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Original Article

Long-Term Follow-up of Bone Mass after Orthotopic Liver Transplantation: Effect of Steroid Withdrawal from the Immunosuppressive Regimen

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Abstract. Glucocorticoids have been suggested to play a major role in transplantation-related osteopenia. In this study we assess the long-term changes and the effect of steroid withdrawal from the standard immunosuppressive regimen on bone mineral density (BMD) after orthotopic liver transplantation (OLT). Sixty-nine non-osteoporotic patients (20 women, 49 men), aged 48 ± 9.5 years (mean ± SD), and with a follow-up of 58.3 ± 23.2 months (range 24–121 months) were studied. Immunosuppressive treatment consisted of prednisone, cyclosporin A and azathioprine. In 41 patients (group A), prednisone was tapered and withdrawn after 36.2 ± 19.3 months (range 13–79 months), whereas in 28 patients (group B) prednisone was maintained. BMD in the spine (L1–L4) was serially measured by dual-energy X-ray absorptiometry (Hologic QDR 1000w) at baseline, before steroid withdrawal and at the end of study. Age- and sex-matched *Z*-scores of BMD were calculated. No differences were found in age, body mass index, time since OLT, or baseline BMD between the two groups. BMD had significantly increased in both groups at the end of follow-up period (group A, +8.1 ± 8.7%; group B, +3.2 ± 8.0%, $p < 0.05$). However, the *Z*-score was significantly higher in group A than in group B at the end of study (–0.44 ± 1.05 vs –0.99 ± 0.77; $p < 0.05$). BMD recovery was lower in pre-OLT biliary cirrhosis patients. Bone mass improvement was independent of the time since OLT in both groups, and of the time of steroid withdrawal in group A. Our data confirm that steroid withdrawal accelerates the recovery of bone mass in patients who have undergone a successful liver transplantation.

Keywords: Bone mineral density; Liver transplantation; Steroids

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Introduction

Bone loss is a clinically significant problem after orthotopic liver transplantation (OLT), and frequently leads to fractures [1]. Reported rate of vertebral fractures is 14% in the first year after OLT and may increase to 33% in the fourth year after transplantation [2]. Although cyclosporin A may induce bone loss in humans, steroids are currently considered the main cause of fast bone loss observed after solid organ transplantation [3]. Contributing factors may include immobility, hypogonadism and the etiology of underlying liver disease which may promote pre-OLT severe bone loss [4]. Several short-term studies have shown that this bone loss mainly occurs in the first 3–6 months after OLT [5,6], further bone loss being controversial. Therefore, the aim of our study was to analyze the effect of steroid withdrawal on BMD in stable liver transplantation patients, and to compare the long-term evolution of BMD in OLT patients with and without steroids.

Patients and Methods

One-hundred and four patients (72 men and 32 women) who underwent OLT between 1987 and 1994 and are currently followed at our Surgical Department, were initially evaluated. After OLT, they were treated with a sequential three-drug protocol (prednisone, cyclosporin A and azathioprine). Lumbar spine bone mineral density (LS BMD) was measured by dual-energy X-ray absorptiometry 15.2 ± 17.6 months (range 1–60 months) after transplantation. Thirty-five patients with low bone mass (LS BMD *Z*-score <–2 SD) were assigned to antiresorptive treatment as previously reported [7]. Sixty-nine OLT patients without osteoporosis were evaluated in this study. The group included 20 women and 49 men aged 48 ± 9.5 years (mean ± SD), with a mean follow-up period of 58.3 ± 23.2 months (range 24–121 months). Indications for OLT included: alcoholic cirrhosis (29%), viral hepatitis (13%), primary biliary cirrhosis (6%), hepatocarcinoma (4%) and a miscellaneous group (48%). After OLT, all patients were given cyclosporin A 2 mg/Kg per day from 36 h post-transplantation, maintaining levels (measured by radio-immunoassay) between 100 and 300 ng/ml; prednisone 500 mg i.v. for induction, repeated at 6 h, slowly reduced to 0.3 mg/kg per day p.o.; and azathioprine 2 mg/kg per day i.v. and 1 mg/kg per day p.o. for 3 months.

To evaluate the effect of steroid withdrawal on bone mass in OLT patients, we started a prospective protocol in 1992. Inclusion criteria were: (1) time since OLT $\geqslant$ 1 year; (2) acute-rejection free period of at least 6 months; (3) stable liver function tests; (4) ability to tolerate a decrease in steroid dosage to a minimum of less than 5 mg/day, maintaining this dose for 3–4 months before final steroid withdrawal; (5) stable cyclosporin A blood levels; (6) not taking any other drug known to interfere with calcium metabolism; (7) optimum compliance with all other medication and visits. Forty-one patients fulfilled these criteria (group A). In these patients, corticosteroids were reduced and finally withdrawn after a 20–30 day period on 5 mg of prednisone on alternate days. The remaining 28 patients (group B) fulfilled all inclusion criteria except for number 4, and they remained on low doses (0.1–0.2 mg/kg per day) of prednisone during the whole follow-up period. All patients were informed of the protocol and gave their consent.

Liver function tests included serum bilirubin, aspartate and alanine aminotransferase (AST and ALT), alkaline phosphatase (ALP), gamma-glutamyltranspeptidase (GGT) and albumin, measured with an autoanalyzer system (DAX 72).

LS BMD (L1–L4) was measured by dual-energy X-ray absorptiometry (DXA) with a Hologic QDR 1000w machine (Hologic, Waltham, MA). The coefficient of variation (CV) of this method is 1.3%. Bone mass measurements were performed after OLT, before steroid withdrawal and at the end of follow-up. The patient's *Z*-score was calculated as $p–m/SD$ (p = measured patient value, m = mean value for sex- and age-matched controls, and SD = standard deviation of the mean value for sex- and age-matched controls).

The control group consisted of healthy subjects of both sexes with no evidence of metabolic bone disease and who were not taking any therapy that could interfere with calcium metabolism [8].

Statistical Analysis

Descriptive statistics [mean, standard deviations (SD) and range] were calculated. Differences between groups were analyzed using Student's *t*-test and chi-square test. Percentage change (Δ) in LS BMD was calculated in each patient. Multiple linear regression analysis (step-wise method) was performed using Δ LS BMD as dependent variable and age, body mass index (BMI), pretransplantation liver disease, liver function tests (AST, ALT, GGT, ALP, bilirubin and albumin), mean steroid daily dose, total follow-up time and steroid withdrawal as independent variables. SPSS 10.0 (SPSS, Chicago, IL) was used for all statistical analysis.

Results

Baseline characteristics are summarized in Table 1. There were no differences in mean age at transplantation or BMI between the two study groups. The percentage of women was higher in group A than in group B, but the difference was not statistically significant. Alcoholic cirrhosis was the most common pretransplant disease when both groups were considered together, but post-viral cirrhosis was more frequent in group B. There were no significant differences in pretransplantation liver function tests between groups.

Table 1. Baseline characteristics of OLT patients

No. of patients	Group A	Group B
No. of patients	41	28
Age at transplantation (years)	49.5 ± 8.7	45.8 ±10.5
Sex (female/male)	14/27	6/22
	34% / 66%	21% / 79%
BMI (kg/m^2)	23.6 ± 3.4	24.8 ± 3.2
Time since OLT to first DXA (months)	16 ± 18.5	14.1 ± 16.5
Pretransplant liver disease		
Alcoholic cirrhosis	13 (32%)	7 (25%)
Biliary cirrhosis	3 (7%)	1 (4%)
Viral cirrhosis	3 (7%)	6 (21%)
Hepatocarcinoma	1 (2%)	2 (7%)
Miscellaneous	21 (51%)	12 (43%)
Pretransplant liver function tests		
AST (IU/l)	293.4 ± 726.4	421.4 ± 916.9
ALT (IU/l)	358.3 ± 1014.8	318.5 ± 777.2
GGT (IU/l)	214.4 ± 271.1	133.3 ± 231.2
Alkaline phosphatase (IU/l)	412.6 ± 620.6	362.2 ± 564.1
Total bilirubin (mg/dl)	7.8 ± 10.3	5.9 ± 8.4
Serum albumin (g/l)	3.5 ± 0.6	3.3 ± 0.6

There was no significant differences betweem groups A and B.

The time from OLT to the first densitometric study was similar in both groups. There were no significant differences in baseline LS BMD or *Z*-scores between the two groups (Table 2). The second densitometric study was performed after 20.8 ± 8.8 months and showed no significant changes in BMD in either group. At this time, there were no significant between-group differences in prednisone dose (14.4 ± 9.4 vs 14.1 ± 8.9 mg/day) or LS BMD. After the second DXA study, prednisone was tapered and eventually withdrawn in group A as previously described (average time of steroid therapy 36.2 ± 19.3 months), whereas in group B it was maintained at low doses (0.1–0.2 mg/kg per day). A third DXA study, performed 25.5 ± 10.2 months after the second, showed a significant increase in LS BMD from baseline in both groups ($p < 0.001$ in group A, and $p < 0.05$ in group B). However, the *Z*-score at the end of the study was significantly higher in group A than in group B ($p < 0.05$) (Table 2, Fig. 1). Mean daily prednisone dose in group B was 9.6 ± 5.6 mg/day at this time. The percent change in LS BMD (Δ LS BMD) was also significantly higher in group A compared with group B (+8.1% vs +3.2%, $p < 0.05$). Similar results were observed when each etiology was considered separately except in the case of biliary cirrhosis (Δ LS BMD +2.4% in group A, –13.3% in group B). Total follow-up time was not significantly different in the groups (60.9 ± 23.2 months in group A vs 53.7 ± 22.9 months in group B).

In multiple linear regression analysis, biliary cirrhosis and steroid withdrawal were predictive factors of Δ LS BMD ($\beta = -0.347$, $p < 0.05$ and $\beta = 0.386$, $p < 0.01$, respectively: $R^2 = 0.238$). No independent effect of age, BMI, prednisone daily dose or baseline liver function tests was found in multiple linear regression analysis. Although bone loss improves over time after OLT (+6.1 ± 8.7% in the whole group), no independent effect of total follow-up time on Δ LS BMD could be found.

Table 2. Mean BMD, percentage changes and Z-scores of lumbar spine in both groups;baseline (1), before steroid withdrawal (2) and at the end of follow-up (3).

	Group A	Group B	*p* value
LS BMD_1 (g/cm^2)	0.888 ± 0.130	0.891 ± 0.091	NS
LS BMD_2 (g/cm^2)	0.889 ± 0.133	0.905 ± 0.084	NS
LS BMD_3 (g/cm^2)	0.946 ± 0.137***	0.917 ± 0.078*	NS
$Z\text{-score}_1$	–1.1 ± 1.06	–1.19 ± 0.96	NS
$Z\text{-score}_2$	–0.84 ± 1.07**	–1.15 ± 0.87	NS
$Z\text{-score}_3$	–0.44 ± 1.05***	–0.99 ± 0.77	< 0.05
Δ_1 (%)	+0.2 ± 5.4	+1.9 ± 5.8	NS
Δ_2 (%)	+6.8 ± 8.5	+1.4 ± 5.2	< 0.01
Δ_{total} (%)	+8.1 ± 8.7	+3.2 ± 8.0	< 0.05

Δ_1, percentage change between the first and second DXA studies; Δ_2, percentage change between the second and third DXA studies; Δ_{total}, percentage change between the first and third DXA studies. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with baseline.

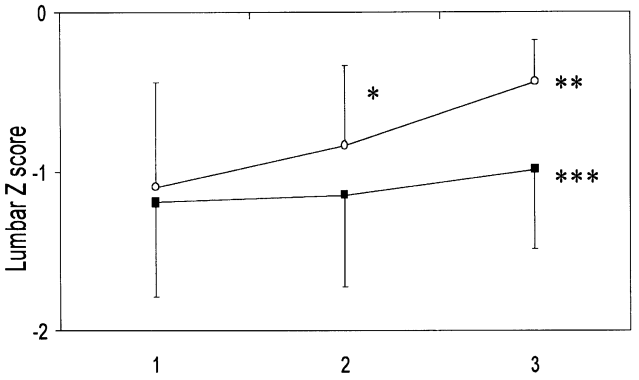

Fig. 1. *Z*-scores of lumbar spine BMD in OLT patients at Baseline (*1*), before steroid withdrawal (*2*) and at the end of follow-up (*3*). *Open circles* denote group A patients, and *filled squares* denote group B patients. Compared with baseline: *$p < 0.01$, **$p < 0.001$; between groups: ***$p < 0.05$.

Discussion

Steroids are the mainstay of the immunosuppressive therapy after solid organ transplantation. However, long-term steroid administration is associated with multiple side effects in transplanted patients, particularly bone loss [9]. In the present study we assessed the impact on BMD of steroid withdrawal from a classical immunosuppressive regimen in OLT patients. We also explored the long-term effect of OLT itself on BMD over a long period of time after transplantation (approximately 4 years).

Patients with a significant bone loss (defined as a *Z*-score below –2 SD) were excluded from this study since they were mandatorily allocated to antiresorptive treatment, according to our protocol [7]. This could explain the modest bone loss observed in our patients at the baseline assessment (approximately 10% or –1 SD) compared with similar studies [5,6].

Few studies have analyzed the long-term impact of liver transplantation on bone mass. Eastell and co-workers [5] reported that women undergoing OLT started to gain bone density by the end of the first year after transplantation, and that 2 years after OLT mean BMD was 5% higher than before transplantation. Likewise, similar studies have found that BMD begins to increase 6 months after liver transplantation, approaching baseline values by the end of the first year [10,11].

Our results suggest that LS BMD improves in the long term after OLT in non-osteoporotic patients. Both groups showed a significant increase in LS BMD at the end of the follow-up period. This positive response in bone mass appears to occur mainly in the second half of the follow-up period (years 3, 4 and 5). These results are consistent with those of Hamburg et al. [12], who recently reported a similar increase (annual increase +4.1%) in lumbar BMD in 45 OLT patients followed with DXA for 5 years. In this case, the highest increase in lumbar BMD was seen in the second postoperative year [12]. Similarly, Feller et al. [13] noted that bone recovery continued in the long term, and BMD returned

to pre-OLT level 85 months after liver transplantation. This late improvement is probably due to multiple factors, including a reduction in immunosuppressive dosage and a normally functioning graft. In addition, the underlying liver disease leading to OLT appears to have an influence on the evolution of BMD after the transplant. Cholestatic diseases may show better long-term results than liver recipients with previous post-necrotic hepatitis [14,15]. In contrast, other authors do not find different BMD outcomes in patients with previous cholestatic or non-cholestatic diseases [12]. In our study, biliary cirrhosis was associated with less bone recovery than other etiologies. However, the limited number of such cases do not allow more specific conclusions to be drawn.

We previously reported that steroid withdrawal is safe in stable OLT patients treated with cyclosporine. Resumption of steroid treatment was not required since acute or chronic rejection was not detected in any case. In addition, improvements in blood pressure and serum cholesterol levels were seen [16]. A significant increase in BMD may occur after discontinuation of exogenous glucocorticoid therapy or reversal of endogenous Cushing's syndrome [17,18]. However, there are no previous studies assessing the effect of steroid withdrawal on BMD in OLT patients. In this study, the increase in LS BMD was significantly higher in steroid-free patients than in patients who were maintained on low prednisone doses. In our opinion, this could reflect the fact that the improvement in BMD after liver transplantation should not be attributed merely to the beneficial effect of stable graft function, and confirms that steroid use has a pivotal role in transplantation-related osteoporosis. On the other hand, one should keep in mind that BMD recovery may be insufficient even after cessation of steroid therapy in patients with a very low basal bone mass or osteoporosis, and antiresorptive treatment should be considered.

Two shortcomings should be considered in this study. First, the possibility that patients with a lower baseline BMD or osteoporosis (Z-score < -2SD) could experience a stabilization or even a further decrease in BMD over time, cannot be ruled out. Second, a deterioration of femoral bone density may exist in spite of an improvement of BMD in the lumbar spine, as postulated by other authors [15]. Unfortunately, femoral BMD could not be measured in our patients.

In conclusion, lumbar bone mass shows a significant improvement in the long term after OLT in non-osteoporotic patients. Our data confirm that steroid withdrawal accelerates bone mass recovery in patients who have undergone a successful liver transplantation.

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