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ORIGINAL ARTICLE

Pamidronate in the prevention of bone loss after liver transplantation: a randomized controlled trial

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Summary

Rapid bone loss and high rates of fractures occur following liver transplantation. To analyze the effect of intravenous pamidronate on bone loss after liver transplantation. A randomized, double-blind, placebo-controlled study was performed. Seventy-nine patients were randomized to two groups of treatment: the pamidronate group ($n = 38$) was treated with 90 mg/IV of pamidronate within the first 2 weeks and at 3 months after transplantation; the placebo group ($n = 41$) received glucose infusions at the same time points. All patients received calcium and vitamin D. Bone mineral density (BMD) at the lumbar spine (L_2–L_4) and proximal femur using dual energy X-ray absorptiometry and also spinal X-rays were performed before, and at 6 and 12 months after liver transplantation. Biochemical and hormonal determinations were performed previous to transplantation, at 24 h before and after treatment, as well as at 6 and 12 months after liver transplantation. At 12 months after transplantation, there were significant differences in lumbar BMD changes (6 months: pamidronate 1.6% vs. placebo 0.8%, P = NS; 12 months: pamidronate 2.9% vs. placebo 1%, $P < 0.05$). Femoral neck BMD decreased in the pamidronate- and placebo groups during the first 6 months (6 months: pamidronate −3.1% vs. placebo −2.9%, P = NS; 12 months: pamidronate −3.2% vs. placebo −3.1%, P = NS). BMD at the trochanter remained stable in the pamidronate group, whilst a reduction was observed in the placebo group at 6 months (6 months: pamidronate −0.7% vs. placebo −3.7%, $P < 0.05$; 12 months: pamidronate −0.5% vs. placebo −1.2%, P = NS). Moreover, no significant differences in the incidence of fractures, serum parathyroid hormone and serum 25-hydroxyvitamin D values between both groups were found. Pamidronate did not increase the risk of serious adverse events. The results of this study show that 90 mg of intravenous pamidronate within the first 2 weeks and at 3 months following liver transplantation preserve lumbar bone mass during the first year, without significant adverse events. However, pamidronate does not reduce bone loss at the femoral neck and furthermore it does not reduce skeletal fractures.

Introduction

Fractures are an important cause of morbidity after organ transplantation [1]. Many risk factors contribute to their development, including the presence of previous chronic disease, immobilization, and long-term immunosuppressive therapy. Those circumstances may cause significant changes in bone remodeling and rapid bone loss during the first months after transplantation [2,3].

The liver, together with the heart and lungs, exhibits one of the highest rates of post-transplant bone disease [4], and about 17–65% of liver recipients suffer fractures during the first year after transplantation [5–12]. Several studies point to pre-existing bone disease and high dose immunosuppressive therapy, especially glucocorticoids, as the most relevant factors in the development of metabolic bone disease in liver graft recipients [7,8,12,13]. In the last few years, new immunosuppressive regimens have been tested in order to prolong graft survival and to minimize their side-effects. However, the effects of these new agents in the development of metabolic bone disease after liver transplantation are controversial.

In the last few years, therapeutic trials with active metabolites of vitamin D as well as with bisphosphonates have been developed in order to prevent bone loss and fractures. However, most of them have methodologic limitations or a low number of patient-participants.

Bisphosphonates are indeed the most promising agents in these patients. Several studies suggest that bisphosphonates increase bone mineral density (BMD) and reduce bone turnover and fracture risk in glucocorticoid-induced osteoporosis [14]. Thus, recent studies with potent bisphosphonates such as zoledronic acid and alendronate suggest that both drugs are able to prevent bone loss during the first year after liver transplantation [15–18]. Moreover, the use of zoledronic acid could also reduce fracture risk after liver transplantation [16]. In addition, four clinical trials have analyzed the effect of disodium pamidronate in liver transplant recipients, showing contradictory results in reducing the risk of fractures and bone loss [19–22].

The aims of this study were to prospectively evaluate the efficacy of two intravenous infusions of pamidronate 90 mg, associated with calcium and calcidiol, in the early post-transplant period, on bone loss in liver transplant recipients, and to assess the safety of this treatment.

Materials and methods

Patient population

A total of 61 men and 18 women, 20–67 years of age, diagnosed with chronic advanced liver disease awaiting orthotopic liver transplantation, were eligible to take part in this study.

Patients excluded from the study were those younger than 18 years, those receiving a multiorgan transplant or retransplant, or patients with a previous allergy to bisphosphonates. Previous treatment with fluoride, estrogens, selective estrogen receptor modulators or bisphosphonates was another reason for exclusion, as well as therapy with glucocorticoids during the last 6 months before transplantation. None of the patients had a previous history of disorders, other than liver disease, known to affect bone metabolism.

Study design and conduct

The study was a 1-year prospective, randomized, double-blind, placebo-controlled trial conducted at 10 centers in Spain. The study was approved by the local Clinical Ethics Committee in each participating center and all patients provided written informed consent. The study was in accordance to the Declaration of Helsinki guidelines concerning to medical research in humans and ICH guides to Good Clinical Practice.

Immunosuppression

All patients received microemulsion cyclosporine A as primary immunosuppressive agent, in combination with glucocorticoids. Additionally, mycophenolate mofetil was added according to the usual clinical practice of each center. In all cases, glucocorticoids were progressively tapered during the first year, with mean prednisone doses of 20, 15, 10, and 5 mg/day at one, 3, 6, and 12 months, respectively.

Intervention

Patients received oral calcium (500 mg twice daily) and oral 25-hydroxy vitamin D (16 000 UI every 15 days), after informed consent for the study was obtained and exploratory screening was done. Within days 7–12 after engraftment, the recruited transplanted patients ($n = 79$) were randomized to either the experimental ($n = 38$) or placebo group ($n = 41$). Patients of the treatment group received a single dose of 90 mg disodium pamidronate within days 7–12 and at 3 months after liver transplantation, diluted in 500 ml of 5% glucose serum and administered as a 4-h continuous intravenous infusion. Patients of the placebo group received 500 ml of 5% glucoside serum infusions. Treatment with oral calcium and vitamin D was maintained for 1 year after transplantation.

Study endpoints

The primary endpoints of the study were changes in BMD, quantified by dual energy X-ray absorptiometry

(DXA) and safety of pamidronate by recording adverse events. Secondary endpoints included the incidence of skeletal fractures, by assessment of radiologic vertebral fractures (symptomatic and asymptomatic), and the development of nonvertebral fractures.

Evaluation

Five study visits were scheduled: before transplantation, within day 7–12 after liver transplantation and at 3, 6, and 12 months after liver transplantation. The development of all parameters in each treatment group throughout the study was analyzed considering the variations registered in each patient and visit, in relation to baseline values, and also before and after pamidronate- or placebo infusions.

Clinical assessment included evaluation of type, severity (Child-Pugh score) and time since the diagnosis of the liver disease, alcohol consumption, concomitant treatments and previous fractures. During the first 24–48 h postinfusion, patients were clinically examined to assess adverse events. In addition, days of immobilization during the year of follow-up were recorded.

Laboratory data included serum calcium and phosphorus, as well as renal and liver function tests, before transplantation and prior to and after both the infusions of disodium pamidronate or placebo, and at 6 and 12 months after liver transplantation. Serum 25-hydroxy-vitamin D (25 OH-D) and parathyroid hormone (PTH) levels were determined before and at 3, 6, and 12 months after transplantation.

The BMD at the lumbar spine (L_2–L_4) and at the proximal femur were measured by DXA before and at 6 and 12 months, using Hologic QDR 2000, 4500, (Hologic, Waltham, MA, USA); Lunar DPX, MD4585, L, -Prodigy (Madison, WI, USA) or Norland XR26 (Norland Corp., Fort Atkinson, WI, USA) instruments. To ensure the highest quality, all centers acquired scans using the same method every time. Before the study, five phantom scans were performed in each center, with the same standard spine phantom being the mean coefficient of variation 0.6% (range 0.05–1.9%). Osteoporosis was considered as a value for lumbar and/or femoral BMD *T*-score of −2.5 or less and osteopenia for a *T*-score between −1 and −2.5. Mean interval between basal BMD testing and transplantation was 109 days. Standard X-rays of the thoracic and lumbar spine were obtained to disclose vertebral fractures, before and at 6 and 12 months after transplant. The evaluation was performed by semi-quantitative approach by an independent observer.

A new vertebral fracture was defined as a reduction of 20% or more in the anterior, middle or posterior height of the vertebral body, as compared with previous X-rays. Only fractures attributable to major trauma (defined as a fracture caused by a fall farther than from standing height, from a car accident or other severe trauma) were not considered in the study.

Statistical analysis

The analysis was based on the intention-to-treat population. Descriptive statistics are provided for all the efficacy variables analyzed. Categorical variables are expressed as percentages and continuous variables as measures of central tendency and dispersion. Basal variables not included in the primary or secondary variables, were subjected to descriptive analysis with list generation, and tested for differences between treatment groups. Continuous variables were subjected to Student's *t*-test or Mann–Whitney test for respectively normal and non-normal data, whereas Chi-squared or Fisher's test was applied to every set of categorical variables.

The number of fractures in the experimental and placebo groups was compared using the chi-squared test. The development of BMD and biochemical parameters of bone metabolism was evaluated through variance analyses (ANOVA) for repeated determinations to detect within group variation. Dropouts were not included in the analysis and missing values were not substituted. These variables were also subjected to *t*-test or Mann–Whitney test to detect possible differences between treatment groups at each visit of the study.

Statistical analyses were conducted using SAS®, version 8.2 (SAS Institute Inc., Cary, NC, USA), and a significance level of 5% ($P = 0.05$) was considered.

Results

Study population

A total of 79 patients awaiting liver transplantation between the ages of 20 and 67 years were included and randomized. The placebo and treatment groups included 41 (52%) and 38 patients (48%), respectively. Sixty-six patients completed the study (83.5%) and 13 patients discontinued prematurely. Discontinuations were attributable to adverse events in seven patients: one patient with lymphoma and one patient with bone metastasis of hepatic carcinoma, as well as five deaths (three patents with sepsis, one with a relapse of a hepatic carcinoma and one patient with metastasis of hemangioendothelioma). Protocol violation and loss of follow-up were recorded as other causes (Fig. 1).

No significant differences were found in respect of basal characteristics between the placebo and pamidronate groups (Table 1). Seven patients in the treatment group

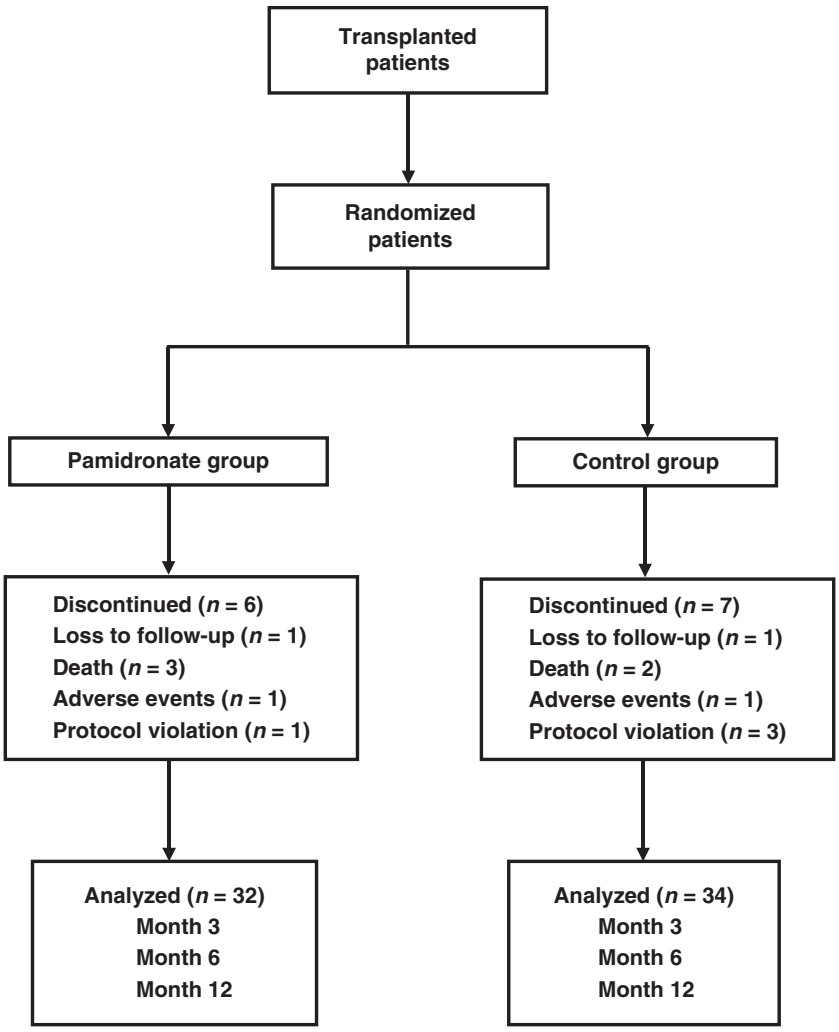

Figure 1 Study flow-chart.

(22%) and 10 in the placebo group (35%) displayed baseline skeletal fractures. Alcohol consumption was the first cause of liver disease in both groups (47–56%) and the most severe liver disease score (Child-Pugh C) was present in (46%) of patients. Finally, according to the T-score values, the prevalence of low bone mass was 86% in the treatment group (55% osteopenia, 31% osteoporosis) and 96% in the placebo group (59% osteopenia, 37% osteoporosis).

After transplant, there were no differences in cumulative doses of glucocorticoids and cyclosporine A between groups (3.385 + 1.902 vs. 4.043 + 2.257 mg of prednisone and 7.3185 + 4.6797 vs. 8.0298 + 5.3871 mg of cyclosporine A; in the pamidronate and the placebo group, respectively). During the study 50% of patients of the pamidronate group and 55% of patients in the placebo group were treated with furosemide. Only one patient in the pamidronate group received thiazides. No patient received hormone therapy.

Bone fractures and bone mineral density

At baseline, there were no differences in BMD at the lumbar spine, femoral neck and trochanter between the pamidronate- and placebo groups (Table 1). The BMD evaluation in both groups is depicted in Fig. 2.

At the end of the study, BMD at the lumbar spine had significantly increased only in the pamidronate group ($P = 0.0160$) as compared with basal values. Percent change from baseline at 12 months was 2.9% in the pamidronate group and 1% in the placebo group (Fig. 2a), and mean values at 12 months were 1.02 (95% CI: 0.95 to 1.09) and 0.93 (95% CI: 0.88 to 0.98) g/cm^2, respectively ($P = 0.0152$ between groups). Moreover, BMD at the trochanter remained stable in the patients who received pamidronate, whilst a significant reduction was observed in the placebo group at 6 months after liver transplantation ($P = 0.0021$). Percent changes were −0.7% and −3.7% in each group, respectively (Fig. 2c).

Table 1. Base-line characteristics of the patients who completed follow-up.

Variable		Pamidronate	Placebo	*P*†
N		32	34	
Age, years		52.8 ± 11.20	53.6 ± 10.0	0.867
Men		26 (81.3)	26 (76.5)	0.635
Postmenopausal women		4 (12.5)	7 (20.6)	0.378
Fractured patients		7/32 (21.9)	10/34 (35)	0.484
Etiology	Alcoholic cirrhosis	15 (46.9)	19 (55.9)	
	HBV and/or HCV	6 (18.8)	4 (11.8)	
	Alcoholic + HCV	2 (6.3)	1 (2.9)	
	Hepatocarcinoma ethanol	3 (9.4)	5 (14.7)	
	Cryptogenic cirrhosis	1 (3.1)	1 (2.9)	
	Primary biliary cirrhosis	2 (6.3)	3 (8.8)	
	Other	3 (9.4)	–	
Child-Pugh grade	Child A	4/24 (16.7)	5/28 (17.9)	
	Child B	9/24 (37.5)	10/28 (35.7)	
	Child C	11/24 (45.8)	13/28 (46.4)	
Lumbar spine (L_2–L_4)	BMD (g/cm^2)	0.99 ± 0.18	0.97 ± 0.15	0.315
	T-score	−1.30 ± 1.45	−1.45 ± 1.29	0.496
	Z-score	−0.71 ± 1.28	−0.89 ± 1.10	0.525
Femoral neck	BMD (g/cm^2)	0.87 ± 0.17	0.82 ± 0.12	0.363
	T-score	−1.01 ± 1.42	−1.37 ± 1.10	0.475
	Z-score	−0.06 ± 1.28	−0.46 ± 1.06	0.242
Trochanter	BMD (g/cm^2)	0.75 ± 0.14	0.73 ± 0.14	0.279
	T-score	−0.76 ± 1.27	−0.88 ± 1.43	0.870
	Z-score	−0.31 ± 1.14	−0.49 ± 1.35	0.617

Values in parentheses are percentages.
HBV, hepatitis B virus; HCV, hepatitis C virus; BMD, bone mineral density.
Results are shown as frequencies in relation to the number of evaluated patients or as mean ± SD depending on the nature of the variable.
†Chi-squared test for categorical variables, and Student's *t*-test or Mann–Whitney *U*-test for continuous variables (normal and non-normal data, respectively).

After 6 months, BMD values increased in patients in the placebo group and by the end of the trial had approached those of the pamidronate group.

Femoral neck BMD significantly decreased by 3.1% and 2.9% during the first 6 months, in the pamidronate- and the placebo groups, respectively ($P = 0.0099$, in the placebo patients and $P = 0.0108$ in treated patients) and variations from baseline persisted thereafter ($P = 0.0041$ and $P = 0.0037$ at 12 months, respectively), without significant differences between both groups (Fig. 2b).

Seven patients in the pamidronate group and three patients in the placebo group developed skeletal fractures in the first year after liver transplantation. In the pamidronate group, five patients developed vertebral, one patient peripheral and one patient both vertebral and peripheral fractures. In the placebo group, two and one patient developed vertebral and peripheral fractures, respectively. Differences in the incidence of fractures were not statistically significant.

No differences were found in the number and duration of immobilization periods between the study groups.

Biochemical and hormonal determinations

Analytical data at baseline and at 6 and 12 months after liver transplantation are listed in Table 2.

After pamidronate infusions, serum calcium levels of patients in the treated group decreased from 8.39 (SD 0.54) to 8.32 (SD 0.54) mg/dl at 24–48 h after the first infusion and from 9.35 (SD 0.43) to 9.04 (SD 0.49) mg/dl after the second one (NS). Postinfusion serum calcium levels were lower in this group than in placebo group, whose average values were 8.53 (SD 0.58) and 9.21 (SD 0.54) mg/dl after the first and second infusion, respectively. Prior to the second infusion, at 3 months, patients in the pamidronate group had significantly lower serum calcium values [9.35 (SD 0.43) vs. 9.62 (SD 0.50) mg/dl] than the placebo group, ($P = 0.0222$). Four patients in the pamidronate group and two patients in the placebo group had asymptomatic hypocalcemia (serum calcium < 8.5 mg/dl), after the second infusion. After correction by albumin, only two patients in the pamidronate group and one patient in the placebo group showed calcium levels under

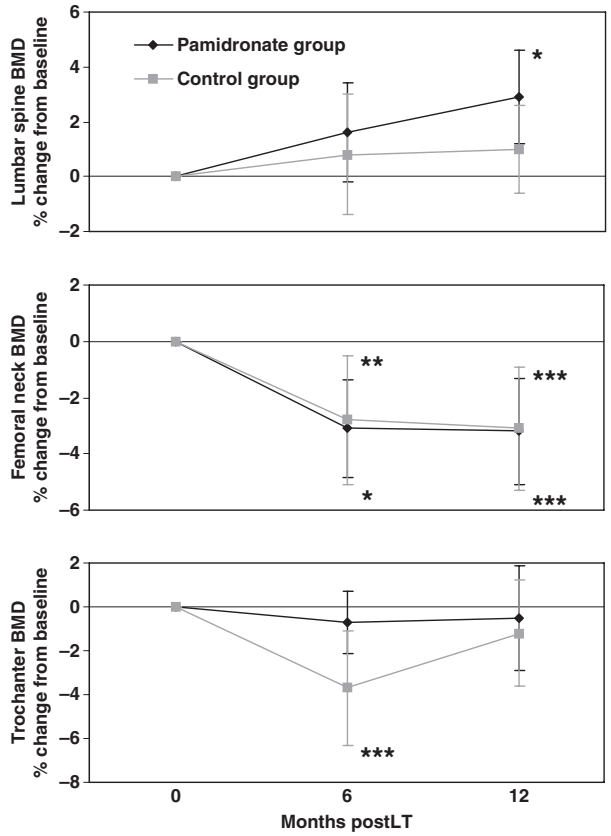

Figure 2 Development of bone mineral denisity (BMD) at lumbar spine (L_2–L_4), femoral neck and trochanter after liver transplantation (mean % change ± 95% CI). *P < 0.05, **P < 0.01, ***P < 0.005 ANOVA repeated measures analysis for within group variation over time.

8.5 mg/dl (pamidronate group: 7.58 and 8.26 mg/dl; placebo group: 7.7 mg/dl).

Nonsignificant differences were found in serum PTH levels between the study groups. Before liver transplantation, 60 out of 61 patients had creatinine clearance values above 30 ml/min, without differences between groups. After transplantation, serum creatinine values experienced a significant increase in both groups. While the mean serum creatinine values were significantly higher in patients from the pamidronate group, significant differences were found in ANOVA for repeated measures analysis (Table 2). Lower total alkaline phosphatase values were observed among treated patients after the first pamidronate infusion; nevertheless, differences between groups were significant only at 3 months [163.6 (SD 101.14) vs. 238.56 (SD 212.23) U/l in the pamidronate- and placebo groups, respectively, $P = 0.044$].

Serum 25 OH-D levels were below 10 ng/ml in 44% of the patients in the pamidronate group and in 33% in the placebo group, before liver transplantation. At 6 months after liver transplantation, a 103% increase in 25 OH-D levels was observed in the pamidronate group and a 128% in the placebo group. No patient with 25 OH-D levels below 10 ng/ml was found at the end of the trial. Nevertheless, 7/24 patients (29.2%) in the pamidronate group and 3/27 patients (11%) in the placebo group still had serum 25 OH-D levels below 20 ng/ml at 1 year after liver transplantation.

Safety

A total of 166 adverse events (Table 3), 35 of which were considered serious events (20 in the pamidronate group and 15 in the placebo group), occurred in 17 patients (11 in the pamidronate- and six in the placebo group). Only in one treated patient with lumbar pain, an association with the drugs was suspected. Likewise, two subjects in the placebo group suffered adverse events presumably related with the treatment (both were renal failure and headache). Therefore, the incidence of serious adverse

Table 2. Laboratory data at baseline and at 6 and 12 months (mean ± SD).

	Pamidronate			Placebo		
Parameter	Baseline	6 months	12 months	Baseline	6 months	12 months
Creatinine (mg/dl)	0.97 ± 0.35	1.48 ± 0.38†,##	1.51 ± 0.44##	0.91 ± 0.25	1.32 ± 0.36†,##	1.40 ± 0.37##
Calcium (mg/dl)	9.12 ± 0.63	9.46 ± 0.64	9.71 ± 0.58	9.00 ± 0.55	9.57 ± 0.39##	9.55 ± 1.01*
Phosphorus (mg/dl)	3.46 ± 0.53	3.95 ± 0.69***	3.78 ± 0.57*	3.40 ± 0.58	4.06 ± 0.61##	3.82 ± 0.45***
Alkaline phosphatase (U/l)	317.70 ± 256.43	142.15 ± 112.28#	182.26 ± 139.72***	295.43 ± 186.77	193.00 ± 216.72*	193.72 ± 147.26***
PTH (pg/ml)	30.41 ± 20.45	47.16 ± 32.09**	46.43 ± 27.02*	36.33 ± 34.74	56.54 ± 74.32	50.85 ± 31.71
25 OH-D (ng/ml)	21.82 ± 21.12	44.45 ± 28.13***	38.70 ± 28.39*	18.87 ± 18.54	43.06 ± 23.08##	37.39 ± 15.34##
sGOT (U/l)	51.81 ± 27.66	44.63 ± 47.27	48.19 ± 43.34	58.80 ± 48.59	37.74 ± 39.91	39.03 ± 31.19
sGPT (U/l)	44.95 ± 31.89	59.80 ± 64.17	66.97 ± 65.47	45.28 ± 37.20	48.65 ± 37.62	47.24 ± 59.89

PTH, parathyroid hormone; 25 OH-D, 25-hydroxyvitamin D; sGOT, serum glutamic-oxaloacetic transaminase; sGPT, serum glutamic-pyruvic transaminase.

†Difference between groups, P < 0.05, Mann–Whitney U-test.

Difference in time within group: *P < 0.05; **P < 0.01; ***P < 0.005; #P < 0.0005; ##P < 0.0001, ANOVA repeated measures analysis.

Table 3. Adverse events occurring in >10% of patients in any treated group.

	Pamidronate	Placebo	*P*
Patients with AE	21 (55)	20 (49)	NS
Numbers AE	70	96	NS
Back pain	5 (19)	0 (0)	
Infections	13 (34)	24 (59)	
Renal insufficiency	9 (24)	7 (17)	
Hypertension	2 (5)	6 (15)	
Biliary complications	12 (32)	8 (18)	
Postoperative complications*	6 (16)	7 (17)	
Neurologic complications	7 (18)	8 (20)	

Values in parentheses are percentages.
AE, adverse events.
*Excluding biliary events.

events related to the study medication was low and comparable across the groups.

Discussion

The present prospective, double-blind, randomized study suggests that the administration of pamidronate disodium, shortly after and at 3 months post-transplantation, reduces bone loss at the lumbar spine in liver transplant recipients. In this study, the use of pamidronate disodium was associated with slight and clinically nonsignificant decreases in serum calcium levels without other relevant adverse events.

In the last few years, new therapies have been tested for the treatment of postmenopausal osteoporosis, bisphosphonates being the most commonly used agents. However, one of the challenges in the treatment of osteoporosis is the evaluation of different bisphosphonates in specific subgroups of patients with secondary bone disease. In this context, the scarce number of randomized studies and the small size of the series of patients have limited the possibility to draw conclusions [15–22].

In our study, the patients who received pamidronate registered a significant increase in lumbar BMD, although patients in the placebo group did not show spinal bone loss. In addition, the average reduction of BMD at the trochanter was nonsignificant in the treated patients, while it significantly decreased by 4% within the first 6 months in the placebo group. As expected, despite the treatment benefits on bone mass, the low number of fractures in both groups precluded finding a protective effect of pamidronate with respect to the fracture incidence. The low fracture rate in the placebo group stands out when compared with previous data reported by our group and others [10,22]. The rationale for such a finding can only be surmised, but the use of immunosuppressive regimens with lower glucocorticoid doses in recent years seems to be the most important reason. Recently, three randomized studies showing positive effects of alendronate and zoledronic acid on BMD after liver transplantation have been published [15–17]. In the first one, patients received 70 mg of alendronate weekly or no alendronate plus 1000 mg of daily calcium and 0.5 μg of calcitriol. Patients receiving alendronate showed at 12 months increases in BMD of 4.7% at the lumbar spine, of 5.4% at the femoral neck and of 4.2% at the total hip when compared with the control group, without significant differences in vertebral or nonvertebral fractures [17]. In the second study, 60 patients were randomized to receive infusions of 4 mg of zoledronic acid or placebo within 7 days of transplantation and at 1, 3, 6, and 9 months after liver transplantation. The estimated differences in BMD changes from baseline between the zoledronic acid- and placebo groups at 12 months were 1%, 2.8%, and 2.3% for the lumbar spine, femoral neck and total hip, respectively. Even so, the trial was not powered to assess differences in skeletal fractures [15]. Recently, Bodingbauer *et al.* [16] reported that treatment with eight infusions of 4 mg of zoledronic acid during the first year after liver transplantation, reduced the risk of fracture (bone fractures in zoledronic acid: 8.5% vs. control group 22.5%: $P = 0.05$, log-rank test) and decrease bone turnover, with small impact on BMD changes. In summary, the data suggest that the more potent bisphosphonates are able to reduce post-transplantation bone loss. Nevertheless, contradictory results have been obtained with intravenous pamidronate. Thus, three nonrandomized studies, without comparison with placebo groups, showed an increase in bone mass and reduction of fractures in liver recipients with pamidronate treatment. [19–21]. The only randomized trial was carried out by Ninkovic *et al.* [22] who administered either 60 mg of intravenous pamidronate prior to liver transplantation or no treatment. This study showed no significant effect on fracture rate or BMD development between patients receiving pamidronate ($n = 45$) or no treatment ($n = 54$). In fact, no significant spinal bone loss was observed in both groups, and femoral neck BMD diminished in both treated and untreated patients [22]. In our study, patients treated with pamidronate showed a significant increase in lumbar BMD and a lower reduction of BMD at the trochanter than the placebo group, suggesting a mild positive effect of pamidronate on bone mass in liver transplant patients. Discrepancies observed between both studies could be attributed to differences in the immunosuppressive and pamidronate regimens. Thus, patients reported by Ninkovic *et al.* [22] received low doses of glucocorticoids and a single 60 mg dose of intravenous pamidronate before liver transplantation. Interestingly, in both the studies, femoral neck BMD decreased at 6 and

12 months despite pamidronate treatment, suggesting a poor effect of this drug on cortical bone. In this regard, differences in the spontaneous development of lumbar and femoral BMD, with a persisting bone loss at the femur during the first year after liver transplantation, has previously been described, and may be attributed to the special characteristics of cortical bone remodeling in these patients [10]. This may contribute to the poor effect on femoral bone loss of less potent bisphosphonates in terms of inhibition of bone resorption.

The observed satisfactory development of vitamin D levels in both cohorts of our study may be because of the concomitant treatment with vitamin D supplements and the improved graft function after transplantation. Thus, the underlying vitamin D deficiency shown by more than one third of the sample at baseline was corrected in most patients at the end of the study. Crosbie *et al.* [3] reported a correlation between serum 25 OH-D levels at 3 months and the increase of BMD between baseline and 6 months after liver transplantation, suggesting that normalization of vitamin D levels may have a positive effect on BMD. In that sense, it should be noted that despite an adequate administration of vitamin D, 19.6% of the patients were below the considered threshold for vitamin D insufficiency. This finding suggests that serum 25 OH-D levels should be measured in all patients after liver transplantation, regardless of supplementation.

Information about the effects of bisphosphonates in kidney function after liver transplantation is scarce. Recently, a case of a 48-year-old liver transplant patient who developed collapsing focal segmental glomerulosclerosis after the initiation of alendronate has been described [23]. In addition, glomerulopathies and acute tubular necrosis have been associated with bisphosphonate therapy, including pamidronate [24–27]. Our study disclosed any adverse event on kidney function and serum creatinine levels similarly increased after liver transplantation in both groups.

An interesting observation is that serum calcium levels gradually increased throughout the follow-up, despite transient decreases following pamidronate infusions. Nevertheless, asymptomatic hypocalcemia episodes were rare in this study, unlike the case in zoledronic acid studies, which showed that postinfusion hypocalcemia was more frequent in treated patients than in the placebo group [15,16].

In conclusion, two infusions of pamidronate disodium in the early post-transplant period, in combination with supplements of calcium and vitamin D, showed beneficial effects on BMD at the lumbar spine and trochanter. Nevertheless, there were no positive effects on bone loss at the femoral neck and, more importantly, in the risk of fractures. These effects in the absence of major adverse events, suggest that pamidronate could only be considered as a second line therapeutic option for osteoporosis in patients after liver transplantation.

Authorship

AM: designed the trial, performed the analysis, and wrote the article. NG: designed the trial, discussed the results, corrected and approved the article. MJS, FS, GC, MGG, MM, TS, FC, ST, and CB: collected the data, discussed the results, and approved the article. MN: designed the trial, discussed the results, corrected and approved the article.

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