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Original Article

Long-Term Persistence of Low Bone Density in Orthotopic Liver Transplantation

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Abstract. We determined bone density and metabolism in 46 patients (35 males, 11 females) who had undergone liver transplantation 1–48 months previously. Twenty-one patients were then followed for the next 24 months. At each visit, blood and urine samples for bone and liver metabolism parameters, as well as spinal and femoral dual-energy X-ray absorptiometry (DXA) scans, were obtained. Basal spinal and femoral density was low ($p < 0.001$). Patients with pre-transplant cholestatic diseases had lower spinal density than all the other subjects ($p < 0.05$) and the cumulative methylprednisolone intake was an independent negative predictor of total hip density ($p < 0.02$). At baseline, urinary hydroxyproline and N-telopeptide were at the upper normal level and decreased only after 24 months of follow-up ($p < 0.05$). During the first year of follow-up, femoral density decreased ($p < 0.05$) and a partial recovery was observed for both spine and femur after 24 months. After 12 months, femoral bone density was negatively associated with serum cyclosporin A levels ($p < 0.005$) and cumulative methylprednisolone intake ($p < 0.05$), while the percent decrease in spinal density after the first 12 months was negatively predicted by mean daily methylprednisolone intake ($p < 0.05$). In patients with pre-transplant cholestatic diseases, femoral and spinal density increased after the first ($p < 0.05$) and second year ($p < 0.05$), respectively. In patients with previous post-necrotic cirrhosis, femoral density decreased after 12 months ($p < 0.05$) and was still lower than baseline after 24 months ($p < 0.05$). However, at the end of the study the cumulative percentage of femoral neck osteoporosis was 43%. In conclusion, an elevated prevalence of spinal and femoral osteoporosis is present even many years after liver transplantation, with immunosuppressive treatment and pre-transplant liver disease being the most important pathogenetic factors.

Keywords: Bone turnover; Immunosuppressive drugs; Liver disease; Liver transplant; Osteoporosis

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Introduction

Orthotopic liver transplantation (OLTx) is now the treatment of choice in patients with end-stage liver disease. Advances in immunosuppressive therapy and surgical techniques have substantially improved the outcome of liver transplantation and increased life expectancy in these patients. In spite of this, transplant recipients still undergo a substantial number of complications, among which bone disease appears to be very frequent [1–3].

It is generally believed that the critical period for the appearance of bone disease occurs within the first 6 months after transplantation, with the trabecular bone of the spine being most at risk. Indeed, vertebral fractures occur very often within the first 12 months after transplantation [1,2,4]. However, very few studies have been performed to elucidate the long-term impact of liver transplantation on bone metabolism and fracture risk [5,6]

The pathogenesis of osteopenia after transplantation is multifactorial. One of the factors contributing to bone loss is the underlying liver disease. End-stage liver failure is generally associated with a reduction in bone density and an increased risk of fractures [7,8]. Nevertheless, although patients with cholestatic diseases, such as primary biliary cirrhosis and primary sclerosing cholangitis, are at higher risk of osteoporotic fractures [9], they seem to have a better long-term outcome of bone status after transplantation [2,5].

Many other conditions may be responsible for the pathogenesis of osteopenia after transplantation. Immobilization, poor nutritional status and hypogonadism may also affect bone metabolism, especially in the early phase after transplantation [10]. However, the high dose of corticosteroids used after the graft is likely to be the major factor affecting bone metabolism in the early post-transplantation period. Glucocorticoid-induced osteoporosis is a well-recognized clinical entity [11]. Corticosteroids affect osteoblast function in several ways [12,13]. At the same time, they stimulate the processes leading to osteoclast activation and, consequently, to bone resorption. They also impair intestinal calcium absorption and their use may be associated with hypogonadism and avascular necrosis of the bone [11].

The effects of cyclosporin A on bone are still debated. An accelerated bone remodeling, with resorption exceeding formation and a consequent net bone loss, has been shown in animals [14]. However, the role of this immunosuppressive agent as a risk factor for osteoporosis in liver transplant recipients has not been fully elucidated.

The aim of this study was to evaluate in a 24-month follow-up bone density and metabolism in patients who had undergone liver transplantation.

Patients and Methods

(a) Cross-sectional Study

Patients

Forty-six patients (35 men, 11 women), aged 47.0 ± 1.4 years, who had undergone orthotopic liver transplantation (OLTx) 1–48 months previously, were consecutively enrolled in this study. These patients represented the total population of subjects who had survived to liver transplantation performed at our Unit, between 1991 and 1996. None of the patients had been previously treated for osteoporosis or endocrine diseases. Among the female population there were 3 postmenopausal women, while the remaining 8 were still experiencing regular menses.

Patients were transplanted for end-stage liver disease related to: post-necrotic cirrhosis (PNC; 23 men, 5 women) resulting from HBV or HCV, primary biliary cirrhosis (PBC; 2 women), primary sclerosing cholangitis (PSC; 2 men, 2 women), alcoholic cirrhosis (OHC; 5 men, 2 women), hepatocellular carcinoma (HCC; 3 men), Budd-Chiari syndrome (BC; 1 man), and α1-antitripsin deficiency (α1T; 1 man).

After transplantation, patients underwent an immunosuppressive regimen consisting of: (a) cyclosporin A (CsA), at a dosage adequate to maintain blood levels of the drug between 240 and 280 ng/ml during the first 3 months and between 120 and 150 ng/ml thereafter; (b) methylprednisolone (MP), at a dose of 1 g intravenously (i.v.) for the first postoperative day, subsequently tapered to 40 mg (i.v.) at day 5 and to 20 mg per os daily after day 6. The dose was then tapered to 8–6 mg daily within 6 months. Eleven of the 46 patients were also treated with azathioprine, 1 mg/kg body weight per day, during the first year after transplantation. Rejection episodes were treated with MP recycles, consisting of 1 g i.v. at day 1, tapered to 40 mg i.v. at day 5 and reduced to 20 mg per os daily after day 6. None of the patients developed steroid-resistant rejection.

The total intake of immunosuppressive drugs was calculated by the sum of the daily doses, including those administered for the treatment of rejection episodes. The mean daily intake was then calculated by dividing the total intake of the drugs for the time (in days) elapsed since transplantation.

All patients gave their informed consent to the study, which was approved by the institute's ethics committee.

Methods

Biochemical Assays. Blood and 24-h urine samples were collected from all patients after an overnight fast. Blood specimens were analyzed for creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (γGT), total alkaline phosphatase and bilirubin (Autoanalyzer, Technicon Instruments, Tarrytown, NY). Serum calcium was assayed by atomic absorption spectrophotometry (Perkin Elmer 3100, Norwalk, CT), while serum phosphate and albumin were analyzed by a colorimetric method (Boehringer Mannheim and Sigma-Aldrich, Milan, Italy, respectively). Bone alkaline phosphatase was determined in catalytic activity by lectin from wheat germ precipitation (Boehringer Mannheim, Milan, Italy), with intra-assay and inter-assay variations of $<4.0\%$ and $<10\%$, respectively. Intact parathyroid hormone (PTH) was analyzed by a commercial radioimmunoassay (RIA) kit (Nichols Institute, San Juan Capistrano, CA), with intra- and inter-assay variations of 3.6% and 5.8%, respectively. 25-OH-vitamin D was analyzed by competitive protein binding assay, with intra- and inter-assay coefficients of variation of 5.1% and 9.7%, respectively. Cyclosporin A concentration in whole blood was determined by RIA method (Incstar, Diasorin, Saluggia, Vercelli, Italy). Twenty-four hour urine samples were assayed for calcium (see above), hydroxyproline (resin-catalyzed hydrolysis, followed by a colorimetric method (Hypronosticon, Organon Teknika, Boxtel, The Netherlands) after 2 days of a collagen-free

diet), and type I collagen cross-linked N-telopeptide (NTx, competitive-inhibition enzyme-linked immunosorbent assay, ELISA; Osteomark, Ostex International, Seattle, WA). The latter had intra- and inter-assay variation coefficients of 7.6% and 14%, respectively. The results of hydroxyproline and NTx were then expressed as a ratio to creatinine excretion.

Bone Densitometry. The bone mass of the lumbar spine (L2–L4) and proximal femur was measured by dual-energy X-ray absorptiometry (DXA; Hologic QDR 1000, Waltham, MA). Our in vivo coefficients of variation, calculated on 8 healthy subjects, were: lumbar spine, 1.06%; total femur, 1.16 %; femoral neck, 1.63%; great trochanter 1.85%. The results were expressed as bone mineral density (BMD, g/cm^2), *T*-score and *Z*-score (number of SD below the mean of young adult healthy subjects or age- and sex-matched normal controls, respectively), when appropriate. The *T*-score values were obtained by comparing the BMD results with the NHANES database. According to the World Health Organization recommendations, osteoporosis is defined as a *T*-score value < -2.5.

(b) Longitudinal Study

Twenty-one of the patients in the cross-sectional study (13 men, 8 women; mean age 43.2 ± 2.3 years; time since graft 7.3 ± 1.9 months) were also studied in a 2-year follow-up, by repeating after 12 and 24 months from baseline the same investigations performed in the cross-sectional study. Sixteen patients were taking only methylprednisolone and CsA, while the remaining 5 were also being treated with azathioprine. These patients had undergone transplantation for the following liver diseases: PNC related to HBV or HCV (8 men, 4 women), PBC (2 women), PSC (1 man, 2 women), OHC (2 men), HCC (1 man) and BC (1 man). In 8 of the 12 patients with a previous PNC, the disease recurred after the transplant.

All patients were given 1000 mg oral calcium supplements (Calcium Sandoz Fortissimum, Milan, Italy).

(c) Statistical Analysis

The results were expressed as mean ± SEM. One- and two-sample *t* tests were used to determine statistical differences between means. One-way analysis of variance (ANOVA) was used for multiple group comparisons. Multifactorial analysis of variance was then employed to adjust for specific variables. Linear regression analysis was used to evaluate the relationships between variables. Stepwise multiple regression analysis was use when appropriate. The α-level for significance was considered as < 0.05.

Results

Cross-sectional Study

The mean daily intake of MP and CsA was 56 ± 6 and 399 ± 25 mg respectively, without differences between sexes.

The main clinical variables are reported in Table 1. As shown, the duration of the graft and the total dose of immunosuppressive drugs were slightly, but not significantly, higher in women.

Mean PTH and 25-OH-D levels, serum calcium, serum phosphate and bone alkaline phosphatase were within the normal range (Table 2). Serum albumin and total alkaline phosphatase were also normal, while the mean values of AST, ALT, γGT and bilirubin were above the normal range. Urine calcium excretion was normal, while mean urinary hydroxyproline values were above the normal range. Hydroxyproline excretion was elevated in 24 patients (52%). Urinary NTx levels were at the upper normal level.

Table 1. Clinical parameters in patients with an orthotopic liver transplant

	Men (n = 35)	Women (n = 11)	p value
Age (years)	48.1 ± 1.5	43.4 ± 3.7	NS
Body mass index (kg/m^2)	24.4 ± 0.6	22.3 ± 0.8	NS
Time since graft (months)	9.8 ± 2.3	14.3 ± 4.3	NS
Total methylprednisolone intake (g)	5.3 ± 0.6	6.5 ± .07	NS
Total cyclosporin A intake (g)	95.2 ± 19.7	128.4 ± 37.7	NS
Total azathioprine intake (g)	7.1 ± 3.1	6.1 ± 2.7	NS

Table 2. Basal biochemical values in subjects with an orthotopic liver transplant

	All patients (n = 46)	Normal range
Plasma or serum level		
Creatinine (mmol/l)	100.8 ± 4.4	62–115
AST (U/l)	46.0 ± 9.6	10–45
ALT (U/l)	68.3 ± 11.0	5–55
γGT (U/l)	95.1 ± 25.7	3–65
Total bilirubin (μmol/l)	23.1 ± 1.9	1.7–17
ALP (U/l)	100.4 ± 8.8	53–128
Albumin (g/l)	41.2 ± 1.7	38–44
Cyclosporin A (ng/ml)	186.5 ± 9.0	100–350
Calcium (mmol/l)	2.25 ± 0.02	2.1–2.6
Phosphate (mmol/l)	1.19 ± 0.03	0.87–1.45
Bone ALP (U/l)	22.9 ± 4.2	10–51
PTH (pg/ml)	35.6 ± 4.0	10–55
25-OH-D (nmol/l)	94 ± 17	25–300
Urinary level		
Calcium (mmol/day)	3.5 ± 0.4	2.5–7.5
OHP/Cr (mmol/mol/day)	22.2 ± 2.6	5.2–19
NTx/Cr (nmol BCE/mmol/day)	91 ± 9	14–95

Table 3. Bone mineral density at different skeletal sites in patients with an orthotopic liver transplant

	Men (n = 35)			Women (n = 11)		
	BMD (g/cm^2)	T-score (SD)	Z-score (SD)	BMD (g/cm^2)	T-score (SD)	Z-score (SD)
Spine (L2–L4)	0.89 ± 0.02	–2.1 ± 0.2	–1.7 ± 0.2	0.84 ± 0.04	–2.1 ± 0.3	–1.7 ± 0.4
Femur						
Neck	0.74 ± 0.02	–2.1 ± 0.2	–1.0 ± 0.2	0.65 ± 0.04	–2.4 ± 0.4	–1.7 ± 0.3
Trochanter	0.63 ± 0.02	–1.5 ± 0.2	–1.0 ± 0.2	0.49 ± 0.03	–2.5 ± 0.4	–2.1 ± 0.4**
Total	0.85 ± 0.02	–1.7 ± 0.2	–1.1 ± 0.2	0.70 ± 0.04	–2.3 ± 0.3	–1.9 ± 0.3*

*$p < 0.05$ versus men; **$p < 0.005$ versus men.

Bone densitometry data are shown in Table 3. A significant reduction in T-score values was observed at all skeletal sites ($p < 0.001$). In the whole population, spinal BMD was below −2.5 standard deviations (mean T-score −3.27 ± 0.13) in 14 patients (30%), while only 7 patients (15%) were completely normal (T-score −0.44 ± 0.23). The percentage of osteoporotic patients was even higher at the femoral neck: 21 patients (46%) had BMD lower than −2.5 SD (T-score −3.17 ± 0.12), while only 6 subjects (13%) were normal (T-score −0.12 ± 0.30). Bone density of the total femur and great trochanter was significantly lower in females (Table 3).

When patients were subdivided according to the pathogenesis of the liver disease, the Z-scores for spinal bone density were significantly different (ANOVA, $p < 0.05$). Consequently, we compared patients with primary cholestatic diseases before transplantation (PBC and PSC; 4 women, 2 men) with all the other subjects (33 men, 7 women). Those with a previous primary cholestasis showed a lower spinal bone mass with respect to all the other patients (Z-score = −2.58 ± 0.60 vs −1.56 ± 0.16, $p < 0.05$). This trend was also present, although not significant, at all femoral sites. Moreover, patients with previous cholestasis did not differ compared with the other subjects as regards the duration of transplant (12.7 ± 5.8 vs 10.6 ± 2.2 months), mean daily dose of MP (45 ± 15 vs 57 ± 7 mg/day) and CsA (341 ± 43 vs 408 ± 28 mg/day), nor as regards any other parameter of liver or skeletal metabolism.

To adjust the correlation analysis for possible confounders, some predictive variables were identified and then put into a stepwise multiple regression model. These were: time since transplant, CsA serum levels, cumulative intake of both CsA and MP, indices of hepatocytolysis and cholestasis, age and weight. Two different multivariate models have been investigated, with bone turnover markers and bone density as dependent variables. Serum levels of CsA ($p < 0.001$) and ALT ($p < 0.005$) were independent predictors of hydroxyproline excretion (R^2 Adj. = 0.66), while weight ($p < 0.01$) and cumulative MP intake (expressed as log n because of a non-normal distribution of this value, $p < 0.02$) predicted bone density of total hip (R^2 Adj. = 0.33) and femoral neck (R^2 Adj. = 0.23).

Longitudinal Study

In Table 4 the mean clinical and biochemical parameters for the liver-transplanted patients followed for 24 months are reported. Mean daily intakes of MP and CsA were significantly lower after 12 and 24 months of follow-up compared with baseline. Serum creatinine, markers of liver function, serum calcium and phosphate and calciotropic hormones did not vary throughout the 2-year study period. Urine hydroxyproline and NTx excretion fell significantly only after 24 months ($p < 0.05$ and < 0.02, respectively). On the other hand, bone alkaline phosphatase tended to increase with respect to the basal value only at the end of the study.

At baseline, a relevant osteopenia was evident at all skeletal sites (T-score values: lumbar spine −2.33 ± 0.27; total femur, −1.83 ± 0.21; femoral neck, −2.06 ± 0.27; greater trochanter, −1.72 ± 0.25).

When all patients were considered together, bone density decreased significantly at the total femur (−3.75 ± 1.64%, $p < 0.05$) and femoral neck (−5.22 ± 1.90, $p < 0.05$) but not at the lumbar spine (−0.40 ± 2.02) after the first year of follow-up. After 24 months of follow-up there were no significant changes in lumbar spine (+2.46 ± 1.95) and total femur (−2.41 ± 1.30) bone density, while femoral neck bone mass continued to be significantly lower compared with the basal value (−5.95 ± 1.74, $p < 0.05$). At the end of the 24-month study period, the percentage of patients with osteoporosis at the femoral neck was 43%.

Because patients entered the longitudinal study at different time-points after transplantation, we also examined the percent changes in bone density according to time since transplant at the baseline visit. Thus, we subdivided patients into those with a baseline time from transplant shorter than 6 months (11 patients, mean time since transplant 3.6 ± 0.2 months) and greater than or equal to 6 months (10 patients, mean time since transplant 16.0 ± 4.0 months; Fig. 1). In patients with a shorter duration of the graft, lumbar bone density decreased after the first year and was unchanged after the second year with respect to baseline. In the same patients, femoral neck bone density was decreased after both 12 and 24 months of follow-up (Fig. 1). In patients with a longer time since transplant, there was a non-

Table 4. Clinical and biochemical parameters in transplanted patients at baseline and at 12 and 24 months follow-up

	Baseline (n = 21)	12 months (n = 21)	24 months (n = 21)
Age (years)	43.2 ± 2.3		
Time since graft (months)	7.3 ± 1.9	19.8 ± 2.5	31.8 ± 2.4
Mean daily methylprednisolone intake (mg)	60.7 ± 1.8	13.7 ± 0.9*	8.9 ± 0.5***
Mean daily cyclosporin A intake (mg)	401 ± 25	303 ± 21	96 ± 11***
Plasma or serum level			
Creatinine (mmol/l)	115 ± 18	107 ± 9	107 ± 5
AST (U/l)	24.7 ± 2.0	45.8 ± 11.3	61.4 ± 20.3
ALT (U/l)	33.6 ± 4.7	76.2 ± 29.0	72.7 ± 21.5
γGT (U/l)	28 ± 4	40 ± 12	72 ± 35
Total bilirubin (mmol/l)	18.8 ± 3.5	18.2 ± 2.2	16.5 ± 1.9
ALP (U/l)	90.3 ± 8.0	82.7 ± 7.5	111 ± 8
Calcium (mmol/l)	2.26 ± 0.05	2.28 ± 0.03	2.24 ± 0.02
Phosphate (mmol/l)	1.23 ± 0.03	1.10 ± 0.06	1.04 ± 0.04
Bone ALP (U/l)	21.7 ± 3.8	22.5 ± 5.0	34.1 ± 6.0
PTH (ng/l)	37.1 ± 4.5	37.9 ± 4.7	45.2 ± 5.3
25-OH-D (nmol/l)	91.8 ± 9.5	95.3 ± 11.3	110.5 ± 16.5
Urinary level			
Calcium (mmol/day)	4.8 ± 0.9	3.3 ± 0.5	3.6 ± 0.7
OHP/Cr (mmol/mol/day)	22.0 ± 2.5	22.4 ± 2.5	15.2 ± 1.1*
NTx/Cr (nmol BCE/mmol/day)	93±8	88±9	67±7**

*$p < 0.05$ versus baseline; **$p < 0.02$ versus baseline; ***$p < 0.001$ versus baseline.

significant increase in spinal bone density after 12 and 24 months, while femoral neck density was substantially unchanged after both the first and second year of follow-up (Fig. 1).

When subdivided according to the positive or negative trend in spinal density observed after the first year of follow-up (Δ% + 9.7 ± 3.1 vs –5.07 ± 1.1), patients losing bone had a higher mean daily MP intake (14.9 ± 0.8 vs 9.3 ± 1.2 mg, $p < 0.005$).

The same multiple regression models reported for the cross-sectional study were applied to the longitudinal study. After the first 12 months of follow-up, cumulative CsA intake ($p < 0.001$), ALT ($p < 0.005$) and γGT ($p < 0.05$) predicted bone alkaline phosphatase values (R^2 Adj. = 0.71), while serum CsA level ($p < 0.005$) was the only predictive variable for hydroxyproline excretion at the end of the study period (R^2 Adj. = 0.36). Correlation analysis between cumulative intake of CsA and bone alkaline phosphatase as well as CsA serum levels and hydroxyproline excretion are reported in Fig. 2. The percent change in spinal density after 12 and 24 months was predicted by mean daily MP intake ($p < 0.05$, R^2 Adj. = 0.21) and by age and γGT ($p < 0.05$ for both, R^2 Adj. = 0.40), respectively. After 12 months of follow-up, bone density of the total hip was associated with serum CsA levels ($p < 0.005$) and cumulative MP intake ($p < 0.05$, R^2 Adj. = 0.53), while, at the end of the study, age was the only predictive variable of the percent change in total hip bone mass ($p < 0.02$, R^2 Adj. = 0.28). Correlation analysis between mean daily MP intake and percent change in bone density as well as cumulative MP intake versus the T-score of the lumbar spine after 12 and 24 months, respectively, are reported in Fig. 3.

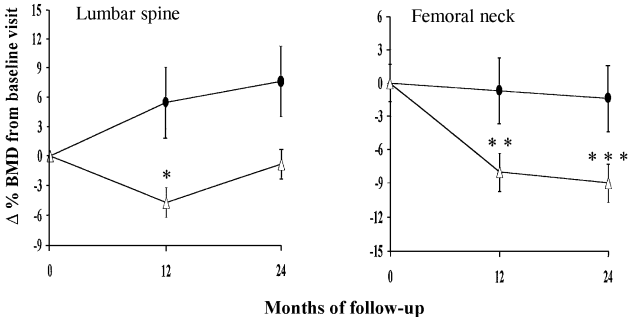

Fig. 1. Changes in bone density in orthotopic liver transplant patients grouped according to the time elapsed since transplantation at the baseline visit. *Ovals* represent patients with a duration of the graft shorter than 6 months, while *triangles* are patients with a duration of the graft greater than or equal to 6 months at the basal observation.

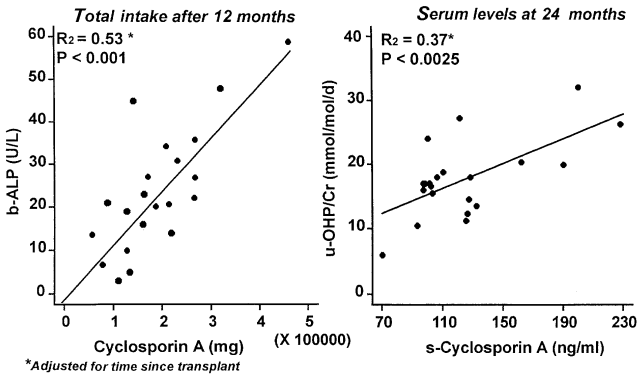

Fig. 2. Cyclosporin A versus bone turnover markers in orthotopic liver transplant patients in the longitudinal study.

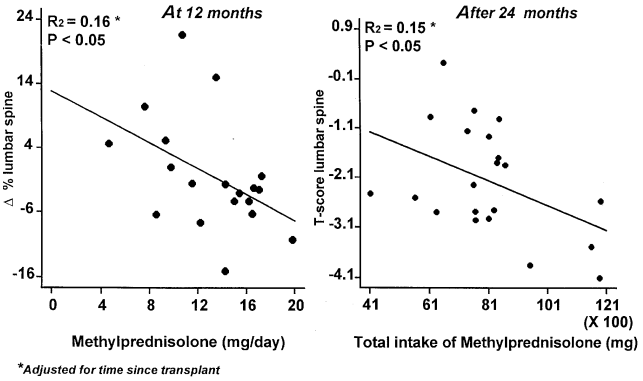

Fig. 3. Methylprednisolone intake versus lumbar bone density in orthotopic liver transplant patients in the longitudinal study.

Table 5 shows the patient data subdivided according to the primary cholestatic or viral origin of their underlying liver disease. The time elapsed since graft was longer in patients with previous cholestasis, while the mean daily intakes of MP were higher in patients with previous viral disease only after the first year ($p < 0.05$). In PNC patients, we observed a significant decrease in total femur and femoral neck bone density after both the first and second year of follow-up as compared with the baseline visit. On the contrary, in patients with previous cholestatic diseases, bone density significantly increased with respect to baseline at the total femur and great trochanter after 12 months and at the lumbar spine after 24 months (Table 5). The changes in femoral bone density differed between the two groups after 12 months from the baseline visit. Adjusting for time since grafting, we observed that this latter parameter did not substanstially influence the above-mentioned changes in bone mass (Table 5).

Finally, only in patients with previous PNC, was a negative correlation between total alkaline phosphatase levels and bone mass change observed ($r = -0.65$; $p < 0.05$).

Discussion

Liver transplantation is associated with low bone mass and increased risk of fractures [1,15]. In the present study, a marked reduction in bone density was observed in patients who had undergone transplantation from a mean of 11 months, and about one-third of our patients demonstrated osteoporotic BMD values of the lumbar spine. These results are in keeping with the finding of a high prevalence of vertebral fractures previously reported in this setting [4,5,16,17]. However, few studies have taken into account the prevalence of appendicular fractures after transplant [3,5]. We found a 46% prevalence of osteoporosis at the proximal femur, which is predominantly constituted by cortical bone. This suggests that also cortical bone is lost after liver transplantation. Considering the relevant morbidity associated with non vertebral fractures, there is a need for a more accurate assessment of peripheral fracture incidence in this setting.

Table 5. Clinical, biochemical and bone densitometry data after 12 and 24 months in subjects with previous primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) or post-necrotic cirrhosis (PNC)

	After 12 months		After 24 months	
	PBC + PSC (n = 5)	PNC (n = 10)	PBC + PSC (n = 5)	PNC (n = 10)
Age (years)	34.2 ± 3.4	49.08 ± 2.5	–	–
Time since graft (months)	28.4 ± 7.0*	16.4 ± 2.1	40.4 ± 7.0*	28.4 ± 2.1
Mean daily methylprednisolone intake (mg)	10.5 ± 2.2*	15.2 ± 0.9	7.2 ± 1.4	9.5 ± 0.5
Mean daily cyclosporin A intake (mg)	274 ± 66	303 ± 22	65.1 ± 16	94.5 ± 14
Plasma or serum level				
Calcium (mmol/l)	2.30 ± 0.05	2.31 ± 0.05	2.27 ± 0.04	2.23 ± 0.03
PTH (pg/ml)	28.8 ± 11.5	42.2 ± 5.7	48.3 ± 20.1	45.5 ± 8.4
Bone-ALP (U/l)	28.0 ± 11.3	15.6 ± 3.3	32.1 ± 15.2	38.6 ± 9.1
ALP (U/l)	73.5 ± 18.5	90.8 ± 9.3	57.3 ± 15.6	131.4 ± 25.6
AST (U/l)	26.4 ± 1.0	59.2 ± 18.7	17.8 ± 3.7	87.75 ± 33.8
ALT (U/l)	33.4 ± 4.4	104.1 ± 49.7	20.8 ± 2.05	105.9 ± 34.7
γGT (U/l)	13.6 ± 2.4	46.3 ± 17.6	15.0 ± 3.2	93.4 ± 58.9
Δ *% BMD from baseline visit*				
Lumbar spine (L2–L4)	7.0 ± 2.7 (6.2)	–2.8±3.1 (–2.4)	9.5 ± 3.1† (9.6)	0.6 ± 2.8 (0.5)
Femoral neck	2.4 ± 3.* (1.7)	–8.7 ± 2.5‡ (–8.4)	0.6 ± 4.3 (–0.01)	–8.6 ± 2.0‡ (–8.3)
Greater trochanter	4.9 ± 1.5*† (4.3)*	–5.9 ± 2.5 (–5.7)	6.0 ± 3.4 (6.5)	–2.9 ± 2.4 (–3.1)
Total femur	3.5 ± 1.2*† (2.6)	–5.6 ± 2.4† (–5.2)	3.7 ± 3.4 (4.2)*	–3.9 ± 1.5† (–4.1)

Values in parentheses are adjusted for time since transplantation.
*$p < 0.05$ verses patients with previous PNC; †$p < 0.05$ versus baseline visit; ‡$p < 0.005$ versus baseline visit.

Many studies have been performed to determine the modifications in bone density and metabolism within the first 6–12 months after liver transplantation, while few studies have focused on the long-term outcome of bone in these patients. Arnold et al. [6] observed a decrease in both trabecular (spine) and cortical (forearm) bone density within the first 12 months after transplant, followed by an inversion of that trend. However, the final values remained well below the baseline levels. McDonald and co-workers [3] described an early decrease in spinal bone density, with only a minor recovery after 12 months. Eastell et al. [5] described an early decrease in lumbar bone density, followed by a restoration of bone integrity within the third year of follow-up. Unfortunately, this study took into account only patients with primary biliary cirrhosis, whose long-term clinical outcome is generally much better compared with most other liver transplant recipients.

Our longitudinal study was carried out on liver transplant recipients with a mean duration of the graft of 7 months at the baseline visit, and approximately 3 years at the end of the 24-month follow-up period. Spinal bone density was unchanged after the first year of follow-up and only slightly increased with respect to baseline at the end of the study. These results are in keeping with only partial restoration of trabecular bone 12–24 months after transplant, observed by others [3,6]. On the other hand, femoral bone density decreased after the first year of follow-up, with a late and only partial trend toward a catch-up after the second year. This suggests different pathways for spinal and femoral bone loss after liver transplantation. Although we did not measure bone density immediately after transplant, it is likely that our patients too experienced the decrease in spinal bone mass expected for that period, followed by a progressive recovery 6–12 months after surgery. At the same time, femoral density might have decreased at a slower rate and for a longer time, with a tendency toward a restoration, starting 12–18 months after transplantation. The most likely explanation for this phenomenon is that bone remodeling is several times higher in trabecular that in cortical bone [18]. The improvement in clinical condition after the transplant was followed by an early trend to the recovery in spinal bone density, while a longer time was necessary for the same process in the proximal femur. This can also contribute to explaining why we found a higher prevalence of osteoporosis at the femoral neck compared with the lumbar spine.

We are well aware that grouping together patients with different times since transplant may affect assessments of the course of bone density in these patients. However, when patients were grouped according to the duration of the graft at the basal observation, lumbar bone mass did not significantly increase over time even in subjects who were at least 6 months post-transplantation at baseline (i.e., mean time since transplant at baseline was 16 months), while in the same patients femoral bone density was unchanged compared with the basal values even at the end of the study. On the whole, these data are consistent with a long-term persistence of an increased fracture risk in liver-transplanted patients.

The pathogenesis of low bone mass after liver transplantation is multifactorial. The underlying hepatic disease before transplant, malnutrition, immobilization and hypogonadism are well-established risk factors for osteoporosis in these patients [10]. However, successful transplantation eliminates most of these conditions within 3–6 months. Immunosuppressive drugs certainly play a major role in this setting [10,11]. Although the alterations seen in bone metabolism after liver transplantation have been mainly attributed to the use of glucocorticoids [4–6,19], most authors failed to demonstrate this relationship. This is probably due to the fact that the majority of studies concentrated on an early phase after transplantation. At this stage, most of the concurrent risk factors for bone loss are still effective, obscuring the impact of corticosteroids on the skeleton. In the present study, we found that MP intake was an independent predictor of low bone mass, and its effects on bone were exerted in a dose-dependent fashion. Accordingly, patients who did not continue to lose bone after the first year of follow-up were taking an average dose of less than 10 mg/day of MP, which is associated with a lower risk of osteoporosis [11]. Furthermore, bone resorption markers decreased after the second year, when corticosteroid intake had been consistently tapered. Also, the tendency to a rise in bone alkaline phosphatase after the second year might have reflected an increase in bone formation due to the lower doses of MP used in this period.

The effects of CsA on bone metabolism are still controversial. In vitro, CsA induces a dose-dependent decrease in osteoclast number [20,21], while in animal models it causes severe bone loss associated with a rise in both bone formation and resorption [10,22]. The few data available suggest an involvement of CsA in the pathogenesis of osteoporosis after liver transplant [19], and our study supports the hypothesis that CsA enhances both bone formation and resorption and might contribute to bone loss in these patients. These results are not surprising. Elevated values of osteocalcin have been observed after liver transplant [16,19], and a link between high bone remodeling, bone loss and CsA has been described even in cardiac-transplant recipients [23,24]. Although further studies are required to better elucidate the effects of CsA on human bone, the potential for a bone-damaging effect due to cyclosporin should be taken into account.

It is well known that cholestasis per se constitutes a risk factor for osteoporosis [25]. In patients with PBC and PSC, bone mass is lower and fracture rate is higher than in patients with other chronic liver diseases, both before and after transplant [2,25]. We also found that subjects transplanted for PBC and PSC had a lower bone mass with respect to all the other patients and that patients with pre-transplant cholestatic diseases had a better outcome in bone density after transplantation. This phenomenon is probably related to the normalization of

hepatic function and the resolution of cholestasis after surgery [2,5]. Indeed, we found that such patients had satisfactory liver function up to the second year of follow-up.

In contrast, it is more difficult to explain the negative pattern in the course of bone density in patients transplanted for PNC related to viral infection. Patients with previous PNC were older than patients with a cholestatic disease before transplant and we found that age was negatively correlated with bone density. The higher daily intake of MP in patients with chronic active hepatitis may have further contributed to our findings, while the shorter duration of the transplant did not seem to be an important factor. Liver function also seemed to be different between these two groups. Due to the high recurrence rate of viral hepatitis after liver transplantation, patients with chronic active hepatitis as their pre-transplant liver disease continued to show high levels of both hepatocytolysis and cholestatis indices after transplantation and we found that these were independent predictors of both bone turnover and density. Thus, it is likely that altered liver function may have contributed to bone loss in these patients.

In conclusion, liver-transplanted patients show an important decrease in bone mass, at both the axial and appendicular skeleton, associated with a rise in bone turnover. This reduction in bone density persists even several years after transplantation, indicating the need for prolonged follow-up in these patients. Corticosteroids, cyclosporin A and the underlying disease are the most important contributing factors. Specific strategies aimed at effectively counteracting bone disease in liver-transplant recipients, both in the short term and in the long term, are needed.

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