased tubular calcium reabsorption as well as increased bone turnover.

Earlier studies have reported 17-88% prevalence of delayed bone mineralization in kidney transplant recipients (2, 17, 20, 21, 25, 28). In our study, more than one-third of transplant candidates had abnormal mineralization, which further declined after kidney transplantation. Since nutritional vitamin D levels improved after kidney transplantation, there is no apparent explanation for defective mineralization. In contrast to kidney transplant recipients, the abnormal mineralization decreased in dialysis patients. Substitution with vitamin D receptor activators and increasing use of nutritional vitamin D substitution over the follow-up period may explain the augmented levels of calcitriol and calcidiol detected in dialysis patients.

As previously noted (2, 20, 26, 27), more than one-third of our transplanted patients presented with low trabecular bone volume at baseline and bone loss showed little change in the late kidney transplantation period

despite the use of glucocorticoids. The proportion of dialysis patients with bone loss doubled in two years. In contrast to previous studies (2, 21), however, cumulative corticosteroid dose was not associated with new bone loss in kidney transplant recipients.

The main strength of this study is the longitudinal design with repeat bone biopsies in both groups. No selection criteria other than being an adult dialysis patient referred to transplantation were applied. Thus, the study population represents common kidney transplant candidates in our clinic. Repeat DXA scans were available for 81% of transplant recipients, allowing comparison with bone histomorphometry. The number of follow-up biopsies was, however, lower than expected. Due to the invasive nature of bone biopsy, 25% of the patients were reluctant to undergo the second sampling without clinical suspicion of bone disease. Single-center data and the limited number of follow-up bone biopsy samples restrict the statistical power of this study and extrapolation of results to all kidney transplant recipients. Another limitation is the lack of analysis of cortical bone, which might have offered additional information about bone metabolism (29-31).

As recently stated, more widespread implementation of bone biopsies in clinical practice would be beneficial for evaluation of the pattern of post-transplant bone disease (32).

Our study indicates that bone turnover decreases in kidney transplant candidates, both in transplant recipients and in those remaining on dialysis. The observation that kidney transplant recipients with hypercalcemic hyperparathyroidism might have normal or low bone turnover is noteworthy in clinical decision-making. The lack of association with biomarkers and bone metabolism emphasizes the importance of bone biopsy in differentiation of post-transplant bone disease.

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The principal investigators (SK, LM, and EH) were solely responsible for the design, execution, analysis, and publication of the study. There were no restrictions on publication and all data were maintained and analyzed solely by the authors. PF, ISB, and HK declared no competing interests.

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Supplemental material

Supplemental Table 1. Clinical characteristics and therapy of participants in a longitudinal bone biopsy study who remained on dialysis.

Supplemental Table 2. Median daily doses of mineral metabolism medication in patients remaining on dialysis.

Supplemental Table 3. Biochemical parameters in 13 patients remaining on dialysis with repeat bone biopsy at baseline and after two further years of dialysis.

Supplemental Table 4. Bone histomorphometric parameters of participants in a longitudinal bone biopsy who remained on dialysis.

Contributors

SK, LM, and EH participated in research design and execution, data analysis, and writing of the article. PF participated in data analysis and writing of the article. ISB and HK participated in data analysis and writing of the article.

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Table 1. Clinical characteristics of participants in a longitudinal bone biopsy study who underwent kidney transplantation

Clinical characteristics	Kidney transplant recipients with repeat bone biopsy At baseline N=27 (%)	Kidney transplant recipients without repeat bone biopsy At baseline N=10 (%)	Kidney transplant recipients with repeat bone biopsy Two years after kidney transplantation N=27 (%)
Men	22 (81)	8(80)	
Median age[IQR], yr	48 [41-60]	58 [52-62]	50 [43-62]
Hemodialysis	12 (44)	4 (40)	
Diabetes	9 (33)	3 (30)	11 (41)
Previous kidney transplantation	4 (15)	0	4 (15)
Previous parathyroidectomy	4 (15)	1 (10)	6 (22)
Left ventricular hypertrophy	17 (63)	5 (50)	17 (63)
Coronary artery disease	4 (15)	4 (40)	5 (19)
CABG/PCI	4 (15)	4 (40)	5 (19)
Heart failure	2 (7)	0	2 (7)
Occlusive arterial disease	4 (15)	2 (20)	6 (22)
Smoking (current and previous)	12 (44)	3 (30)	12 (44)
Transient ischemic attack/stroke	4 (15)	1 (10)	6 (22)
Previous fracture	5 (19)	0	9 (33)
Calcium carbonate	23 (85)	9 (90)	13 (48)
Non-calcium containing phosphate binder	12 (44)	1 (10)	2 (7)
Active vitamin D	24 (89)	7 (70)	9 (33)
Cinacalcet	2 (7)	0	4 (15)
Bisphosphonate	2 (7)	1 (10)	2 (7)
Calcineurin inhibitor	0	0	27 (100)
Current corticosteroid use	2 (7)	2 (20)	4 (15)

IQR, interquartile range; CABG, coronary artery bypass graft; PCI, percutaneous intervention

Table 2. Bone histomorphometric parameters of participants in a longitudinal bone biopsy study who underwent kidney transplantation

		Kidney transplant recipients with repeat bone biopsy (N=27)		
Bone parameter	**All kidney transplant recipients At baseline N=37**	**At baseline**	**Two years after kidney transplantation**	**P value ***
Bone formation rate/bone surface (mm^3/cm^2/year)	21.1 (13.7- 44.2)[a]	20.3 (13.4- 43.4)[c]	10.4 (6.1- 27.1)	0.02 *
Activation frequency ($^{-}$ 1/year)	0.6 (0.2-1.1)[a]	0.5 (0.1-1.0)[c]	0.3 (0.1-0.6)	0.13
Osteoblastic surface/bone surface (Z-score)	0.2 (-3.2-5.5)[b]	1.6 (-2.5-6.2)	-2.2 (-2.6-(-0.6))	0.008*
Osteoclastic surface/bone surface (Z-score)	3.2 (-2.1-13.8)[b]	9.9 (-1.5-13.8)	-0.2 (-2.3-2.5)	0.002*
Osteoid surface/bone surface (%)	39.1(32.9-53)[b]	40.2 (34-53.9)	34.1 (20.4-46.1)	0.08
Osteoid thickness (µm)	6.9 (5.7-8.6)[b]	7.3 (6.3-9.1)	8.9 (7.6-10.6)	0.02*
Mineralization lag time (d)	40.6 (27.6-110.2)[a]	47.7 (28.7-117.5)[c]	69.3 (42.8-162.8)	0.66
Bone volume/ tissue volume (%)	22.3 (14.3-26.5)[b]	22.2 (13.9-26.8)	20.7 (13.6-27.8)	0.98
Lumbar spine T-score	-0.7 (-1.6 to 0.4)[d]	-0.8 (-1.6 to 0.3)[e]	-0.4 (-1.3 to 0.5)[f]	0.41
Femoral neck T-score	-1.1 (-1.8 to -0.1)[d]	-1.6 (-2.0 to -0.8)[e]	-1.9 (-2.7 to -1.2)[f]	0.03*

All values are expressed as median + (interquartile range)

* P- value compares values two years after transplantation to values at baseline among kidney transplant recipients with repeat bone biopsy

a n=25; b n=32; c n=23; d n=36; e n=26, f n=22

Table 3. Biochemical parameters in 27 kidney transplant recipients with repeat bone biopsy at baseline and two years after kidney transplantation.

Variables (normal range)	**Baseline**	**Two years after kidney transplantation**	**P value**
creat mg/dl (0.68-1.13)	N/A	1.38 (1.09-1.98)	N/A
GFR mL/min/1.73m²	N/A	54 (38-70)	N/A
Ca^{2+} mg/dl/pH7.4 (4.64-5.2)	4.8 (4.48-5.08)	5.04 (4.88-5.28)	0.005*
inorganic phosphate mg/dl (2.19 -3.81)	6.04 (5.51-7.09)	2.97 (2.51-3.60)	<0.001*
PTH pg/ml (15-65)	248 (142-394)	90 (50-168)	0.001*
25-hydroxyvitamin D ng/ml (>16)	12.8 (5.8-23.6)	23.2 (16.8-31.8)	<0.001*
1,25 vitamin D pg/ml (22.9-76.3)	12.1 (8.8-18.3)	54.8 (18-67.5)	<0.001*
bone-spesific alkaline phosphatase µg/l (6-15)	12 (9.4-18.5)	14 (10.5-27)	0.14
osteocalcin ng/ml (14-46)	230 (94-290)	41 (28-66)	<0.001*

All values are expressed as median + (interquartile range)

*Statistically significant

N/A, not applicable; creat, creatinine; GFR, glomerular filtration rate; Ca^{2+}, ionized calcium; PTH, parathyroid hormone; 1,25 vitamin D, 1, 25-dihydroxyvitamin.

Conversion factors for units: creat in mg/dl to µmol/l x 88.4, plasma ionized calcium in mg/dl to mmol/l x 0.25, inorganic phosphate in mg/dl to mmol/l x 0.3229. Intact parathyroid hormone levels pg/ml and ng/l are equivalent. 25-hydroxyvitamin D in ng/ml to nmol/l x 2.496, 1,25-dihydroxyvitamin D pg/ml to pmol/l x 2.4. Osteocalcin ng/ml and µg/l are equivalent.

FIGURE LEGENDS

Fig. 1

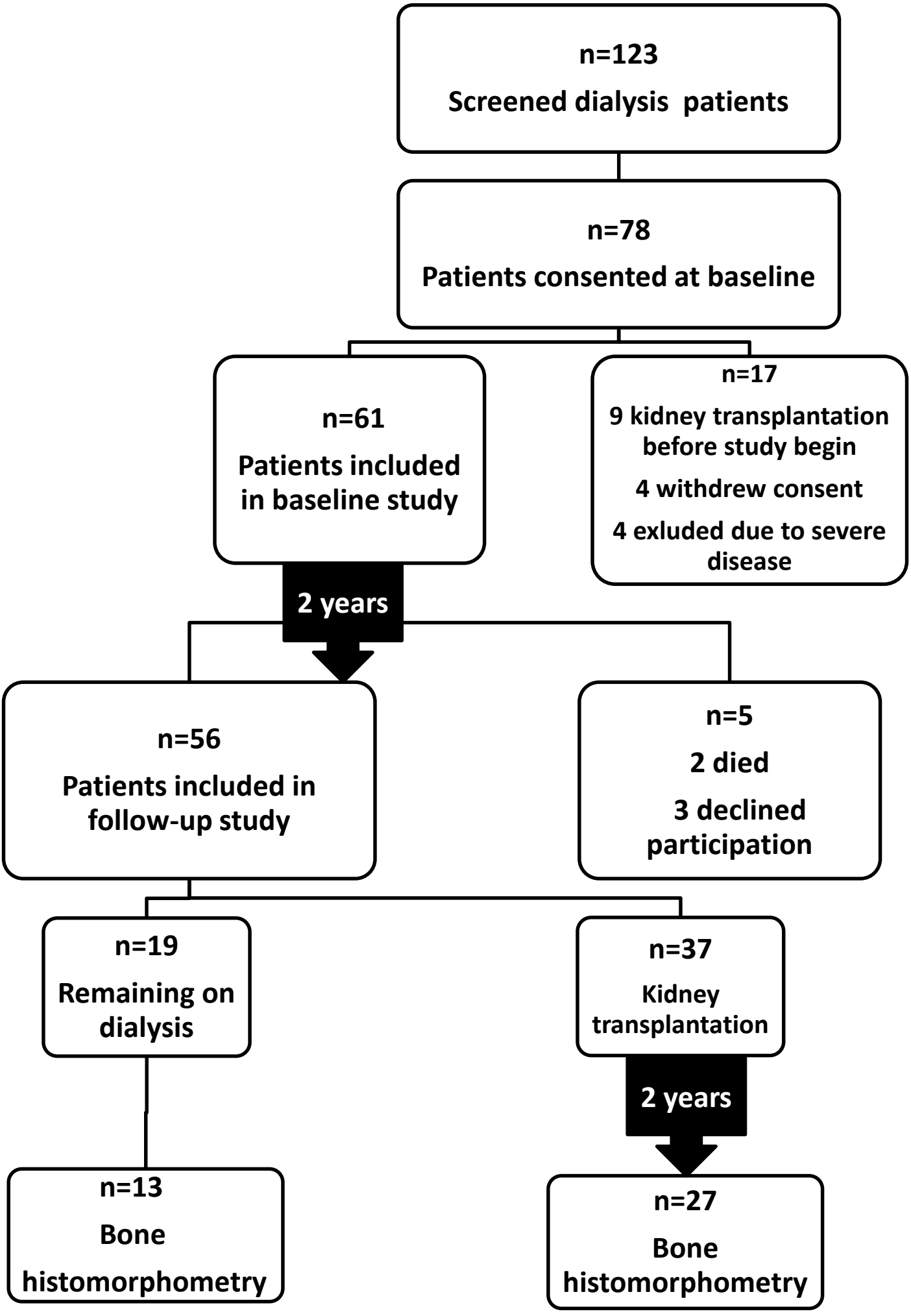
Patient recruitment and participation

Fig. 2

TMV classification at baseline and two years after kidney transplantation (n=27)

Fig. 3

TMV classification at baseline and after two further years of dialysis (n=13)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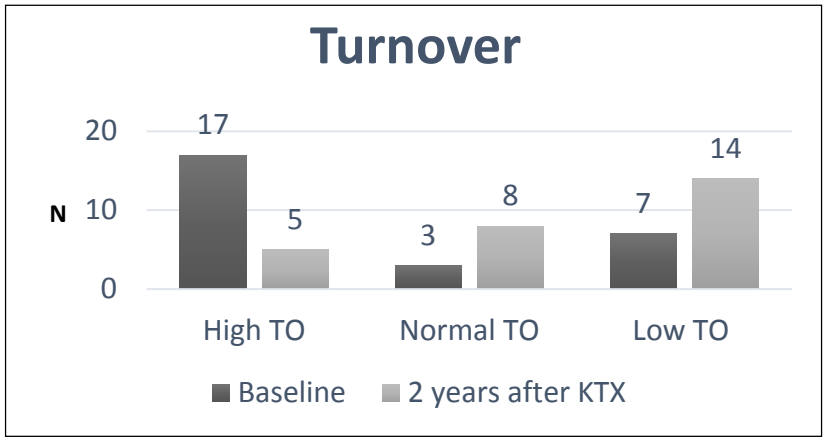
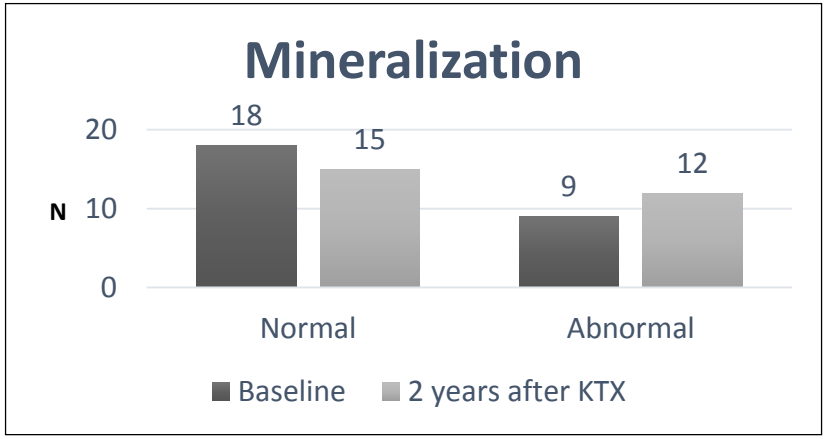
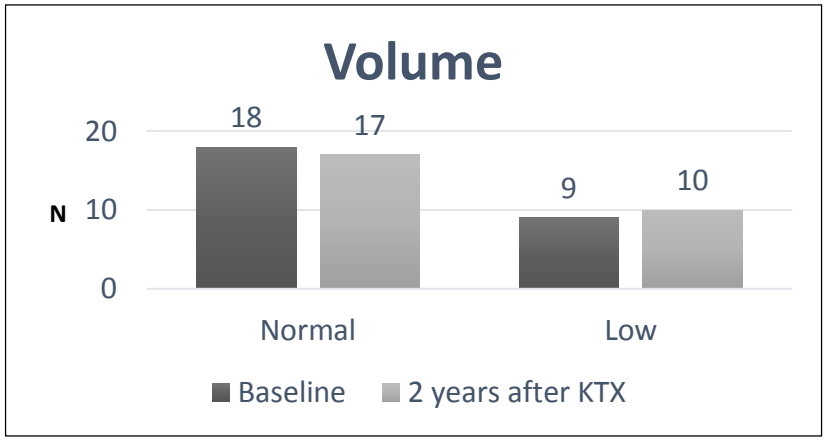
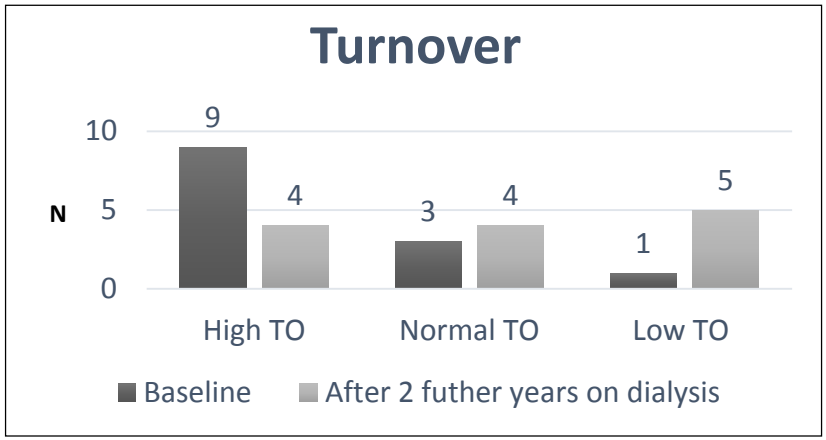
Screened dialysis patients
n=78
Patients consented at baseline
n=61
Patients included in baseline study
n=17
9 kidney transplantation before study begin
4 withdrew consent
4 exluded due to severe disease
2 years
n=56
Patients included in follow-up study
n=5
2 died
3 declined participation
n=19
Remaining on dialysis
n=37
Kidney transplantation
2 years
n=13
Bone histomorphometry
n=27
Bone histomorphometry

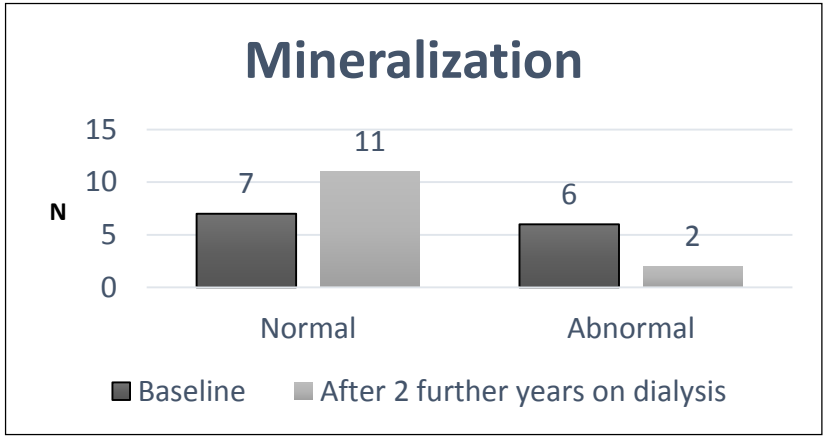


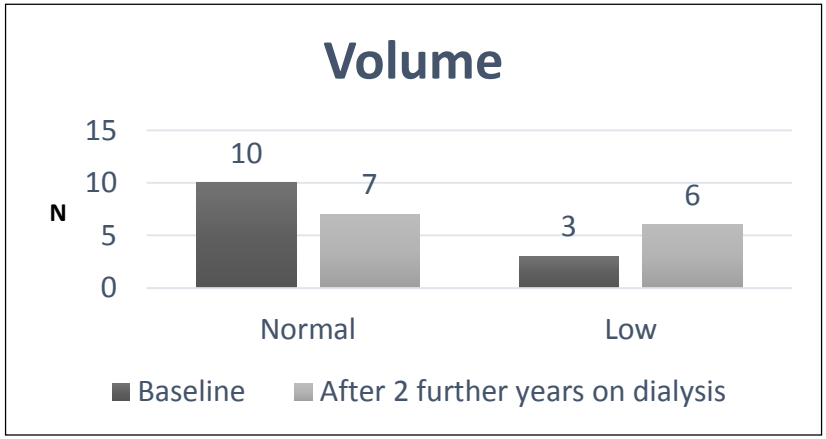




N=27 at baseline and after kidney transplantation; TO, turnover; KTX, kidney transplantation







n=13 at baseline and after 2 further years on dialysis; TO, turnover

Supplemental material –Table of contents

Supplemental Table 1. Clinical characteristics and therapy of participants in a longitudinal bone biopsy study who remained on dialysis

	Patients remaining on dialysis with repeat bone biopsy (N=13)		Patients remaining on dialysis without repeat bone biopsy (N=6)
Variables (%)	**At baseline**	**Two years after baseline**	**At baseline**
Men	8 (62)		3 (50)
Median age [IQR], yr	53 [46-65]	55 [48-67]	47 [32-60]
Hemodialysis	9 (69)	9 (69)	4 (67)
Diabetes	3 (23)	3 (23)	1 (17)
Previous kidney transplantation	6 (46)	6 (46)	1 (17)
Previous parathyroidectomy	2 (15)	6 (46)	1 (17)
Left ventricular hypertrophy	4 (31)	4 (31)	3 (50)
Coronary artery disease	0	0	0
CABG/PCI	0	0	0
Heart failure	0	0	0
Occlusive arterial disease	2 (15)	2 (15)	0
Smoking (current and previous)	8 (62)	8 (62)	3 (50)
Transient ischemic attack/stroke	1 (8)	2 (15)	0
Previous fracture	4 (31)	4 (31)	0
Calcium carbonate	11 (85)	10 (77)	5 (83)
Non-calcium containing phosphate binder	8 (62)	9 (69)	3 (50)
Active vitamin D	9 (69)	10 (77)	6 (100)
Cinacalcet	1 (8)	3 (23)	0
Bisphosphonate	0	1 (8)	2 (33)
Current corticosteroid use	1 (8)	2 (15)	1 (17)

IQR=interquartile range, CABG=coronary artery bypass graft, PCI= percutaneous coronary intervention

Supplemental Table 2. Median daily doses of mineral metabolism medication in patients remaining on dialysis

	Patients remaining on dialysis with repeat bone biopsy (N=13)		**Patients remaining on dialysis without repeat bone biopsy (N=6)**	
Variable	**At baseline**	**Two years after baseline**	**At baseline**	**Two years after baseline**
Calcium carbonate (mg)	750 (500-1500)[a]	1250 (500-1625)[b]	2000 (1000-2500)[i]	1500 (1250-2500)[i]
Alphacalcidol (µg)	0.5 (0.2-0.6)[c]	0.3 (0.2-0.5)[d]	0.3 (0.1-1.0)[i]	0.4 (0.1-0.4)[j]
Paricalcitol (µg)	1.4 (0.9-3.2)[e]	1.8 (1.4-1.8)[f]	1.4[g]	0.9[g]
Cinacalcet (mg)	120mg[g]	60(15-60)[h]	N/A	60[g]

All values are expressed as median + (interquartile range)

N/A= not applicable

a n=11; b n=10; c n=4; d n=8; e n=5; f n=2; g n=1; h n=3; i n=5; j n=3

Supplemental Table 3. Biochemical parameters in 13 patients remaining on dialysis with repeat bone biopsy at baseline and after two further years of dialysis.

Variables **(normal range)**	**At baseline**	**Two years after baseline**	**P value**
Ca $^{2+}$ mg/dl/pH7.4 (4.64-5.2)	4.72 (4.3-4.86)	4.60 (4.32-4.98)	0.46
Inorganic phosphate mg/dl (2.19 -3.81)	5.73 (3.86-7.40)	6.07 (4.74-6.88)	0.28
PTH pg/ml (15-65)	355 (144-636)	132 (92-246)	0.10
D-25-OH ng/ml (>16)	9.2 (7.61-25.6)	25.6 (13.2-34.5)	0.12
D-1,25 pg/ml (22.9-76.3)	7.9 (3.1-14.6)	11.7(7.1-25.4)	0.03*****
bone specific alkaline phosphatase µg/l (6-15)	9.3 (8.5-19)	14(10.5-21.5)	0.45
osteocalcin ng/ml (14-46)	150 (65-260)	130(70-206)	0.53

All values are expressed as median + (interquartile range)

*Statistically significant

Ca $^{2+}$, ionized calcium; PTH, parathyroid hormone; 25(OH) D, 25-hydroxyvitamin; D-1,25 $(OH)_2$ D, 1,25-dihydroxyvitamin.

Conversion factors for units: plasma ionized calcium in mg/dl to mmol/l x 0.25; inorganic phosphate in mg/dl to mmol/l x 0.3229. Intact parathyroid hormone levels pg/ml and ng/l are equivalent. 25-hydroxyvitamin D in ng/ml to nmol/l x 2.496; 1,25-dihydroxyvitamin D pg/ml to pmol/l x 2.4. Osteocalcin ng/ml and µg/l are equivalent.

Supplemental Table 4. Bone histomorphometric parameters of participants in a longitudinal bone biopsy who remained on dialysis

	All patients remaining on dialysis (N=19)	Patients remaining on dialysis with repeat bone biopsy (N=13)		
Bone parameter	**At baseline**	**At baseline**	**Two years after baseline**	**P value***
Bone formation rate/bone surface (mm^3/cm^2/ year)	24.5 (8.6-32)[a]	28.8 (7.2-32)[e]	23.6 (13.5-32.9)[g]	0.69
Activation frequency (¯ 1/year)	0.47 (0.18-0.84)[a]	0.45 (0.14-0.88)[e]	0.68 (0.33-1.02)[g]	0.5
Osteoblastic surface/bone surface (Z- score)	0.1 (-1.6-9.9)[b]	-0.6 (-1.9-12.6)[f]	-1.3 (-2.8-(-0.6))	0.11
Osteoclastic surface/bone surface (Z- score)	10.8 (1.9-19.6)[b]	12.3 (1.4-20.1)[f]	0.02 (-2.0- 3.3)	0.13
Osteoid surface/bone surface (%)	46.3 (36.6-57.2)[c]	48.6(35.2-58.8)	43.1(14.5-49.2)	0.13
Osteoid thickness (µm)	8 (6.2-9.9)[c]	8.3 (6.7-10.6)	7 (6.6-12.1)	0.42
Mineralization lag time (d)	77.2 (46.7-183.2)[a]	60.3 (49.8-179.5)[e]	61.6 (26-90.5)[g]	0.89
Bone volume/ tissue volume (%)	21.4 (17.3-30.5)[d]	21 (17.4- 30.4)	18.5 (14.2-25.4)	0.08
Lumbar spine T-score	-1.3 (-2.2-0.2)[b]	-0.9 (-2.2-1.0)[f]	-0.3 (-1.7-1.1)[f]	0.96
Femoral neck T- score	-0.8 (-1.7-0.3)[b]	-0.6 (-2.5 to -0.4)[f]	-1.6 (-2.4 to -0.1)[f]	0.04*

All values are expressed as median + (interquartile range)

* P -value compares values after two further years of dialysis to values at baseline among patients remaining on dialysis with repeat bone biopsy

a n=12; b n=16; c n=18; d=17 ; e n=7; f=11; g=8