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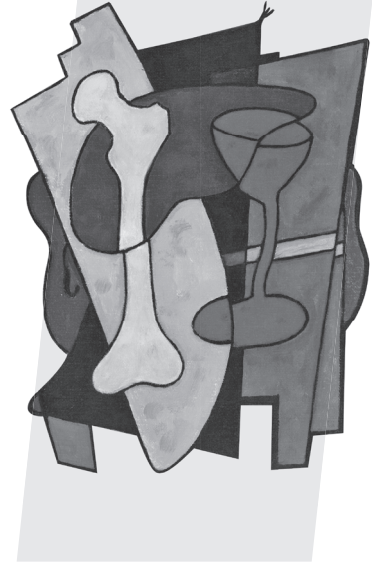


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Bisphosphonates in the prevention of bone loss after liver transplantation

a comprehensive review of the literature and a case series from the Leiden Liver Transplantation Cohort Study

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ABSTRACT

Introduction Bone loss and fractures are common after orthotopic liver transplantation (OLT). Data on the efficacy of bisphosphonates to decrease skeletal complications are not concordant and therapeutic strategies to decrease skeletal morbidity after OLT remain to be established.

Materials and methods A review was undertaken of published studies on the effect of bisphosphonates on bone loss and fracture risk in liver transplant recipients. Changes in bone mineral density (BMD) measurements routinely obtained before and 6 months and one and two years after OLT were analyzed in consecutive recipients of a successful OLT at the Leiden University Medical Centre between 2000 and 2011, who were treated with the bisphosphonate alendronate within the first year after OLT. Non bisphosphonate-treated transplant recipients were used as controls. Conventional spinal radiographs available at time of screening for OLT and at six and twelve months post-OLT were assessed for vertebral fractures using the Genant's semi-quantitative method.

Results Review of the published literature generated 16 studies, in all but 3 of which bisphosphonates were started around the time of OLT at various doses, frequency of administration and duration. Decreased fracture risk was the primary end point in only one study. A short-term beneficial effect of bisphosphonate treatment on BMD was observed in most studies. Data on long-term effect of treatment are conflicting.

Leiden case series: Data of 39 OLT recipients (mean age 49 yrs, 72% male) treated with oral alendronate within the first year after OLT (median 185 days, range 22-365) were compared to those of 104 controls (mean age 53 yrs, 68% male) transplanted within the same period and not receiving bisphosphonates. At baseline, BMD was lower in the bisphosphonate-treated group ($p < 0.005$) at one or both sites measured with osteoporosis observed in 41% and osteopenia in 56% of patients compared to 39 and 11% respectively in the control group. Radiological vertebral fractures were equally prevalent in both groups (61% of patients in the treatment group and 55% in the control group). LS BMD increased equally in treated and non-treated patients. FN BMD remained stable in the treatment group, but decreased in the control group. There was no significant difference in changes in BMD at either site between treated and untreated patients or between patients in whom treatment was initiated between 3 and 6 months or more than 6 months after OLT.

Conclusion Our data suggest that although preventing further bone loss, bisphosphonate treatment not initiated shortly after OLT does not significantly

influence post-transplantation changes in BMD compared to controls. Whether bisphosphonate use is associated with a significant decrease in fracture risk remains to be established by long-term randomized controlled studies using these agents.

INTRODUCTION

Vertebral fractures are prevalent in patients with end-stage liver disease awaiting orthotopic liver transplantation (OLT).⁽¹⁾ Accelerated bone loss is observed in the first six months after transplantation despite correction of the metabolic abnormalities associated with disturbed liver function, and fracture risk remains high despite post-OLT recovery of bone mineral density (BMD).⁽¹⁻¹²⁾ The initial rapid decline in BMD after OLT has been consistently shown to be followed by a recovery of bone mass to pre-transplant values one year after OLT.^(1;4-7;9;10;13) However, we and others have demonstrated that this recovery in BMD is not associated with a corresponding decrease in the incidence of new fractures which have been documented in 25-34% of transplant recipients at one year after OLT.^(1;8;10;12;14-17) A meta-analysis conducted in 2011 provides evidence that treatment with bisphosphonates generally prevents or decreases bone loss and decrease fracture risk in patients with solid organ transplantation.⁽¹⁸⁾ The rapid decline in bone mass observed within the first 6 months after OLT combined with the increased bone turnover demonstrated by the histomorphometric analysis of paired bone biopsies obtained at transplantation and 3-6 months after OLT as well as the prevention of this increased bone turnover by very early use of bisphosphonates provide the rationale for the use of bisphosphonates in OLT recipients to prevent or decrease the associated high fracture risk observed within the first year after OLT.^(19;20) However, data on the potential beneficial effect of bisphosphonates to decrease or prevent bone loss and fracture risk after OLT remain scarce with available data obtained from 16 studies with short follow-up duration or including a small number of patients, with a new fracture being the primary end-point in only one randomized controlled trial (RCT).^(19-34;34) A meta-analysis conducted in liver transplant recipients did demonstrate that bisphosphonates had a beneficial effect on bone loss but fracture risk could not be evaluated because of heterogeneity of available data.⁽³⁵⁾ Three studies have previously addressed the efficacy of the orally administered nitrogen-containing bisphosphonate alendronate to decrease or prevent bone loss and fracture risk within the first year after OLT.^(31;34;36)

The aims of our study were to review published data on the effect of bisphosphonate treatment on bone loss and fracture risk after OLT, and to evaluate the effect of bisphosphonate treatment (oral alendronate administered weekly) initiated within the first year after OLT on BMD in liver transplant recipients.

MATERIALS AND METHODS

A. Comprehensive review of the literature

Objective

To review available data on the effect of bisphosphonate treatment to decrease or prevent bone loss and fracture risk when administered to liver transplant recipients before, at or after OLT.

Design

Review of all RCT's and controlled or open observational studies published until May 2014 on the effect of a bisphosphonate of any type administered at any time around OLT, at any dose or schedule, and for any duration after OLT, on BMD and on fracture risk after OLT.

Data source

Pubmed, Embase, reference lists of published papers.

Method

The comprehensive search strategy included the following terms: liver transplantation, ibandronate, bisphosphonates, alendronate, etidronic acid, zoledronic acid, risedronic acid, alendronate, bone mineral density, fractures.

The search was conducted in May 2014 through a search of two databases (Pubmed and Embase) and screening reference lists of published papers. All recovered abstracts were screened. All search generated published papers were carefully read to extract details of treatment and its efficacy.

B. Case series from the Leiden Liver transplantation cohort study

Patients and methods

Patients

The electronic hospital records of all consecutive patients who underwent a first OLT at the Leiden University Medical Centre between January 1st 2000 and January 1st 2011 were screened for treatment with bisphosphonates initiated at any time within the first year after transplantation. Only patients with available BMD measurements before start of treatment and at one and two years after OLT were eligible for entry in the study. In our unit, treatment with bisphosphonates uniformly consisted of oral alendronate at a dose of 70 mg per week in addition to calcium- and vitamin D supplementation at a

combined fixed dose of 500 mg elemental calcium and 400 IU colecalciferol. There was no preplanned protocol for, or prerequisites or time for initiation of treatment with these agents after OLT, which was left at the discretion of the treating physician. The remaining consecutive patients who were transplanted within the same 10-year period and who also had BMD data available at one and two years after OLT, but who were never treated with bisphosphonates before or after OLT were used as controls. Patients in whom treatment with bisphosphonates was initiated before OLT were excluded from the study because of the lack of baseline data before or at start of treatment

Immunosuppressive regimens consisted of corticosteroids for at least 6 months in all patients, with a calcineurin inhibitor: tacrolimus or cyclosporine in the majority, and with mofetil mycophenolate (MMF) or sirolimus or everolimus as additional immunosuppressive agent in a few. International guidelines for immunosuppression were complied with in the case of newly transplanted patients, but established successful immunosuppressive regimen were seldom altered in individual transplant recipients. Basiliximab 20 mg was given in the anhepatic phase and on day 4. Corticosteroid schedules included methylprednisolone at a dose of 500 mg given during the OLT procedure, followed by oral prednisolone at a dose of 20 mg daily for one week, 10 mg daily for 3 months, slowly tapering to complete discontinuation of corticosteroids between 3 and 6 months after OLT in all but a few patients who required maintenance doses of prednisolone of 2.5 – 10 mg/day. Treatment of rejection episodes consisted of methylprednisolone at a single dose of 1000 mg or – in severe cases- of three doses of 1000 mg, followed by oral prednisolone at a dose of 140 mg tapered by 20 mg a day until a dose of 20 mg daily was reached. The number of rejection episodes were recorded in individual patients.

Methods

Demographic and clinical data

Demographic and clinical data were obtained from individual patients' electronic hospital records. Data on age, gender, smoking, height, weight, primary liver disease and date and cause of death were extracted from the records. Concomitant or later renal transplantation was documented. Data on immunosuppressive regimen, calcium and vitamin D supplements and date of initiation and discontinuation of alendronate were also recorded.

Laboratory investigations

Laboratory data were also obtained from electronic hospital records. Biochemical data collected included serum calcium, corrected for an albumin of 40 g/L, phosphate, creatinine, parathyroid hormone (PTH) and 25-hydroxy-vitamin D (25-OHD) concentrations, which were measured at screening for OLT and at various time points

thereafter in the two years after OLT. Bone turnover markers were not routinely measured in all patients so that because of the scarcity of available data these were not used in the analysis. Severity of liver disease was determined using the laboratory-Model for End-stage Liver Disease (MELD) scores (37) calculated on the basis of serum creatinine, bilirubin and INR measurements obtained at time of screening for OLT. The following accepted formula was used to calculate the scores:

$$MELD = 10 * (0.957 * \ln(Creatinine/88.4)) + (0.378 * \ln(Bilirubin/17.1)) + (1.12 * \ln(INR))) + 6.43$$

Bone mineral density measurements

BMD was measured at the lumbar spine and at the femoral neck using dual energy X-ray absorptiometry (DXA- Hologic QDR 4500, Hologic inc. Waltham, MA, USA, equipped with reference values based on the National Health and Nutrition Examination Survey (NHANES III)). Absolute measurements of BMD in g/cm² as well as T-scores (matched to a young adult reference population at peak bone mass) and Z-scores (age- and sex-matched reference population) were recorded. World Health Organization (WHO) criteria were used to define osteoporosis (T-score of -2.5 SD or less) and osteopenia (T-score between -1 SD and -2.5 SD). BMD measurements were performed at time of screening for OLT (baseline) and at 6, 12 and 24 months after OLT.

Vertebral fracture assessment

Conventional spinal radiographs were performed as part of the standard protocol for evaluating skeletal complications at screening before OLT and at six and twelve months after OLT.

All radiographs of the thoracic and lumbar spine were blindly assessed for the prevalence of vertebral fractures by two independent observers, using the Genant's semi-quantitative method.(38)

Laboratory, BMD and fracture data collected at time of screening for OLT were considered to be baseline data.

The study was approved by the Medical Ethics Committee of the Leiden University Medical Centre.

Statistical analysis

For descriptive statistics, categorical variables were expressed as numbers and as percentages. Continuous variables were summarized using mean and standard deviation in case of normal distribution of data. Median and 5th and 95th percentiles were used otherwise.

Patients in whom treatment was initiated in the first year after OLT were analyzed on an intention to treat basis. BMD changes were compared with those of patients not

receiving treatment with bisphosphonates, using an extension of a Generalized Linear Model, Generalized Estimation Equations (GEE) with correction for BMD, change in BMD compared to screening, fractures prevalent before OLT and incident fractures after OLT, age, gender and bilirubin levels (reflecting hepatobiliary function).

Prevalence of fractures was calculated by dividing the number of patients with at least one vertebral fracture by the total number of patients with available spinal radiographs. The incidence of fractures was defined as the occurrence of a new vertebral fracture or an increase in grade of a prevalent fracture between serial radiographs before and in the first year after OLT. A GEE model was used to evaluate the influence of change of BMD on fracture risk, corrected for age and sex and accounting for repeated measurements. P-values < 0.05 were considered significant.

Calculations were performed using STATA/SE 12.0 software (Stata Corp LP, TX, USA).

RESULTS

A. Comprehensive review of the literature

Review of the literature generated sixteen studies, including nine RCT's and seven observational studies, and a total of 729 patients treated with bisphosphonates orally or intravenously after OLT. Details of the studies elicited by the literature search are shown in Table 1.

1. Details of bisphosphonate treatment

Bisphosphonates used were largely nitrogen-containing bisphosphonates including oral alendronate and risedronate and intravenous zoledronate, ibandronate and pamidronate. Two early studies reported results with the use of the first generation bisphosphonate etidronate.(29;30) Mode of administration was intravenous in most studies (total number of patients treated 499)(19-23;25-30;32;33) and oral in 4 studies (total number of patients treated 235)(24;26;31;34). Time of initiation of treatment was before OLT in 3 studies (total number of patients treated 66 patients with exact number of patients treated not mentioned in one study)(28;29;31), at or shortly after OLT in 10 studies (total number of patients treated 488)(19-22;24-26;32-34;39), and 1-2 years after OLT in 3 studies (total number of patients treated 82)(23;27;30). Duration of treatment was one year in 7 studies (total number of patients treated 303 patients) (19;21;22;25;26;30;32;34) or 2-3 years in 5 studies (total number of patients treated 251) (23;24;27;31;34). Details of duration of other treatments are shown in Table 1. Follow-up duration was 9 months in one study (total number of patients treated 13)(28), one year in 9 studies (total number of patients treated 413)(19-22;24;26;29;30;32-34) and 2-3 years in 6 studies (total number of patients treated 280)(21;23;25;27;31;34).

Table 1: Available data on the effect of various bisphosphonates to prevent bone loss and fracture risk after liver transplantation

Source	Study type	No. of patients (treatment/controls)	Bisphosphonate used	Time start treatment	Duration of treatment (months)	Follow-up after OLT (months)	Effect on BMD	Effect on fracture risk	Effect on bone turnover	Bone markers	Histo-morphometry
Valero et al. 1995 (30)	RCT	40 (17/23)	Calcitonin or Etdronate	17 mo (range 1-74)	12	12	+	NA	NS	NS	NA
Riemsens et al. 1996 (29)	Open label	53 (53/0)	Etdronate	Pre-OLT (median 4 mo, r.20-0)	Until OLT	12	NS	NS	NS	NS	NA
Reeves et al. 1998 (28)	Open label	29 (13/16) ^a	Pamidronate	2-10 weeks before OLT	9 post-OLT	9	NA	+ ^{a,b}	NA	NA	NA
Vedi et al. 2002 (20)	RCT	99 (45/54)	Pamidronate	At OLT	Single dose	12	NS	NA	NA	NA	↓
Dodidou et al. 2003 (23)	Obs	128 (29/99) ^a	Pamidronate	Mean 2 yrs after OLT	24	24	+	+ [*]	↓	NA	NA
Millonig et al. 2005 (31)	Open label	136 (98/38)	Alendronate	Pre-OLT, at OLT, 4 mo after OLT	27	27	+	+	+	NA	NA
Atamaz et al. 2006(34;39)	Open label	98 (49/49) ^a	Alendronate	< 7 days after OLT	24	24	+	+	↓	NA	NA
Crawford et al. 2006 (22)	RCT	62 (32/30)	Zoledronate	< 7 days after OLT	12	12	+	NA	↓	NA	NA
Bodlingbauer et al. 2007 (19)	RCT	96 (47/49)	Zoledronate	At OLT	12	12	+	+	↓	↓	↓
Pennis et al. 2007 (32)	Open label	85 (43/42)	Pamidronate	At OLT	12	12	+	NA	↓	↓	NA
Monegal et al 2009 (33)	RCT	79 (41/38)	Pamidronate	< 14 days after OLT	3	12	+	NS	↓	↓	NA
Bodlingbauer et al. 2010 (21)	RCT	57 (28/29)	Zoledronate	At OLT	12	36	NS	NS	NS	NS	NA
Kaemmerer et al. 2010 (25)	RCT	114 (74/40)	Ibandronate	At OLT	12	24	+	+ ^b	NS	NS	NA
Guadalix et al. 2011 (24)	RCT	89 (45/44)	Risedronate	< 14 days after OLT	24	12	NS	NS	↓	↓	NA
Wagner et al. 2012 (27)	Open label	54 (30/24) ^a	Ibandronate	1-2 yrs after OLT	36	36	+	NS	↓	↓	NA
Shane et al. 2012 (26)	RCT	111 (84/27)	Alendronate Zoledronate	<30 days after OLT	12	12	+	+ [*]	NS	NS	NA

RCT = randomized controlled trial; obs = observational study; OLT = orthotopic liver transplantation; yrs= years; + = beneficial effect of bisphosphonate treatment on BMD or fracture incidence; NS = non-significant effect of bisphosphonate treatment; NA = not available; mo = months; ↓ decrease of bone turnover ↑ increase of bone turnover * p-value not mentioned; ^a historical controls used; ^b only clinical fractures documented.

BMD was sequentially evaluated in 15 studies (total number of patients treated 693) (19-27;29-34;39), and incidence of new fractures was reported in 13 studies (total number of patients treated 608)(19-21;23-29;31;33;34;39), in 2 studies on the basis of clinical fractures (25;28), and in 10 studies the basis of sequential X-rays.(19;21;23-27;29;31;33;34;39) Only one study had incidence of new fractures as a primary endpoint(19;21).

2. *Effect of bisphosphonate treatment on bone turnover*

Most studies have evaluated the effect of bisphosphonates on bone turnover using changes in bone turnover markers. The bone turnover marker used as bone formation marker was osteocalcin in most studies, bone alkaline phosphatase in some, and in later years procollagen type 1 N-terminal propeptide (P1NP). Bone resorption markers used were mostly urinary deoxypyridinoline and in later years serum beta crosslaps (CTX). A decrease in bone turnover markers is reported after the initiation of treatment with bisphosphonates in the majority of studies (21-27;29;32;34;39). This decrease in bone turnover has also been histologically confirmed by histomorphometric analysis of paired bone biopsies obtained at start of bisphosphonate treatment shortly after transplantation , and 3-6 months after transplantation.(19;20)

3. *Effect of bisphosphonate treatment on bone loss*

Most studies show a short-term beneficial effect of treatment initiated at or shortly after OLT on BMD.(22;24-26;31-34;39) The conclusion reached by authors of a meta-analysis published in 2010 was that bisphosphonate treatment administered during the critical first months after OLT appeared to reduce accelerated bone loss and to improve lumbar spine (LS) BMD.(35) Data on long-term effect of treatment with bisphosphonates administered at or shortly after OLT on BMD are scarce and conflicting. Treatment with bisphosphonates in the form of pamidronate administered as a single infusion before OLT did not prevent post-transplantation bone loss in the long-term.(29) Three studies showed a persistent beneficial effect on BMD up to 27 months after OLT (25;31;34) but this was not confirmed in a fourth study.(21) Three studies in which treatment was initiated longer than a year after OLT show no bone loss after start of treatment. (23;27;30)

4. *Effect of bisphosphonate treatment on fracture risk*

Data on both short-term and long-term effect of bisphosphonate treatment on fracture risk are conflicting, with studies showing a decreased fracture incidence in treated patients with oral as well as intravenous bisphosphonates, when treatment is initiated before, at or shortly after transplantation, or 1-2 years after OLT with a range of treatment duration of 9-24 months (23;25;26;28;31;34;39), whereas other studies do

not show any beneficial effect on fracture risk.(21;24;27;29;33) Of note is that 2 studies report only clinical fractures (25;28) whereas 10 studies radiographic fractures (19;21;23-27;29;31;33;34;39) and 4 studies report only on BMD changes (20;22;30;32). In the only published meta-analysis, the effect of bisphosphonate treatment on fracture risk could not be analysed because of the heterogeneity of the available data.(35)

Conclusion

A comprehensive review of published literature up to May 2014 suggests that bisphosphonate treatment when administered at or shortly after OLT is able to decrease the rapid early post-transplantation bone loss and to improve bone mass in the short term, irrespective of type of bisphosphonate used or duration of treatment. Data on long-term effects of these agents particularly when started 6 months or longer after OLT on BMD and fracture risk are conflicting.

B. Leiden Case Series

Baseline data

Demographic characteristics

223 patients with end-stage liver disease received an orthotopic liver transplant at the Leiden University Medical Centre between 2000 and 2011 and were eligible for the study (Figure 1). Twenty-one patients were excluded from analysis because of use of bisphosphonates at time of screening for OLT. Bisphosphonate treatment was initiated within the first year after OLT (median 185 days after OLT, range 22-365 days) in 44 patients, of whom 39 patients had available BMD measurements before start of treatment and at one and 2 years after OLT. Bisphosphonate treatment was initiated within the first 6 months after OLT (median 100 days after OLT, range 22-179) in 15 out of these 39 patients. The control group consisted of 104 patients not treated with bisphosphonates in whom BMD measurements were also available at the same time points. Demographic details of bisphosphonate treated and untreated OLT recipients are shown in Table 2. 72% and 68% of patients were men in the treatment and control group respectively, with a respective median age of 49 and 53 years. Most common primary liver disease was viral disease in both groups (33 and 29% in the treatment group and control group respectively), followed by alcohol liver disease (23% and 25% respectively) and cholestatic liver disease (15 and 14% respectively). Median time between screening and transplantation was 9 and 10 months in the treatment and control group respectively.

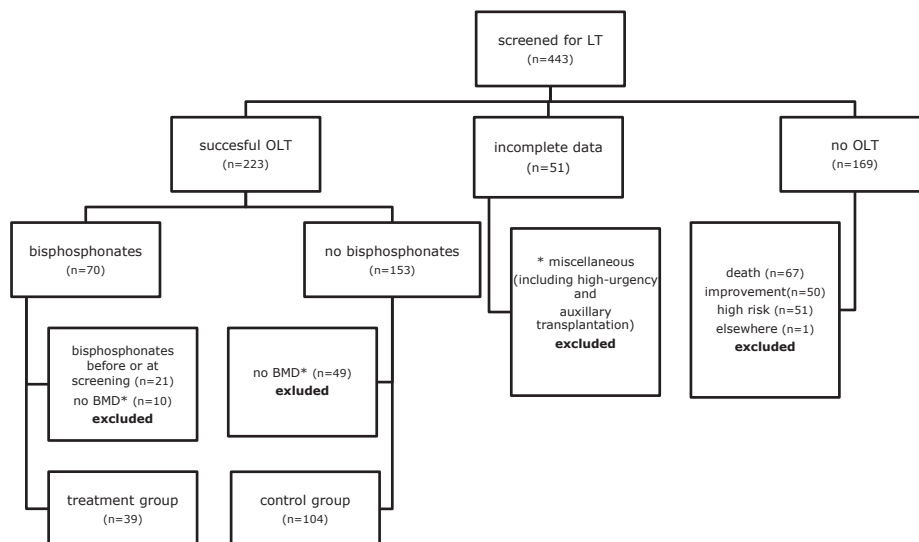


Figure 1: Flow-chart of the study. OLT = orthotopic liver transplantation; BMD = bone mineral density measurement; * none availability of BMD measurement before start of treatment and at one and two years after OLT.

Laboratory data

Vitamin D levels were significantly lower in bisphosphonate treated patients before OLT, with a mean vitamin D level of 24 ± 17 nmol/L compared to a mean level of 35 ± 24 nmol/L in the control group (normal 50-250 nmol/L, $p = 0.043$). In the treatment group, 88% of patients had vitamin D levels < 50 nmol/L, and 53% < 25 nmol/L, compared to the control group, in which 80% of patients had vitamin D levels below 50 nmol/L, and 38% below 25 nmol/L. There was no significant difference in any other laboratory parameter measured at time of screening for OLT between bisphosphonate treated and non-treated OLT recipients, including mean serum creatinine, bilirubin and MELD scores, reflecting severity of liver disease.

BMD data

BMD data were available as per inclusion criterion in all patients studied but LS data were not interpretable in a number of patients ($n = 10$) because of residual oral contrast material from abdominal CT-scans, required as part of the screening protocol for OLT, but chronologically performed by error before DXA in these patients. BMD measurements were significantly lower in the bisphosphonate treated group, with a mean LS BMD of 0.85 ± 0.15 g/cm² compared to 1.00 ± 0.17 g/cm² in the non-treated control group ($p < 0.005$). Femoral neck (FN) BMD was 0.71 ± 0.12 g/cm² in the bisphosphonate treated group compared to 0.85 ± 0.13 g/cm² in the non-treated group

Table 2: Demographic data at time of screening and after liver transplantation

Demographic data	Treatment group	Control group	P-value
Number of patients	39	104	
Gender – number of male patients (%)	28 (72%)	71 (68%)	0.684
Age at the time of OLT- Median (range), years	49 (18-68)	53 (19-67)	0.488
Death during follow-up – no of patients (%)	0	0	
BMI – mean (SD), kg/m2	25 ± 4	26 ± 5	0.105
Smoking – number of patients (%)	6 (17%)	38 (38%)	0.026 [†]
Primary liver disease – number of patients (%)			
Viral	13 (33%)	30 (29%)	0.133
Alcoholic	9 (23%)	26 (25%)	0.002 [†]
Cholestatic (PSC / PBC/ overlap syndrome)	6 (15%)	15 (14%)	0.058
Auto-immune hepatitis	3 (8%)	4 (4%)	0.706
Malignancy	2 (5%)	5 (5%)	0.273
Metabolic	0 (0%)	3 (3%)	-
Other*	6 (15%)	21 (20%)	0.007 [†]
Previous or concomitant renal transplantation			
- number of patients (%)	2 (5%)	3 (3%)	0.502
Time between screening and OLT			
- median (5 th – 95 th percentile), months	9 (1-19)	10 (1-25)	0.371
Second OLT			
- number of patients (%)	4 (10%)	16 (15%)	0.445
- time to second OLT – median (5 th – 95 th percentile), months	6 (1-11)	4 (0-23)	
Rejection episodes – number of episodes (cumulative incidence, %)			
6 months after OLT	14 (37%)	10 (10%)	< 0.005 [†]
12 months after OLT	0 (0%)	1 (11%)	0.498 [#]
24 months after OLT	2 (45%)	5 (17%)	0.773 [#]
Calcium – and/or vitamin D supplements at time of screening – number of patients (%)	5 (13%)	11 (11%)	0.918
Corticosteroids at time of screening - number of patients (%)	2 (5%)	4 (4%)	0.760
Immunosuppressive medication initiated at OLT			
- number of patients (%)			
Prednisolone, tacrolimus	15 (39%)	42 (40%)	0.834
Prednisolone, cyclosporine	10 (26%)	26 (25%)	0.937
Prednisolone, cyclosporine and MMF	5 (13%)	12 (12%)	0.833
Prednisolone, tacrolimus and MMF	3 (8%)	11 (11%)	0.607
Other	6 (15%)	13 (13%)	-

OLT = orthotopic liver transplantation; BMI = body mass index; PSC = primary sclerosing cholangitis; PBC = primary biliary cirrhosis; LS = lumbar spine; FN = femoral neck; MMF = mycophenolate mofetil.

* including polycystic liver disease, Budd Chiari, portal vein thrombosis, acute fatty liver of pregnancy, cryptogenic cirrhosis, cystic fibrosis, Rendu Osler Weber syndrome; † = p-value < 0.05; # = p-value concerning incident rejection episodes

($p < 0.005$). Mean LS and FN T-scores were respectively -1.98 ± 1.28 and -1.78 ± 1.12 in the bisphosphonate treated group and -0.70 ± 1.23 ($p < 0.005$) and -0.63 ± 1.08 ($p < 0.005$) in the control group. The prevalence of LS osteoporosis was higher in the treatment group than in the control group (35 vs 8%, OR 5.6, 95%CI 1.6-15.9, $p = 0.001$) whereas the prevalence of osteoporosis at the FN did not differ between groups (19 and 8%, OR 2.9, 95%CI 0.89-9.40, $p = 0.078$). Osteopenia was also more prevalent in treated patients, with a prevalence of 52% at the LS compared to 31% in untreated patients (OR 2.3, 95%CI 1.0-5.4, $p = 0.050$), and a prevalence of 59% at the FN compared to 26% in untreated patients (OR 4.3, 95% CI 1.8-10.0, $p = 0.001$).

Prevalent vertebral fractures at screening for OLT

Conventional spinal radiographs were available and evaluable in 33 of 39 patients in the bisphosphonate treated group and in 92 of 104 patients in the non-treated control group at time of screening for OLT. Vertebral fractures were prevalent in 20 patients in the bisphosphonate treated group (61%) and in 51 patients in the control group (55%) with no significant difference between groups ($p = 0.607$). Vertebral fractures were thus equally prevalent in treated and untreated patients at screening for OLT despite significantly lower BMD measurements both at LS and FN in the bisphosphonate treated group.

Follow-up data after transplantation

Clinical course

Immunosuppression included prednisolone in all patients, with the majority also receiving tacrolimus (39% in the treatment group and 40% control group) or tacrolimus and MMF (8% and 11%). Cyclosporine or cyclosporine and MMF were given to 26% and 15% of patients in the treatment group and to 25% and 12% of patients in the control group. Rejection episodes occurred mainly in the first year after OLT, with a significantly higher cumulative incidence of rejection episodes in bisphosphonate treated patients (37% versus 10% in the first six months after OLT), consequently reflecting significantly higher cumulative doses of corticosteroids in bisphosphonate treated than in non-treated patients ($p = 0.002$). All patients in the treatment group were receiving bisphosphonate treatment at 12 months after OLT. At the end of follow-up at 24 months after OLT, 34 out of the 39 patients (87%) in the treatment group were still using alendronate. Reasons for discontinuation of treatment were not clearly documented.

Laboratory data

There was a comparable significant improvement in bilirubin levels during the first year after OLT reflecting improvement in hepatobiliary function with mean bilirubin levels

significantly decreasing from 70 ± 60 mmol/L to 30 ± 66 mmol/L at six months after OLT ($p = 0.038$) in the bisphosphonate treated group and from 88 ± 174 mmol/L to 15 ± 11 mmol/L ($p < 0.005$) in the control group, remaining stable thereafter in both groups. There was no difference in mean bilirubin levels between groups at screening for OLT, or thereafter at any time point measured. There was no significant difference in changes in bilirubin levels between groups ($p = 0.928$).

Changes in renal function were also comparable between groups ($p = 0.337$). Serum creatinine levels increased significantly in the control group with a mean level of 107 ± 44 $\mu\text{mol/L}$ at 3 months after OLT ($p = 0.003$) but remained stable thereafter. There was also an increase in creatinine levels albeit not significant in the bisphosphonate treated group, reaching levels of 97 ± 31 $\mu\text{mol/L}$ at 3 months, 102 ± 32 $\mu\text{mol/L}$ at 6 months and 107 ± 32 $\mu\text{mol/L}$ at 12 months after OLT ($p = 0.179$). There was no significant difference in serum creatinine concentrations between groups at any time point measured.

Vitamin D levels increased significantly after OLT in both groups, reaching significance at three months after OLT in the bisphosphonate treated group ($p = 0.020$), with a mean level of 36 ± 24 nmol/L increasing to a mean level of 58 ± 25 nmol/L at 12 months after OLT ($p < 0.005$ compared to 3 months after OLT) and remaining stable thereafter with levels of 60 ± 29 nmol/L at 24 months after OLT ($p = 0.573$ compared to 12 months after OLT). In the control group, vitamin D levels increased significantly at 12 months after OLT ($p < 0.005$) with a mean value of 43 ± 24 nmol/L, and increasing further until 24 months after OLT with a mean value of 63 ± 36 nmol/L ($p = 0.001$ compared to 12 months after OLT). There was no significant difference in changes in vitamin D levels between groups ($p = 0.870$).

Sequential changes in BMD

Changes in BMD after OLT are shown in Figure 2 and 3. LS BMD decreased in all patients in the first six months after OLT, albeit significant only in the patients who subsequently did not receive treatment with bisphosphonates ($p = 0.009$), with no further decrease between 6 and 12 months after OLT. LS BMD increased significantly thereafter in both the bisphosphonate-treated and control groups, with values reaching pre-transplantation values in the control group ($p = 0.382$) and exceeding pre-transplant levels after OLT in the bisphosphonate treated group ($p = 0.016$) at 24 months after OLT. Changes in BMD at the LS between 12 and 24 months after OLT did not differ significantly between the treatment and control groups ($p = 0.574$). There was no difference in changes in BMD over the first 2 years of treatment between patients in whom treatment was initiated within the first six months after OLT compared to patients in whom treatment was initiated between six and twelve months after OLT ($p = 0.282$). (Figure 3) There was also no difference in changes in BMD between the group

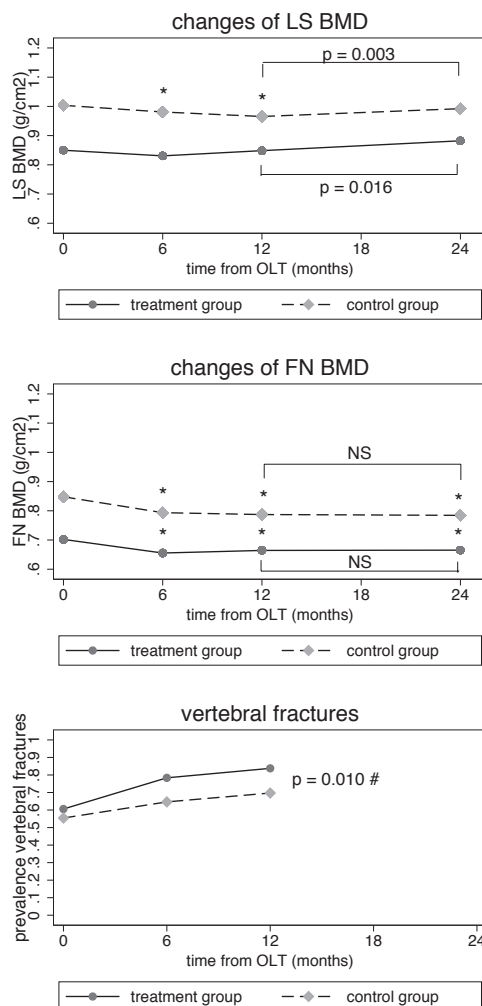


Figure 2: Changes of BMD at the LS (upper panel) and FN (middle panel) and incidence of vertebral fractures (lower panel). P-values concerning increase of BMD between 12 and 24 months after OLT in bisphosphonate treated patients and untreated controls. BMD = bone mineral density; LS = lumbar spine; FN = femoral neck; * = significantly lower when compared to screening ($p < 0.05$); NS = not significant. # = p-value concerning difference in incidence of new vertebral fractures between groups.

of patients in whom treatment with bisphosphonates was initiated within the first 6 months after OLT and the untreated control group ($p = 0.613$).

FN BMD decreased significantly between screening and six months after OLT in both bisphosphonate-treated and untreated patients ($p < 0.005$)(Figure 2). In the bisphosphonate treated group, FN BMD stabilized after start of treatment (mean 167 days after OLT, range 22-364), whereas FN BMD declined further in the control group,

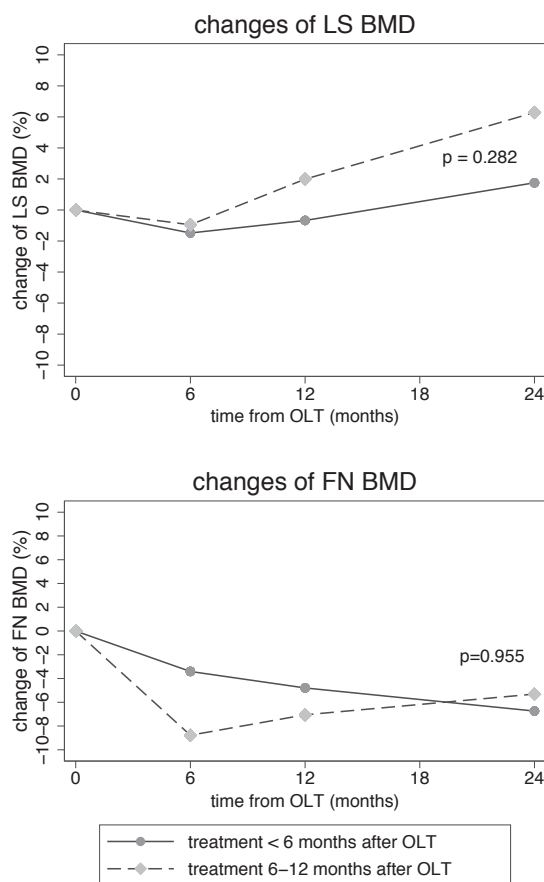


Figure 3: Percentage changes in BMD at the LS (upper panel) and FN (lower panel) in bisphosphonate treated patients in whom treatment was initiated within the first six months after OLT ($n=15$) or between six and twelve months after OLT ($n = 24$). OLT = orthotopic liver transplantation; BMD= bone mineral density; LS lumbar spine; FN = femoral neck; p - value reflecting difference in changes of BMD between patients in whom treatment was initiated before or after 6 months after OLT.

with levels being significantly lower at 24 months than at 6 months after OLT (12 months: $p = 0.091$; 24 months: $p=0.024$). In the treatment group, changes in FN BMD did not differ whether treatment was initiated within the first 6 months after OLT or between 6 and 12 months after transplantation (at 12 months $p = 0.955$)(Figure 3). There was no significant difference in changes in BMD between the treated and control group between 12 and 24 months after OLT ($p = 0.545$), also when comparing the control group with that of patients in whom bisphosphonate treatment was initiated within the first 6 months after OLT ($p = 0.361$).

Incident vertebral fractures during the first year after transplantation

Within the first year after transplantation, the incidence of fractures was significantly higher at 54% in the bisphosphonate treated group compared to 32% in the non-treated control group ($p = 0.010$). At 12 months after OLT, the prevalence of fractures was 84% in the treatment group after an overall median time of initiation of treatment of 180 days (range 343-0), and 70% in the control group ($p = 0.070$) (Figure 2). Data on vertebral fractures were not available beyond the first year after OLT.

DISCUSSION

Our findings from this retrospective analysis of a single centre case series of OLT recipients treated with bisphosphonates indicate that treatment with bisphosphonates initiated within the first year after liver transplantation with a median time of 185 days after OLT and given for a median time of 17 months (range 4-23) does prevent bone loss after start of treatment although there was no significant difference in BMD at one and two years after OLT between treated and untreated patients.

Data on the protective effect of bisphosphonates on post-transplantation bone loss are scarce in liver transplant recipients, with studies conducted in small numbers of patients, treatment initiated either before, at or shortly after OLT or more than a year or later after transplantation. (22;23;25-29;34;39) Our study addresses the effect of bisphosphonate treatment initiated within the first year after OLT, not directly after transplantation, but after a median time of 185 days after OLT. Whereas treatment initiated within the first six months after OLT did decrease to some extent the accelerated bone loss characteristic of that period, it appears that it was not started soon enough after liver transplantation to prevent it all together. Notwithstanding, our findings do show an increase in LS BMD and a stabilization of FN BMD after initiation of bisphosphonate treatment, regardless of when treatment was initiated after OLT. Intriguingly, BMD changes were not significantly different between bisphosphonate treated and control patients at one and two years after transplantation. We and others have previously shown the spontaneous recovery of BMD to pre-transplant levels in liver transplant recipients not receiving bone-modifying treatment. (1;2;4;6;8;10;13;15;16;40) This recovery in BMD is believed to be at least partly due to the improvement of gonadal function and of vitamin D status as a result of restoration of hepatobiliary function as well as a result of the tapering and rapid discontinuation of corticosteroids within the first few months after transplantation. We have also shown, however, that this spontaneous recovery in BMD is not associated with a parallel decrease in fracture risk (1), suggesting that bone loss may not be the sole determinant of increased fracture risk, and that altered bone quality may persist and be a significant contributor to fracture

risk after liver transplantation. In our study, the incidence of new vertebral fractures was higher at one year after transplantation in the bisphosphonate treated group, which had also a higher prevalence of osteoporosis and fracture before OLT, with the caveat that at the end of the first year after OLT, only few patients had been treated long enough with bisphosphonates, and more rejection episodes had occurred within the first six months after OLT in treated patients than in non-treated controls, resulting in higher cumulative doses of corticosteroids thereby predisposing to more severe bone loss.

Most studies show a beneficial effect of bisphosphonate treatment on BMD when treatment is initiated at or very shortly after transplantation.(19;22;25;26;34;39) This is in keeping with the beneficial effect of bisphosphonates on the prevention of glucocorticoid-induced bone loss, which is associated with a decrease in fracture risk when these agents are initiated shortly after start of glucocorticoids.(41;42) However, a single peri-operative pamidronate infusion (within 3 months of OLT), or treatment administered only before OLT failed to prevent post-transplantation bone loss (20;29), which suggests that longer treatment may be necessary to achieve bone protection, particularly if high dose prednisolone is used as part of the immunosuppressive regimen in the first months after transplantation when rejection episodes are the most frequent. Only three studies, including one RCT, have previously evaluated the effect of the oral bisphosphonate alendronate in liver transplant recipients, showing an increase in BMD during the first year after OLT.(26;31;39) Data on the effect of treatment with other bisphosphonates are heterogeneous and results are conflicting.(19-30;32-34) Our data are in keeping with a study using oral risedronate treatment, which showed a temporary beneficial effect of treatment at 6 months on LS BMD but not on FN BMD and no effect at either site at 12 months after transplantation.(24) A number of studies have reported beneficial effects of intravenous zoledronate or ibandronate on BMD.(19;22;23;25-27) Only one study compared an oral and intravenous bisphosphonate (alendronate and zoledronate respectively) (26), showing a similar increase in LS BMD and stabilization of FN BMD with the use of either agent. In our study, BMD changes were not different in patients in whom treatment with alendronate was initiated during the first 6 months after OLT compared to those in whom treatment was started 6-12 months after OLT. However, in the majority of the patients in whom treatment was initiated within the first 6 months after OLT, it was started at least 3 months post-transplantation and therefore after the early and most vulnerable postoperative period when the highest corticosteroid dosages are likely to have been administered.

Our study has a number of strengths and limitations. Its main strength lies in the availability of sequential BMD measurements and serial conventional spinal radiographs in the most vulnerable first year after transplantation, and in the blinded semi-quantitative assessment of all spinal radiographs for vertebral fractures by two experienced independent observers. A further strength of the study is the availability of

data in a relatively large and representative group of patients not receiving treatment with bisphosphonates, which was used as control group, and which was largely comparable to the treatment group in every respect except for an expected lower BMD in the bisphosphonate-treated group, which was corrected for in the final analysis of data.

Our study has also a number of limitations. There was no pre-defined protocol for bisphosphonate therapy. The decision to treat and when to start treatment was left to the clinical judgment of the treating physician which may have provided some degree of bias. Even though treatment was not randomly allocated and patients with the lowest BMD and the more prevalent fractures received treatment, we were able to compare treated and untreated patients by correction for BMD as well as for other parameters. Another limitation of our study is the relatively small sample size of bisphosphonate treated patients, although comparable to that of previously published studies. Because of the small number of patients in whom treatment with a bisphosphonate was initiated in referring hospitals before OLT, the lack of data at start of treatment in these patients, and the variable duration of treatment before OLT, we decided to exclude these patients from analysis and were thus unable to assess the effect of starting treatment before OLT on BMD and fracture risk after transplantation. Another limitation of our study is the lack of data on fracture incidence beyond the first year after transplantation, because of which we were unable to evaluate the long-term effect of bisphosphonate treatment on fracture risk. Non-vertebral fractures were poorly documented so that we could also not examine any potential beneficial effect of bisphosphonates on their occurrence.

In conclusion, our data suggest that the incidence of vertebral fractures increases in the first year after transplantation and that treatment with bisphosphonates initiated 3 months or later but not immediately after transplantation may be too late to prevent or decrease the crucial and rapid bone loss characteristic of the early post-OLT period and its associated increased long-term fracture risk despite recovery of BMD. Whether this increased fracture risk may be modulated by the earlier and longer use of bisphosphonates after OLT to decrease or prevent skeletal morbidity after OLT remains to be established using RCT's addressing the incidence of new fractures in the long-term as primary end-point.

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