Fibroblast Growth Factor-23 and Parathyroid Hormone Are Associated with Post-Transplant Bone Mineral Density Loss

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Background and objectives: Among the multiple factors contributing to bone mineral density (BMD) loss after renal transplantation, hypophosphatemia is increasingly recognized to play an important role. Hypophosphatemia occurs in up to 90% of the renal transplant recipients in the early post-transplant period and is caused by renal phosphate wasting. We hypothesized that a high pretransplant level of the recently described phosphaturic hormone fibroblast growth factor 23 (FGF-23) is a risk factor for accelerated BMD loss occurring within the first post-transplant year.

Design, setting, participants, & measurements: We performed a two-center observational retrospective cohort study in 127 incident renal transplant recipients. Serum full-length FGF-23, parathyroid hormone (PTH), and parameters of mineral metabolism were determined at the time of transplantation. BMD was assessed by osteodensitometry at the time of transplantation and 1 year later.

Results: A moderate decrease of BMD was observed during the first post-transplant year. High FGF-23 levels were associated with BMD loss at the lumbar spine and total hip region, whereas low PTH levels were associated with BMD loss at all three regions. Cumulative doses of prednisone and post-transplant serum phosphate level were not correlated with BMD changes.

Conclusion: Our data indicate that patients with a high serum FGF-23 level and/or a low PTH level at the time of transplantation are at risk for increased BMD loss during the first post-transplant year.


Accelerated bone mineral density (BMD) loss is an almost universal complication in transplant recipients. As in the general population, a low BMD in dialysis patients confers an increased risk of fractures and inherent morbidity and mortality (1). The accelerated BMD loss in organ transplant recipients is mainly considered a side effect of immunosuppressive drugs, especially glucocorticoids. It is, however, well established that the fracture risk is higher in renal transplant recipients compared with recipients of other organs. This points to specific risk factors. Among these, pre-existing renal osteodystrophy, hypogonadism, and metabolic acidosis undoubtedly play an important role (2). Post-transplant disturbances in phosphate homeostasis should be accounted for as well. Hypophosphatemia is a frequent finding in the early post-transplant period, occurring in up to 90% of the renal transplant recipients. Renal phosphate wasting is the main mechanism contributing to this complication. It is recognized that both hypophosphatemia and renal phosphate wasting may have a detrimental impact on bone mineralization (3,4). We and others recently reported that renal phosphate wasting in the post-transplant period is caused, at least partly, by the persistence of inappropriately high levels of fibroblast growth factor 23 (FGF-23) (5,6). This condition is often referred to as “tertiary hyperphosphatoninism.” As opposed to serum parathyroid hormone (PTH) levels, FGF-23 levels return to normal by 1 year after transplantation in the majority of the patients. This evolution goes along with the regression of hypophosphatemia and renal phosphate wasting in these patients (7). Because pretransplant FGF-23 levels are the most important determinant of FGF-23 levels up to 1 year after transplantation (7), we formed the hypothesis that a high pretransplant FGF-23 level is a risk factor for accelerated BMD loss in this period. To test this hypothesis, we performed a retrospective observational cohort study.

Materials and Methods

Study Population
We performed a two-center (University Catholic Louvain and Katholieke Universiteit Leuven) observational retrospective cohort study in incident renal transplant recipients. Between 2001 and 2004 (University Catholic Louvain) and 2006 and 2008 (Katholieke Universiteit Leuven), each center performed an osteodensitometry within the first 14 days and at 12 months after transplantation in all incident renal transplant recipients. Inclusion criteria were as follows: recipient of a single kidney transplant, age older than 18 years, functional renal transplant at 1...
year, osteodensitometry assessed at the time of transplantation and 1 year later, and availability of a spare pretransplant serum sample. To exclude a potential selection bias, demographic characteristics (age, gender, and dialysis vintage) were compared between selected and excluded patients and did not show any significant difference between both groups.

Procedures and Assays

Relevant clinical and biochemistry data were extracted from the electronic medical files. Serum samples were collected immediately before transplantation, centrifuged, aliquoted, and stored at −25°C until analysis. Serum full-length FGF-23 levels were determined with a sandwich ELISA using two kinds of monoclonal antibodies requiring the simultaneous presence of both the N-terminal and C-terminal portions of FGF-23 (Kainos Laboratories, Tokyo, Japan). This assay differs from the C-terminal assay (Immunotopics) that recognizes both full-length and processed C-terminal fragments of FGF-23 (8). FGF-23 levels determined in healthy controls (n = 58) with the full-length FGF-23 assay amounted to 26.3 ± 0.82 ng/L (9). Serum calcitriol and 25(OH)D3 (calcidiol) levels were measured using an RIA (10,11). Serum concentrations of PTH were determined by an immunoradiometric assay (IRMA), as described elsewhere (12). In contrast to most other commercially available IRMAs for PTH, this assay detects full-length human PTH but not N-terminal truncated fragments and thereby resembles recently introduced third-generation PTH IRMAs (biointact PTH or whole PTH). This also explains its lower normal range of 3 to 40 pg/ml (ng/L).

BMD was assessed by dual-energy x-ray absorptiometry (DXA) with a DQR 2000 densitometer (Hologic, Bedford, MA). Tests were performed within the first 14 days and at 1 year after transplantation. Measurements were taken at the lumbar spine, femoral neck, and total hip. The reproducibility of the DXA expressed as a coefficient of variation averages 0.82% at the lumbar spine and 0.79% at the hip (13). Results are expressed in grams per square centimeter, as well as T scores, i.e., the difference in SD compared with the mean value of healthy young sex-matched controls, and Z scores, i.e., the difference in SD compared with the mean value of healthy age-and-sex matched controls. BMD changes were expressed as the percentage of the baseline measurement value.

Statistical Analyses

Parametric and nonparametric parameters are expressed as mean ± SD and median (interquartile range), respectively. Correlations were assessed by Spearman’s test. Simple linear regression analyses were used to examine the associations between BMD changes at the different sites and pretransplant serum calcidiol, calcium, PTH, and FGF-23 concentration, pre- and post-transplant phosphorus, age, and dialysis vintage. Correlations between T and Z scores were assessed by simple regression. Multivariate regression analysis was performed including all previous variables, gender, and center. After excluding colinearity, the best subset of variables was selected by backward elimination. Assumptions for linear regression were justified. The best a priori assumptions for linear regression were justified. The best model was achieved with square root-transformed PTH and FGF-23.

The Statview (SAS Institute) version 5.0.1 software program was used for the statistical analysis. Two-sided P < 0.05 was considered statistically significant.

Results

Patient Characteristics

One hundred twenty-seven patients were included (Table 1). Mean age was 51.1 ± 13.3 years (52 years for men and 50 years for women); 62% were men. The median time on dialysis was 22.5 months (14.5 to 35.5 months). Ninety-six percent had been transplanted with a kidney from a deceased donor.

Induction therapy consisting of anti-thymocyte globulin or basiliximab was given in 31% of patients. Maintenance immunosuppression consisted of glucocorticoids (98%), calcineurin inhibitor (tacrolimus 83% or cyclosporine 16%), and an antimitabolite agent (mycophenolate mofetil 70% or azathioprine 5%). Eighteen percent of the patients received sirolimus. The cumulative prednisolone dose during the first post-transplant year amounted to 2515 mg (2258.8 to 2811.3).

Specific drug treatment to prevent BMD loss was not routinely prescribed. At 1 year after transplantation, calcium supplements, calcidiol, and calcitriol were prescribed to 28, 8, and 7% of the patients. Bisphosphonates were taken by 1.5%. Eight percent of the postmenopausal women were on estrogen replacement therapy.

Biochemistry and Bone Mineralization Data

The median PTH and calcidiol serum levels at transplantation were 43.1 pg/ml (14.9 to 109.9 pg/ml) and 31.2 μg/L (18.0 to 41.9 μg/L), respectively. The median FGF-23 serum level at the time of transplantation was 6494 pg/ml (1660.5 to 19,278.8 pg/ml); calcium and phosphorus levels were, respectively, 9.8 (9.2 to 10.6 mg/dl) and 4.9 mg/dl (4.0 to 5.6 mg/dl). The phosphorus level at month 3 after transplantation was 2.7 mg/dl (2.2 to 3.2 mg/dl), T scores and Z scores at the time of transplantation were −1.11 (−2.02 to −0.045) and −0.795 (1.50 to 0.390) at the lumbar spine, −1.57 (−2.19 to −0.778) and −0.795 (−1.325 to −0.045) at the femoral neck, and −1.095 (−1.94 to −0.36) and −0.700 (−1.40 to 0.00) at total hip regions, respectively. Correlations between T and Z scores were excellent (R² = 0.862). FGF-23 was positively correlated with serum phosphate level at the time of transplantation and negatively correlated with serum phosphate level at 3 months after transplantation.

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics (n = 127)</th>
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<tr>
<td>Age (years) (mean ± SD)</td>
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<td>Sex (M/F)</td>
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<tr>
<td>Center University Catholic Louvain/Katholieke Universiteit Leuven (%)</td>
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<tr>
<td>Dialysis duration (median) (months)</td>
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<tr>
<td>Deceased donor (%)</td>
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<tr>
<td>Causes of ESRD (%)</td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
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<td>Interstitial nephritis</td>
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<td>Vascular</td>
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Factors Associated with BMD Changes

BMD change, expressed as the percentage of the pretransplant value, was $-2.2\%$ at the lumbar spine, $-2.9\%$ at the femoral neck, and $-2.2\%$ at the total hip. In univariate analysis, low PTH levels were significantly associated with BMD loss at the total hip and femoral neck regions, whereas high FGF-23 was significantly associated with BMD loss at the lumbar spine and total hip region. Phosphorus level at month 3 after transplantation was not correlated with BMD loss at any region. In multivariate analysis, low PTH levels were significantly associated with BMD loss at all three regions, whereas high FGF-23 levels were significantly associated with BMD loss at the lumbar spine and total hip region (Table 2). Adjustment for age, gender, dialysis vintage, transplant center, 1-year cumulative prednisolone dose, and pre- and post-transplant serum phosphorus level did not affect the association. Figure 1 shows the association between PTH and BMD loss, as well as FGF-23 and BMD loss at the lumbar and total hip regions.

Discussion

The key findings of this study are as follows: (1) BMD loss during the first post-transplant year is limited in contemporary kidney transplantation; (2) high FGF-23 levels and low PTH levels at time of transplantation are associated with BMD loss within the first year after renal transplantation. Opposite to previous studies where BMD change reached $-5$ to $-7\%$ (14–17), we observed a rather modest decrease of BMD during the first year after successful kidney transplantation. The BMD change, expressed as the percentage of the pretransplant value, was $-2.2\%$ at the lumbar spine, $-2.9\%$ at the femoral neck, and $-2.2\%$ at the total hip. There is broad consensus that glucocorticoids are the main cause of post-transplant bone loss. Glucocorticoids have many osteoporotic effects: (1) they decrease intestinal calcium absorption and increase renal calcium excretion, with consequent secondary hyperparathyroidism; (2) they decrease the effect of IGF 1, (3) they suppress osteoblastogenesis, and (4) they inhibit preosteoblast to osteoblast transformation. Secondary hypogonadism further reduces bone formation (18). Several studies correlate bone loss to glucocorticoid exposure (4,14,15), with high cumulative and maintenance doses of prednisone correlating negatively with bone volume and bone turnover (19). In this study, cumulative doses of prednisolone were not associated with BMD loss. Our patients received a daily prednisolone dose of 20 mg/day after transplantation, rapidly tapered to 5 mg/day at week 6. They were therefore receiving a low daily dose of prednisolone, resulting in a low cumulative dose over 1 year. In addition, they were receiving the same steroids prescription, with, as a consequence, very few interpatient variations. This could explain the lack of association between BMD loss and

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<th>Table 2. Univariate and multivariate regression with ΔBMD at the lumbar spine, total hip, and femoral neck regions</th>
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<td>Independent Variables</td>
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<tr>
<td><strong>Univariate models</strong></td>
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<td>Age (years)</td>
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<td>Dialysis vintage (m)</td>
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<td>Serum calcium (mg/dl)</td>
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<td>Serum phosphorus T3 (mg/dl)</td>
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<td>Square root PTH (ng/L)</td>
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<td>Square root FGF-23 (ng/L)</td>
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<td>Serum 25OH vitamin D</td>
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<td><strong>Multivariate model</strong></td>
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<td>Square root PTH</td>
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<td>Square root FGF-23</td>
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Figure 1. Simple linear regression showing the association between PTH and BMD loss and FGF-23 and BMD loss at lumbar and total hip regions.
steroid administration. Indeed, previous studies have shown that higher cumulative prednisone dose (4000–6000 mg/yr compared with 2500 mg/yr in our patients) and higher maintenance prednisone dose (> 10 mg/day) correlated negatively with BMD loss (15,20). Thus, other causes might contribute to BMD loss.

Hypophosphatemia is a common complication of kidney transplantation, occurring in up to 90% of the renal transplant recipient within the first 3 months after transplantation. Hypophosphatemia and phosphate deficiency has been shown to cause defective osteoblastic bone formation and defective mineralization (2–4,21). FGF-23, a recently described 30-kD protein, is a phosphaturic hormone produced by osteocytes. It promotes renal phosphate wasting through internalization of the sodium phosphate cotransporter IIa and IIc from the proximal tubular apical membrane. By month 3 after successful renal transplantation, FGF-23 levels were shown to remain elevated in the majority of patients, despite a 95% decrease from pretransplant levels (6). Moreover, increased FGF-23 levels, but not PTH levels, were independently associated with the serum phosphate nadir and increased phosphate wasting (6). We found an association between FGF-23 levels and BMD loss at the lumbar spine and total hip mixed trabecular and cortical bone but not at the femoral neck (mainly cortical bone). This is not surprising because changes in bone mass are always more obvious in trabecular compared with cortical bone (22). This observation conflicts with a recent study showing no association between serum FGF-23 levels and BMD loss in incident renal transplant recipients (23). It should be of note that the small patient number (n = 44) and short follow-up (10 weeks) in that study confers an increased risk for a type II statistical error. Our data failed to show a correlation between serum phosphate level at month 3 after transplantation and BMD loss at any of the three regions. However, FGF-23 could affect BMD in a serum phosphate–independent manner. Indeed, using fgf-23– and Npip2a-null mice, Sitara et al. (24) have reported a phosphate-independent effect of FGF-23 on skeletogenesis. Moreover, Wang et al. (25) have shown that adenoviral overexpression of FGF-23 in rat calvarial cells inhibits bone mineralization independent of systemic effects on phosphate homeostasis. In children with secondary hyperparathyroidism and high phosphorus level treated with peritoneal dialysis, plasma FGF-23 levels were reported to be inversely correlated with both static and dynamic indices of osteoid mineralization, further supporting the hypothesis of a phosphate independent effect of FGF-23 on bone architecture (26).

Besides high pretransplant FGF-23 levels, low PTH levels were found to be associated with increased BMD loss in this study. Previous studies have yielded conflicting data on the significance of pretransplant PTH levels as a predictor of post-transplant BMD loss: both high (20,27–29) and low (15) pretransplant PTH levels have been correlated with BMD loss. Similar to FGF-23, pretransplant PTH levels are directly associated with post-transplant levels (7). It is recognized that the main alteration in bone remodeling after renal transplantation is a decrease in bone formation in the face of persistent bone resorption. This produces an imbalance in remodeling favoring resorption. Defective bone formation may be the consequence of alterations in osteoblast function, decreased generation, or increased osteoblast death rates (4). A recent bone biopsy study showed a positive correlation between osteoblast surface and serum levels of pre- and post-transplant PTH, supporting an important role of PTH in preserving osteoblast number and activity after transplantation. High PTH levels might thus oppose the detrimental effect of glucocorticoids (4). These findings are in agreement with previous animal data (30).

Vitamin D status in our patients did not correlate with BMD loss. However, the underlying status of vitamin D might have affected serum levels of FGF-23 and/or PTH levels. FGF-23 induces reduction of 1,25(OH)2D by inhibiting 1α-hydroxylase, which converts 25OHD to 1,25(OH)2D, and stimulating 24-hydroxylase, which converts 1,25(OH)2D to metabolites with lesser biologic activity (31). In patients with ESRD, treatment with low-dose calcitriol analogs resulted in an increased trend of FGF-23 levels and a decrease in PTH levels (32). In patients on dialysis, active vitamin D therapy was shown to increase serum FGF-23 levels in patients with secondary hyperparathyroidism and to decrease significantly serum intact PTH levels (33). In our patients, the mean 25OH vitamin D was at the upper end of the range of insufficiency, implying that ~50% of the patients were at least vitamin D deficient. It is possible that more aggressive correction of vitamin D status could have resulted in an increase of FGF-23 levels and a decrease of PTH levels. However, in the absence of biologic evidence of calcitriol deficiency in this study, this latter point remains speculative.

An aspect of this study that could be considered both as a limitation and a strength is that it was conducted in the course of routine patient care and therefore represents “real life” medical care. Another strength is the relatively large number of subjects included. The limitations of the study are mainly related to the retrospective design of the study. Finally, post-transplant changes in BMD were measured by DXA. DXA provides a measure of BMD as an estimate of the total quantity of mineral in a scanned area of bone and does not distinguish between changes in bone volume and mineralization density of the bone matrix. In addition, DXA cannot distinguish between cortical or cancellous bone. Finally, particularly in patients with chronic kidney disease, tissue or vascular calcification can interfere with DXA measurements and yield falsely high values. Post-transplant mobilization of calcification would decrease BMD without actually changing the bone mineral content. In the absence of more accurate clinical alternatives, however, DXA remains the most eligible technique for the longitudinal assessment of changes in BMD.

In conclusion, the results of this preliminary study indicate that patients with a high serum FGF-23 level and/or a low PTH level at the time of transplantation are at risk for increased BMD loss during the first post-transplant year. The associations, however, are weak and independent of serum phosphorus level, raising the hypothesis that the clinical impact of FGF-23 or PTH on bone changes after renal transplantation could be small and partially independent of hypophosphatemia. Further studies involving a larger number of patients
are needed to further delineate the role of FGF-23 and PTH in the pathogenesis of bone in kidney transplant recipients.

**Disclosures**

None.

**References**

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