Transpl Int (1993) 6: 99–107

Transplant International

Cyclosporin A-induced transient rise in plasma alkaline phosphatase in kidney transplant patients

V. A. Briner[1]*, J. Landmann[2], F. P. Brunner[1], G. Thiel[1]

[1] Renal Division, Department of Medicine, University Hospitals Basel, CH-4031 Basel, Switzerland
[2] Department of Surgery, University Hospitals Basel, CH-4031 Basel, Switzerland

Received: 16 December 1991/Received after revision: 27 May 1992/Accepted: 2 June 1992

Abstract. In 1981 cyclosporin A (CyA) became available and replaced azathioprine (Aza) as the immunosuppressive agent in kidney transplantation at the University Hospitals in Basel, Switzerland. Patients on CyA and prednisone (CyA/p) therapy frequently demonstrated an isolated rise in bone-derived serum alkaline phosphatase (aP) concentration, but patients on Aza and prednisone (Aza/p) therapy did not. On the basis of long-term aP concentration and using noninvasive means, the present retrospective study was designed to investigate biochemical markers and radiographic signs of bone disease after successful kidney transplantation in patients on Cya/p treatment. Similar investigations were performed in patients on Aza/p and the results were compared. Follow-up examinations included clinical examination, radiography of the hand, and biochemical analysis of serum and urine. In 139 renal transplant patients on CyA/p, aP increased transiently after successful grafting (at transplantation 84 ± 43 U/l; on day 90, 112 ± 82 U/l). In 50 patients aP levels were higher at the time of transplantation (120 ± 80 U/l) and aP peaked after 8 ± 6 months, at a mean concentration of 242 ± 103 U/l. In these patients aP concentrations exceeded the normal range for 16 ± 10 months. None of the patients on CyA/p showed symptoms of bone disease when aP was increased. Radiological surveys revealed more pronounced osteodystrophy in patients at the time of transplantation, which increased aP to above the normal range after transplantation. Despite this rise in aP, over the long term bone lesions improved radiographically while bone mass remained stable. In contrast, patients treated with Aza/p demonstrated a significant decrease in aP level after transplantation from 75 ± 33 U/l to 54 ± 29 U/l on day 90. In addition, radiographic bone changes persisted and bone mass decreased significantly. After a 2-year follow-up, serum parathyroid hormone, 1,25-$(OH)_2$-vitamin D_3, calcium, and phosphorus concentrations, urinary excretion of hydroxyproline, and tubular reabsorption of phosphate did not differ between patients on CyA/p and controls on Aza/p. We conclude that after successful kidney transplantation and initiation of CyA/p therapy, a transient increase in bone-derived aP frequently occurred. These patients more often demonstrated radiographic signs of pre-existing osteodystrophy. However, over the long term, these changes improved.

Key words: Cyclosporin A, alkaline phosphatase – Alkaline phosphatase, CyA – Kidney transplantation, CyA – Osteodystrophy, kidney transplantation

In the years 1968–1981, kidney transplant patients at the University Hospitals in Basel received azathioprine and prednisone (Aza/p) as immunosuppressive therapy. When cyclosporin A (CyA) became available in 1981, it replaced azathioprine in subsequent transplantations. Under the new immunosuppressive regimen, an increase in serum alkaline phosphatase (aP) concentration was frequently noticed. Increased aP was derived from the bone [15]. In the majority of patients this rise was unaccompanied by changes in other chemical indices. We reported these findings as early as 1983 [15]. Results of the follow-up reports of the European Multicentre Trial confirmed our observations. Patients on CyA/p treatment at several centres demonstrated significantly higher serum aP levels of 186%, 133%, and 117%, 1 [9], 3 [13], and 5 years [6], respectively, after kidney transplantation when compared to aP levels of 100%, 103%, and 93% at similar times after grafting in patients on Aza/p (aP level in Aza/p-treated patients 1 year after transplantation was estimated as 100%). Despite these significant differences in aP levels between the two treatment groups, no attention was paid to these changes in aP levels and no further characterization of aP has been performed thus far. The fact that aP levels increased and remained elevated in many of our patients on CyA/p for several months despite stable graft function, and the observations of accelerated bone turnover in animals treated with CyA [17] raise the question of

* *Present address:* Department of Medicine, University Hospital Bern, CH-3010 Bern, Switzerland
Correspondence to: G. Thiel

whether an aP increase in kidney transplant patients on CyA/p is a manifestation of progressive bone disease.

The aim of the present retrospective study was to investigate aP concentration over a 2-year period in 139 patients treated with CyA/p after kidney transplantation, as well as to analyze the significance of increased aP in kidney transplant recipients immunosuppressed with CyA/p. The latter was accomplished by noninvasive means, including: (1) blood and urine chemistry, (2) radiographic survey of the hand, and (3) clinical examination. The data derived from this analysis were compared to data derived from similar investigations in patients on conventional immunosuppression with Aza/p.

Patients and methods

From 1981 to 1986, 220 patients with end-stage renal failure received cadaveric kidney transplants. One hundred thirty-nine of these patients met the following criteria for inclusion in this study: graft survival longer than 6 months, stable transplant function, no evidence of acute or chronic hepatitis, no evidence of malignant disease, and CyA/p as immunosuppressive therapy. The mean age of these 71 women and 68 men was 48 ± 12 years (range 18–67 years). A group of controls included 32 patients (17 women and 15 men) who had received transplants between 1978 and 1982. The patients in this group met the aforementioned criteria, except that they received Aza/p as immunosuppressive therapy.

Patient groups

CyA 1 group. Fifty of the 139 patients on CyA/p with a transient rise in aP above 120 U/l (normal range < 106 U/l) for at least two consecutive measurements were assigned to the CyA 1 group. Their mean age was 49 ± 2 years and they included 30 women and 20 men.

CyA 2 group. Eighty-nine kidney transplant recipients treated with CyA/p and who demonstrated aP levels within the normal range at any time tested were assigned to the CyA 2 group. Their mean age was 47 ± 1 years and they included 41 women and 48 men.

Aza/p group. Thirty-two patients on conventional immunosuppressive therapy with Aza/p comprised the Aza/p group. Their mean age was 50 ± 9 years and they included 17 women and 15 men.

Laboratory tests

Up to 2 years after transplantation, regular clinical examinations and chemical analyses were carried out simultaneously. Blood was drawn after overnight fasting and urine was collected for the preceding 24 h. Blood and urine chemistries were determined using the hospital's central laboratory multichannel analyzers (Hitatchi 705 and 737, Boehringer, Mannheim, FRG; Flammenphotometer IL 543 and IL 943, Instrumentation Laboratory Milano, Italy; ACA SX, Du Pont, Boston, Mass.). The bone-derived isoenzyme of aP was determined quantitatively by precipitation with wheat germ lectin [2] (Institute Viollier, Basel, Switzerland). Whole blood CyA levels were assayed using polyclonal antibodies (Sandoz RIA kit).

In addition, in 38 patients on CyA/p (16 patients with aP > 120 U/l, 22 patients with normal aP levels) and 21 patients on Aza/p, two series of tests were carried out at an interval of 1 year. The following parameters were determined: serum C- and N-terminal parathyroid hormone (C-/N-PTH) by radioimmunoassay [4] (I. Fischer and M. A. Dambacher, University Orthopaedic Hospital, Zürich, Switzerland), and 1,25-$(OH)_2$-vitamin D_3 by radioimmunoassay according to Scharla et al. [21] (E. Ritz, University Hospital Heidelberg, FRG). Hydroxyproline excretion was determined in a sample collected after 24 h (D. J. Vonderschmitt, Chemical Laboratory of the University Hospital Zürich). Blood was drawn after 12 h of fasting. The urinary collection period was 24 h. For these tests patients continued with their usual food intake and were not placed on a diet. Tubular reabsorption of phosphate was calculated using the formula: (UP × PCreat × 100)/(PP × UCreat), where PCreat represents morning plasma creatinine, PP plasma phosphorus, UCreat urinary excretion of creatinine, and UP urinary phosphorus. Urinary phosphate excretion per day divided by glomerular filtration rate (GFR) was also calculated.

Radiographic examinations

X-rays of the hand were recorded on a sensitive film (Kodak ORTHO MA 1824) and examined for the presence of the following three features: acro-osteolysis of the terminal phalanges, subperiosteal resorption, and cortical striation in the second phalanges. A severity score was established on all occasions by the same investigator (G. T.), who was not aware of the date of the examination or of the group to which the patients belonged. The changes were rated as follows: 0 = no change, 1 = suspected changes, 2 = apparent changes, and 3 = very pronounced changes. Hence, a total score of 0 represented a normal radiographic appearance and a score of 9 represented the most severe pathological bone changes. Cortical thickness of the left second metacarpal shaft was assessed by measuring the narrowest total diameter minus the width of the medullary cavity. Cortical thickness has been shown to correlate with total cortical bone mass [19].

At transplantation, hand X-rays were made of 78 patients on CyA/p (44 with an increased aP level and 34 with a normal aP concentration) and of 32 patients on Aza/p. Follow up X-rays were recorded at intervals of 6 months.

Changes in the immunosuppressive treatment

In addition to the 32 Aza/p-treated patients described above, 14 patients on Aza/p were switched to CyA/p, irrespective of the time of transplantation. Over a period of 2–3 months, prednisone was tapered in these patients in order to reduce prednisone-induced side effects in six diabetic patients, four patients with progressive weight gain, two patients with necrosis of the femur head, one patient with intestinal symptoms and ulceration, and one patient with recurrent carcinoma of the skin. Eleven of these patients had stable kidney graft function, no evidence of hepatitis, and no signs or symptoms of wasting disease. Nine patients initially treated with CyA/p developed CyA-induced nephrotoxicity and were switched to conventional immunosuppression with Aza/p, 3–18 months after transplantation. They met the abovementioned criteria of no hepatitis, no wasting disease, and stable graft function. Blood chemistry was monitored in these patients.

Statistics

The results are expressed as mean ± SD. Between-group comparisons of the variables were carried out using the Mann-Whitney U-test. Wilcoxon's test or analysis of variance (ANOVA with Bonferroni correction) was used for statistical comparison of subsequent values of the same subject. A P value of less than 0.05 was considered significant.

Results

Alkaline phosphatase concentration

The majority of the 139 patients immunosuppressed with CyA/p demonstrated an increase in aP concentration within the 1st few months after kidney transplantation (at

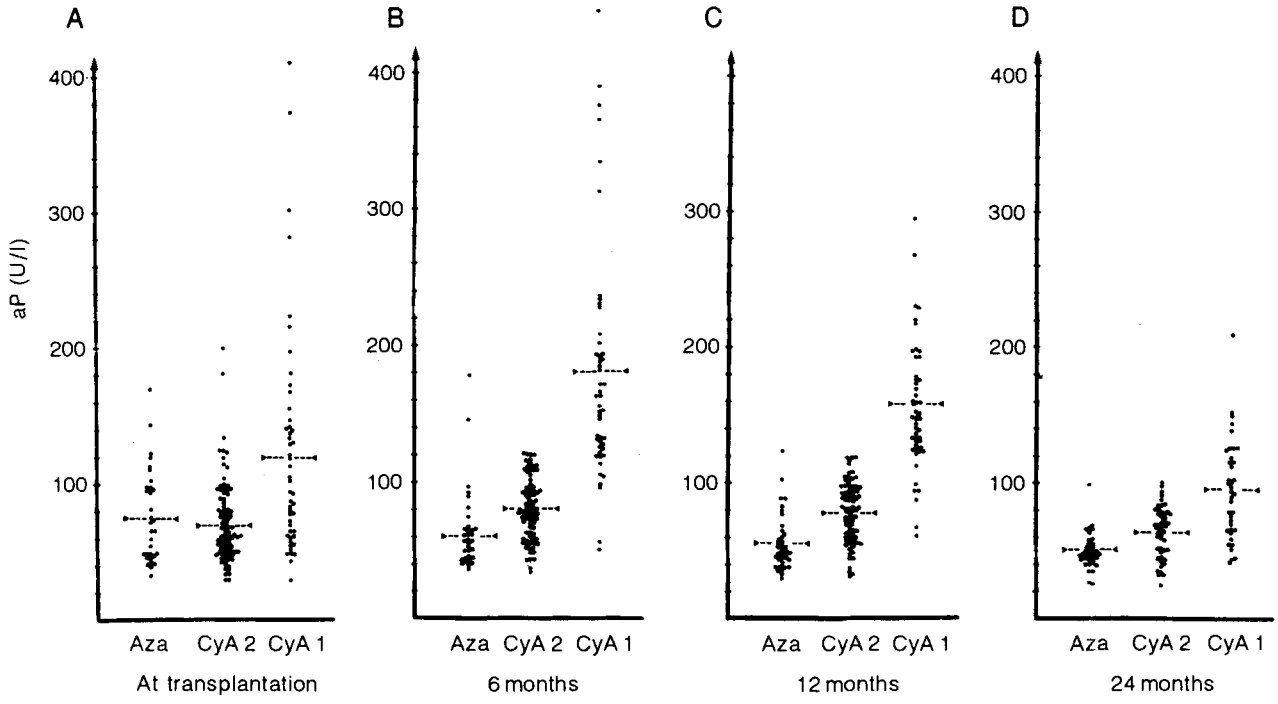

Fig. 1 A–D. Alkaline phosphatase (aP) concentration in cyclosporin/prednisone (CYA/p)- and azathioprine/prednisone (Aza/p)-treated patients at transplantation (**A**) and at 6, 12, and 24 months thereafter (**B–D**). Patients on CyA/p with a rise in aP above 120 U/l (normal range < 106 U/l) = CyA 1 group; patients on CyA/p with aP concentration within the normal range = CyA 2 group; and patients on conventional immunosuppressive therapy = Aza/p group. In 45 of the 89 patients in the CyA 2 group, aP increased by more than 20 U/l but peaked within the normal range

transplantation 84 ± 43 U/l; on day 90, 112 ± 82 U/l; $P < 0.0005$). Isoenzyme analysis demonstrated that the increase in aP under CyA/p therapy was due to bone-derived aP. Fifty (36%) of the 139 transplant recipients showed peak aP levels above the upper limit of the normal range on at least two subsequent measurements within the first 2 years after transplantation. These patients were assigned to group CyA 1 (Fig. 1). At transplantation, in the CyA 1 group, the mean aP level was 120 ± 80 U/l; in the CyA 2 group it was 71 ± 30 U/l ($P < 0.001$). In the CyA 1 group, a rise in aP levels above 120 U/l occurred 3 ± 3 months after grafting (range 0–12 months). A peak concentration of 242 ± 103 U/l (range 129–660 U/l) was reached 8 ± 6 months (range 0–40 months) post-transplantation. The mean duration of raised serum aP levels in CyA 1 patients was 16 ± 10 months (range 1–48 months). In 5 of the 50 patients, aP levels were still elevated after 2 years, when the study was completed.

Eighty-nine patients on CyA/p (CyA 2 group) demonstrated no rise in aP concentration above the normal range (106 U/l) after transplantation. Forty-five of these patients also showed an increment in aP rise of more than 20 U/l. However, peak aP levels did not exceed the normal range (Fig. 1). In contrast, in Aza/p-treated patients, aP levels did not rise after transplantation but decreased significantly over the 1st few months after grafting (at transplantation 75 ± 33 U/l; on day 30 after grafting 66 ± 38 U/l; on day 60 after grafting 58 ± 28 U/l; on day 90 after grafting 54 ± 29 U/l; analysis of variance $P < 0.005$; Fig. 1, Table 1). Figure 2 shows an isolated transient increase in bone-derived aP concentration in a 51-year-old woman with analgesic nephropathy on CyA/p therapy (CyA 4.9 mg/kg body weight = 200 mg/day; prednisone 7.5 mg, 6 months after transplantation). The patient had no symptoms.

CyA dose in relation to aP levels

In CyA/p-treated patients the increment of aP rise was a dose-dependent phenomenon. In patients with increased aP levels (CyA 1 group) the dose of CyA/kg body weight was higher than in patients in the CyA 2 group ($P < 0.05$; Table 1). A correlation between CyA and aP concentration is further suggested by the finding of a high incidence (45%) of increased aP levels (> 120 U/l) in patients initially treated with 12–17 mg/kg body weight compared to only 28% in patients on low-dose CyA (3–5 mg/kg body weight).

Kidney transplant function

In the 139 CyA/p-treated patients, blood creatinine concentrations were significantly higher after day 60 post-transplantation than they were in Aza/p-treated patients (on day 60: 159 ± 57 vs 140 ± 54 μmol/l, NS.; on day 90: 155 ± 45 vs 131 ± 46 μmol/l, $P < 0.01$). However, creatinine concentration did not correlate with aP levels. An example showing marked changes in isolated aP concentration post-transplantation despite stable graft function is illustrated in Fig. 2. Thus, impaired kidney

Table 1. Blood chemistry 30, 60 and 90 days after kidney transplantation in patients on cyclosporin A and prednisone (CyA/p) with increased aP (CyA 1; $n = 50$), patients on CyA/p with aP in the normal range (CyA 2; $n = 89$), and patients on azathioprine and prednisone (Aza/p; $n = 32$). * $P < 0.05$, CyA 1 vs CyA 2; ** $P < 0.02$, Aza/p vs CyA 1; Aza/p vs CyA 2; *** $P < 0.001$, CyA 1 vs CyA 2; CyA 1 vs Aza/p (Mann-Whitney U-test for unpaired values) **** $P < 0.005$, aP on days 30, 60, and 90 after transplantation in Aza/p-treated patients (ANOVA)

	CyA 1			CyA 2			Aza/p		
	Day 30	Day 60	Day 90	Day 30	Day 60	Day 90	Day 30	Day 60	Day 90
aP (U/l)	133 ± 61***	146 ± 53***	153 ± 55***	62 ± 19	66 ± 33	70 ± 18	****66 ± 38	****58 ± 28	****54 ± 29
Creatinine (μmol/l)	185 ± 135	152 ± 52	156 ± 42	175 ± 93	164 ± 61	155 ± 47	175 ± 149	140 ± 54	131 ± 46**
Bilirubin (μmol/l)	13 ± 8	9 ± 3	8 ± 4	10 ± 4	8 ± 4	8 ± 4	7 ± 3	7 ± 3	7 ± 3
AST (U/l)	12 ± 6	12 ± 7	13 ± 6	17 ± 14	11 ± 5	11 ± 5	17 ± 11	10 ± 6	12 ± 5
ALT (U/l)	22 ± 7	16 ± 13	14 ± 8	25 ± 18	16 ± 12	16 ± 9	44 ± 39	16 ± 10	17 ± 11
S-Ca (mmol/l)	2.44 ± 0.22	2.53 ± 0.22	2.61 ± 0.23	2.38 ± 0.16	2.39 ± 0.16	2.45 ± 0.15	2.4 ± 0.23	2.52 ± 0.21	2.55 ± 0.23
S-Ph (mmol/l)	0.86 ± 0.41	0.91 ± 0.25	0.95 ± 0.24	0.86 ± 0.34	0.88 ± 0.22	0.97 ± 0.21	0.85 ± 0.38	0.84 ± 0.28	1.08 ± 0.40
CyA (mg/kg)	9.9 ± 4.0*	8.0 ± 3.4*	7.4 ± 3.5*	7.8 ± 2.9	6.3 ± 2.6	6.0 ± 2.5			

function did not account for the more pronounced aP rise in CyA 1 patients than in CyA 2 patients at any time (Tables 1, 2).

Blood and urine chemistry

Serum phosphorus tended to be lowest early after transplantation. However, there were no differences in mean serum phosphorus between the groups at any time the patients were examined during the first 2 years after grafting (Tables 1, 2). Moreover, in five patients in whom aP remained elevated for more than 2 years, phosphorus levels did not differ either. After postoperative day 30, calcium concentrations were lower in patients with normal aP concentrations than in CyA-treated patients with an increase in aP above the normal range (> 120 U/l; $P < 0.01$). However, in all groups mean calcium concentrations still remained within the normal range. Calcium concentrations were similar in CyA 1 group patients and in Aza/p-treated patients.

In patients in the CyA 1 group, the following chemical analyses did not differ from those in the CyA 2 group or Aza/p group at the time aP levels exceeded the normal range: serum phosphorus, calcium, 1,25-$(OH)_2$-vitamin D_3, and C- and N-terminal PTH (Table 2). There was a trend towards higher C-terminal PTH in patients on CyA with increased aP. This was accounted for in part by one extremely high reading (patient B.C., C-terminal PTH 5680 ng/ml).

Bone turnover was assessed by measuring urinary excretion of hydroxyproline. The difference in hydroxyproline excretion per 24 h and urinary hydroxyproline per excreted creatinine was small and SD large in both CyA/p and Aza/p-treated patients. Therefore, the results were not statistically different (Table 2). The present results do not allow conclusions to be drawn about the equilibrium of the ongoing process. Destruction or reconstruction may predominate or balancing may occur. Neither estimated tubular reabsorption of phosphate (CyA 1 62 ± 12% CyA 2 65 ± 12%; Aza/p 63 ± 13%), nor urinary phosphate excretion divided by GFR (CyA 636 ± 186; Aza/p 602 ± 236), nor urinary calcium excretion was significantly different. CyA/p-treated patients with and without increased aP concentrations had lower calcium loss in the urine than Aza/p-treated patients (CyA 1 group vs Aza/p, $P < 0.02$; CyA 2 group vs Aza/p, $P < 0.02$). The difference disappeared within half a year after transplantation. Urine was collected over a 24-h period without any dietary restrictions at that time.

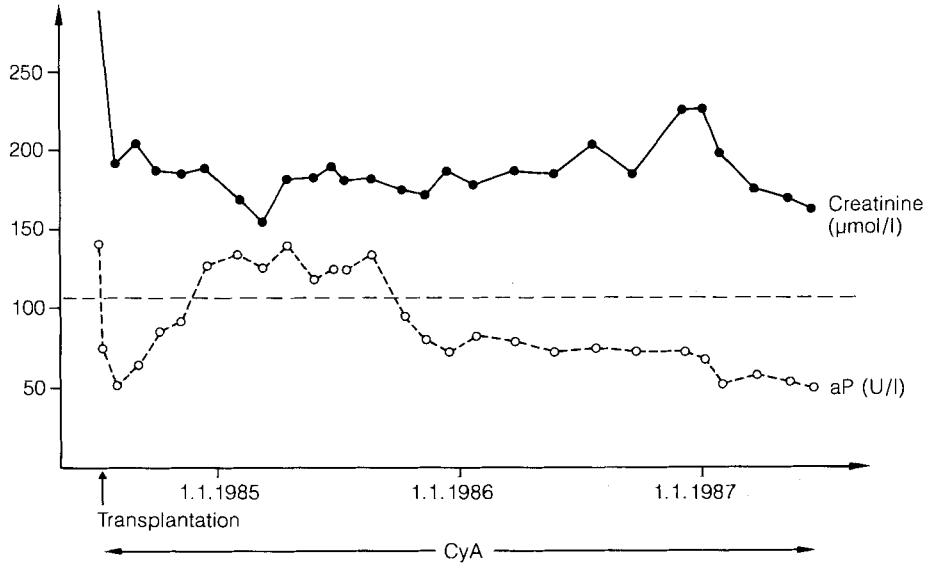

Fig. 2. Transient rise in serum aP concentration in a 51-year-old female kidney transplant recipient on CyA/p (CyA 4.9 mg/kg body weight = 200 mg/day; 7.5–10 mg prednisone) therapy. The woman had analgesic nephropathy and received her transplant after 2 years of hemodialysis treatment. There were no symptoms of bone disease at any time

Table 2. Blood and urine chemistry and radiological score in kidney transplant patients, including CyA 1 patients with increased aP (> 120 U/l; $n = 16$), CyA 2 patients with aP in the normal range ($n = 22$), and Aza/p patients on conventional immunosuppressive therapy ($n = 21$). Two series of tests at an interval of 1 year are presented. * $P < 0.05$, CyA 1 vs CyA 2; ** $P < 0.02$, CyA 1 vs CyA 2; CyA 1 vs Aza/p; *** $P < 0.001$, CyA 1 vs CyA 2; CyA 1 vs Aza/p; **** $P < 0.02$, Aza/p vs CyA 1; Aza/p vs CyA 2 (Wilcoxon's R test for paired values, Mann-Whitney U-test for unpaired values)

		CyA 1		CyA 2		Aza/p	
		Series 1	Series 2	Series 1	Series 2	Series 1	Series 2
Creatinine	< 110 µM/l	166 ± 39	170 ± 47	176 ± 46	172 ± 48	166 ± 72	145 ± 72
Bilirubin	< 25 U/l	11 ± 9	9 ± 6	10 ± 4	10 ± 4	9 ± 3	10 ± 3
AST	< 35 U/l	14 ± 3	14 ± 5	14 ± 5	13 ± 6	12 ± 4	17 ± 12
ALT	< 36 U/l	15 ± 6	19 ± 11	15 ± 8	19 ± 9	13 ± 9	17 ± 11
Gamma-GT	< 65 U/l	15 ± 14	19 ± 17*	10 ± 15	10 ± 15	19 ± 15	17 ± 15
aP	< 106 U/l	185 ± 133***	129 ± 40***	82 ± 17	77 ± 24	58 ± 24	54 ± 24
S-Ph	0.65–1.32 mM/l	1.14 ± 0.24	1.11 ± 0.15	1.13 ± 0.22	1.07 ± 0.25	1.08 ± 0.22	1.16 ± 0.33
S-Ca	2.2–2.64 mM/l	2.55 ± 0.18	2.57 ± 0.20	2.48 ± 0.16	2.48 ± 0.12	2.52 ± 0.27	2.58 ± 0.16
PTH-N-term.	< 0.25 ng/ml	0.57 ± 0.49	0.52 ±	0.42 ± 0.46	0.39 ± 0.48	0.36 ± 0.42	
	median	0.54	0.38	0.39	0.26	0.27	Not
PTH-C-term.	< 40 ng/ml	465 ± 1304	184 ± 158	286 ± 637	212 ± 290	145 ± 119	done
	median	167	104	120	128	98	
$1,25OH_2D_3$	43–67 pM/l	238 ± 178	–	196 ± 120	–	184 ± 127	
U-Ca/d	0.25–7.5 mM/d	1.95 ± 2.04	2.36 ± 2.48	1.72 ± 1.25	1.56 ± 1.01	3.0 ± 2.56****	2.32 ± 2.24
% TRP		62 ± 12	58 ± 9	65 ± 12	62 ± 9	63 ± 15	63 ± 16
U-OH-Prolin/d	10–50 mg/d	283 ± 121	Not	251 ± 118	Not	253 ± 324	Not
U-OH-Prolin/U-Creat		25.2 ± 12.8	done	20.2 ± 8.0	done	18.2 ± 19.6	done
CyA	mg/kg	5.1 ± 2.1	5.2 ± 1.8	5.1 ± 1.9	4.6 ± 1.6	–	–
X-ray score	0	2.41 ± 2.06**	–	0.71 ± 0.91	–	1.09 ± 1.14	–

Effect of changing the immunosuppression on aP

Eight of the 11 kidney transplant recipients who were switched from Aza/p to CyA/p later showed a significant rise in aP concentration. In six of these patients aP levels peaked above 120 U/l 1.5–6 months after the switch. Initiation of CyA treatment not only caused a rise in aP but also an increase in creatinine concentration. However, a decrease in kidney function is not the sole cause of aP change. The aP increase was transient while the creatinine concentration remained stable. Figure 3 shows aP and creatinine concentrations in a 42-year-old man with diabetic nephropathy who received Aza/p (azathioprine 150 mg, prednisone 10 mg) after transplantation. Three months later, pelvic X-ray revealed a spontaneous fracture of the ischiopubic bone. To reduce steriod side effects on blood glucose and to stop progression in weight gain, 18 months after transplantation Aza/p was switched to CyA/p (CyA 6.1 mg/kg body weight = 350 mg, prednisone 10 mg). Prednisone was tapered off within 2 months after initiation of CyA/p treatment. The increase in aP level did not correlate with N-terminal PTH (unmeasurably low) or 1,25-$(OH)_2$ vitamin D_3 concentration. Before death, bone-derived aP was normalized. At autopsy, bone histology demonstrated normal morphology.

Nine patients developed severe nephrotoxicity under CyA/p therapy and were therefore switched to Aza/p 4–18 months after grafting. The mean aP concentration during initial CyA/p therapy was 70.4 ± 21 and thereafter, under Aza/p therapy, 52.7 ± 7 ($P < 0.05$). Seven of these nine patients demonstrated a decrease of more than 10% and four patients had a decrease of more than 20% in aP concentration. These findings of an increase in aP levels after changing from Aza/p to CyA/p administration and a decrease after the switch from CyA/p to Aza/p is highly suggestive of an interrelation between CyA and aP increase.

Radiographic hand examination

At transplantation, radiographic signs of renal bone disease, i.e., acro-osteolysis of the terminal finger phalanges, subperiosteal resorption, and cortical striation in the second phalanges, were more pronounced in patients on CyA with increased aP after transplantation (CyA 1 group) than in CyA-treated patients with aP in the normal range after grafting (CyA 2 group; CyA 1 group vs CyA 2 group $P < 0.01$; Table 3). In the CyA 1 group, the X-ray score was high at transplantation (2.9 ± 2.3) and fell subsequently (1.8 ± 5, $P < 0.01$; Table 3, Fig. 4). Irrespective of the date of transplantation, at the time aP concentration exceeded 120 U/l, patients demonstrated more severe bone lesions than those with normal aP levels (Table 2). In patients on Aza/p, the X-ray score was also higher at transplantation (1.4 ± 4), though to a lesser degree. Increased scores in CyA/p-treated patients diminished. In contrast, in patients on Aza/p, a high X-ray score remained elevated and did not improve, with one exception only (Fig. 4). Patients on CyA/p with normal aP concentration and without radiographic signs of osteopathy at transplantation did not regularly have follow-up X-rays taken. However, in 15 patients, subsequent radiographs were recorded (1–5 years after grafting) for several reasons. They did not demonstrate a significant increase in the radiographic score. Even though there were remarkable changes in the X-ray scores in CyA 1 patients ($n = 44$), follow-up examinations demonstrated unchanged cortical thickness (4.69 ±

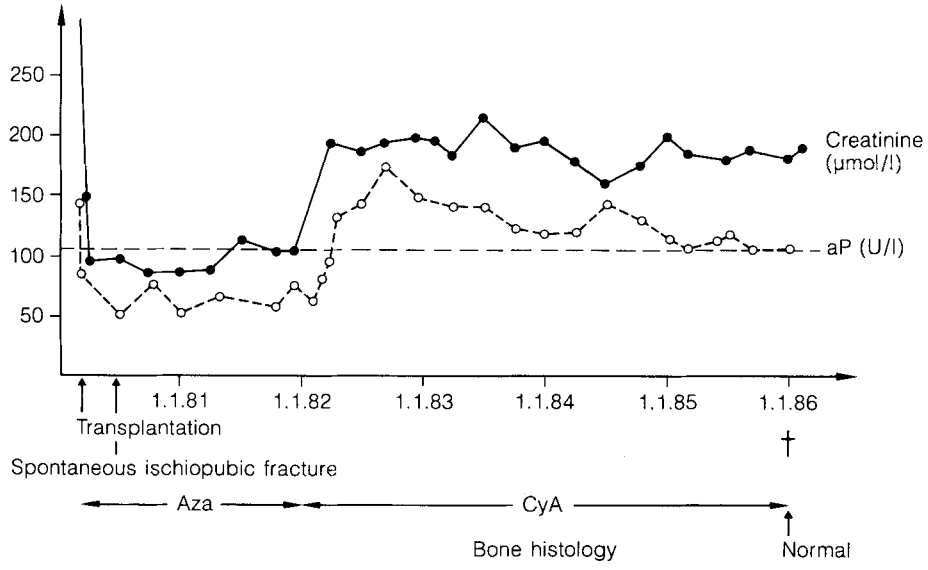

Fig. 3. A 42-year-old man with diabetic nephropathy received a kidney transplant and was first immunosuppressed with Aza/p (150 mg/10 mg). One month after renal grafting the pelvis X-ray was normal. Three months later a spontaneous fracture of the pubic bone occurred. Immunosuppression was changed from Aza/p to CyA/p (6.1 mg/kg/10 mg). Prednisone was tapered off within 2 months after changing to CyA/p. Thereafter, bone-derived aP concentration increased transiently. Autopsy revealed normal histology of the bone

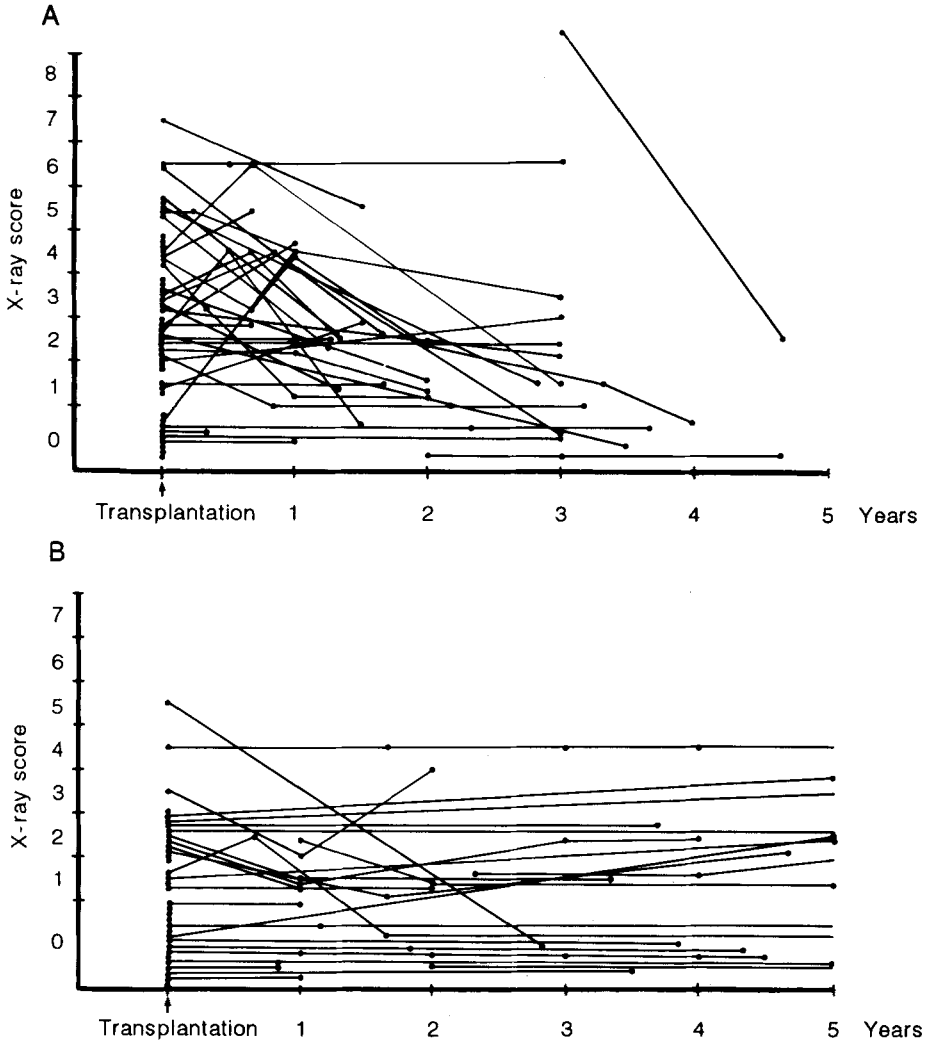

Fig. 4 A, B. Radiographic score in kidney transplant patients **A** on CyA/p with aP above the normal range and **B** on Aza/p. The score was rated for acro-osteolysis of the terminal finger phalanges, subperiosteal resorption, and cortical striation in the second phalanges. High radiographic scores diminished in the majority of patients treated with CyA/p but remained elevated in most patients on Aza/p

0.86 mm vs 4.73 ± 0.79 mm). The latter is regarded as a measure of cortical bone mass [19]. In contrast, bone mass decreased progressively in patients receiving Aza/p ($n = 32$; at transplantation 4.94 ± 0.96; at follow-up 4.78 ± 0.95; $P < 0.05$).

Discussion

Serum aP is a biochemical marker often used as an indirect measure of parathyroid activity and renal bone disease [19, 22]. aP is synthesized by osteoblasts. Kidney

Table 3. X-ray score at transplantation and 1.5–5 years later. Scores ranged from 0 (no change) to 3 (very pronounced changes) for acro-osteolysis of the terminal finger phalanges, subperiosteal resorption, and cortical striation in the second phalanges in CyA/p-treated patients with increased aP (> 120 U/l; CyA 1), with aP in the normal range (CyA 2), and patients on azathioprine/prednisone (Aza/p). * $P < 0.01$ CyA 1 vs CyA 2; CyA 1 vs Aza/p; ** $P < 0.01$ CyA 1 at transplantation vs CyA 1 at follow-up

Score	CyA 1	CyA 2	Aza/p
At transplantation	2.9 ± 2.3*	0.9 ± 1.5	1.4 ± 1.4
1.5–5 years later	1.8 ± 1.5**	Not done	1.3 ± 1.2

transplant patients on immunosuppressive therapy with Aza/p who show an isolated rise in aP have very likely developed autonomous hyperparathyroidism [1]. At the University Hospitals in Basel, fewer than 5 % of patients with stable transplant function who are on Aza/p therapy demonstrate such a complication. In contrast, patients receiving CyA/p for immunosuppression have frequently demonstrated an isolated increase in bone-derived aP concentration [5, 15]. This observation and results from animal studies suggesting increased bone turnover in rats treated with CyA [18] and impaired bone repair after experimental fracture [26] have given rise to concern about the long-term effects of CyA on bone in kidney transplant recipients. Moreover, the administration of CyA to patients with pre-existing osteodystrophy, as it is seen in most patients with chronic renal failure, may be deleterious.

The present analysis demonstrated a significant and isolated transient rise in bone-derived aP concentration in more than 70 % of 139 kidney transplant patients. In 50 patients aP exceeded the upper limit of the normal range. It is striking that 50 % of the remaining 89 patients also showed a transient rise in serum aP by more than 20 U/l, although the peak level never exceeded the normal range. These findings suggest that after successful kidney transplantation, aP rises in the majority of patients on CyA/p, but that the level varies.

The frequency of aP rising above the normal range correlated with the CyA dose. The aP increased in 45 % of the patients given a dose of 12–17 mg/kg body weight while it increased above the normal range in only 28 % of those given a dose of 5 mg/kg (or less) body weight. A correlation between aP rise and CyA therapy is further supported by findings in patients treated with Aza/p after transplantation who subsequently had to be switched to CyA/p. More than two-thirds of these patients demonstrated a rise in aP levels after initiation of CyA/p treatment. On the other hand, when the treatment was changed from CyA/p to Aza/p within the first 3–18 months after grafting, aP concentration decreased significantly. Patients demonstrating a marked rise in aP showed a preponderance of women over men (30 women, 20 men). End-stage renal failure due to analgesic nephropathy was more common in these women. Renal osteodystrophy has been shown to be more pronounced in this type of nephropathy [12]. The difference in hormone pattern may also have an effect on osteopathy. In particular, many female transplant recipients were premenopausal. However, we did not carry out hormone analysis to elucidate this point.

Patients with chronic renal failure invariably develop abnormalities in bone histology [3, 8, 10, 20]. However, only 50 % of patients with positive bone biopsies of renal osteodystrophy show increased aP levels [8] and only one-third of patients demonstrate radiographic signs of renal osteodystrophy [8, 10, 17]. Thus, aP concentrations in the normal range and normal radiography of the bone at the time of transplantation do not exclude renal osteodystrophy. Still, three facts argue in favor of a more pre-existing renal osteopathy among those CyA/p-treated patients who demonstrated an aP rise above the normal range: (1) a higher radiographic score of osteodystrophy (acro-osteolysis of the terminal phalanges, subperiosteal resorption, and cortical striation in the second phalanges) at transplantation and at the time aP concentration exceeded 120 U/l; (2) higher levels of aP before initiation of CyA/p therapy; and (3) a tendency towards higher plasma PTH levels in patients with increased aP. However, aP concentration did not correlate with N-terminal PTH concentration. Moreover, in three patients on CyA/p, PTH concentrations remained unmeasurably low for more than 1 year while aP was increased. Therefore, persistence of secondary hyperparathyroidism does not solely account for the transient aP rise in renal transplant patients on CyA/p therapy.

After successful kidney transplantation and initiation of CyA/p treatment, the X-ray score did not deteriorate despite the aP rise. At long-term follow-up, the X-ray score even improved and cortical bone mass remained unchanged. Thus, there is no radiographic evidence for progressive bone disease in patients treated with CyA/p. In contrast, the majority of patients treated with Aza/p demonstrated a decrease in aP levels after grafting. Patients with a high X-ray score at transplantation also demonstrated a decrease in aP concentration. Despite better kidney function in Aza/p-treated patients, the long-term X-ray score did not improve and bone mass decreased. Thus, earlier reports of progressive bone resorption and loss of cortical bone volume by as much as 0.07 % per month under Aza/p therapy [17] and Huffer et al.'s suggestion [11] of more severe bone disease in kidney transplant patients on Aza/p than patients on chronic dialysis therapy are confirmed. Therefore, good transplant function seems insufficient to normalize pre-existing renal osteodystrophy in patients treated with Aza/p. We are aware of the fact that hand radiography is neither a very specific nor a very sensitive method to detect distinct changes in renal bone disease. Only severe cortical lesions are shown, but early signs of renal osteodystrophy and osteopenia occur in trabecular bone. Computerized axial tomography imaging or dual-beam photon densitometry are more valuable methods to detect changes in trabecular bone [10, 16, 25]. Still, diagnostically, they are not specific.

Glucocorticoids modulate osteoblast function and thus serum aP concentration [7]. A decrease in aP concentration immediately after kidney transplantation may, therefore, result in part from inhibitory effects of glucocorticoids. Increased renal calcium loss and diminished

intestinal calcium absorption may occur [7]. During the 1st month after grafting, the cumulative steroid dose was highest in the Aza/p group, which may account for the highest urinary calcium excretion rate at that time. aP increased significantly when the prednisone dose was 15 mg/day or less. In more than 50% of the CyA/p-treated patients, steroids were tapered off 6–18 months after grafting. The remaining patients received maintenance prednisone doses that were significantly lower than the dose administered in Aza/p therapy. A recent follow-up study [14] in kidney transplant recipients on CyA/p reported normal aP levels after transplantation. This finding contrasts with our observation and those reported in the European Multicentre Trial [6, 9, 13]. The difference may be explained in part by differences in the mean prednisone dose of 10–17.5 mg/day 6 months after grafting, while our patients received 0–15 mg/day. Still, the aP increase cannot be explained solely by steroid withdrawal in CyA/p patients. CyA/p-treated patients received 10 mg/day or more of prednisone when aP levels rose, on the average, 3 ± 3 months after transplantation. This prednisone dose was similar to the dose administered to patients on Aza/p who did not show an aP rise but in fact showed a decrease in aP concentration after transplantation. In addition, when immunosuppression was switched from Aza/p to CyA/p, or vice versa, patients received the same prednisone dose before and after this change. Still, aP concentration rose only under CyA/p treatment.

The mechanism of CyA effects on bone is not known. However, there is increasing evidence that cells of the immune system are involved in the regulation of bone turnover. In vitro lymphocyte- and macrophage-derived cytokines have been shown to stimulate bone resorption. Thus, it is conceivable that the inhibition of immune responses by agents such as CyA or Aza may elicit bone effects. Indeed, in vitro CyA inhibited PTH-, interleukin-1- and lipopolysaccharide-stimulated bone resorption in mice calvaria [24]. Analogues of CyA without immunosuppressive effect did not affect bone resorption [23].

In conclusion, after successful kidney transplantation, bone-derived aP increased in the majority of patients on CyA and low-dose prednisone therapy. The early onset and transient nature of the increase in aP levels suggest that pre-existing renal bone disease may contribute to these changes in aP. Radiographic signs of osteopathy did not deteriorate; in contrast, the X-ray score improved over the long term. Symptoms and clinical signs of progressive bone disease did not develop. Information about CyA effects on aP and bone in patients with nonsystemic diseases such as psoriasis are not available. However, the lack of an aP rise after heart and liver grafting, despite CyA treatment (personal communication, Prof. P. Krupp, Sandoz, Basel), can well be explained if pre-existing osteopathy is a requirement for CyA-induced increase in bone aP levels. An isolated, transient rise in aP after kidney transplantation in patients on CyA/p, therefore, does not seem to be the result of progressive bone disease but rather reflects repair mechanisms of pre-existing osteodystrophy. Only bone biopsies taken at transplantation and subsequently will shed light on the effects of CyA/p in humans.

Acknowledgements. The authors would like to thank Prof. E. Ritz, University Hospital Heidelberg, FRG, for his generous support in measuring 1,25 $(OH)_2$ vitamin D_3; Prof. I. Fischer and Prof. M. A. Dambacher, University Orthopaedic Hospital, Zürich, Switzerland, for measuring serum C- and N-terminal parathyroid hormone; and Prof. D. J. Vonderschmitt, Chemical Laboratory of the University Hospital, Zürich, for measuring hydroxyproline. The authors would also like to thank Ms. Evelyne Graf and Ms. Christa Haenel for their excellent assistance in the preparation of the manuscript.

References

1. Alfrey AC, Jenkins D, Groth CG, Schorr WS, Gecelter L, Ogden DA (1968) Resolution of hyperparathyoidism, renal osteodystrophy and metastatic calcification after renal homotransplantation. N Engl J Med 279: 1349
2. Behr W, Barnert J, Kanitz W, Vogt H (1986) Quantitative Bestimmung der alkalischen Knochenphosphatase durch Ausfällung mit Weizenkeimlektin. Aerztl Lab 32: 159
3. Binswanger U, Sherrard DJ, Rich C, Curtis FK (1973) Dialysis bone disease: a quantitative histologic study. Nephron 12: 1
4. Born W, Dambacher MA, Meyrier A, Ardaillou R, Fischer JA (1982) Parathyroid suppressibility in hyperparathyroidism due to chronic renal failure: studies with autotransplanted parathyroid tissue. Clin Endocrinol 17: 333
5. Bourbigot B, Moal MC, Cledes J (1988) Bone histology in renal transplant patients receiving cyclosporin (letter). Lancet I: 1049
6. Calne RY (1987) Cyclosporine in cadaveric renal transplantation: 5-year follow-up of a multicentre trial. Lancet II: 506
7. Cundy T, Kanis JA (1987) Rapid suppression of plasma alkaline phosphatase activity after renal transplantation in patients with osteodystrophy. Clin Chim Acta 164: 285–291
8. Delmez JA, Fallon MD, Bergfeld MA, Gearing BK, Dougan CS, Teitelbaum SL (1986) Continuous ambulatory peritoneal dialysis and bone. Kidney Int 30: 379
9. European Multicentre Trial Group (1983) Cyclosporin in cadaveric renal transplantation: one-year follow-up of a multicentre trial. Lancet II: 986
10. Haef JG, Joffe P, Podenphant J, Andersen JR (1987) Noninvasive diagnosis of uremic osteodystrophy: uses and limitations. Am J Nephrol 7: 203
11. Huffer WE, Kuzela D, Popoutzer MM, Starzl TE (1975) Metabolic bone disease in chronic renal failure. II. Renal transplant patients. Am J Pathol 78: 385
12. Jaeger P, Burckhardt P, Wauters JP, Trachsel U, Bonjour JP (1985) Evidence for a particularly severe secondary hyperparathyroidism in analgesic abuse nephropathy. Am J Nephrol 5: 342
13. Johnson RWG for the European Multicentre Trial Group (1986) Cyclosporine in cadaveric renal transplantation: three-year follow-up of a European multicentre trial. Transplant Proc 18: 1229
14. Julian BA, Lawskow DA, Dubovsky J, Dubovsky EV, Curtis JJ, Quarles LD (1991) Rapid loss of vertebral mineral density after renal transplantation. N Engl J Med 325: 544
15. Lörtscher R, Thiel G, Harder F, Brunner F (1983) Persistent elevation of alkaline phosphatase in cyclosporine-treated renal transplant recipients. Transplantation 36: 115
16. Malluche HH, Faugere MC (1990) Renal bone disease 1990: an unmet challenge for the nephrologist. Kidney Int 38: 193
17. Mora Palma FJ, Ellis HA, Cook DB, Dewar JH, Ward MK, Wilkinson R, Kerr DNS (1983) Osteomalacia in patients with chronic renal failure before dialysis or transplantation. Q J Med New Series 207: 332

18. Movsowitz C, Epstein S, Fallon M, Ismail F, Thomas S (1988) Cyclosporine A in vivo produces severe osteopenia in the rat: effect of dose and duration of administration. Endocrinology 123: 2571
19. Posen S, Cornish C, Kleerckoper M (1974) Alkaline phosphatase and metabolic bone diseases. In: Avioli LV, Krane SM (eds) Metabolic bone disease. Academic Press, New York
20. Ritz E, Malluche HH, Bommer J, Mehls O, Krempien B (1974) Metabolic bone disease in patients on maintenance hemodialysis. Nephron 12: 393
21. Scharla S, Schmidt-Gayk H, Reichel H, Mayer E (1984) A sensitive and simplified radioimmunoassay for 1,25-dihydroxy vitamin D_3. Clin Chim Acta 142: 325
22. Siede WH, Seiffert UB, Bundschuh F, Malluche HH, Schoeppe W (1980) Alkaline phosphatase bone isoenzyme activity in serum in various degrees of micromorphometrically assessed renal osteopathy. Clin Nephrol 13: 277
23. Stewart PJ, Stern PH (1989) Interaction of cyclosporin A and calcitonin on bone resorption in vitro. Horm Metab Res 21: 194
24. Stewart PJ, Green OC, Stern PH (1986) Cyclosporine A inhibits calcemic hormone-induced bone resorption in vitro. J Bone Miner Res 1: 285–291
25. Torres A, Lorenzo V, Gonzales Posada JM (1986) Comparison of histomorphometry and computerized tomography of the spine in quantitating trabecular bone in renal osteodystrophy. Nephron 44: 282
26. Warren SB, Pelker RR, Friedlaender G (1985) Effects of short-term cyclosporin A on biomechanical properties of intact and fractured bone in rat. J Orthop Res 3: 96