Kidney International, Vol. 55 (1999), pp. 2021–2029

Bone loss in long-term renal transplantation: Histopathology and densitometry analysis

Alfonso M. Cueto-Manzano, Shaikh Konel, Alastair J. Hutchison, Vivion Crowley, Michael W. France, Anthony J. Freemont, Judith E. Adams, Barbara Mawer, and Ram Gokal

Departments of Renal Medicine and Clinical Biochemistry, Manchester Royal Infirmary, and Departments of Osteopathology, Diagnostic Radiology, and Medicine, School of Medicine, University of Manchester, Manchester, England, United Kingdom

Bone loss in long-term renal transplantation: Histopathology and densitometry analysis.

Background. There is little information of the spectrum and factors implicated in the bone loss in long-term renal transplantation, and virtually no data using both histomorphometric and densitometric analysis.

Methods. Twenty-three males and 22 females (13 postmenopausal) were studied with a bone biopsy and densitometry. Sixteen patients were on cyclosporine A monotherapy, 20 on azathioprine + prednisolone, and 9 on cyclosporine A + prednisolone or triple therapy. The mean time after transplantation was 127 ± 70 months.

Results. No group had a significant decrease in bone mineral density (BMD) of the axial skeleton compared with an age- and sex-matched normal population. Compared with sex-matched young controls, osteopenia was observed in all groups at the femoral neck (except premenopausal women and triple therapy) and in the triple-therapy group at the L1–L4 spine region. At the distal radius, osteopenia was found in all the groups. Histopathological diagnosis was mixed uremic osteodystrophy in 46.5%, adynamic bone in 23.2%, hyperparathyroid disease in 13.9%, and normal bone in 16.3%. The diagnosis was not different according to immunosuppressive therapy, but men tended to show more mixed uremic bone disease. There was no significant difference in BMD between histopathological subtypes. In general, patients showed slight osteoclast function increase, osteoblast function decrease, and marked retardation of dynamic parameters. The cyclosporine A monotherapy group had a significantly lower appositional rate than azathioprine + prednisolone. Men had a significantly lower bone volume than women, and premenopausal women had a significantly lower mineralizing surface than postmenopausal women and men. In the multivariate analysis, male gender, time after transplantation, old age, and time on dialysis prior to transplantation were significant predictive factors for a negative effect on bone mass.

Conclusions. Long-term renal transplant patients showed reduced BMD in both trabecular and cortical bone. This reduction in BMD was not as severe as in short-term reports and was associated with osteoclast stimulation, osteoblast suppression, and retardation of mineral apposition and bone formation rates. Bone mass loss was not different between the immunosuppression therapy groups. Male gender and age were the strongest predictive factors for low bone mass.

Key words: bone mineral density, cyclosporine A, glucocorticoids, osteoclast, osteoblast, immunosuppression, renal osteodystrophy.

Received for publication June 5, 1998
and in revised form December 21, 1998
Accepted for publication December 22, 1998

Disorders of mineral and bone metabolism have an impact on significant morbidity in patients with end-stage renal disease, and play a prominent role in the development of renal osteodystrophy [1, 2]. Kidney transplantation improves the metabolic environment and restores the glomerular filtration and renal production of 1,25-dihydroxyvitamin D_3 impaired during chronic renal failure. In spite of this, patients with kidney transplantation, as well as heart and liver transplant recipients, have been shown to be at risk for bone loss [3–6]. Osteopenia, avascular bone necrosis, fractures, and persisting hyperparathyroid bone disease are common after transplantation [3, 4, 7]. It has repeatedly been demonstrated that bone loss is highest within the first 6 to 18 months after renal transplantation [8–10]. Cross-sectional data suggest that beyond three years after transplantation, the bone mineral density (BMD) does not change or might increase slightly, but remains below the normal population reference values [11]. Others, however, have suggested that a continuous demineralization process is present in long-term renal transplant recipients [12].

Before the introduction of cyclosporine A (CsA) as an immunosuppressive agent, bone loss following transplantation was attributed primarily to glucocorticoids [13, 14]. As CsA reduces the requirements for glucocorticoids, an improvement of the bone loss in the post-transplant period was expected with its wide use. However, this expectation has not been fulfilled, as bone alterations remain a problem [8–10, 15–20]. In humans, several immunosuppressive regimens, including CsA and

prednisolone (PRED), have been shown to decrease lumbar BMD [8–10, 18–20]. Moreover, conflicting results have been reported about the role of age, sex, postmenopausal status, type and duration of dialysis treatment, immunosuppressive therapy, graft rejection episodes, persistent hyperparathyroidism, and decreased creatinine clearance as risk factors for the bone loss related to kidney transplantation [10, 11, 18–20].

Mineralized bone histopathology provides the best information regarding bone abnormalities; however, this technique is underused because of perceived constraints [21]. There is scarce histopathological information on the bone loss associated with short-term renal transplantation and virtually no data on long-term adult renal recipients. Recently, one study performed in children and adolescents (employing histopathological and bone mineral content analysis) suggests that reductions in bone mass and post-transplant osteoporosis are not prominent findings in this renal transplant population when the influence of growth retardation on bone mass is carefully considered [22]. In adults, to the best of our knowledge, no study has been undertaken in the long-term setting, employing both histomorphometry and densitometry analysis. Therefore, our cross-sectional study was designed, employing bone histomorphometry and densitometry, to investigate the spectrum of the bone disease in adult patients with long-term, successful renal transplantation. The association between these histomorphometric and densitometric parameters with some biochemical, clinical, and immunosuppression variables was also analyzed.

METHODS

Patients

Forty-five patients were studied: 23 male and 22 female (13 postmenopausal; time since menopause 11.2 ± 8.3 years). The mean age was 48 ± 12 years, and the time after transplantation was 127 ± 70 months. The cause of renal failure was chronic primary glomerulonephritis in 18, reflux nephropathy in 8, adult polycystic kidney disease in 7, unknown in 4, obstructive nephropathy in 3, diabetes mellitus in 2, and other cause in 3. Only four patients were on furosemide, with a mean cumulative dose of 262 ± 225 g. Patients were selected randomly if they had a first kidney transplant performed over two years previously, had stable graft function (serum creatinine of less than 180 μmol/liter), and gave informed consent. Exclusion criteria included any condition (prolonged immobilization, systemic illness, or malignancy) or intake of drugs (other than immunosuppression) that could affect bone metabolism. No patient received calcium carbonate or calcitriol after transplantation.

Patients were classified into groups according to gender and menopausal status and according to the immunosuppressive therapy received. In our renal unit, immunosuppression protocols were as follows.

CsA monotherapy. CsA was infused intravenously at a dosage of 5 mg/kg at days 0 and 1, continued orally at a dose of 15 mg/kg/day, and progressively reduced according with serum CsA levels (100 to 150 pg/ml).

Azathioprine (AZA) + PRED dual therapy. PRED 20 mg/day was given orally for the first 90 days and then reduced monthly to a maintenance dose of 5 to 10 mg/day. AZA was started at 2 mg/kg/day and then progressively decreased to a maintenance dose of 1 mg/kg/day.

Triple therapy (CsA + AZA + PRED). After the CsA intravenous infusion of the first two days, this drug was given orally at a dose of 15 mg/kg/day and then tapered off to a maintenance dose of 5 mg/kg/day (accordingly with serum levels). AZA and PRED were given as before.

Rejection episodes were treated with three daily intravenous methylprednisolone boluses (1.0 g each). Intravenous monoclonal antibodies against T-lymphocytes (OKT3) or anti-thymocyte globulin (ATG) was given in case of steroid-resistant rejection. Cumulative doses of CsA, PRED, and AZA were calculated, considering both intravenous and oral doses. Methylprednisolone and PRED were considered as equipotent.

Twelve patients were changed from one immunosuppressive group to another: eight patients from CsA monotherapy transferred to conventional, CsA + PRED, or triple-therapy group because of repeated rejection episodes, and four patients from the triple therapy to a dual or monotherapy group to decrease side-effects. All patients were receiving their final immunosuppressive therapy (as classified in this study) for at least two years prior to the study. PRED and CsA have been considered the most relevant drugs for bone loss after transplantation; thus, patients on triple immunosuppression and on CsA + PRED were considered as one group.

Bone histological evaluation

After double tetracycline labeling, bone biopsies were taken from the anterior iliac crest using an 8 mm Bordier trephine biopsy needle as described previously [23]. Specimens were processed as described in detail elsewhere [24] and were analyzed by a single pathologist (A.J.F.), who was blinded to patients' details. Bone histomorphometry was performed according to guidelines of the ASBMR histomorphometry nomenclature committee [25]. Results were compared with age- and sex-matched normal values (Z scores) derived from the database biopsies for 234 normal subjects (including 84 individuals with double tetracycline labeling) and necropsies currently employed in our department [24].

Bone mineral density measurements

Within two weeks of the bone biopsy, BMD of the axial and appendicular skeleton was determined by den-

sitometry. BMD of the forearm (distal radius) was analyzed by single-energy x-ray absorptiometry (SXA) using a bone densitometer DTX-100 (Osteometer; MediTech, Roedrove, Denmark). The density of the right femoral neck and lumbar spine (L1–L4) was evaluated by dual-energy x-ray absorptiometry (DXA) using a DPX scanner (Lunar Corporation, Madison, WI, USA). Results were compared with values of age- and sex-matched reference population (Z score) and with values of sex-matched peak bone mass of a young control population (T score) currently employed in the Department of Diagnostic Radiology, University of Manchester. Densitometers were calibrated every morning against a phantom provided by dealers. The SXA densitometer was autocalibrated using an integrated computer program. In the case of the DXA densitometer, the coefficient of variability for femoral neck was 2.07% and 1.57% for L1–L4.

Laboratory tests

Blood samples were obtained on the day of the bone biopsy. Ionized calcium, phosphate, and creatinine were measured by standard techniques. Bone specific alkaline phosphatase was assayed by colorimetry and carboxyterminal telopeptide of type I collagen and propeptide of type I procollagen by radioimmunoassay. Intact molecule of parathyroid hormone (iPTH) was measured by immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), and 1,25-dihydroxyvitamin D_3 was evaluated by radioimmunoassay on a 1217 Rackbeta Scintillation Spectrometer (Crownhill Wallac, UK).

Statistical analysis

Results are shown as mean ± SD. Comparisons of dimensional variables between two groups were performed by the independent samples *t*-test or Mann–Whitney *U*-test as appropriate and between three groups by analysis of variance. In case of a significant model obtained in the latter analysis, all pair-wise multiple comparisons were analyzed by the Student–Newman–Keuls method. A comparison of nominal variables was performed by χ^2 or Fisher's exact test as appropriate. Univariate association analysis was done by Pearson's or Spearman's correlation coefficients (as appropriate). Multivariate analysis was carried out by stepwise multiple linear regression. All of the univariate and multivariate analyses, in which densitometric and histomorphometric parameters were regarded as dependent variables, were performed considering Z scores. We consider these scores as the best way to control for the influence of age and gender, which are known risk factors for bone loss in the normal population [24, 26]. Age, sex, creatinine clearance, iPTH, rejection episodes, type and time on dialysis before transplantation, time after transplantation, and cumulative dose of PRED and CsA were considered independent variables. Collinearity between independent variables was tested by Durbin–Watson test. A two-tail $P < 0.05$ was accepted as significant, but the exact value is preferentially shown.

RESULTS

At the time of the study, 16 patients were on CsA monotherapy, 20 on AZA + PRED, 6 on CsA + PRED, and 3 on triple therapy. One patient had a history of hip replacement (avascular necrosis). Two had a history of peripheral bone fracture. Three had vertebral disc space narrowing. One had chronic knee bone pain. Two had generalized bone pain, and one had loss of the hip joint space and sclerosis. Relevant clinical information according to the immunosuppressive therapy and sex is shown in Table 1. The time after transplantation was significantly longer in the AZA + PRED group than in the other two immunosuppressive groups, probably because this scheme was the first employed in our renal unit but has been subjected to a restricted use in the last five years. A cumulative dose of PRED in the CsA monotherapy group represents only steroids given during graft rejection episodes.

Detailed biochemical characteristics are shown in Table 2. The mean propeptide of type I procollagen was higher in premenopausal and postmenopausal women than reference values for women (50 to 170 μg/liter). All groups showed a mean telopeptide of type I collagen higher than reference values (1.6 to 4.6 μg/liter).

Table 3 shows the bone densitometry data. Compared with an age- and sex-matched normal population (Z score), no group had a significant reduction of BMD at L1–L4 and femoral neck, and none was in the range for fracture risk [26]. However, when compared with the peak bone mass of sex-matched young normal controls (T score), osteopenia (according to the World Health Organization diagnostic criteria) [27] was observed at the femoral neck in all groups except premenopausal women and patients on triple therapy. In the appendicular skeleton, a greater reduction of BMD was evident as osteopenia was found in all the groups at the distal radius. No significant differences of BMD measurements between groups according to immunosuppressive drug therapy, gender, or menopausal status were observed. However, men seemed to display BMD T scores as decreased as postmenopausal women and lower than premenopausal women (although this difference was not statistically significant).

Two bone biopsy samples were not useful for histopathological diagnosis. In the remaining 43 patients, the histopathological analysis revealed mixed uremic bone disease in 20 (46.5%) patients, adynamic bone in 10 (23.2%), hyperparathyroid bone disease in 6 (13.9%), and normal bone in 7 (16.3%). Histopathological diagno-

Table 1. Clinical characteristics according immunosuppression therapy, sex and menopausal status

	Immunosuppression therapy			Sex		
Clinical	CsA mono ($N = 16$)	AZA + PRED ($N = 20$)	Triple ($N = 9$)	Premenopausal Female ($N = 9$)	Postmenopausal Female ($N = 13$)	Male ($N = 23$)
Premenopausal/postmenopausal/men	3/5/8	4/5/11	2/3/4			
Age *years*	50.6 ± 12.1	48.3 ± 11.2	42.2 ± 13.2	34.9 ± 7.0[c]	57.5 ± 5.9[d]	47.6 ± 11.4
Weight *kg*	73.7 ± 18.6	73.9 ± 13.9	66.9 ± 7.3	73.1 ± 18.7	65.9 ± 10.3	75.2 ± 14.8
Type of dialysis before the graft						
Nil/HD/PD (*N*)	4/3/9	6/10/4	1/3/5	1/4/4	4/3/6	6/9/8
Time on dialysis *months*	17.4 ± 24.1	9.1 ± 11.4	12.3 ± 8.3	19.9 ± 22.3	15.5 ± 22.7	8.3 ± 7.2
Time since transplantation *months*	80 ± 30	186 ± 59[a]	78 ± 36	117 ± 70	114 ± 64	138 ± 75
Rejection episodes	0.50 ± 0.63	1.00 ± 1.08	1.22 ± 1.20	1.33 ± 1.0	0.92 ± 1.04	0.65 ± 0.93
Cumulated dose of CsA *g*	725 ± 274	70 ± 141[a]	701 ± 387	418 ± 3986	448 ± 414	423 ± 431
Cumulated dose of PRED *g*	2.1 ± 3.4[b]	43.8 ± 12.4	35.4 ± 47.7	25.1 ± 21.2	30.8 ± 43.5	26.2 ± 22.3

Abbreviations are: CsA, cyclosporine A; AZA, azathioprine; PRED, prednisolone; HD, hemodialysis; PD, peritoneal dialysis; CD, cadaveric donor; LRD, living related donor.

[a] $P < 0.05$ vs. CsA monotherapy

[b] $P < 0.05$ vs. AZA + PRED and triple therapy groups

[c] $P < 0.05$ vs. postmenopausal female and male

[d] $P < 0.05$ vs. male

Table 2. Biochemical characteristics according immunosuppression therapy, sex and menopausal status

	Immunosuppression therapy			Sex		
Biochemical variable	CsA mono ($N = 16$)	AZA + PRED ($N = 20$)	Triple ($N = 9$)	Premenopausal Female ($N = 9$)	Postmenopausal Female ($N = 13$)	Male ($N = 23$)
iPTH *pg/ml*	85.0 ± 45.1	69.0 ± 40.2	65.2 ± 35.0	64.8 ± 32.3	90.1 ± 40.1	68.3 ± 43.4
Ionised calcium *mmol/liter*	1.26 ± 0.07	1.25 ± 0.07	1.24 ± 0.03	1.22 ± 0.08	1.26 ± 0.04	1.26 ± 0.06
Phosphate *mmol/liter*	0.90 ± 0.18	0.85 ± 0.21	0.92 ± 0.25	0.88 ± 0.25	0.92 ± 0.21	0.85 ± 0.19
Telopeptide of Type I collagen *µg/liter*	8.3 ± 3.0[a]	5.9 ± 3.0	8.9 ± 4.2	8.4 ± 3.9	7.2 ± 3.7	7.0 ± 3.2
Propeptide of Type I collagen *µg/liter*	182 ± 79	173 ± 90	196 ± 92	202 ± 84	185 ± 104	169 ± 76
1,25-Dihydroxyvitamin D_3 *pg/ml*	32.6 ± 8.3	35.1 ± 15.1	32.0 ± 15.0	29.4 ± 9.2	31.2 ± 11.8	36.5 ± 14.2
Bone alkaline phosphatase *U/liter*	39.1 ± 22.4	35.5 ± 24.9	38.9 ± 21.0	36.4 ± 27.5	39.1 ± 30.5	36.9 ± 16.1
Creatinine *µmol/liter*	133 ± 23	118 ± 22	135 ± 23	129 ± 25	124 ± 21	127 ± 25
Creatinine clearance *ml/min*	50.7 ± 10.3[a]	68.4 ± 22.2	55.3 ± 13.0	62.7 ± 23.5	47.0 ± 12.2[b]	67.3 ± 17.4

[a] $P < 0.05$ vs. AZA + PRED group

[b] $P < 0.05$ vs. Male

Table 3. Bone densitometry data according the immunosuppression therapy, sex and menopausal status

	Immunosuppression therapy			Sex		
Densitometric variable	CsA mono ($N = 16$)	AZA + PRED ($N = 20$)	Triple ($N = 9$)	Premenopausal Female ($N = 9$)	Postmenopausal Female ($N = 13$)	Male ($N = 23$)
Single energy X-ray absorptiometry						
Distal						
Z score	−0.8 ± 0.8	−1.0 ± 1.0	−1.1 ± 1.1	−1.0 ± 0.7	−0.6 ± 0.9	−1.1 ± 1.0
T score	−1.7 ± 1.1	−1.9 ± 1.1	−1.7 ± 1.3	−1.0 ± 0.7	−1.9 ± 1.2	−2.0 ± 1.1
Dual energy X-ray absorptiometry						
Femoral neck						
Z score	−0.7 ± 0.9	−0.5 ± 1.1	−0.5 ± 1.2	−0.7 ± 1.3	−0.2 ± 1.1	−0.7 ± 0.9
T score	−1.4 ± 1.2	−1.1 ± 1.1	−1.0 ± 1.3	−0.6 ± 1.3	−1.3 ± 1.4	−1.4 ± 0.9
L1-L4						
Z score	0.3 ± 1.2	−0.2 ± 1.4	−0.8 ± 1.5	−0.5 ± 1.2	0.4 ± 1.6	−0.4 ± 1.3
T score	−0.4 ± 1.3	−0.8 ± 1.4	−1.3 ± 1.4	−0.5 ± 1.1	−0.8 ± 1.8	−0.9 ± 1.3

sis according to immunosuppressive drug therapy was not different (Fig. 1A). Men tended to show a higher proportion of mixed uremic bone disease than women (Fig. 1B). Bone marrow was normal in 24 cases. In 17, there was mild fibrosis and moderate fibrosis in 2. In only one case (diagnosed as adynamic bone disease), aluminum was present in bone, cement lines and diffusely (not restricted to the mineralizing front); this was considered to be biologically inactive. Iron was not observed in any biopsy. A trend to a better preserved BMD

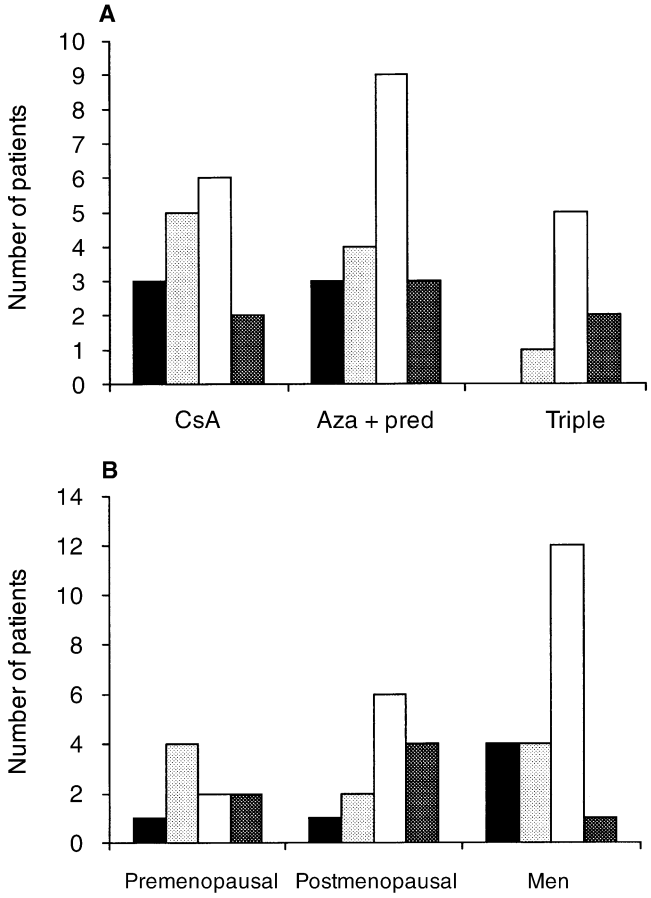

Fig. 1. Histopathological diagnosis according to the immunosuppression drug therapy (*A*) and gender and menopausal status (B). Symbols are: (■) predominant hyperparathyroid bone disease; (▨) adynamic bone disease; (□) mixed uremic bone disease; (▩) normal bone.

was observed in patients with normal bone histology (mainly at the L1–L4 level); however, there were no significant differences between the histopathological subtypes (Table 4). Detailed results of the histomorphometric analysis are shown in Table 5. In general, patients showed a slight to moderate increase in osteoclast function and a decrease of osteoblast function, as well as a marked retardation of mineral apposition and bone formation rates. There was a wide variance in all of the histomorphometric parameters; however, some differences were found. Adjusted for age and sex, patients on CsA monotherapy had a lower appositional rate compared with the AZA + PRED group, and men displayed lower bone volume than premenopausal and postmenopausal women, whereas premenopausal women had a lower mineralizing surface than postmenopausal women and men.

The CsA monotherapy and premenopausal women groups showed a trend toward higher serum iPTH and significantly lower creatinine clearance values. Although the serum iPTH levels were not particularly high, in order to identify the effect of CsA clearly, we decided to remove the data of those patients with an iPTH higher than the reference values (10 to 60 pg/ml). This also removed those patients with the lowest creatinine clearance, as the latter was negatively correlated with iPTH ($r = –0.40$, $P = 0.01$). There were 24 patients with iPTH levels higher than 60 pg/ml: 98.0 ± 29.1 pg/ml (range 63 to 168). A new analysis was performed in 21 patients with normal PTH who were on CsA monotherapy ($N = 6$), AZA + PRED ($N = 9$), and triple therapy ($N = 6$). Figure 2 shows the main significant results of this further analysis. CsA monotherapy was associated with a significantly more pronounced reduction in wall thickness compared with the AZA + PRED and triple-therapy groups and in trabecular appositional rate compared with the AZA + PRED group. In this same analysis, males showed a lower bone volume than postmenopausal women (Fig. 2).

As in any clinical conditions in which multiple variables interact at the same time in a given patient, renal transplant bone mass reduction has a multifactorial origin. To identify clearly which factor(s) was independently associated with the bone mass reduction in our study, we performed a multiple regression analysis once collinearity between the independent variables was excluded. Table 6 shows only the significant models obtained in this multivariate analysis. There was no significant model predicting BMD. Regarding the histomorphometric variables, sex (female 0, male 1) and time after transplantation were the most significant factors predicting bone volume and mineralizing surface, whereas age and time on dialysis prior to the transplantation were the only significant predictive factors for eroded surface and osteoclast number, respectively.

DISCUSSION

This study showed a mild to moderate BMD reduction in both axial and appendicular skeleton of patients with long-term renal transplantation, which was not significantly different in patients receiving CsA alone, AZA + PRED, or triple therapy. Although there was a trend to display a lower BMD compared with age- and sex-matched normal population or with sex-matched young controls, the lumbar spine seemed to be less affected. The magnitude of reduction in the axial skeleton BMD in our study is in keeping with that reported by Grotz et al [11], but contrasts with the severe demineralization of the spine reported by Pichette et al in long-term kidney transplant survivors [12]. Our results in the distal radius BMD (predominantly cortical bone) also contrast with those reported by Julian et al in the short term after transplantation, as they found an increase in BMD probably caused by resolution of mild secondary hyperparathyroidism [8]. The BMD reduction in our patients

Table 4. Comparison of bone mineral density (BMD) measurements between the different histopathological subtypes

Densitometric variable	Normal ($N = 7$)	Mixed ($N = 20$)	HPT ($N = 6$)	Adynamic ($N = 10$)
Single energy X-ray absorptiometry				
Distal				
Z score	-0.7 ± 1.0	-0.9 ± 0.9	-1.2 ± 1.1	-1.2 ± 0.8
T score	-1.4 ± 0.7	-1.9 ± 1.2	-1.8 ± 1.5	-1.9 ± 0.9
Dual energy X-ray absorptiometry				
Femoral neck				
Z score	-0.5 ± 1.2	-0.5 ± 1.2	-0.3 ± 0.5	-0.8 ± 0.9
T score	-1.1 ± 1.1	-1.4 ± 1.3	-0.9 ± 0.7	-1.3 ± 1.2
L1-L4				
Z score	0.8 ± 1.3	-0.3 ± 1.4	-0.3 ± 0.8	-0.4 ± 1.7
T score	0.1 ± 1.3	-1.0 ± 1.4	-0.8 ± 1.1	-0.8 ± 1.6

Abbreviations are: Normal, normal bone; Mixed, mixed uremic bone disease; HPT, predominant hyperparathyroid bone disease; Adynamic, adynamic bone.

Table 5. Histomorphometric analysis according immunosuppression therapy, sex and menopausal status

	Immunosuppression therapy			Sex		
Histomorphometric variable (trabecular bone)	CsA mono ($N = 16$)	AZA + PRED ($N = 19$)	Triple ($N = 8$)	Premenopausal Female ($N = 9$)	Postmenopausal Female ($N = 13$)	Male ($N = 21$)
Z score						
Bone volume	-1.6 ± 1.8	-2.6 ± 2.0	-1.9 ± 0.8	-1.1 ± 1.5	-1.6 ± 1.3	-2.8 ± 1.9[c]
Osteoblast surface	-0.9 ± 6.4	-1.5 ± 4.7	-2.7 ± 2.0	-1.8 ± 5.2	-1.1 ± 4.0	-1.6 ± 5.7
Osteoid surface	-1.2 ± 3.8	-2.2 ± 3.2	-2.8 ± 2.8	-2.8 ± 3.4	-1.7 ± 3.3	-1.7 ± 3.5
Wall thickness	-4.2 ± 5.7	-3.9 ± 3.1	-2.5 ± 1.6	-3.0 ± 2.4	-2.5 ± 2.6	-4.8 ± 5.1
Osteoclast number	1.7 ± 4.3	1.8 ± 3.4	0.9 ± 2.6	-0.02 ± 3.4	2.3 ± 2.8	1.8 ± 4.0
Osteoclast surface	3.5 ± 11.2	1.7 ± 3.3	2.2 ± 2.9	3.5 ± 15.0	2.2 ± 2.5	2.2 ± 3.7
Eroded surface	1.3 ± 6.1	2.2 ± 4.4	4.9 ± 8.8	0.6 ± 5.6	2.0 ± 2.8	3.4 ± 7.4
Mineralizing surface	-5.6 ± 5.3	-7.1 ± 9.9	-4.3 ± 3.5	-11.8 ± 12.6[b]	-3.4 ± 3.7	-5.2 ± 4.9
Bone formation rate	-4.4 ± 4.1	-5.6 ± 16.1	-2.9 ± 2.0	-3.4 ± 5.6	-3.6 ± 3.6	-5.8 ± 15.1
Appositional rate	-6.5 ± 2.7[a]	-4.4 ± 3.2	-4.2 ± 2.4	-6.0 ± 2.7	-4.1 ± 2.5	-5.5 ± 3.4

[a] $P < 0.05$ vs. AZA + PRED
[b] $P < 0.05$ vs. female postmenopausal and male
[c] $P < 0.05$ vs. female pre- and postmenopausal

could theoretically be related to hyperparathyroidism, but we did not find any correlation between PTH and distal radius BMD. Alternatively, a technical difference could be implicated, as we used SXA, which has better spatial resolution (and therefore, enhanced precision) than single-photon absorptiometry [28] as employed by Julian et al [8].

Our bone histopathological findings of an imbalance in bone remodeling favoring net bone loss, that is, increased osteoclast function, decreased osteoblast function, and retardation of mineral apposition, and bone formation rates clearly explain the densitometry data. Few studies have addressed the histopathological picture in patients receiving combined regimens of CsA and PRED in short-term renal transplantation. The histomorphometric features displayed in our study population have been described previously in bone biopsies after six months of transplantation and were attributed to a toxic effect of glucocorticoids [8]. Others have found increased osteoclast and osteoblast activity one- to two-years post-transplantation, attributing these effects to delayed bone repair due to CsA [16] or resistance of bone to normal circulating levels of 1,25-dihydroxyvitamin D_3 [17]. In another study, a higher frequency of non–aluminum-related adynamic bone disease was reported in a Mexican population after 84 months of transplantation [29]; however, a discriminative analysis of the associated factors was not performed. In our study, the most frequent histopathological diagnosis was mixed uremic bone disease. Interesting to note is the lack of differences in BMD between the histopathological subtypes, even when patients with normal bone tended to show a slightly better BMD. This may suggest that even patients with adynamic bone suffer from bone demineralization.

Bone loss and fracture rates after renal transplantation may be lower than after liver [5] or cardiac [6] transplantation, probably because lower doses of immunosuppressive drugs are used [30]. Nevertheless, a triple fracture rate after renal transplantation has been reported that is related to low lumbar BMD [7]. The long bones appear to be more commonly affected than the vertebral bodies in kidney transplant recipients [7, 30], as was the case in this study.

In humans, the study of the role of different immunosuppressive drugs has produced contradictory results. Some authors have suggested PRED as the main factor

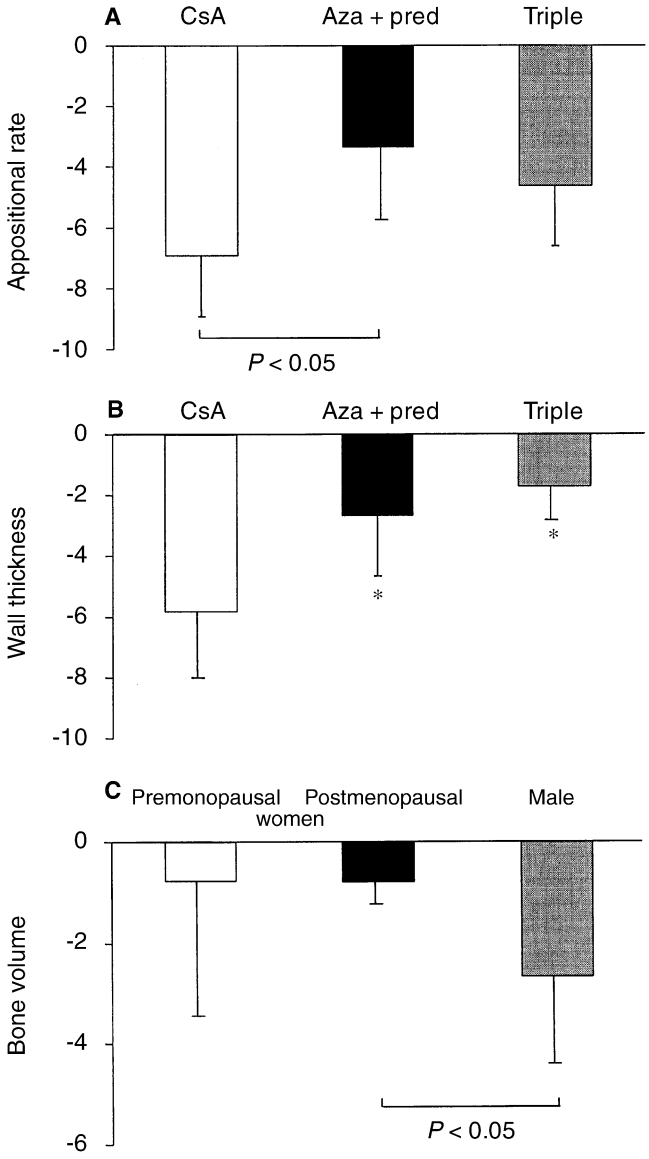

Fig. 2. The Z score of trabecular appositional rate, wall thickness, and bone volume in 21 patients with normal PTH level, according to the immunosuppressive therapy (*A* and *B*) and gender (*C*). $*P < 0.05$ vs. CsA monotherapy.

implicated in BMD loss after renal transplantation [11, 12, 20]. Others have claimed a positive impact of CsA on the lumbar BMD [19, 20]. Nevertheless, CsA may be related to bone loss because, in *in vivo* animal studies, CsA causes high-turnover osteopenia with trabecular bone loss and a transient rise in serum osteocalcin [31, 32] and 1,25-dihydroxyvitamin D_3 [32], whereas *in vitro*, CsA inhibits bone resorption elicited by calcemic hormones or cytokines [33, 34] and fusion of osteoclast precursors [35]. Our data show that in the long term, PRED, as well as CsA alone or triple-drug therapy, is associated with osteoclast activation, osteoblast suppression, and retardation of bone formation parameters. Moreover, all

Table 6. Significant multiple linear regression models predicting histomorphometric parameters (only significant independent variables are shown)

Dependent variable	Independent variables	β	*P*
Bone volume	Sex	−0.36	0.01
R 0.54, R^2 0.30, $P = 0.002$	Time after transplantation	−0.36	0.02
Eroded surface	Age	0.36	0.03
R 0.36, R^2 0.13, $P = 0.03$			
Osteoclast number	Time on dialysis	−0.38	0.02
R 0.38, R^2 0.14, $P = 0.02$			
Mineralizing surface	Time after transplantation	−0.40	0.01
R 0.50, R^2 0.25, $P = 0.006$	Sex	0.37	0.02

Independent variables included: age, sex (female 0, male 1), creatinine clearance, iPTH, rejection episodes, type and time on dialysis before transplantation, time after transplantation, and cumulative dose of PRED, CsA, and AZA.

of the immunosuppressive drug schemes were associated with bone loss to roughly the same degree. High serum PTH levels have been implicated in bone loss related to renal transplantation whether as persistent hyperparathyroidism [10] or secondarily related to the development of mild to moderate renal failure [17]. As our group on CsA monotherapy showed a slightly higher iPTH and lower creatinine clearance, it could be speculated that the observed effects were due to secondary hyperparathyroidism. In a further analysis, eliminating those patients with an iPTH level above the normal range (hence, eliminating the patients with the poorest renal function), the negative effect of CsA monotherapy on the trabecular appositional rate and wall thickness were still evident. In addition, no correlation between PTH levels and the histomorphometric parameters was observed. Likewise, the trabecular bone loss and imbalance of bone formation and resorption observed in our patients are not usually present in the PTH excess [36, 37]. Thus, the histomorphometric picture observed in our patients does not seem to be explained by PTH alone. The low levels of bone specific alkaline phosphatase (reference values 30 to 150 U/liter) displayed in this study agree with the general osteoblast hypoactivity observed. The higher serum levels of telopeptide of type I collagen in the CsA monotherapy group suggest higher resorption; however, this might have been affected by the poorer renal function in this group, as it has been reported that creatinine clearance below 75 ml/min increases the serum concentration of this small peptide [38]. This is supported by a negative correlation between serum concentration of telopeptide of type I collagen and creatinine clearance ($r = –0.37$, $P = 0.02$).

Age, sex, and postmenopausal status are factors affecting natural bone loss in the normal population [24, 26], but their role in renal transplantation remains controversial. Our data show a trend for postmenopausal women to exhibit a lower BMD than premenopausal women when compared with a sex-matched young population. However, when compared with age- and sex-matched

peers, premenopausal women tended to have lower BMD. Furthermore, men tended to have low BMD, as well as bone volume significantly lower than postmenopausal women. Consequently, it was expected that male gender is a negative predictive factor for bone volume, and its association with a low BMD seems clear in this setting. Age predicted a higher eroded surface, which may be considered as exceeding its natural effect, as this effect was observed employing Z scores. Time after transplantation negatively predicted trabecular bone volume; therefore, its main implication is that reduced bone mass is still present in long-term renal transplantation.

In summary, long-term renal transplant patients showed reduced BMD in both trabecular and cortical bone. This reduction in BMD was not as severe as has been reported in short-term studies and appears to result from an imbalance in bone remodeling, characterized, in turn, by osteoclast stimulation, osteoblast suppression, and retardation of mineral apposition and bone formation rates. Bone mass loss was not different between the CsA monotherapy, AZA + PRED dual therapy, or triple immunosuppressive therapy groups. Male gender and age were the strongest predictive factors for a negative outcome on bone mass.

ACKNOWLEDGMENTS

Dr. Cueto-Manzano had an ISN fellowship award from December 1996 to June 1998.

Reprint requests to Dr. Ram Gokal, Department of Renal Medicine, Manchester Royal Infirmary, Oxford Road, Manchester M13 9WL, England, United Kingdom.
E-mail: rgokal@renal.cmht.nwest.nhs.uk

REFERENCES

1. Malluche H, Faugere MC: Renal bone disease 1990: An unmet challenge for the nephrologist. *Kidney Int* 38:193–211, 1990
2. Delmez JA, Slatopolsky E, Martin KJ, Gearing BN, Harter HR: Minerals, vitamin D and parathyroid hormone in continuous ambulatory peritoneal dialysis. *Kidney Int* 21:862–867, 1982
3. Huffer WE, Kuzela D, Popovtze MM, Starzl TE: Metabolic bone disease in chronic renal failure. II. Renal transplant patients. *Am J Pathol* 78:385–400, 1975
4. Main J, Velasco N, Catto GR, Fraser RA, Edward N, Adami S, O'Riodan JL: The effect of hemodialysis, vitamin D metabolites and renal transplantation on the skeletal demineralization associated with renal osteodystrophy: A computerized histomorphometric analysis. *Clin Nephrol* 26:279–287, 1986
5. Shane E, Rivas M, Silverberg S, Kim T, Staron R, Bilezikian J: Osteoporosis after cardiac transplantation. *Am J Med* 94:257–264, 1993
6. McDonald J, Dunstan C, Dilworth P, Sherbon K, Sheil AG, Evans RA, McCaughan GW: Bone loss after liver transplantation. *Hepatology* 14:613–619, 1991
7. Grotz WH, Mundinger FA, Gugel B, Exner V, Kirste G, Schollmeyer PJ: Bone fracture and osteodensitometry with dual energy x-ray absorptiometry in kidney transplant patients. *Transplantation* 58:912–915, 1994
8. Julian BA, Laskow DA, Dubovsky J, Curtis J, Quarles D: Rapid loss of vertebral mineral density after renal transplantation. *N Engl J Med* 325:544–550, 1991
9. Horber FF, Casez JP, Steiger U, Czeerniak A, Montandon A, Jaeger PD: Changes in bone mass early after kidney transplantation. *J Bone Miner Res* 9:1–9, 1994
10. Almond MK, Kwan JTC, Evans K, Cunningham J: Loss of regional bone mineral density in the first 12 months following renal transplantation. *Nephron* 66:52–57, 1994
11. Grotz WH, Mundinger FA, Rasenack J, Speidel L, Olschewski M, Exner VM, Schollmeyer PJ: Bone loss after kidney transplantation: A longitudinal study in 115 graft recipients. *Nephrol Dial Transplant* 10:2096–2100, 1995
12. Pichette V, Bonnardeaux A, Prudhomme L, Gagné M, Cardicnal J, Ouimet D: Long-term bone loss in kidney transplant recipients: A cross-sectional and longitudinal study. *Am J Kidney Dis* 28:105–111, 1996
13. Slatopolsky E, Martin K: Glucocorticoids and renal transplant osteonecrosis. *Adv Exp Med Biol* 171:353–359, 1984
14. Luckert BP, Raisz LG: Glucocorticoid-induced osteoporosis: Pathogenesis and management. *Ann Intern Med* 112:352–364, 1990
15. Epstein S: Cyclosporin A: Friend or foe? *Calcif Tissue Int* 49:232–234, 1991
16. Aubia J, Masramón J, Serrano S, Lloveras J, Mariñoso LL: Bone histology in renal transplant patients receiving cyclosporin. *Lancet* 1:1048-1049, 1988
17. Briner VA, Thiel G, Monier-Faugere MC, Bognar B, Landmann J, Kamber V, Malluche HH: Prevention of cancellous bone loss but persistence of renal bone disease despite normal 1,25 vitamin D levels two years after kidney transplantation. *Transplantation* 10:1393–1400, 1995
18. Wolpaw T, Deal CL, Fleming-Brooks S, Bartucci MR, Schulak J, Hricik DE: Factors influencing vertebral bone density after renal transplantation. *Transplantation* 58:1186–1189, 1994
19. Torregrosa JV, Campistol JM, Montesinos M, Pons M, Marines de Osaba MJ: Evolution of bone mineral density after renal transplantation: Related factors. *Nephrol Dial Transplant* 10(Suppl 6):111–113, 1995
20. Aroldi A, Tarantino A, Montagnino G, Cesana B, Cocucci C, Ponticelli C: Effects of three immunosuppressive regimens on vertebral bone density in renal transplant recipients. *Transplantation* 63:380–386, 1997
21. Malluche HH, Monier-Faugere MC: The role of bone biopsy in the management of patients with renal osteodystrophy. *J Am Soc Nephrol* 4:1631–1642, 1994
22. Sanchez CP, Salusky IB, Kuizon BD, Ramirez JA, Gales B, Ettenger RB, Goodman WG: Bone disease in children and adolescents undergoing successful renal transplantation. *Kidney Int* 53:1358–1364, 1998
23. Hutchison AJ, Whitehouse RW, Boulton HF, Adams JE, Mawer EB, Freemont TJ, Gokal R: Correlation of bone histology with parathyroid hormone, vitamin D_3 and radiology in end-stage renal disease. *Kidney Int* 44:1071–1077, 1993
24. Rehman MTA, Hoyland JA, Denton J, Freemont AJ: Age related histomorphometric changes in bone in normal British men and women. *J Clin Pathol* 47:529–534, 1994
25. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR: Bone histomorphometry: Standardization of nomenclature, symbols and units. *J Bone Miner Res* 2:595–610, 1987
26. Riggs BL, Wahner W, Seemann E: Changes in bone mineral density of the proximal femur and spine with aging. *J Clin Invest* 70:716–723, 1982
27. World Health Organisation: *Assessment of Fracture Risk and Its Application to Screening for Postmenopausal Osteoporosis.* Technical report series, Geneva, WHO, 1994, p 843
28. Adams JE: Single- and dual-energy: X-ray absorptiometry, in *Dual Energy X-ray Absorptiometry in Osteoporosis and Bone Densitometry*, edited by Genant HK, Guglielmo G, Jergas M, Berlin, Springer-Verlag, 1997, pp 305–334
29. Velazquez-Forero F, Mondragon A, Herrero B, Peña JC: Adynamic bone lesion in renal transplanted recipients with normal renal function. *Nephrol Dial Transplant* 11(Suppl 3):58–64, 1996
30. Rodino MA, Shane E: Osteoporosis after organ transplantation. *Am J Med* 104:459–469, 1998
31. Movsowitz C, Epstein S, Fallon M, Ismail F, Thomas S:

Cyclosporin-A *in vivo* produces severe osteopenia in the rat: Effect of dose and duration of administration. *Endocrinology* 123:2571–2577, 1988

32. Stein B, Halloran BP, Reinhardt T, Engstrom GW, Bales CW, Drezner MK, Curria KL, Takizawa M, Adams JS, Epstein S: Cyclosporin-A increases synthesis of 1-25 dihydroxyvitamin D3 in the rat and mouse. *Endocrinology* 128:1369–1373, 1991
33. Stewart PJ, Green OC, Stern PH: Cyclosporin-A inhibits hormone induced bone resorption in-vitro. *J Bone Miner Res* 1:285–291, 1986
34. Klaushofer K, Hoffman O, Stewart PJ, Czerwenka E, Koller K, Peterlik M, Stern PH: Cyclosporin-A inhibits bone resorption in cultured neonatal mouse calvariae. *J Pharmacol Exp Ther* 243:584–590, 1987
35. Orcel P, Denne MA, De Vernejoul MC: Cyclosporine-A in-vitro decreases bone resorption, osteoclast formation and the fusion of cells of the monocyte-macrophage lineage. *Endocrinology* 128: 1638–1646, 1991
36. Bilezikian JP: Primary hyperparathyroidism, in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, edited by Favus MJ, Philadelphia, Lippincott-Raven, 1996, pp 181–186
37. Kronenberg HM: Parathyroid hormone: Mechanism of action, in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, edited by Favus MJ, Philadelphia, Lippincott-Raven, 1996, pp 68–73
38. Risteli J, Elomaa I, Niemi S, Novamo A, Risteli L: Radioimmunoassay for the pyridinoline cross-linked carboxyterminal telopeptide of type I collagen: A new serum marker of bone collagen degradation. *Clin Chem* 39:635–640, 1993