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Maintenance low dose systemic glucocorticoids have limited impact on bone strength and mineral density among incident renal allograft recipients: a pilot prospective cohort study

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Disclosures

Maria José Pérez-Sáez, Sabina Herrera, Laia Vilaplana, Xavier Nogués, Dolores Redondo-Pachón, Marisa Mir, Roberto Güerri, Marta Crespo, and Julio Pascual declare that they have no conflict of interest.

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Abstract

Soon after kidney transplant (KT), a decrease in parathormone and bone mineral density (BMD) occur, but little is known on the impact of KT on novel bone quality parameters including trabecular bone score (TBS) and bone material strength index (BMSi). We aimed to study BMD, TBS and BMSi in the first year after KT, in patients not treated with any bone therapy. A cohort including 36 patients underwent KT on a low-glucocorticoid-dose protocol (5 mg daily-prednisone from post-operative-day 42 onwards) and was observed for 12 months prospectively. At 3 months, phosphorus and parathormone decreased, while calcium increased. We also observed at 3 months a transient mild 2.9% bone loss at femoral neck (BMD change 0.752 ± 0.15 vs 0.730 ± 0.15 ; $p=0.004$), but no change at either spine or total hip. Both TBS and BMSi remained stable. At 12 months, lumbar (but not total hip or femoral neck) BMD slightly decreased by 2.1% vs. baseline (0.950 ± 0.15 vs 0.930 ± 0.5 ; $p=0.046$), whilst TBS and BMSi remained unmodified.

In KT patients on low-dose glucocorticoids and no bone therapy, there were small BMD decreases at femoral neck (at 3 months) and lumbar spine (at 12 months), but no change in either TBS or BMSi. Low-dose post-KT glucocorticoid treatment shows limited impact on bone, supporting steroid-restrictive protocols.

Introduction

Bone and mineral metabolism disturbances usually experience changes soon after kidney transplantation (KT) [1]. Particularly, in the first few months there is a decrease in bone mineral density (BMD) and a readjustment of secondary hyperparathyroidism parameters [2]. Previous studies have reported up to 20% BMD loss in the first 6 months after transplant, with posterior stabilization [3-6]. However, patients from these studies received a cumulative dose of glucocorticoids of 4-5 grams in the first year [5,6]. According to this, a high risk of fracture in KT population is noticed, predominantly in these first years following transplant when the cumulative dose of glucocorticoids is very impactful [7]. Therefore, a correct management of patient's bone health pre and posttransplantation is essential for preventing these fractures [8].

Bone disease in KT recipients is usually studied by measuring BMD using dual-energy x-ray absorptiometry (DXA). But bone strength is not only driven by bone density [9], and other specific non-invasive methods for estimating bone structure and tissue-level properties (i.e. bone quality) are lacking in this population. In recent years, the development of new techniques that may improve the estimation of the bone strength (and, therefore, the fracture risk) in different populations has been a line of investigation in our group [8,10-13]. Trabecular bone score (TBS) estimates bone microarchitecture [14] and has already been correlated with fractures in a KT cohort [15]. Reference-point indentation (RPI) directly measures the mechanical properties of bone at a tissue level [10]. RPI tests the resistance of cortical bone tissue to the opening of micro-cracks with a very fine needle, closely mimicking the initiating crack of the starting fracture [10]. In previous studies, RPI has demonstrated better performance than DXA to discriminate the risk of osteoporotic fractures from healthy controls [10], in patients with osteopenia [16] or with type 2 diabetes mellitus [17] as well as a better clinical correlation with fractures in cases of atypical fractures [11] compared with BMD values. Two previous reports from our group have described TBS and RPI in long-term KT recipients and KT candidates, respectively [13,8]. Long-term KT recipients presented

with similar values than healthy controls after adjustment by age, gender and body mass index (BMI) [13]. In contrast, those properties were damaged in KT candidates [8] at the time of the transplantation.

We aimed to study the natural history of bone disease soon after KT considering all the parameters that determine bone strength: BMD (measured by DXA), bone microarchitecture (measured by TBS) and tissue mechanical properties (measured by RPI) in a cohort of incident KT recipients prospectively assessed during the first posttransplant year.

Materials and Methods

Study design

This is a pilot prospective cohort study including a series of patients receiving KT in a university general hospital.

Study population

Patients receiving a first KT at Hospital del Mar, Barcelona, Spain, were eligible to participate if they were incident KT recipients older than 18 years and signed the informed consent. Figure 1 shows patient flow-chart during the study. The immunosuppression regimen consisted on antibody induction (basiliximab or thymoglobulin) and maintenance with tacrolimus, mycophenolic acid or mTOR inhibitor and steroids. Steroid dose was low and quickly tapered (250 mg iv methylprednisolone at the operation room and 125 mg day 1, oral prednisone 20mg days 2-14, 15 mg days 15-28, 10 mg days 29-42 and 5 mg day 43 onwards), with a cumulative dose of 2360 mg in the first 12 months. None of them received any other treatment that potentially could impact on their bone and mineral status. Written informed consent was obtained from each patient and the Ethics Review Board in our institution approved the study protocol.

Laboratory workout

Routine laboratory measurements (including 2-hour fasting sample and 24h urine sample) were performed. At baseline, month 3 and at month 12, plasma levels of intact parathormone (iPTH) (electrochemiluminescence, ElecsysSystem; Roche DiagnosticsGmbH, Penzberg, Germany), and 25-OH vitamin D (ELISA, IDS, Boldon, UK) were also measured.

Bone assessment

BMD was measured by DXA at lumbar spine and proximal hip at baseline, three months and one year after transplantation. All measurements were obtained with the same machine, a Hologic QDR 4500 SR[®] (Hologic Inc. Bedford, MA, USA). DXA-based TBS was evaluated on the lumbar spine BMD measurements using iNsight[®] v 2.1 (Med.Imaps, Merignac, France). Bone microindentation was performed at the anterior face of tibia with a hand held reference-point indenter device, Osteoprobe[®] (Active Life Scientific, Santa Barbara, CA, USA) following the recently described protocol [13,18]. In brief, after local anesthesia, a preload of 10 Newton (N) followed by a 30N indentation was performed with a test probe with a conic edge of 4 μ . Average values of 8 indents were calculated by a computerized algorithm. Five calibration indents were then performed in a polymethylmethacrylate block (BMSi-100 Reference Material). Ratio between both tibia and the reference material measurements yield the final parameter of bone mineral strength index (BMSi) as previously described [10].

Statistics

Statistical analyses were performed with SPSS v 22.0 (IBM[®]) by using Student T-test for paired samples for normally distributed parameters, and Wilcoxon (non-parametric) test for repeated samples for non-normally distributed ones. The comparisons were done two by two (baseline and 3 months, baseline and 12 months, and 3 and 12 months).

Results

Fifty-four consecutive KT recipients who agreed to participate were initially enrolled in the study. Thirty-six (66.7%) of them completed 1-year (4 patients missed their DXA test at 3 months, 4 lost their graft before 1 year, 6 withdrew consent, 3 were retired from the study for other reasons and 1 patient died) follow-up and were finally included in the analysis. Mean age was 54.9 ± 11.6 years, 83.3% were Caucasians and 47.2% were female. Table 1 summarizes the details of the baseline characteristics of the cohort.

Two patients presented an acute rejection episode easily reversed after an extra dose of steroids (iv methylprednisolone, 250 mg daily for 3 days). Patients had good kidney function and were free of significant proteinuria (less than 500 mg/dL) at 3 and 12 months after KT. At 3 months, phosphorus and iPTH levels decreased (4.5 vs 3.2 mg/dl and 183 vs 93 ng/ml, respectively), while calcium increased (9.7 vs 9.9 mg/dl) and 25-OH vitamin D remained stable (Table 2).

Regarding bone parameters, we observed a transient slight decrease in BMD values at femoral neck at 3 months (0.752 vs 0.730 gr/cm²; 2.9% of bone loss) and a minor at lumbar spine at 12 months (0.950 vs 0.930 gr/cm²; 2.1% of bone loss). We did not find any difference in TBS or RPI values when measured at baseline, 3 and 12 months after transplant (Table 3).

Discussion

Immunosuppression regimens in KT always include variable glucocorticoids exposure, an important treatment to avoid organ rejection. However, steroid minimization is an essential goal in most units, to avoid adverse events associated to these drugs, particularly infection [19]. Another potential benefit associated to steroid minimization would be a better bone health maintenance.

BMD decreases after KT and this may have an impact on future fractures in KT recipients [3,4,20]. However, here we present the results of a pilot prospective study performed in our transplant population, treated with low dose of glucocorticoids and complete absence of any calcium/vitamin D supplementation or bisphosphonates treatment, showing stable bone health. Although there was a mild BMD decrease at femoral neck in the first three months and at lumbar spine at one year, the other two components of bone strength, bone microarchitecture by TBS and mechanical tissue properties by RPI did not capture significant changes in the first year after transplantation.

Secondary hyperparathyroidism related to chronic kidney disease is usually reversed in the first months after successful KT. Initially, the patients show hypercalcemia and hypophosphatemia with high levels of PTH. After three months, calcium and phosphorus levels are returned to normal range and PTH experiences an important decrease in many patients, although a relevant proportion of them persist with high PTH levels even 1 year after KT [21-23]. Our patients presented with initial hypophosphatemia and progressive correction of PTH levels, although the latter remained elevated at 12 months. Calcium levels increased slightly in the first three months with low levels of 25-OH-vitamin D that did not change at one year after transplantation [24].

On the other hand, it has been well established that the loss of bone mineral density occurs largely in the first 12 months after KT. Repeated measurements by DXA show the most rapid BMD decrease in the first 6 months after transplantation (5.5-19.5% [3]), and seems to slow down later on (2.6-8.2% in months 6 to 12 after transplant [4], and 0.4-4.5% thereafter [25]). This observation can be explained by the high doses of glucocorticoids administered during the first 6 months after transplantation, inducing accelerated bone remodeling [26]. Although glucocorticoids are essential for normal osteoblasts development, at non-physiological doses, osteoblasts and osteocytes are glucocorticoid targets [27]. Notably, the studies with the highest reported rate of BMD loss are those using high doses of glucocorticoids, up to 5 - 6 grams of cumulative dose in the first year after transplantation [3,4,18]. Other more recent studies primarily focused in the early loss of BMD [28] or testing interventions with vitamin D or bisphosphonates to prevent bone loss [5,6] have also used

high doses of glucocorticoids, around 4 grams of cumulative dose. In contrast, studies with withdrawal or early tapering of steroids after transplant have demonstrated beneficial effects on BMD [29,30] although even low doses of corticosteroids are able to increase fracture risk in the general population [31]. Our patients, with 2 grams of cumulative dose of glucocorticoids showed a slight decrease in BMD at the femoral neck, recovered at one year. The mild, transitory decrease in femoral neck bone density with no detectable changes in total hip has little or no clinical impact. Therefore, we can assume that a reduced dose glucocorticoid regimen may have less impact on BMD loss.

The most important skeletal consequence of transplantation is an increase in the risk of fracture [7]. BMD measured by DXA is currently the clinical standard for diagnosing bone disease and monitoring the effects of treatments in KT patients. However, the resistance that bone opposes to fracture is not solely given by BMD (or bone quantity) but also by bone microarchitecture and bone quality [9]. DXA is two-dimensional and thus cannot discriminate between cortical and trabecular bone regions. Its limitations go further in cases of chronic kidney disease where measurements might also be affected by extra-skeletal calcifications, common in this population, or by osteomalacia and osteosclerosis [9,32]. Attempting to capture all bone properties, other techniques have recently emerged. TBS estimates bone microarchitecture by analyzing vertebral DXA images with a specific software and has been correlated with higher risk of fracture [33]. Lower TBS values compared to general population have been described in hemodialysis patients [34] and short-term KT recipients [15]. Our group has recently reported lower values of TBS among waitlisted KT candidates on dialysis compared to healthy controls [8]. However, these findings were not confirmed in a cohort of very long-term KT recipients [13], where bone microarchitecture seemed to be recovered.

As stated above RPI is a minimally invasive technique for measuring tissue-level bone strength. The feasibility of RPI in KT recipients was proven in a first study, where long-term recipients showed similar BMSi values compared to healthy controls [13]. In addition, we performed RPI in a

cohort of dialysis patients waitlisted for KT. They had lower BMSi values than controls [8]. In the present study we have prospectively assessed BMSi evolution during the first year after transplantation, showing stability in the setting of low dose of glucocorticoids. BMSi measurements have been consistently found independent of DXA measurement either in lumbar spine or hip. Although no clinical studies comparing BMSi and peripheral DXA have been published, the correlation between DXA measurements in different skeletal areas makes unlikely any relationship between them.

Altogether, our results show a transient, mild decrease in BMD, while the other two drivers of bone strength are not deteriorated by this regimen of low-dose glucocorticoids.

The limitations of the study are the small number of patients and its observational nature. Although the individual data do not suggest the existence of different patterns, the low number of patients deserves caution. Without a comparison group on higher doses of steroids it is hard to be certain that the diminished bone loss (compared with historic studies) is due to or only due to the steroid dose. It is possible that changes in pre-transplant bone disease management might be affecting post-transplant bone loss as well. In addition, we did not perform BMD in peripheral skeleton and RPI or TBS could not be sensitive enough to capture bone damage in this population, although RPI has been able to early detect bone material properties changes in patients receiving higher doses of glucocorticoids [12]. However, the clinical translation of these data has to be made with caution because normal reference values for this technique are not available yet and, furthermore, there is intra- and inter-individual variation. Given the limited sample size, we were not able to show any interaction with diabetes mellitus, any data on fractures nor find a pattern of patients that could improve or worse bone health after KT. However, this is the first prospective study exploring bone properties beyond the common BMD measurements in KT recipients, in order to cover different aspects of bone strength. Moreover, the lack of intervention with medications that potentially might affect bone and mineral status allows the accurate description of bone disease natural history after transplantation.

In summary, our data show that with a low glucocorticoid dose regimen, without any bone and mineral therapeutic intervention, KT recipients experienced a mild decrease in BMD, but measurements of microarchitecture and bone tissue resistance to microindentation remained stable both at 3 and 12 months. We postulate that a comprehensive bone assessment, including BMD, TBS and RPI, might be needed in order to detect those KT patients at risk of fracture that warrant pharmacological intervention.

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Table 1. Baseline characteristics of the patients included in the study

Age (years, mean \pm sd)	54.9 \pm 11.6
Sex (female, %)	47.2
Postmenopausal status (%)	97.2
Race (%)	
<i>Caucasian</i>	83.3
<i>Hispanic</i>	8.3
<i>Asian</i>	8.3
BMI (mean \pm sd)	26.7 \pm 5.2
Diabetes mellitus (%)	
<i>Pre-KT</i>	19.4
<i>Post-transplant DM</i>	8.3
Smoking (current/ex/no, %)	16.6/30.6/52.8
Previous fracture (%)	16.7
Treatment for secondary hyperparathyroidism at the time of transplantation (%)	
<i>Native vitamin D</i>	33.3
<i>Active vitamin D</i>	22.2
<i>Vitamin D analogs</i>	19.4
<i>Phosphate binders</i>	75
<i>Calcimimetics</i>	22.2
<i>Parathyroidectomy</i>	0
Time on the KT waiting list (months, median [IQR])	18 [8-35]
Preemptive KT (%)	22.2
Living donor (%)	22.2
IS regimen	
<i>Induction (basiliximab/thymoglobulin, %)</i>	94.4/5.6
<i>Maintenance* (MPA/imTOR)</i>	83.3/16.6
Steroids (mg/kg, median [IQR])	35.6 \pm 6.2
Acute rejection (n)	2

sd, standard deviation; BMI, body mass index; DM, diabetes mellitus; KT, kidney transplant; IQR, interquartile range; MPA, micophenolic acid; mTORi, mTOR inhibitors.

*All patients were also receiving calcineurin inhibitors.

Table 2. Evolution of analytical parameters during the study period

	Baseline	3 months	12 months
Serum Creatinine^a (mg/dl)	-	1.42 ± 0.4	1.39 ± 0.5
eGFR MDRD-4 IDMS^a (ml/min/1.73/m²)	-	54 ± 21	55 ± 22
Proteinuria 24h^b (mg)	-	307 [257-483]	277 [258-480]
Calcium^a (mg/dl)	9.7 ± 0.7	9.9 ± 0.5 [*]	9.9 ± 0.5
Phosphorus^a (mg/dl)	4.5 ± 1.3	3.2 ± 0.6 ^{***}	3.5 ± 0.5 ^{***#}
25-OH vitamin D^b (nmol/L)	11.4 [4.3-22.4]	10.1 [6.9-11.9]	10.2 [6.9-13.8]
PTH^b (pg/ml)	183 [106-342]	93 [66-155] ^{**}	87 [47-143] ^{**}

eGFR, estimated glomerular filtration rate; MDRD-4 IDMS, isotope dilution mass spectrometry (IDMS) traceable Modification of Diet in Renal Disease (MDRD); PTH, parathormone

Calcium levels were corrected by albumin levels.

Values are expressed as mean ± standard deviation or median [interquartile range].

We used T Student paired test for parametric variables (a) and Wilcoxon test for repeated samples for non-normally distributed ones (b).

* p<0.05; ** p<0.005; ***p<0.001 compared to baseline

p<0.05; ## p<0.005; ###p<0.001 compared to 3 months

Table 3. Evolution of bone parameters during the study period

	Baseline	3 months	12 months
	N=36	N=36	N=36
Bone mineral density by dual-energy x-ray absorptiometry^a			
Lumbar			
gr/cm ²	0.950 ± 0.15	0.938 ± 0.15	0.930 ± 0.15*
Total Hip			
gr/cm ²	0.865 ± 0.17	0.857 ± 0.17	0.858 ± 0.19
Femoral Neck			
gr/cm ²	0.752 ± 0.15	0.730 ± 0.15*	0.734 ± 0.14
Trabecular bone score^a	1.198 ± 0.15	1.198 ± 0.15	1.169 ± 0.15
	Baseline	3 months	12 months
	N=14	N=14	N=14
Reference-point indentation bone mineral strength index^b (units)	79.2 [73.2-85.4]	75.9 [67.3-81.2]	80.1 [73-85.4]

Values are expressed by mean ± standard deviation or median [interquartile range].

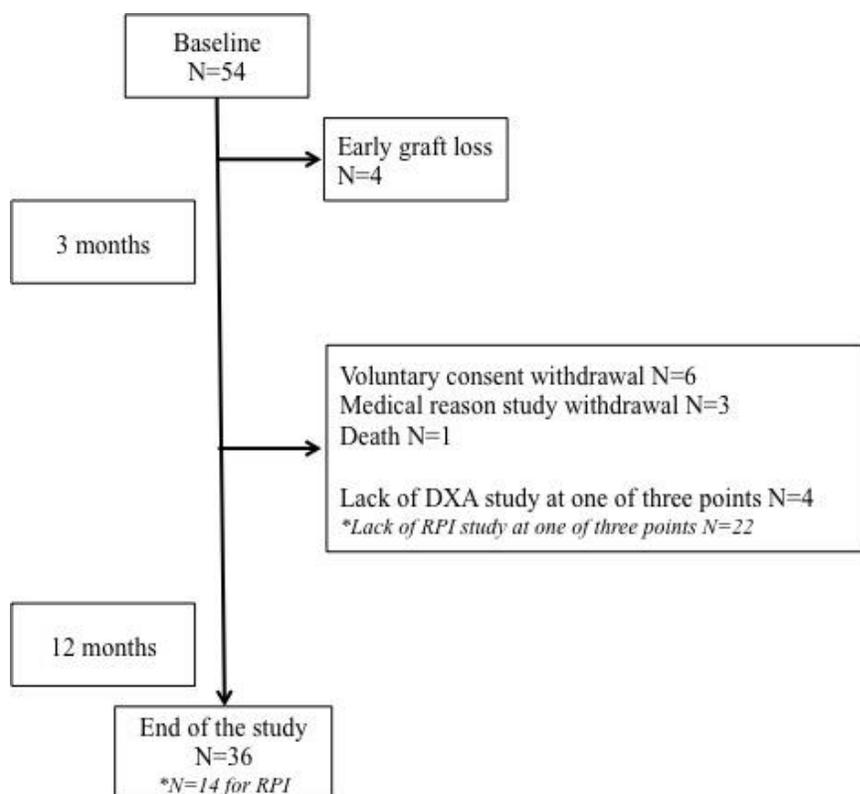
We used T Student paired test for parametric variables (a) and Wilcoxon test for repeated samples for non-normally distributed ones (b).

* p<0.05; ** p<0.005; ***p<0.001 compared to basal

Table 4. Bone mineral strength index values of the 14 kidney transplant recipients who underwent referent point indentation at 3 points (baseline, 3 and 12 months after transplantation).

Patient Age, Sex	BMSi at baseline	BMSi at 3 months	BMSi at 12 months
34, male	89.2	68.12	68.4
43, male	87.8	84	91
54, female	87.3	65.8	86.33
51, female	84.8	75.7	83.3
34, male	81.5	81.8	86.4
71, male	79.5	66.17	80.9
65, female	79	81	79.3
61, female	79	59.7	78
50, male	77.7	79.1	71.2
50, female	75.3	77.5	78.9
64, female	75	72	80.9
61, female	67.8	76.2	85.1
77, female	58.4	85.5	73.6
55, female	58	69.6	60.3

BMSi, bone mineral strength index

Figure 1. Flow-chart of patients included in the study.

Six patients voluntarily withdrew the consent due to visits and time spent on the clinic

Three patients were withdrawn by the doctor due to medical reasons: frequent hospitalizations, anticoagulant treatment, etc.

One patient died during the first year after transplantation due to cardiovascular reasons.

Highlights

- KT recipients experience a decrease in BMD over the 1st year after transplantation
- BMD loss leads to an increased risk of fractures in this population
- TBS and BMSi measure bone properties that contribute to bone fragility and fractures
- BMD decreases very slightly in KT recipients on low-dose glucocorticoids
- TBS and BMSi remain stable during 1st year after transplantation

ACCEPTED MANUSCRIPT