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Cyclosporine bone remodeling effect prevents steroid osteopenia after kidney transplantation

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Cyclosporine bone remodeling effect prevents steroid osteopenia after kidney transplantation.

Background. It is well established that prednisone above 7.5 mg/day may induce osteopenia in association with decreased bone formation. In contrast, the effect of cyclosporine on bone remodeling and bone mineral density (BMD) is controversial. Multiple confounding factors explain this controversy, especially after renal transplantation.

Methods. Fifty-two renal transplanted patients never exposed to aluminum while on dialysis were selected because they had no rejection and no hypercalcemia for 24 months while being treated with low dose prednisone/cyclosporine A (daily dose at 10 mg and 4.8 mg/kg, respectively, beyond 3 months). Bone remodeling markers (BRMs; plasma osteocalcin, bone and total alkaline phosphatases for formation, and urinary pyridinolines for resorption) were sequentially measured together with plasma creatinine, intact parathyroid hormone (PTH) and 25 OH vitamin D and cyclosporine from day 0 to 24 months. BMD was measured at 3, 6, 12, and 24 months by quantitative computerized tomography (QCT) at the lumbar spine and by double-energy x-ray absorptiometry (DEXA) at this site, as well as at the femoral neck, radius shaft, and ultradistal (UD) radius.

Results. Plasma concentrations of creatinine, PTH, and 25 OH vitamin D initially decreased and stabilized beyond three months at 137 μmol/L, 1.5 the upper limit of normal (ULN) and 11 ng/mL, respectively. All BRM increased significantly above the ULN at six months and then decreased. The BMD *Z* score at three months was low at all sites measured by DEXA and QCT. Follow-up measurements showed stability of absolute value and of *Z* score at all sites measured by DEXA. A comparison of the lumbar QCT *Z* score, which was available in 42 patients at 3 and 24 months, showed an increase in 28 and a decrease in 14, so that the increase for the whole group was significant ($P < 0.04$). Compared with patients with a decreased *Z* score, those with an increased *Z* score had significantly higher cyclosporine and lower prednisone dosages and a greater BRM increase at six months, whereas age, sex ratio, and plasma creatinine, PTH and 25 OH vitamin D were comparable and stable from months 3 through 24. The mean trough level of cyclosporine for the first six months was positively correlated to osteocalcin and total alkaline phosphatase increase at six months, and both bone formation and resorption marker increases were significantly correlated to the lumbar QCT *Z* score increase at 24 months.

Conclusions. Combined low-dose prednisone and cyclosporine immunosuppression are associated with a stabilization of BMD measured at all sites with DEXA 3 to 24 months after renal transplantation and with a prevention of age-related loss of vertebral trabecular bone, as shown by the significant increase in lumbar spine QCT *Z* score. It is suggested that cyclosporine, together with the decrease of prednisone dosage but independent of renal function, PTH, and vitamin D status, contributes to a transient stimulation of bone remodeling at six months, which counterbalances the deleterious effect of prednisone on bone formation and BMD.

Key words: osteopenia, kidney transplant, bone remodeling, prednisone, cyclosporine.

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It is well established that long-term prednisone therapy above 7.5 mg/day may induce osteopenia because of decreased bone formation (in relationship to the decrease in osteoblast differentiation and proliferation and to increased apoptosis of osteoblasts and osteocytes) and an increased bone resorption (caused by secondary hyperparathyroidism induced by calcium malabsorption) [1–4]. In contrast, the effect of cyclosporine on bone remodeling is controversial. In vitro studies show that cyclosporine A inhibits resorption of organotypic bone cultures, whereas most in vivo animal studies [5, 6], with the exception of those of Orcel et al [7] and Klein, Shaffer, and Wolfe [8], show that cyclosporine induces an accelerated high-turnover osteopenia by stimulating T-cell secretion of cytokines like interleukin-1 and -6 [5].

The clinical data on the bone effect of cyclosporine in transplantation also support a stimulating effect on bone turnover because, in contrast to the precyclosporine era [4], bone formation and resorption markers are increased at least transiently during the first year [9–12].

Bone histopathological data have confirmed the contrast between the decreased bone formation observed 6 to 12 months after transplantation with prednisone ± azathioprine treatment [13–15] and the stimulation of bone formation and resorption with cyclosporine + prednisone treatment. The difference was not explained by variations in prednisone therapy, time after transplantation, and renal function in a prospective comparative study [15].

In spite of the fact that cyclosporine is a glucocorticoid-sparing agent and may therefore protect against prednisone-induced ostopenia, the net result of combined therapy on bone mineral density (BMD) after renal transplantation is not judged to be beneficial in most studies, even though the decrease of BMD attenuates over the long-term [16–20]. The suspicion of a deleterious effect of cyclosporine on BMD is reinforced by the observation that in heart transplant patients treated with low-dose prednisone/cyclosporine, BMD decreases in association with increased osteocalcin and alkaline phosphatase levels [21, 22]. However, recent studies have shown that cyclosporine in monotherapy may have no harmful effect [23–25] or even a beneficial effect on BMD [26], as well as on trabecular bone volume assessed on bone biopsy [27]. In the latter study, the disappearance of heavy aluminum overload was a confounding issue, and multiple other factors may explain these discrepancies: (*1*) heterogeneity in previous vitamin D deficiency and hyperparathyroid bone diseases developed during long-term dialysis and their variable regression rate after transplantation due to heterogenous renal graft function; (*2*) population heterogeneity regarding other osteopenia risk factors such as diabetes and postmenopausal status; (*3*) the heterogeneity in immunosuppressive regimen and specifically in the glucocorticoid dose, which critical importance in early bone loss is well established [6, 13, 14]; (*4*) interference of other drugs, like ketoconazole, which decreases cyclosporine metabolism, potentiates prednisone effects, and decreases calcitriol synthesis [28]; and (*5*) heterogeneity in BMD measurements regarding technique, timing after transplantation, and site [29].

We herein report a prospective long-term follow-up (from 3 months to 2 years post-transplantation) of sequential BMD measurements performed not only at the lumbar spine [by absorptiometry and quantitative axial computerized tomography (QCT)], but also at the radius and femoral neck level (by absorptiometry only) in a population of 52 normocalcemic renal transplant patients. These patients were selected to assess the effect of low-dose prednisone combined with cyclosporine A, without interference of the previously mentioned confounding factors.

METHODS

Patients

Seventy-nine patients received a cadaveric kidney allograft and gave informed consent for measurement of BMD and bone remodeling markers (BRMs) for two years. Twenty-seven patients were excluded because of menopause (5 patients), poor renal function (plasma creatinine >200 μmol/L, 2 patients), acute rejection (14 patients), diabetes (3 patients), and lack of data (3 patients). A total of 52 patients (42 men and 10 women) was therefore included in this study. Their mean age ± SD was 39 ± 11 years. The causes of end-stage renal failure were glomerulonephritis (22 cases) interstitial nephritis (7 cases), polycystic kidney disease (10 cases), hereditary disease (8 cases), undetermined nephropathy (4 cases), and vascular nephropathy (1 case). The duration on dialysis was 12 ± 6 months. During dialysis, the patients were never exposed to aluminum in the dialysate, since reverse osmosis-treated water was used or by the oral aluminum phosphate binders because of exclusive use of $CaCO_3$. The lack of aluminum exposure was confirmed by plasma aluminum values on maintenance dialysis, which were always below 0.50 μmol/L. Parent vitamin D and $CaCO_3$ as phosphate binders were prescribed before transplantation, but not afterward. No patient received ketoconazole or aluminum hydroxide. None complained of bone pain.

Immunosuppression

Immunosuppressive therapy consisted of antithymocyte globulin (ATG) from day 0 to day 10 (Thymoglobulines Imtix Pasteur Merieux with initial dosage of 1.25 mg/kg daily), cyclosporine A (Sandimmun Sandoz) started at day 0 (1 mg/kg/day) and increased to 8 mg/kg/day when ATG was discontinued. Cyclosporine dosage was adjusted to achieve trough serum levels at 200 to 300 during the three first months and 150 to 200 ng/mL at one year. After a preoperative bolus of 500 mg of methylprednisolone, oral prednisone was started at 1 mg/kg/day and was progressively tapered to 0.5 mg/kg/day (1 month) and 10 mg/day after three months post-transplantation (Table 1). Cyclosporine dosage was stable during the first six months (7 to 6.5 mg/kg/day) and slowly decreased until two years after engraftment (to 4.8 mg/kg/day).

Laboratory measurements

Patients were studied on day 0 (just before surgery) and sequentially at 14, 30, 90, 180, and 365 days, and 24 months after transplantation.

Plasma concentrations of calcium, phosphorus, creatinine, total alkaline phosphatase activity, and urinary concentrations of calcium, phosphate, and creatinine were measured using standard techniques in the central labo-

Table 1. Immunosuppressive treatment

	Day 14	Day 30	3 months	6 months	12 months	24 months
Cyclosporine dosage *mg/kg/day*	7.7 ± 1	7.7 ± 0.6	7 ± 0.7	6.5 ± 0.8	5.7 ± 1	4.8 ± 0.9
Cyclosporine trough levels *ng/mL*	149 ± 77	190 ± 85	246 ± 98	222 ± 86	194 ± 73	160 ± 51
Oral prednisone *mg/day*	52 ± 11	33 ± 10	13 ± 4	10 ± 0.8	10 ± 0.4	10 ± 0.4

Data are mean ± SD of 52 patients.

ratory in our institution. Urinary pyridinolines were measured by high-performance liquid chromatography [30, 31]. Serum intact parathyroid hormone (PTH) was measured with the Chiron two-site immunochemiluminometric assay [32]. Serum bone alkaline phosphatase concentration was measured according to a previously described method (Ostase kit from Hybritech Laboratory) [33]. Osteocalcin (bone GLA protein) was measured with an immunoluminometric method [34]. Plasma 25 OH vitamin D was measured by a competitive protein-binding assay [35].

Bone densitometry

Patients were studied 3, 6, 12, and 24 months after transplantation. BMD was measured using two techniques: dual-energy x-ray absorptiometry (DEXA) with the Hologic QDR 2000 instrument (Hologic, Inc., Waltham, MA, USA) and by quantitative computerized tomography (QCT) (General Electric).

Dual-energy x-ray absorptiometry measured the anteroposterior BMD of the second, third, and fourth lumbar vertebrae (trabecular bone), as well as the BMD at the radius ultradistal (UD) site (where bone is mainly trabecular) and at its shaft (the junction between distal third and proximal two thirds) where bone is mainly cortical. BMD was also measured by DEXA at the femoral neck (mixed bone). Quality control of the machine was performed by daily scanning of an anthropomorphic phantom supplied by the manufacturer. The interday in vitro variability was 0.4%. DEXA BMD is an areal density and is expressed either in absolute value in grams of hydroxyapatite divided by the projected area in square centimeters (g/cm^2) or by the number of standard deviations from the mean of age- and sex-matched controls (Z score) [36].

Quantitative computerized tomography was used to measure trabecular mineral density of L2-L3-L4 lumbar vertebrae. QCT BMD is a true volumetric density expressed in absolute value in mg/cm^3. It is also expressed in Z score, the normal mean of lumbar QCT BMD being given by the following equations [37]: for men, $225 - 0.62 \times \text{age}$; and for women: $-6.1 + 13.8 \times \text{age} - 0.317 \times \text{age}^2 + 0.00197 \times \text{age}^3$.

Statistical analysis

The Wilcoxon matched-pairs test was used to compare chronological changes in the same whole group. Analysis of variance was used to compare the two groups in which lumbar QCT Z score increased or decreased from 3 to 24 months for the various parameters of Table 5.

Regression analysis was used to assess the associations between biochemical changes, BMD changes, and treatment. $P < 0.05$ was considered to indicate statistical significance. The results are expressed as mean ± SD.

Even though 52 patients were included in the study, not all data are available at all points for each parameter. Therefore, the number of patients for each comparison or each correlation has been given.

RESULTS

Bone mineral density

Vertebral BMD measured with QCT was stable from three months to two years (when expressed in absolute values) and was increased when expressed as Z scores (from −1.37 to −1.19; Table 2). This increase between 3- and 24-month Z scores was significant by the paired Wilcoxon test (0.18, $P < 0.04$). Paired data were available only in 42 patients and showed an increase in 28 and a decrease in 14 patients. The vertebral BMD Z score measured by DEXA increased, but not significantly. Vertebral Z scores obtained with QCT and DEXA were positively correlated ($r = 0.76$, $P < 0.0001$).

Bone mineral density absolute absorptiometric data at the UD radius, radius shaft, and femoral neck were stable from three months to two years. At three-months post-transplantation, the mean Z score was negative at all sites (−0.95, −0.62, and −1.34) and did not change significantly for two years.

Biochemical determinations

Table 3 shows that mean serum creatinine concentration significantly decreased from day 0 to day 14 and then remained constant around 137 μmol/L. The plasma total calcium concentration was always in the normal range, but presented a slight and transient decrease at days 14 to 30 and then stabilized after six months at 2.46 mmol/L. Plasma phosphate rapidly decreased to the lower limit of normal at days 14 and 30 and then stabilized after six months to approximately 1.1 mmol/L. The mean serum concentration of intact PTH decreased from 184 pg/mL on day 0 to 96 pg/mL at day 14, and stabilized after six months at 87 pg/mL, corresponding to 1.5-fold

Table 2. Bone mineral density from 3 months to 2 years post-transplantation

Measurement method and sites	Units[a]	3 months	6 months	1 years	2 years	Difference 24–3 months
Quantitative computerized tomography						
Vertebra (L2-L3-L4)	mg/cm^3	122.6 ± 33	122.5 ± 35	125.13 ± 31	123.7 ± 28	
	Z score	−1.37 ± 1.09	−1.36 ± 1.19	−1.29 ± 0.99	−1.19 ± 1.03	+0.18[b]
Dual x-ray absorptiometry						
Vertebra L2-L3-L4 (frontal absorption)	g/cm^2	0.93 ± 0.2	0.91 ± 0.1	0.93 ± 0.1	0.94 ± 0.1	
	Z score	−1.40 ± 1.4	−1.42 ± 1.3	−1.41 ± 1.2	−1.35 ± 1.16	+0.05
Ultradistal radius	g/cm^2	0.44 ± 0.1	0.42 ± 0.6	0.42 ± 0.1	0.415 ± 0.1	
	Z score	−0.95 ± 1.4	−1 ± 1.1	−1 ± 1.2	−1.1 ± 1.4	−0.15
Radius shaft	g/cm^2	0.72 ± 0.1	0.74 ± 0.1	0.75 ± 0.1	0.75 ± 0.1	
	Z score	−0.62 ± 1	−0.6 ± 1.1	−0.58 ± 1	−0.53 ± 1.2	+0.09
Femoral neck	g/cm^2	0.74 ± 0.1	0.71 ± 0.1	0.74 ± 0.1	0.75 ± 0.1	
	Z score	−1.34 ± 1.2	−1.56 ± 0.9	−1.39 ± 0.9	−1.22 ± 0.9	+0.12

Data are mean ± SD of 52 patients.
[a] mg/cm^3 and g/cm^2 mean milligram and gram equivalent of hydroxyapatite
[b] None of the measurements after 3 months significantly differs from that at 3 months at the exception of the lumbar QCT Z score at 3 and 24 months for which paired Wilcoxon test showed a significant increase ($P < 0.04$)

Table 3. Follow-up of plasma creatinine, calcium, phosphate and intact parathyroid hormone (PTH)

Plasma concentration *normal range*	Day 0	Day 14	Day 30	3 months	6 months	12 months	24 months
Creatinine *70–120 μmol/L*	800 ± 200	145 ± 81[b]	121 ± 35	128 ± 30	137 ± 30	137 ± 30	137 ± 25
Calcium *2.2–2.6 mmol/L*	2.54 ± 0.3	2.27 ± 0.2[b]	2.33 ± 0.2	2.46 ± 0.1	2.47 ± 0.1[c]	2.46 ± 0.2	2.45 ± 0.1
Phosphate *0.7–1.3 mmol/L*	1.57 ± 0.6	0.87 ± 0.2[b]	0.76 ± 0.2	0.98 ± 0.2	1.08 ± 0.2[c]	1.17 ± 0.2	1.13 ± 0.2
Intact PTH *10–55 pg/mL*	184 ± 153	96 ± 55[a]	122 ± 79	92 ± 46	87 ± 41	94 ± 40	82 ± 43
Plasma 25 OH vitamin D *10–40 ng/mL*	18 ± 12	11.3 ± 10[a]	11 ± 5	11 ± 6	15.3 ± 9	10.2 ± 6	12.5 ± 7

Data are mean ± SD. None of the changes is significant when comparing 6 months with 24 months
[a] $P < 0.01$, [b] $P < 0.001$, comparison of Day 0 with Day 14
[c] $P < 0.001$, comparison of Day 14 to 6 months

Table 4. Follow-up of the bone remodeling markers

Markers *normal range*	Day 0	Day 14	Day 30	3 months	6 months	12 months	24 months
Markers of bone formation							
Bone alkaline phosphatase *3.2–20.4 μg/L*	12.5 ± 9.4	11.3 ± 9.7	16.9 ± 9.7	21.7 ± 10.4	26.8 ± 11.9[c]	18.5 ± 9.1	14.3 ± 7.1[e]
Total alkaline phosphatase *40–120 IU/L*	84 ± 57	72 ± 48[a]	86 ± 46	104 ± 52	119 ± 61[c]	93 ± 36	74 ± 26[e]
Osteocalcin (or bone GLA protein) *4–12 ng/mL*	28 ± 20	7 ± 11[a]	7 ± 6	13 ± 9	20 ± 13[c]	21 ± 17	14 ± 8[d]
Markers of bone resorption							
Urinary deoxypyridinoline *3–12 μmol/mol creatinine*		9.3 ± 5.7	9.4 ± 6.4	9.4 ± 4.6	12.8 ± 6.7[b]	10.6 ± 5.1	9 ± 5.4[e]
Urinary pyridinoline *15–50 μmol/mol creatinine*		51 ± 26	51 ± 29	50 ± 19	56 ± 25	45 ± 17	34 ± 24[e]

Data are mean ± SD.
[a] $P < 0.001$, comparison of Day 0 to Day 14
[b] $P < 0.01$, [c] $P < 0.001$, comparison of Day 14 to 6 months
[d] $P < 0.01$, [e] $P < 0.001$, comparison of 6 months to 24 months

the upper limit of normal (Table 3). Plasma 25 OH vitamin D levels decreased significantly from day 0 to day 14 (from 17 to 11 ng/mL) and then remained stable up to 24 months after transplantation.

Table 4 shows the absolute values of the BRMs during follow-up. At day 14, there was a significant decrease in plasma osteocalcin and total alkaline phosphatase, but not in bone alkaline phosphatase. These markers of bone formation showed a progressive increase up to six months, starting from day 30 for total and bone alkaline phosphatase and from month 3 for osteocalcin. After six months, these markers progressively decreased until two years, with the exception of osteocalcin, which decreased only after 12 months. Figure 1 shows the data expressed

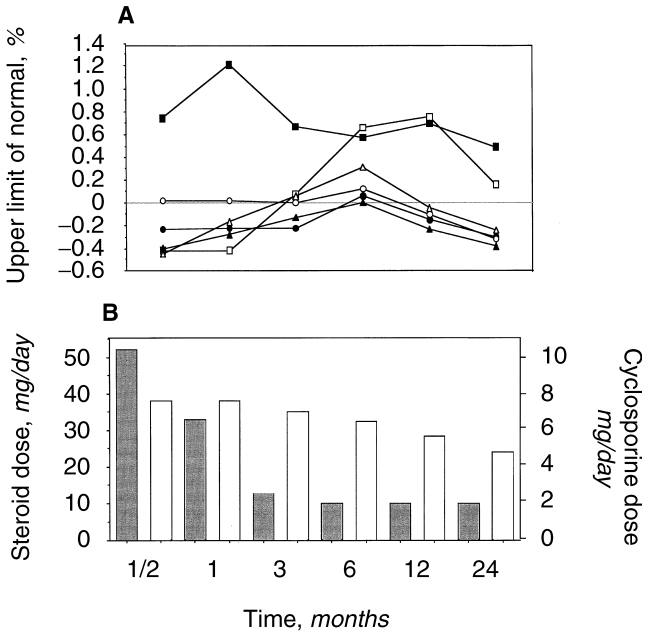

Fig. 1. Follow-up of bone remodeling markers (BRMs) (*A*) and treatment (*B*). Symbols are: (■) parathyroid hormone; (□) osteocalcin; (▲) total alkaline phosphatase; (△) bone alkaline phosphatase; (○) pyridinoline; (●) Deoxypyridinoline. Black boxes represent steroid doses.

in percentage of the upper limit of normal; at six months, bone alkaline phosphatase and osteocalcin levels were above the upper limit of normal. At this point, the dose of cyclosporine was still high (6.5 ng/kg/day), whereas the dose of prednisone had been decreased to 10 mg/day. The decrease in the bone formation markers to normal levels at two years occurred, while the dose of cyclosporine decreased to 4.8 ng/kg/day and that of prednisone remained stable at 10 mg/day.

The markers of bone resorption were measured only in 24-hour urine specimen and therefore were not available before transplantation. Deoxypyridinolinuria was 80% of the upper limit of normal, and pyridinolinuria was at this limit during the three first months. Both increased slightly above normal, but only at six months, and then decreased to normal. The increase at six months was significant only for deoxypyridinoline.

It is noteworthy that plasma intact PTH and 25 OH vitamin D levels stabilized between day 14 and two years, while the markers of bone remodeling transiently increased above normal at six months.

Factors connected with vertebral BMD changes

Table 5 shows that lumbar QCT Z scores increased in 28 patients and decreased in 14. Patients whose Z score increased had significantly lower prednisone and higher cyclosporine doses (therefore, higher cyclosporine/prednisone dosage ratio) than those in whom Z scores decreased. Age (35 ± 8 vs. 40.7 ± 11.6 years, mean ± SD) and sex ratio (11/3 vs. 23/5 males/females) did not differ between the two groups. Plasma concentrations of creatinine, PTH, and plasma 25 OH vitamin D were comparable in both groups at 3, 6, 12, and 24 months. The levels of BRMs at six months were higher in the patients with an increased Z score at 24 months, but the difference was significant only for pyridinolines and total alkaline phosphatase.

Correlatives studies

The mean daily dose of prednisone at three months was significantly negatively correlated to the lumbar QCT score changes between 3 and 6 or 24 months ($r = -0.37$, $N = 43$, $P < 0.02$).

Mean trough cyclosporine levels for the first six months were significantly correlated to osteocalcin (0.48, $N = 28$, $P < 0.01$; Fig. 2) and to total alkaline phosphatase increases from 0.5 to 6 months ($r = 0.40$, $N = 25$, $P < 0.05$), but not to the pyridinoline increases.

Table 6 shows that the increases of all remodeling markers between 0.5 and 6 months were correlated to the changes of lumbar QCT *Z* score between 3 and 24 months.

DISCUSSION

We evaluated BMD in a highly selected renal transplant population to discard most of the recognized confounding factors. We found that low-dose prednisone and cyclosporine therapy is associated with a stabilization of BMD measured by DEXA at all sites beyond three months. An increase of the lumbar QCT Z score suggests prevention of age-related loss of vertebral trabecular bone. The fact that only the lumbar QCT Z score shows an increase between 3 and 24 months but not the DEXA Z score may be explained by the larger surface of trabecular bone involved in bone remodeling than that of the cortical bone. Schober et al have indeed evaluated that an increase of 25 μm of bone thickness on the entire trabecular surface would result in a 30 to 40% increase of mineralized bone volume, whereas the same thickness increase at the entire endosteal surface would result in only a 3% increase of mineralized cortical bone [38]. Indeed, QCT used in this study measured a true volumetric density of trabecular bone, and its results are expressed in mg of calcium per cm^3, whereas DEXA measures only an areal density related to the calcium content not only of the trabecular bone, but also of two corticals.

This favorable effect was observed while all BRMs transiently increased to values at/or above normal at six months. A comparison of the 28 patients whose lumbar QCT Z scores increased to the 14 patients whose Z scores decreased showed that lower prednisone and higher cyclosporine dosages were the sole distinctive

Table 5. Comparison of treatment, plasma concentrations of creatinine, PTH and vitamin D and of bone remodeling markers in patients with increasing or decreasing lumbar QCT Z score between 3 and 24 months post-transplantation

Parameters	Lumbar QCT Z score		*P*
	Increasing ($N = 28$) ($+ 0.85 \pm 0.70$)	Decreasing ($N = 14$) (-0.77 ± 0.69)	
Treatment dosage for 3–24 months			
Prednisone *mg/day*	10.5 ± 0.5	11.2 ± 1.3	0.02
Cyclosporine *mg/kg/day*	6.70 ± 0.5	6.35 ± 0.6	0.05
Ratio cyclosporine/prednisone	0.59 ± 0.07	0.53 ± 0.08	0.02
Plasma creatinine *μmol/L*			
3 months	124 ± 25	133 ± 35	NS
6 months	134 ± 27	143 ± 32	NS
12 months	134 ± 29	141 ± 32	NS
24 months	137 ± 25	143 ± 25	NS
PTH status *pg/mL*			
3 months	93 ± 52	91 ± 36	NS
6 months	92 ± 46	80 ± 34	NS
12 months	96 ± 45	88 ± 33	NS
24 months	85 ± 48	81 ± 34	NS
Plasma 25 OH vitamin D *ng/mL*			
3 months	10 ± 6	13 ± 6	NS
6 months	15 ± 8	16 ± 10	NS
12 months	11 ± 6	9 ± 7	NS
24 months	12 ± 5	11 ± 6	NS
Bone remodeling marker at 6 months			
Urinary deoxypyridinoline *μmol/mmol creatinine*	14.6 ± 73	8.5 ± 3.3	0.01
Urinary pyridinoline *μmol/mmol creatinine*	62 ± 25	40 ± 11	0.01
Total alkaline phosphatase *IU/L*	174 ± 47	109 ± 29	0.01
Bone alkaline phosphatase *μg/L*	28 ± 11	22 ± 11	NS
Osteocalcin *ng/mL*	22 ± 14	16 ± 11	NS

Data are mean ± SD. ANOVA was used to determine *P* values. Abbreviations are: PTH, parathyroid hormone; QCT, quantitative computerized tomography.

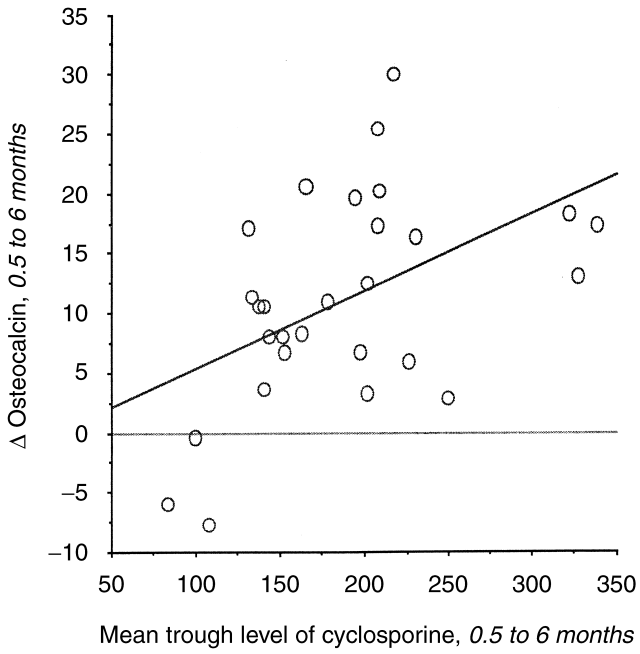

Fig. 2. Correlation of plasma osteocalcin increase at six months and mean trough level of cyclosporine during the first six months ($P = 0.01$; $r = 0.48$; $N = 28$).

characteristic between the two groups. The groups were comparable for age, sex ratio, and plasma concentrations of creatinine, PTH, and 25 OH vitamin D for 3 to 24 months after transplantation. At six months, patients with an increased QCT Z score had higher levels of urinary pyridinolines and plasma total alkaline phosphatase. The mean trough cyclosporine level for the first six months correlated to the increase at six months of the bone formation markers, whereas increases in bone formation and resorption markers correlated with the lumbar QCT Z score increase at 24 months.

Although we did not have a control group with a comparable dose of prednisone and a different dose of cyclosporine, all of these comparisons and correlations suggest that cyclosporine contributed to the transient increase of bone formation, independently of lowering the prednisone dosage. Indeed, the prednisone dose was still supraphysiologic and in the range expected to decrease osteoblast differentiation and proliferation [2]. Thus, the elevation of alkaline phosphatase and osteocalcin above normal cannot be explained solely by a decrease in factors inhibiting bone formation (such as prednisone, a uremic factor inhibiting osteoblasts, or aluminum overload). Stability of renal function excludes an increase of osteocalcin levels simply due to decreased urinary excretion. Stability of plasma PTH and 25 OH vitamin D levels excludes hyperparathyroidism worsening and changes in

Table 6. Correlations of lumbar spine QCT Z score increase at 24 months and remodeling factor changes between 0.5 and 6 months

Z score change period	Remodeling marker *6–0.5 months*	*r*	*N*	*P*
24–3 months	Osteocalcin	+0.54	20	0.01
	Bone alkaline phosphatase	+0.40	40	0.01
	Total alkaline phosphatase	+0.80	18	0.0001
	Deoxypyridinoline	+0.34	37	0.04
	Pyridinoline	+0.30	38	0.06

vitamin D status as explanations for the increase of bone remodeling parameters.

Because cyclosporine increased biochemical and histologic bone formation markers in animal [7, 8] and clinical studies [9, 11, 12, 15], and the cyclosporine mean trough level for the first six months in this study correlated to the six-months increase in osteocalcin and total alkaline phosphatase, we suggest that cyclosporine was responsible for the increase in the bone formation markers in this study.

The suggestion that cyclosporine contributed to the beneficial effect on BMD comparatively to age- and sex-matched control by stimulating preferentially bone formation is further supported by the observation that the lumbar QCT Z score increases at 24 months were correlated with all BRM increases at six months, while in contrast, prednisone at 10 mg/day would be expected to increase bone resorption and to decrease bone formation and BMD [2, 3]. The lumbar QCT Z score increase, in spite of stimulation of both formation and resorption, is not surprising. It has been also documented during the prevention of bone loss in patients with therapeutical estrogen deficiency for endometriosis [39] or glucocorticoid-induced osteoporosis [40] by daily injection of PTH (1-34). The principal mechanism of this anabolic effect on bone of intermittent PTH administration is the prevention of the osteoblast and osteocyte apoptosis [1]. Interestingly, while Movsowitz et al have stressed the osteopenic effect of high bone turnover in rats induced by cyclosporine, they have also reported that combined therapy with cyclosporine and cortisone acetate stabilized their bone mass [41]. However, the rat model for osteopenia is not a good model for human glucocorticoid osteoporosis since glucocorticoids stimulate bone formation in rat.

In clinical renal transplantation, most long-term BMD data beyond two years document an attenuation of the bone loss in patients treated with cyclosporine + prednisone ± azathioprine. This loss becomes parallel to the age-related bone loss [18, 20]. As the same is observed on the very late, after 80- to 120-months post-transplantation, in patients treated with prednisone and azathioprine alone [25], this long-term favorable outcome may only reflect the decrease in prednisone dose. This possibility stresses the contribution of recent controlled studies with a cyclosporine monotherapy group and a control group. However, the interpretation of these studies is confounded by important issues: severe hyperparathyroidism in that of Torregrosa et al [23], improvement of the bone aluminum overload in that of Briner et al [27], and lack of information on pretransplant renal osteodystrophy and on prevalence of methylprednisolone-treated rejections in that of Parry et al [24]. Most informative was the prospective study by Aroldi et al, which showed that lumbar DEXA *Z* scores were significantly greater in 13 patients with cyclosporine monotherapy than in 20 patients receiving cyclosporine + prednisone and in 20 patients on triple therapy, despite very large standard deviations [26]. This study is therefore in agreement with our study showing that cyclosporine with low-dose prednisone can prevent the age-related trabecular bone loss before two-years post-transplantation and supports a proper bone-sparing effect of cyclosporine.

The clinical data on bone effect of cyclosporine in other organ transplantations are generally interpreted as evidence for a deleterious effect on BMD of cyclosporine bone hyper-remodeling effect, but again are clouded by numerous confounding factors. In heart transplantation, previous smoking, prolonged physical inactivity, and high-dose cyclosporine induced nephrotoxicity are problematic. In the study by Thiebaud et al, high-turnover bone loss was related to cyclosporine or hyperparathyroidism [21]. Indeed, severe bone loss at six months occurred with an increase of osteocalcin, whereas PTH levels stabilized at lower level after month 2, but the lack of significant BMD increase at 12 months in spite of corticotherapy discontinuation was related to an increase of plasma creatinine, PTH, and total alkaline phosphatase, suggesting a role of hyperparathyroidism induced by renal insufficiency caused by cyclosporine toxicity.

After liver transplantation with combined prednisone and cyclosporine therapy, Vedi et al showed by histomorphometry an increase in bone turnover in the first three post-transplant months without a significant disruption of cancellous bone [42]. They further speculated, without other long-term data, that this increase in bone remodeling predisposes to trabecular penetration and decreased bone strength.

In hemopoietic stem cell transplantation, Eberling et al have suggested that cyclosporine played a role in bone loss, but only on the basis of correlation between BMD

decreases and duration of cyclosporine treatment given with prednisone for graft versus host (GVH) disease [43]. However, bone loss was also correlated with daily and cumulative prednisone dose and was not observed in autologous transplantation. Therefore, the correlation with cyclosporine duration may simply reflect the severity of the GVH disease with its direct toxicity on bone cells, rather than the intensity of immunosuppressive treatment.

Thus, none of these observations in other transplantation settings unequivocally support the concept that cyclosporine bone hyper-remodeling effect has a deleterious long-term effect on BMD.

Conversely, the single placebo-controlled study of cyclosporine effect on BMD and bone remodeling (performed in patients with primary biliary cirrhosis with decreased bone turnover) has shown that cyclosporine treatment was associated with stable lumbar BMD after 24 months, whereas the placebo group lost bone mass [44]. This protective effect of cyclosporine was associated with higher plasma osteocalcin and urinary hydroxyprolinine. However, because plasma PTH levels were also higher in the cyclosporine group (in spite of insignificantly higher plasma concentrations of 25 OH vitamin D and calcitriol), it cannot be inferred that the protection of BMD was due to a direct skeletal effect of cyclosporine.

Even though our study does not provide direct evidence for a protective effect of cyclosporine on bone, it suggests that cyclosporine may reduce the risk for glucocorticoid-induced osteopenia in transplant patients not only as being a glucocorticoid-sparing agent but also by transiently stimulating bone formation more than bone resorption.

Conclusion

Our study shows that in renal allograft recipients without confounding factors, low-dose prednisone and cyclosporine were associated with a stable BMD measured by DEXA beyond three months after transplantation. Furthermore, the lumbar QCT *Z* score increased, suggesting that age-related loss of vertebral cancellous bone was prevented. This increase in *Z* score was associated with lower prednisone and higher cyclosporine dosages, and higher BRMs at six months and was independent of renal function, PTH, and vitamin D levels. Because the increase in the BRMs correlated with cyclosporine trough levels, it is suggested that cyclosporine may counterbalance the depressive effect of prednisone 10 mg/day on bone formation and BMD by stimulating bone remodeling.

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