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ORIGINAL ARTICLE

Effect of early risedronate treatment on bone mineral density and bone turnover markers after liver transplantation: a prospective single-center study

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Summary

The aim of this study was to investigate the effect of risedronate (RIS) on bone loss and bone turnover markers after liver transplantation (LT). Patients with osteopenia or osteoporosis within the first month after LT were randomized to receive RIS 35 mg/week plus calcium 1000 mg/day and vitamin D_3 800 IU/day ($n = 45$) or calcium and vitamin D_3 at same dosages ($n = 44$). Primary endpoint was change in bone mineral density (BMD) 6 and 12 months after LT. Secondary endpoints included changes in serum β-CrossLaps (β-CTX) and procollagen type 1 amino-terminal peptide (P1NP) and fracture rate. Spine X-rays were obtained at baseline and after 12 months. There was no significant difference in BMD changes between both treatment groups at any sites; either at 6 or 12 months. Spine BMD increased in both groups at 12 months vs. baseline ($P = 0.001$). RIS patients had a significant increase in intertrochanteric BMD at 12 months ($P < 0.05$ vs. baseline). Serum β-CTX decreased in both groups ($P < 0.01$), with significant differences between groups at 3 months. No significant difference in vertebral fracture incidence was found. After 12 months, BMD improved at lumbar spine and did not change at hip in both groups. Significant differences between both groups were not found. Other factors (calcium and vitamin D replacement, early prednisone withdrawal) seem to have also positive effects in BMD.

Introduction

Liver transplantation (LT) is a well-established procedure in the treatment of end-stage liver diseases. Improved outcome for these patients has allowed us to study some of the complications. One of these is metabolic bone disease, which can hinder their long-term survival and quality of life. There are a number of risk factors contributing to bone loss in these patients: hypogonadism, vitamin D deficiency, malabsorption, low body weight, low level of physical activity before LT and immunosuppressive therapy after LT, being this the main one reported [1–3].

Reports suggest that management of the pretransplant risk factors has improved, resulting in better bone mineral density (BMD) levels before LT [2].

After transplantation, rapid and marked bone loss is observed in the first 3–6 months [4–6]. The speed of the bone loss suggests that corticosteroids are heavily

involved. Greater bone loss at vertebral and hip sites and high rates of incident fragility fractures have been reported [4,5].

Prevention and management of post-transplantation bone loss includes appropriate life-style measures and adequate vitamin D and calcium replacement. To reduce bone loss and the risk of fracture after LT, therapeutic intervention is vital with modifications in the immunosuppressive regimen and glucocorticoids dose kept at a minimum. Several antiresorptive agents have been evaluated with small number of patients [7–13]. Most extensively studied drugs for post-transplantation bone loss are aminobisphosphonates (alendronate, pamidronate and zoledronate). To our knowledge, risedronate (RIS) has not been tested in liver transplant patients. RIS has been indicated for postmenopausal [14] and glucocorticoid osteoporosis treatment [15,16]. An early effect (fracture reduction within 6–12 months of starting therapy) and a sustained effect has been described for RIS in patients with postmenopausal osteoporosis [17,18].

The aim of this study was to evaluate the efficacy and safety of RIS treatment on early post-transplantation bone loss and bone turnover markers in LT recipients with low bone mass.

Patients and methods

This is a prospective randomized open-label 1-year trial performed in liver recipient patients at ''12 Octubre'' University Hospital, Madrid, between January 2006 and March 2008. In this period, 212 LT were performed.

Exclusion criteria were age <18 years; T-score > −1 SD at lumbar spine and hip; positive HIV serology; severe gastrointestinal disease (such as oesophageal diseases, gastritis and ulcers); creatinine clearance <30 ml/min; previous treatment with antiresorptive agents; other metabolic bone disease; and LT more than 15 days before recruitment in the study. Informed consent, previously approved by our Independent Ethics Committee (IEC) was obtained from each patient before inclusion in the study. The main reason for exclusion (62%) was normal BMD (T-score > −1 SD at lumbar spine and hip).

The study was designed to detect a difference of 3% (a standard deviation of 5%) in the percentage change between the two groups, from baseline to 12 months in BMD at the spine and femoral neck; a power of 80% and a two-tailed *P*-value of 0.05. Forty patients were needed per group. Estimating a loss of 10% patients during the study, 89 patients were included (Fig. 1). The patients were randomized to risedronate (RIS group, $n = 45$) or no risedronate (control group, CON, $n = 44$). RIS group received oral risedronate (Actonel; Sanofi-aventis, Paris, France) 35 mg once weekly, plus oral calcium (500 mg twice daily) and vitamin D_3 (800 IU daily), whereas CON group received only calcium and vitamin D_3 at same dosages. No placebo RIS was given according to our IEC regulations. Randomization was performed by a simple random sampling from Survey select of SAS enterprise guide. As a result of the small sample size, female patients were not stratified by menopausal status or BMD. Patients were asked about general compliance but medication was not counted in a systematic way.

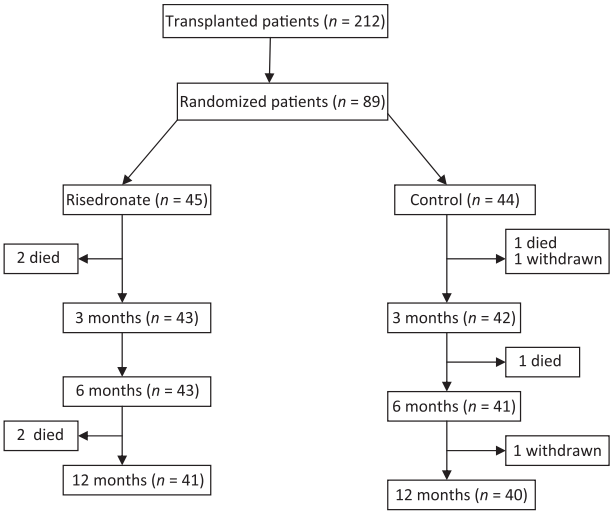

Figure 1 Study flow-chart.

All patients received immunosuppression after LT with tacrolimus and glucocorticoids. In selected patients, mycophenolate mofetil or cyclosporine was added as per protocol. Perioperative intravenous methylprednisolone (500 mg) was followed by oral prednisone doses of 20 mg/day. Glucocorticoids were progressively tapered, aiming to withdraw after 3 months. Tacrolimus was initially given at 0.15 mg/kg/day divided into two doses, thereafter adjusting for level (5–10 ng/ml).

Study endpoints

Primary endpoint was change in lumbar spine and femoral BMD (total hip, femoral neck, trochanteric and intertrochanteric). Secondary outcomes included changes in bone turnover markers, incidence of fractures (morphometric vertebral and clinical fractures) and adverse events.

Bone mineral density measurement

Bone mineral density was measured at 0, 6 and 12 months post-LT, at lumbar spine (L1–L4) and femoral sites, with a DXA densitometer (QDR 4500; Hologic, Waltham, MA, USA). Precision error was <1.5%. Osteoporosis and osteopenia were defined according to WHO

criteria. Osteopenia is defined as T-score between −1.0 and −2.5. Osteoporosis is defined as T-score −2.5 or lower. T-score is the number of standard deviations above or below the mean for a healthy 30-year-old adult of the same gender and ethnicity as the patient's. Z-score is the number of standard deviations above or below the mean for the patient's age, gender and ethnicity. Mean interval between basal DXA and transplantation was <15 days.

Fracture assessment

Standard X-rays of thoracic and lumbar spine were obtained at baseline and 12 months after transplantation. Evaluation was performed using the Genant semiquantitative approach [19].

Biochemical parameters

Biochemical parameters were determined at baseline, 3, 6 and 12 months after LT. Routine serum parameters were measured by an autoanalyser (Modular P; Roche Diagnostics, Basel, Switzerland). Serum samples were obtained between 08:00 and 09:00 hours after overnight fasting and were immediately processed and kept frozen at −70 °C until the assays were performed. Serum intact parathyroid hormone (PTH) was measured using chemiluminescent immunometric assay (DPC) with reference values of 7–57 pg/ml. Serum 25-hydroxyvitamin D_3 (25(OH)D) was assessed by enzyme immunoassay (IDS). Serum bone remodelling markers, procollagen type 1 amino-terminal propeptide (P1NP) and β-CrossLaps (β-CTX), were analysed using electrochemiluminescence technique (Roche Diagnostics) with reference values of 20–100 ng/ml and 0.200–0.704 ng/ml respectively. The coefficient of variation for each one of these assays was <10%.

Adverse events

At each visit, a single investigator (SG) assessed medications use, presence of side effects of the study drugs (such as nausea, dyspepsia, myalgia and arthralgia) and any other adverse events.

Statistical analysis

Categorical variables are described as percentage and continuous variables as mean and standard deviation. For between-group comparisons, unpaired Student's *t*-test, Mann–Whitney *U*-test or chi-square test was used accordingly. ANOVA followed by multiple comparison test was applied to test for differences in variables between groups. Correlation analysis between various parameters was performed by simple linear regression analysis.

Changes in parameters compared with baseline (e.g. BMD or biochemical parameters) were analysed using paired Student's *t*-test.

Changes in BMD, biochemical parameters of bone metabolism, PTH and 25(OH)D were evaluated through mixed effects ANOVA (group × time design with repeated determinations in time factor). Dropouts were not included in the analysis and missing values were not replaced. Adjustments for gender were made by double classification ANOVA to control gender factor. BMD changes between baseline, 6 and 12 months were presented as a percentage. Data were analysed under the intention-to-treat principle.

Statistical analyses were conducted using the SPSS program (version 11.0; SPSS, Chicago, IL, USA).

Results

Study population

Demographic details of the included patients are shown in Table 1. The higher percentage of postmenopausal women at baseline in RIS group explains the lower lumbar spine BMD and T-score, without significant differences in Z-scores (Table 2). Osteoporosis at either lumbar spine or femoral level was present at baseline in 39% of patients (lumbar spine and/or femoral T-score < −2.5). No differences were found between groups in the number of osteoporotic patients (44.4% RIS group, 34.1% CON group; $P = 0.317$) (Table 2).

Disease aetiology, years since diagnosis and Child-Pugh grade showed no relationship with baseline BMD.

Six patients died during follow-up because of liver transplantation-related complications (four in RIS group, two in CON group). Two CON group patients withdrew from study, one after bone anabolic therapy was started and the other because of recent diagnosis of CREST syndrome. Overall, 91% of patients finished the study, without differences between groups. Hospitalization periods were similar in both groups: 34.2 ± 36.7 days RIS group; 40.0 ± 31.6 days CON group ($P = 0.467$).

Immunosuppressive treatment

Glucocorticoids were withdrawn in 53% of RIS patients and 42% of CON group at the end of the third month. At 6 months 83% (RIS) and 80% (CON) of patients were put on a steroid-free treatment. Six months after LT mean cumulative dose of prednisone was 1394.39 ± 1508.38 mg in RIS group and 1638.50 ± 1622.99 mg in CON group ($P = 0.485$). Twelve months after LT mean cumulative dose of prednisone was 3931.2 ± 2129.4 mg in RIS group and 4584.0 ± 2638.6 mg in CON group ($P = 0.224$). Mean cumulative doses of tacrolimus and mycophenolate mofetil were also similar in both groups. There were no significant differences between groups in the number of acute rejection episodes requiring IV

Table 1. Baseline characteristics of patients randomized at inclusion.

	Risedronate (n = 45)	Control (n = 44)	P-value
Age (years)	57.9 ± 6.5	54.6 ± 8.8	0.052
Gender, males/females (%)	32 (71)/13 (29)	38 (86)/6 (14)	0.079
Postmenopausal women (%)	13/13 (100)	4/6 (67)	**0.028**
BMI (kg/m^2)	24.6 ± 4.5	25.2 ± 4.3	0.524
Aetiology (%)			
Alcoholic	15 (37)	11 (30)	0.142
HCV	14 (35)	12 (32)	
HBV	2 (5)	6 (16)	
Miscellaneous	9 (23)	8 (22)	
Duration of liver disease (years)	8.3 ± 8.8	11.9 ± 11.7	0.133
Prevalent vertebral fractures (%)	19 (44)	13 (30)	0.181
Creatinine (mg/dl)	1.14 ± 0.36	1.13 ± 0.42	0.889
GFR MDRD-4 (ml/min/1.73 m^2)	84.10 ± 41.51	77.69 ± 40.42	0.537

Data are represented as mean ± SD. Boldface indicates statistically significant values.
HBV, hepatitis B virus; HCV, hepatitis C virus; GFR MDRD-4, glomerular filtration rate (calculated with MDRD-4 formula).
Miscellaneous includes: viral-alcoholic, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune and cryptogenic liver disease.

Table 2. Baseline bone mineral density (BMD) T-score and Z-score for study population (mean ± SD).

	Risedronate (n = 45)	Control (n = 44)	P-value
BMD (g/cm^2)			
Lumbar spine	0.792 ± 0.104	0.844 ± 0.089	**0.012**
Femoral neck	0.691 ± 0.106	0.713 ± 0.135	0.389
Total hip	0.798 ± 0.130	0.842 ± 0.153	0.150
Trochanter	0.617 ± 0.111	0.641 ± 0.123	0.331
Intertrochanter	0.937 ± 0.168	0.987 ± 0.198	0.259
T-score			
Lumbar spine	−2.61 ± 0.87	−2.14 ± 0.75	**0.008**
Femoral neck	−1.68 ± 0.86	−1.58 ± 0.99	0.611
Total hip	−1.48 ± 0.92	−1.26 ± 1.03	0.269
Trochanter	−1.20 ± 0.93	−1.06 ± 1.00	0.507
Intertrochanter	−1.32 ± 0.96	−1.08 ± 1.06	0.610
Z-score			
Lumbar spine	−1.78 ± 0.91	−1.67 ± 0.84	0.577
Femoral neck	−0.67 ± 0.88	−0.71 ± 1.04	0.827
Total hip	−0.89 ± 0.93	−0.82 ± 1.03	0.719
Trochanter	−0.69 ± 0.93	−0.72 ± 1.02	0.879
Intertrochanter	−0.88 ± 0.97	−0.77 ± 1.05	0.610
Lumbar spine and/or femoral (%)			
Osteoporosis	20 (44.4)	15 (34.1)	0.317
Osteopenia	25 (55.6)	29 (65.9)	

BMD, bone mineral density. Boldface indicates statistically significant values.

corticosteroid bolus: RIS 10 (24%), CON 16 (40%) (P = 0.132). In the first year after LT, five patients in RIS group and one patient in CON group required oral doses of cyclosporin A (because of tacrolimus-induced neurotoxicity). No relationship was found between BMD change or incident vertebral fracture and doses of immunosuppressive drugs (steroids, tacrolimus or mycophenolate mofetil).

Bone mineral density

An increase in spine BMD was seen at 6 months compared with baseline values in RIS group (P = 0.014). Twelve-month lumbar spine BMD increased significantly in both groups (Fig. 2). Although 12-month BMD increase at lumbar spine was numerically higher in the RIS group, the difference was not statistically significant.

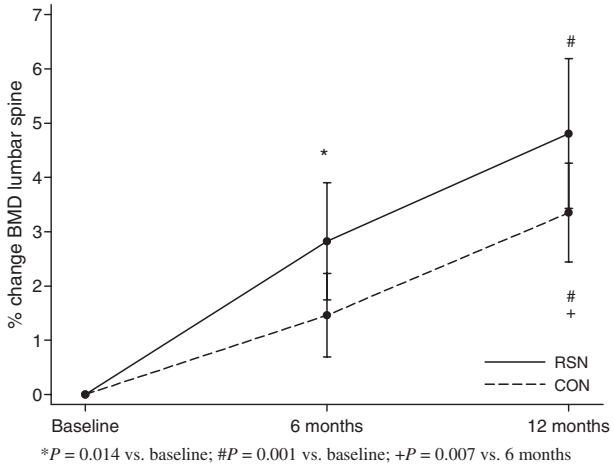

Figure 2 Lumbar spine bone mineral density (BMD) change (%) at 6 and 12 months from baseline. Data are represented as mean ± SEM.

There were no significant changes in femoral neck or total hip BMD in either group (Fig. 3a, b). Compared with baseline, trochanteric BMD significantly decreased by 2.9 ± 8.0% ($P < 0.05$ vs. baseline) at 6 months in control group (Fig. 3c), whereas at intertrochanteric site, patients receiving RIS significantly gained bone mass at 12 months (2.5 ± 7.3%, $P < 0.05$ vs. baseline) (Fig. 3d).

Differences in BMD changes between groups were [mean (95% confidence interval)]: Lumbar spine BMD 1.47% (−1.88%, 4.81%), femoral neck BMD 2.11% (−1.17%, 5.39%), total hip 2.03% (−1.42%, 5.47%) in favour of RSN. But there were no significant differences between groups in BMD at any site at either 6 or 12 months.

To control the possible effect of gender factor on the results, they were adjusted by double classification ANOVA (gender-treatment). Gender factor did not modify the treatment effect on BMD change.

Biochemical markers of bone turnover and laboratory analyses

Compared with baseline, albumin-corrected serum calcium levels in RIS group decreased significantly at 6 and 12 months (Table 3).

No significant differences between groups were found in serum calcium, phosphorus, PTH or vitamin D levels during follow-up. There was a trend to an increase in PTH in both groups after 12 months, without statistical significance between groups. However, 25(OH)D levels significantly increased at 3 months ($P < 0.0001$) and remained stable throughout the study (Table 3). At baseline, serum 25(OH)D was below 30 ng/ml in 91% of patients, while at the end of the study 52% of patients showed vitamin D sufficiency (>30 ng/ml). In the whole group, 25(OH)D values at 12 months positively correlated with the percentage change in BMD at femoral neck ($r = 0.259$, $P = 0.028$) and total hip ($r = 0.287$; $P = 0.015$).

Procollagen type 1 amino-terminal peptide (P1NP) levels significantly increased in control group after 6 months compared with baseline, whereas in RIS group remained stable throughout the study. At 6 months, P1NP levels

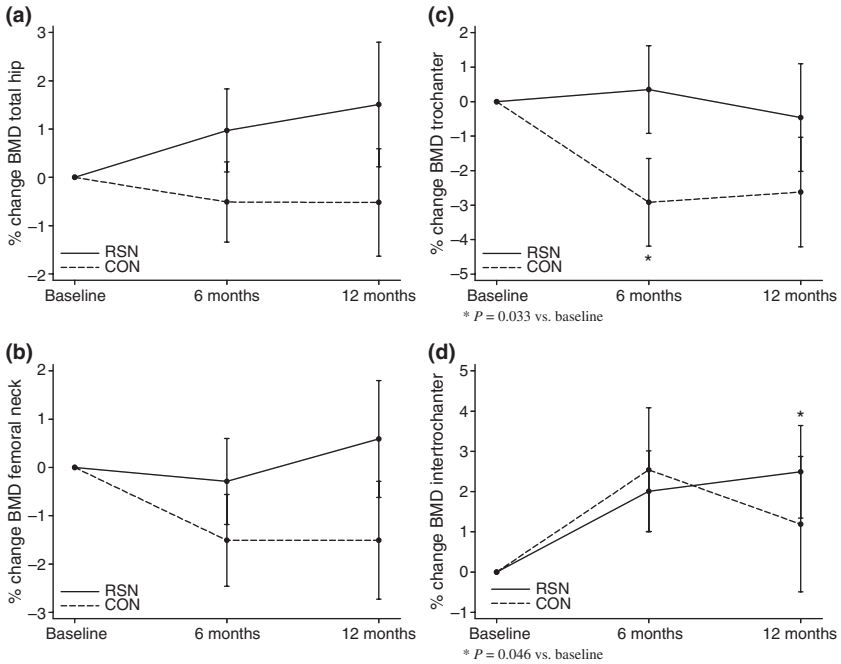

Figure 3 Bone mineral density (BMD) change (%) at 6 and 12 months from baseline at total hip (a), femoral neck (b), trochanter (c) and intertrochanter (d). Data are represented as mean ± SEM.

Table 3. Serum calcium, phosphorus, 25(OH) vitamin D and PTH at baseline, 3, 6 and 12 months.

	Risedronate (n = 45)	Control (n = 44)	P-value
Calcium (mg/dl)			
Baseline	9.40 ± 0.44*	9.44 ± 0.48	0.755
3 months	9.24 ± 0.50	9.33 ± 0.40	0.545
6 months	9.10 ± 0.45*	9.15 ± 0.43	0.823
12 months	9.09 ± 0.48*	9.19 ± 0.34	0.864
Phosphorus (mg/dl)			
Baseline	4.04 ± 0.77	3.87 ± 0.68	0.314
3 months	3.90 ± 0.79	3.80 ± 0.51	0.527
6 months	3.73 ± 0.69	3.66 ± 0.58	0.602
12 months	3.46 ± 0.65	3.41 ± 0.54	0.725
PTH (pg/ml)			
Baseline	52.69 ± 35.15	50.86 ± 29.20	0.804
3 months	48.15 ± 25.58	45.90 ± 29.38*	0.595
6 months	51.92 ± 30.25	52.66 ± 34.44	0.921
12 months	55.69 ± 31.46	55.36 ± 30.87*	0.966
25(OH) vitamin D (ng/ml)			
Baseline	18.82 ± 6.61	17.39 ± 7.46	0.371
3 months	33.60 ± 14.45**	32.60 ± 16.12**	0.773
6 months	30.42 ± 10.72**	34.92 ± 13.46**	0.104
12 months	32.59 ± 14.09**	30.27 ± 11.10**	0.462

*P < 0.05 intragroup, **P < 0.0001 intragroup.

were higher in CON group than in RIS group ($P < 0.05$) (Fig. 4a).

β-CrossLaps significantly decreased in both groups at 3, 6 and 12 months of treatment compared with baseline ($P < 0.01$). Differences between groups were only found at 3 months (0.34 ± 0.30 ng/ml in RIS vs. 0.48 ± 0.27 ng/ml in CON group; $P = 0.045$) (Fig. 4b). However, significant lower levels of β-CTX in RIS group were found during the study when only patients with good treatment adherence were taken into account (RSN $n = 30$, CON $n = 26$) (3 months: RIS 0.24 ± 0.17 vs. CON 0.52 ± 0.29 ng/ml, $P < 0.001$; 6 months RIS 0.27 ± 0.18 vs. CON 0.55 ± 0.42 ng/ml, $P = 0.003$; 12 months RIS 0.25 ± 0.18 vs. CON 0.46 ± 0.24 ng/ml, $P = 0.001$).

Fractures

Four patients (10%) in RIS group and eight patients (21%) in CON group developed vertebral fractures in the first year after LT, but no statistically significant differences were found between groups ($P = 0.178$). All vertebral fractures were diagnosed morphologically. Fracture analyses according to severity (Genant classification) also showed no inter-group differences. There were no peripheral fragility fractures.

Adverse events

Six patients died during the study (four RIS, two CON) because of major LT complications (acute rejection, liver failure or infection). Ten patients in RIS group (24%) and 16 in CON group (40%) had at least one episode of acute rejection (not statistically significant difference). Chronic graft dysfunction or infection was similar in both groups and treated in accordance with local protocols. Most frequent nonserious adverse events were nonspecific gastrointestinal symptoms (eight RIS patients, three CON patients) and skin erythema (two CON patients). One patient in CON group was diagnosed with CREST syndrome. There were no treatment-related patient dropouts or differences with regard to adverse events between the two groups.

Discussion

The main findings of our study are that LT patients with low BMD who receive either RIS combined with calcium and vitamin D3 or vitamin D3 and calcium alone showed a significant increase in spine BMD at 12 months compared with baseline values. In addition, RIS patients showed a significant increase in intertrochanteric BMD, but we were not able to find any significant differences between groups. Hence, these results suggest that weekly RIS after LT combined with 1000 mg/day of calcium and 800 IU/day of vitamin D are not superior to the administration of calcium and vitamin D alone.

Therapeutic interventions both to improve bone mass and reduce the risk of fracture after LT are recommended. As yet, the most effective treatment for this purpose has not been established. Given the fact that low

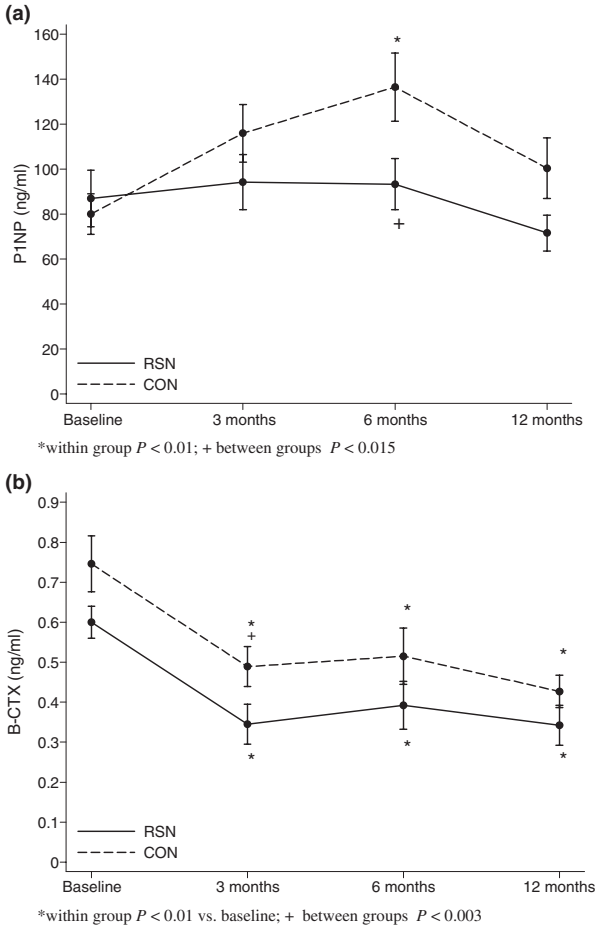

Figure 4 Changes in procollagen type 1 amino-terminal peptide (P1NP) (a) and β-CrossLaps (β-CTX) (b) values during follow-up. Data are represented as mean ± SEM.

bone mass and vertebral fractures have been reported before LT and they are predictors of post-transplant fragility fractures, early treatment should be initiated for their prevention.

To the best of our knowledge, this is the first study with RIS in LT patients. RIS has demonstrated in two randomized, double-bind, placebo-controlled studies (224 and 290 patients respectively) to improve long-term BMD in users of high-dose glucocorticoids [15,16]. Recently, in patients receiving glucocorticoids (>0.5 mg/kg/day prednisolone) for various medical diseases, RIS produced a significant gain in lumbar BMD at 6 months of therapy [20]. Moreover, RIS seems to have a different binding affinity profile and antiresorptive potency that could result in an early effect on fracture reduction [18]. In a 6-month analysis of an observational database with 7081 osteoporotic patients, RIS was more effective than calcitonin and alendronate in reducing non-vertebral fractures [17].

Previous studies on the efficacy of bisphosphonates in preventing bone loss after LT have provided conflicting results. Several studies have reported beneficial [7,8,11–13] effects whereas others found limited [10] or no effect [9]. In a trial conducted by our group in 40 LT recipients, Calcitonin was compared with cyclic etidronate. All patients received 1000 mg/day of elemental calcium. Both treatments increased lumbar BMD, although the increases were higher with bisphosphonate [7].

In the first randomized controlled study with alendronate in 98 liver transplant patients, weekly oral alendronate plus calcium and calcitriol increased lumbar and femoral neck BMD at 24 months when compared with the control group (who received calcium and calcitriol in the same doses). Despite the significant increase in bone mass, alendronate, given for 24 months, did not appear to offer protection against fractures [8].

On the other hand, Ninkovic *et al.* found in 99 patients that bone loss at femoral neck was not prevented in patients who received a single 60-mg dose of IV pamidronate before liver transplantation. No significant changes were observed in lumbar spine BMD. Neither calcium nor vitamin D was administered to these patients [9]. Monegal *et al.*, conducted a multicentre randomized placebo-controlled study with pamidronate 90 mg IV (two infusions in 1 year) in 79 LT receptors. All patients received also calcium and 25(OH)vitamin D. Early significant differences were shown in lumbar BMD changes at 6 and 12 months, while femoral neck BMD decreased at 6 and 12 months in both groups [10].

With regard to zoledronic acid, Crawford *et al.* found in 62 patients that intravenous infusions at 1, 3 and 9 months post-LT plus calcium and vitamin D3 decreased bone loss at lumbar spine, femoral neck and total hip, but the differences only remained significant at the total hip at 12 months. As in our study, average BMD at the lumbar spine recovered above pretransplant levels by 12 months in both the intervention and control groups. Side effects were not infrequent, with myalgia, arthralgia, pyrexia and hypocalcaemia in zoledronic treated group [11].

In a 96 patient study, Bodingbauer *et al.*, administered eight zoledronic acid infusions of 4 mg over the first 12 months postliver transplantation in addition to calcium and vitamin D3. Primary endpoint was the appearance of fractures 2 years after transplantation. 8.5% patients in zoledronic group and 22.5% in control group had fragility fractures ($P = 0.05$). Significant differences in bone mass between groups were only found at 6 months in femoral neck [12].

Ibandronate has been other bisphosphonate recently used in LT. Ibandronate (2 mg IV/3 months) plus calcium and vitamin D3 for 24 months post-LT allows

better recovery of BMD and significantly reduces post-transplant fractures in a 74-patient study [13].

A recent meta-analysis in 364 LT patients from six randomized controlled trials have found that bisphosphonate therapy improved lumbar spine BMD by 0.03 g/cm^2 (95% CI 0.01–0.05 g/cm^2, $P = 0.02$) at 12 months post-LT compared with the control group. However, a statistically significant change in femoral neck BMD could not be demonstrated in this meta-analysis. Data on fractures could not be analysed [21].

In our study, β-CTX decreased as from 3 months in both groups and persisted throughout follow-up, reflecting a reduction of bone resorption after transplantation. Good adherence to oral RSN treatment in LT patients ensures adequate inhibition of bone resorption. Greater reductions in β-CTX may be obtained with intravenous bisphosphonates. Shane *et al.*, showed a significant decrease in urinary deoxypyridinoline in heart transplant recipients after intravenous pamidronate treatment [22]. Misof *et al.*, described a significant decrease in β-CTX levels 6 months after LT in 13 patients treated with intravenous zoledronic acid [23].

Procollagen type 1 amino-terminal peptide levels significantly increased in CON group at 6 months, but did not change in RIS patients. Other groups have previously reported an increase in bone turnover markers such as osteocalcin after LT [7,24]. As in our study, that increase seems to be attenuated in patients who have been treated with bisphosphonates such as zoledronate [23] or pamidronate [25].

Inadequate levels of vitamin D have been described in patients with end-stage liver diseases prior to LT and this may play a role in the aetiology of lower mineralization after transplantation. At baseline, 91% of patients had insufficient serum levels of 25(OH)D and after adequate supplementation, both groups increased serum 25(OH)D levels from 3 months onwards. Crosbie *et al.* [26] found correlation between serum 25(OH)D levels at 3 months and BMD increase at 6 months, suggesting that this vitamin has a positive effect on mineralization. In our study, serum 25(OH)D levels showed positive correlation with the percentage change in total hip and femoral neck BMD at 12 months of treatment. These results and the fact that we could not demonstrate significant differences between RSN and CON group suggest that vitamin D could have a main role in bone loss prevention after LT.

Our study has several limitations. The most important one is that sample size was calculated to detect 3% points in the change from baseline to 12 months in BMD between groups. We found differences of 1.47% in lumbar BMD spine and 2.11% in femoral neck BMD between groups. With this information, we conclude that the true difference was probably <3% because there was an 80% power chance of achieving statistical significance (that is, ''detecting a difference'') if a 3% true difference had existed.

Another limitation is that although this is a randomized and controlled study, a placebo group does not exist. Significant improvement in BMD at the lumbar spine was also observed 12 months after LT in the control group. This response may be related to the administration of calcium and vitamin D3 itself, but also to improvement in general health, mobility, muscle mass and nutrition as a consequence of better liver function. Furthermore, we have previously reported that steroid withdrawal in LT accelerates the recovery of lumbar spine bone density in patients who have undergone a successful LT [27].

Moreover, the higher percentage of postmenopausal women in RIS group could have skewed the results. Different results might have been obtained if the treatment groups were balanced for postmenopausal patients. The small sample size did not allow us to stratify female patients by menopausal status. However, results adjustment by gender factor did not show modification of the treatment effect. Last, our study has not statistical power to assess effects on fractures.

Our results do not let us recommend systematic bisphosphonate therapy in all liver transplant recipients. However, we advocate its use in LT patients with osteoporosis or in those with long-term glucocorticoid treatment.

In summary, RIS therapy in the early post-LT period in combination with calcium and vitamin D appears to have a rapid beneficial effect on lumbar BMD at 6 months. However, at 12 months the increase was not significantly different to the obtained with calcium and vitamin D without bisphosphonate. No significant changes were found at femoral neck or total hip BMD in either group. The limitations of the trial include a relatively small sample of patients and inadequate power to assess fractures. Longer follow-up studies are necessary to establish the role of this bisphosphonate in preventing fractures post-LT.

Authorship

SG: was involved in patient data management, analysis and writing of the manuscript. GM and FH: conceptualized the original idea, protocol draft, analysis and writing of the manuscript. CV: was involved in laboratory analysis. DL: was the statistical advisor. MG and BC: provided patient data management and technical support. EM: was involved in liver transplantation and critical review.

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