

Zoledronic Acid Prevents Bone Loss after Liver Transplantation

A Randomized, Double-Blind, Placebo-Controlled Trial

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Background: Clinically important rapid bone loss occurs within 3 to 6 months after liver transplantation and may be associated with osteoporotic fractures.

Objective: To determine whether bisphosphonate treatment with zoledronic acid reduces transplant-related bone loss more than placebo in adults having liver transplantation for chronic liver disease.

Design: 12-month randomized, double-blind, placebo-controlled trial.

Setting: 2 large liver transplantation centers in Australia.

Patients: 62 adults having liver transplantation for chronic liver disease.

Interventions: Infusions of zoledronic acid, 4 mg ($n = 32$), or saline ($n = 30$) were given within 7 days of transplantation and again at months 1, 3, 6, and 9 after transplantation. All patients received supplementation with calcium carbonate, 600 mg/d, and ergocalciferol, 1000 U/d.

Measurements: The primary outcome was bone mineral density (BMD) measured by dual x-ray absorptiometry before transplantation and 3, 6, and 12 months later. Secondary outcomes included bone turnover markers that were measured before transplantation and 1, 3, 6, 9, and 12 months later.

Results: There were statistically significant interactions between treatment effects and time for BMD measurements at the lumbar

spine ($P = 0.002$), femoral neck ($P = 0.001$), and total hip ($P < 0.001$). Differences in acute bone loss 3 months after transplantation favored zoledronic acid over placebo. Differences between groups in percentage change from baseline adjusted for baseline weight and serum parathyroid hormone (PTH) level were 4.0% (95% CI, 1.1% to 7.0%) for the lumbar spine, 4.7% (CI, 1.9% to 7.6%) for the femoral neck, and 3.8% (CI, 1.7% to 6.0%) for the total hip. At 12 months after transplantation, the difference in percentage change from baseline between the 2 groups adjusted for baseline weight and serum PTH level was 1.1% (CI, -2.1% to 4.4%) for the lumbar spine, 2.7% (CI, 0.0% to 5.4%) for the femoral neck, and 2.4% (CI, 0.1% to 4.7%) for the total hip. Treatment with zoledronic acid induced temporary secondary hyperparathyroidism and postinfusion hypocalcemia statistically significantly more often than did placebo.

Limitations: The trial was not powered to assess fractures, and 10 of 62 (16%) patients were not included in adjusted analyses because of missing weight or serum PTH measurements.

Conclusion: Treatment with zoledronic acid can prevent bone loss within the first year after liver transplantation.

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Osteoporosis is a frequent complication of end-stage liver disease of various causes. Initially described in patients with cholestatic liver disease (1–4), it also occurs in association with cirrhosis secondary to hepatitis B and C virus infection and alcohol abuse (5–8). Although contributing factors that are common to postmenopausal osteoporosis have been identified, such as age, gonadal status, and vitamin D deficiency, cirrhosis is an independent risk factor for osteoporosis (8, 9). Some patients with cirrhosis eventually need liver transplantation, a procedure that is associated with accelerated bone loss, particularly within the first 3 to 6 months (10–13). Investigators report fractures rates of 16% to 40% during this period, predominantly of the vertebrae and ribs (12–16). Pain and restricted mobility after a fracture delay rehabilitation of patients and are severe enough at times to require hospitalization.

It is not clear whether bisphosphonate drugs, which inhibit osteoclast-mediated bone resorption, reverse bone loss after transplantation. One uncontrolled study reported variable results (17). A recent controlled study, however, did not find any benefit of intravenous pamidronate in liver transplant recipients who had unusually little acute

post-transplantation bone loss and few fractures in both the treatment and control groups (18). In our experience, bone disease and fractures after transplantation are major problems. Thus, we performed a randomized, double-blind, placebo-controlled trial of a potent intravenous bisphosphonate, zoledronic acid, in patients undergoing liver transplantation. The primary end point was bone density change at 3 months after transplantation; secondary end points were bone density change at 12 months and biochemical variables of bone turnover.

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Summary for Patients I-37

Web-Only

Appendix Figures
Conversion of tables and figures into slides

Context

Rapid bone loss occurs in the first few months after liver transplantation and may be associated with fractures.

Contribution

This randomized, double-blind trial found that infusing zoledronic acid within 1 week of liver transplantation and again 1, 3, 6, and 9 months after transplantation prevented bone loss more effectively than did placebo infusions. Differences between groups lessened over time as some patients receiving placebo regained bone after several months. Zoledronic acid sometimes caused postinfusion hypocalcemia and temporary secondary hyperparathyroidism.

Cautions

The trial was not powered to assess differences in fracture outcomes.

Implications

Zoledronic acid infusions can prevent bone loss after liver transplantation.

—The Editors

METHODS**Setting and Patients**

From July 2000 to July 2003, we recruited adults undergoing transplantation for chronic liver disease from 2 large transplantation centers within the Australian and New Zealand Liver Transplant program. We based recruitment primarily on convenience for individuals, including whether they resided close to the transplantation center and were not already taking part in other research studies. We excluded patients if they had a serum creatinine level greater than 1.5 times the upper limit of normal (for example, $>165 \mu\text{mol/L}$ [$>1.86 \text{ mg/dL}$]), had had treatment within the previous 12 months with agents known to affect bone metabolism (bisphosphonates, calcitriol, or sex hormones), or had hypocalcemia (corrected serum calcium level $<2.2 \text{ mmol/L}$ [$<8.8 \text{ mg/dL}$]). We discussed the study and obtained written informed consent from patients at their pretransplantation assessment. After transplantation, just before the first infusion, we asked the patients to verbally re-consent. Recruited patients were similar to adult patients in the Australian Liver Transplant Database (www.cs.nsw.gov.au/gastro/livertransplant) with respect to age, sex, Child–Pugh score, and cause of cirrhosis. The ethics review committees at both institutions approved the study protocol.

Randomization and Interventions

As soon as was feasible after liver transplantation, patients were randomly assigned centrally to receive zoledronic acid or placebo. Random assignment was done by using the method of stratified minimization (19) on the

basis of the following variables: age, sex, baseline bone mineral density (BMD), and primary immunosuppressive therapy (cyclosporine or tacrolimus). Of note, all but 4 patients were recruited from 1 center; these 14 patients were randomly allocated to 2 per treatment group. Hospital pharmacists prepared infusions and coded them to maintain blinding. Five infusions of zoledronic acid (4 mg in 100 mL of normal saline, administered over 15 minutes) or saline were given over a 12-month period. The first infusion was administered within 7 days of liver transplantation and then again at months 1, 3, 6, and 9 after transplantation. All patients received calcium carbonate, 600 mg/d, and vitamin D supplementation (ergocalciferol, 1000 U/d) from the time of transplantation listing and throughout the study. The standard immunosuppression regimen after transplantation at both centers consisted of tacrolimus or cyclosporine, azathioprine, and methylprednisolone at 500 mg on day 1, decreasing to 20 mg by day 12. Alternative immunosuppressive therapy included the use of mycophenolate instead of azathioprine in some patients. Biopsy-proven cellular rejection was treated with pulses of methylprednisolone, 1000 mg/d for 3 days, followed by reduced doses of prednisone similar to those used after transplantation, aiming to reach a prednisone dosage of 20 mg/d by day 14.

Assessments and Follow-up

Before transplantation, baseline data, including the Child–Pugh score, were re-collected every 6 months (designated as time 0 months). Bone mineral density at L2 to L4 of the lumbar spine, femoral neck, and total hip was measured by using dual x-ray absorptiometry on a Prodigy densitometer (Lunar, Madison, Wisconsin) in both centers at baseline and at months 3, 6, and 12 after transplantation. In vitro and in vivo coefficients of variation for BMD at the lumbar spine, hip, spine, and total body were less than 1.0 and less than 2.0%, respectively. The BMD data were expressed as T-scores (that is, SDs removed from the average BMD of young healthy controls) using the North American reference ranges provided by the densitometer manufacturer. Radiographs of the thoracolumbar spine were taken at the baseline assessment and at 12 months after transplantation. New vertebral fractures were identified by radiology report.

Blood and urine samples were collected in the morning at the pretransplantation assessment and were subsequently taken every 6 months until liver transplantation and at months 1, 3, 6, 9, and 12 months after transplantation. Serum testosterone levels (in men), parathyroid hormone (PTH) levels, and urinary free deoxyypyridinoline levels, corrected for creatinine, were measured on the Immulite 2000 autoanalyzer (Diagnostic Products Corp., Los Angeles, California). Both 25- and 1,25-hydroxyvitamin D levels were measured by radioimmunoassay (Diasorin, Stillwater, Minnesota) after an initial extraction from serum with acetonitrile and a second solvent column extrac-

tion step for 1,25-hydroxyvitamin D. Serum levels of insulin-like growth factor I were measured by using a double antibody radioimmunoassay after acid-ethanol extraction (Bioclone, New South Wales, Australia). The coefficients of variation, measured at 2 levels, were less than 8% for total testosterone, intact PTH, and 25- and 1,25-hydroxyvitamin D; less than 11.0% for insulin-like growth factor I; and 17.7% and 6.9% for urinary deoxypyridinoline at 27 nmol/L and 107 nmol/L, respectively. Bone-specific alkaline phosphatase (ALP) was measured by using competitive immunocapture assay on an Access analyzer (Beckman Coulter, Inc., Fullerton, California).

Adverse events were assessed at each follow-up point by open-ended questions asked by the treating physician and by review of charts by the research nurse. Physicians and nurses were blinded to treatment group. Physicians rated events as expected or unexpected and assessed potential attribution to therapy. Fractures or renal impairment and serum creatinine levels greater than 165 $\mu\text{mol/L}$ (>1.86 mg/dL) resulted in discontinuation of therapy. Hypocalcemia (corrected serum calcium) was graded according to the following criteria: grade 1, 1.90 to 2.14 mmol/L (7.6 to 8.6 mg/dL); grade 2, 1.70 to 1.89 mmol/L (6.8 to 7.6 mg/dL); grade 3, 1.50 to 1.69 mmol/L (6.0 to 6.8 mg/dL); and grade 4, less than 1.50 mmol/L (<6.0 mg/dL).

Statistical Analysis

The primary end points of change in BMD at 3 months for the hip and the lumbar spine were used as the basis for a priori sample size calculations. On the basis of a 10% difference in BMD change, a 2-tailed *P* value of 0.05, and 80% power, 34 patients per group were required. Anticipating a 10% noncompletion rate, we estimated that 75 patients would need to be recruited.

Linear mixed-effects models were fitted to BMD at the lumbar spine, femoral neck, and total hip and to biochemical data. Treatment (zoledronic acid or placebo), time, and their 2-way interaction were considered as fixed effects, as were the baseline body weight, serum PTH level, and testosterone level (in men). These baseline variables showed some imbalance between treatment groups. Differences between treatments were estimated as both unadjusted and adjusted for potential confounding variables, baseline weight, and serum PTH level. Because estimates of treatment differences in men further adjusted for testosterone changed by less than 10%, this covariate was not included in any final models. All tabulated results refer to men and women combined. The unadjusted treatment differences are based on intention-to-treat analyses of all 62 patients. Because 10 patients had missing baseline weight or serum PTH measurements, the adjusted analyses represent modified intention-to-treat analyses of the remaining 52 patients. Unadjusted and adjusted treatment differences for the percentage change in BMD from baseline were calculated by analyzing the log-transformed BMD mea-

surements and then back-transforming the variable estimates and their 95% CIs. All results pertaining to percentage change from baseline were calculated in this manner, whereas results for absolute change in BMD were based on models fitted to the untransformed data. Identical conclusions were reached by using either the untransformed or log-transformed BMD models.

Nested models allowing random intercepts and slopes, random intercepts only, or no random effects were fitted by using maximum likelihood estimation and were compared by using likelihood ratio tests. In all instances, the most parsimonious models required random intercepts only. The time linear and quadratic terms were considered as continuous fixed covariates. To obtain point estimates of between-treatment differences in change from baseline together with 95% CIs at 3, 6, and 12 months, time was considered as a factor with 4 levels (0, 3, 6, and 12 months). A banded correlation structure of order 2 for measurements made over time within patients was, in all instances, the most appropriate within-patient correlation structure for the time factor identified through likelihood ratio tests.

Diagnostic plots were used to assess the adequacy of the fitted models. For each fitted model, box plots of residuals by patient were used to verify that the errors were centered at 0 and to identify obvious outlying observations. Scatter plots of standardized residuals by fitted values by treatment were used to check whether there was more variability among patients receiving zoledronic acid than among patients receiving placebo. Observed versus fitted values were plotted to assess the magnitude of the residuals relative to the value being estimated. Normal quantile plots (Q-Q plots) of residuals and of estimated random effects were used to check the assumption of normality for the within-patient errors and for the random effects.

Two-tailed tests were used throughout the study. To achieve an overall 5% level of significance for the primary outcome variables of change from baseline BMD at the hip and at the lumbar spine at 3 months, a significance level of 2.5% was required for each. All other analyses were purely exploratory with a 5% level of significance. These exploratory analyses were not adjusted for multiple comparisons, and any observed statistically significant effects need to be treated with caution because they could be type I errors. The statistical software package S-PLUS, version 6.2 (Insightful Corp., Seattle, Washington), was used to analyze the data. Data are presented as means and SDs.

Role of the Funding Source

This study was investigator-initiated, and the principal investigators developed the protocol and conducted the study. The study sponsors were the respective hospitals. Novartis Pharmaceuticals Pty Ltd., Australia, supplied the drug, zoledronic acid, and some financial support. All analyses were done by statisticians independently from the pharmaceutical company and by the principal investigators.

Table 1. Baseline Values*

Variable	Zoledronic Acid Group (n = 32)	Placebo Group (n = 30)
Mean age (SD), y	47.4 (9.7)	49.0 (6.8)
Sex (male/female), n/n	26/6	23/7
Mean Child–Pugh score (SD)	9.8 (2.7)	9.4 (2.5)
Underlying disease, n		
Hepatitis C virus infection	11	15
Hepatitis B virus infection	3	3
Alcoholism	6	7
Chronic autoimmune hepatitis	2	1
α 1-Antitrypsin deficiency	2	0
Nonalcoholic steatorrheic hepatitis	1	0
Cryptogenic or cryptohepatic carcinoma	1	0
Wilson disease	1	0
Primary biliary cirrhosis	2	2
Primary sclerosing cholangitis	3	2
Body weight (SD), kg	81.5 (16.5)	73.8 (13.0)
Smoking status, n		
Current	2	6
Previous	20	16
Never	10	8
25-Hydroxyvitamin D level (SD), nmol/L†	34.4 (21.0)	38.1 (27.9)
Parathyroid hormone level (SD), pmol/L‡	5.4 (3.4)	4.0 (2.4)
Deoxypyridinoline/creatinine ratio (SD), nmol/mmol§	11.4.0 (4.9)	10.4 (5.8)
Bone-specific ALP level (SD), μ g/L	30.8 (22.4)	29.3 (14.2)
Insulin-like growth factor I level (SD), nmol/L¶	6.9 (4.5)	6.7 (9.0)
Testosterone level in men only (SD)**		
nmol/L	11.2 (7.4)	13.7 (9.8)
ng/dL	322 (213)	395 (282)
Bone mineral density (SD), g/cm ²		
Lumbar spine	1.092 (0.146)	1.125 (0.180)
Femoral neck	0.909 (0.133)	0.940 (0.168)
Total hip	0.944 (0.137)	0.990 (0.175)
T-score††		
Lumbar spine	−1.2	−0.9
Femoral neck	−1.1	−0.9
Total hip	−1.0	−0.6

* ALP = alkaline phosphatase.

† Normal range: 39 to 120 nmol/L.

‡ Normal range: 2.5 to 6.5 pmol/L.

§ Normal range: 3.0 to 7.4 nmol/mmol.

|| Normal range: 4 to 21 μ g/L.

¶ Normal range: 3.3 to 30 nmol/L.

** Normal range: 11 to 35 nmol/L (317 to 1009 ng/dL).

†† SDs from the average bone mineral density of healthy controls.

Novartis Pharmaceuticals Pty Ltd., Australia, did not have access to the database but reviewed the manuscript for comment without modifying it.

RESULTS

Baseline Characteristics

Treatment groups were similar with respect to all baseline variables (Table 1) except for weight, PTH level, and testosterone level in men, which showed some imbalance. There was no difference between groups in the timing of collection of baseline data before transplantation (mean, 3.3 months [SD, 2.2] for the zoledronic acid group vs. 3.4 months [SD, 2.0] for the placebo group). Of the 13 women, 9 were postmenopausal, whereas the others (3 who received placebo and 1 who received zoledronic acid), who were 34 to 50 years of age, had varying degrees of oligomenorrhea or amenorrhea. Only the 3 patients with

chronic autoimmune hepatitis had received prolonged glucocorticoid therapy before transplantation. By World Health Organization criteria, osteoporosis of the lumbar spine or hip at baseline was present in 18% of patients receiving zoledronic acid and in 10% of patients receiving placebo, and osteopenia was present in 32% and 50% of patients, respectively. Compared with the normal reference range, levels of 25-hydroxyvitamin D, insulin-like growth factor I, and testosterone in men were low, and levels of biochemical markers of bone turnover (deoxypyridinoline and bone-specific ALP) were high.

The mean length of the hospitalization for liver transplantation was similar in both groups (33.0 days [SD, 18.4] for the zoledronic acid group vs. 27.1 days [SD, 12.5] for the placebo group; $P = 0.143$). The first infusion of zoledronic acid or saline was given at a median of day 3 (range, 1 to 13 days) in both groups. Most patients ($n =$

54) received tacrolimus as primary immunosuppressive therapy; the 8 patients who received cyclosporine were equally distributed (4 per group). Although the average daily dose of prednisone was similar in the 2 groups at 1, 3, and 6 months (Table 2), the patients in the zoledronic acid group received higher total glucocorticoid exposure because of more episodes of rejection requiring glucocorticoid pulse therapy and because 1 patient required a second liver transplantation.

Discontinuations and Adverse Events

Figure 1 summarizes the progress of the study. There were no missed treatments for patients who remained in the study. Six patients in the zoledronic acid group and 7 patients in the placebo group withdrew from the study because of adverse events: death (4 patients), fractures (4 patients), sepsis (2 patients), and the nephrotic syndrome (1 patient). Two additional patients also discontinued therapy, 1 because of poor adherence and 1 because androgen therapy was required. The fractures all involved minimal trauma; 2 patients in the placebo group had multiple vertebral fractures (1 had a subsequent wrist fracture), and 2 patients in the zoledronic acid group had a single nonvertebral fracture each (rib and humerus). No asymptomatic vertebral fractures were identified by radiology. Complete data were obtained and analyzed in all patients with fractures and for 2 additional patients (1 with the nephrotic syndrome and 1 with androgen deficiency).

Hypocalcemia developed within 2 to 3 days after the first infusion in 3 patients from the placebo group (grade 1 hypocalcemia) and in 13 patients from the zoledronic acid group (9 with grade 1 hypocalcemia and 2 each with grades 2 and 3 hypocalcemia) ($P = 0.023$ [Fisher exact test]). Patients with grade 3 hypocalcemia required treatment with intravenous calcium for 24 to 48 hours. Patients with grade 2 and 3 hypocalcemia had secondary hyperparathyroidism and lower levels of 25-hydroxyvitamin D (21 nmol/L [SD, 13]) before transplantation than those with grade 1 hypocalcemia (45 nmol/L [SD, 37]) ($P = 0.25$).

Bone Density

There were treatment-by-time interactions at all sites (spine, femoral neck, and total hip) in both the unadjusted analyses of BMD and those adjusted for baseline weight and serum PTH levels. In particular, for the adjusted models of BMD, the interaction of treatment with the linear time term was statistically significant only for BMD at the hip ($P = 0.050$), whereas the interaction of treatment with the quadratic time term was significant for BMD at the hip ($P < 0.001$), lumbar spine ($P = 0.002$), and femoral neck ($P = 0.001$).

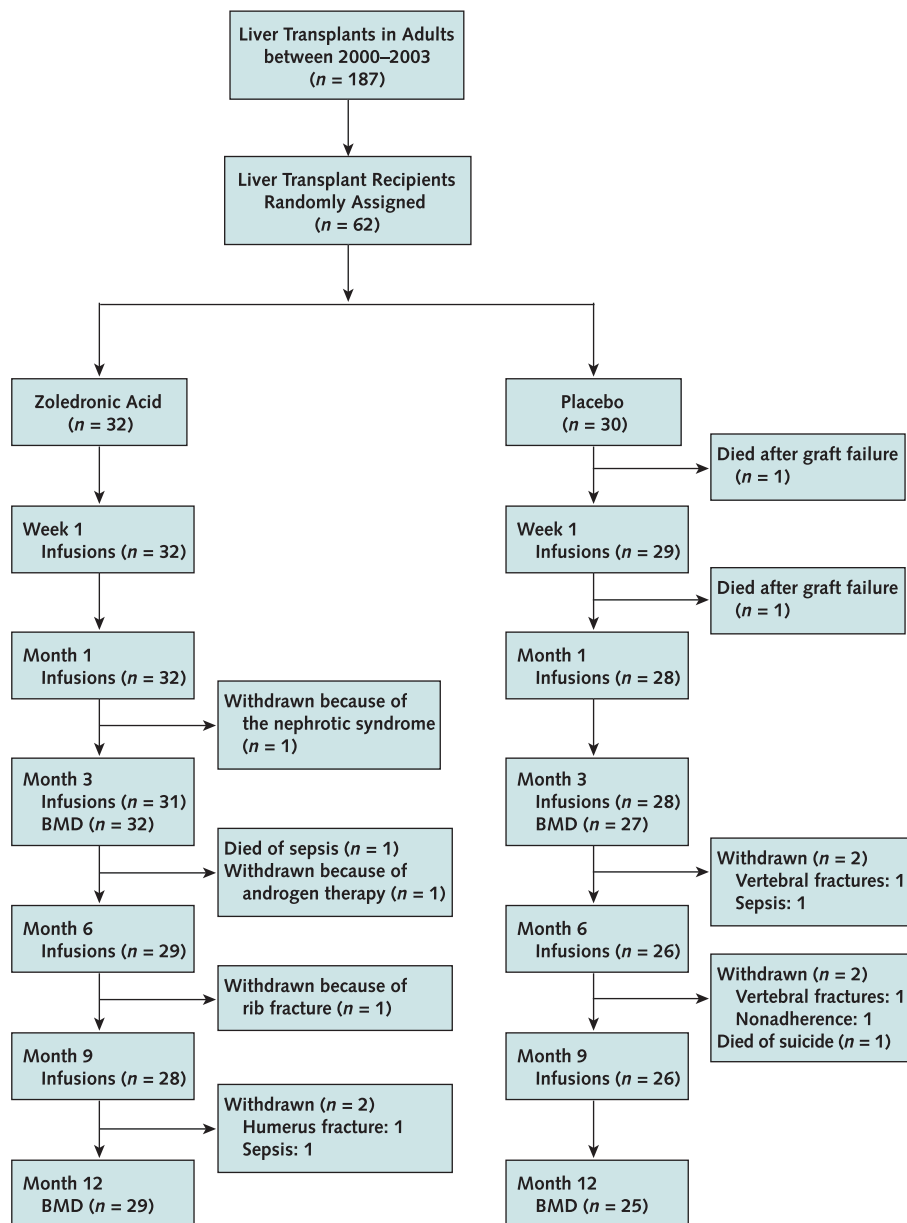
The difference in changes from baseline in BMD between the zoledronic acid and placebo groups at the different time points (3, 6, and 12 months) is shown in Table 3. Figure 2 shows the unadjusted post-transplantation percentage changes from baseline over time for each group. The primary outcome variables of change from baseline BMD at the hip and lumbar spine 3 months after transplantation each differed between the zoledronic acid and placebo groups at the 2.5% level of significance whether unadjusted or adjusted for baseline weight and serum PTH levels ($P < 0.001$ for hip and $P = 0.012$ for lumbar spine for adjusted values). The differences at each site between the zoledronic acid and placebo groups for the change from baseline BMD (either unadjusted or adjusted for baseline weight and serum PTH level) were always significant at the 5% level at 3 and 6 months after transplantation. The size of the difference in changes from baseline BMD decreased after 6 months at all sites. In the case of the lumbar spine, the difference in change from baseline BMD between the zoledronic acid and placebo groups was no longer significant at 12 months after transplantation ($P = 0.54$). The percentage change in BMD from baseline between groups, adjusted for baseline weight and serum PTH level, was 4.0% (95% CI, 1.1% to 7.0%) for the lumbar spine, 4.7% (CI, 1.9% to 7.6%) for the femoral neck, and 3.8% (CI, 1.7% to 6.0%) for the total hip at 3 months after transplantation and 1.1% (CI, -2.1% to 4.4%) for the lumbar spine, 2.7% (CI, 0.0% to 5.4%) for the femoral neck, and 2.4% (CI, 0.1% to 4.7%) for the total hip at 12 months

Table 2. Mean Daily Prednisone Dose at Each Post-Transplantation Visit and Pulses of Glucocorticoid Therapy Given for Acute Cellular Graft Rejection*

Variable	Month				
	1	3	6	9	12
Zoledronic acid group					
Mean daily prednisone dose (SD), mg	16.4 (4.5)	9.1 (3.8)	6.3 (3.2)	5.5 (3.5)	5.9 (5.1)
Additional glucocorticoid therapy	Second transplant (1 patient)	6 pulses (4 patients)	1 pulse (1 patient)	1 pulse (1 patient)	–
Placebo group					
Mean daily prednisone dose (SD), mg	16.4 (3.5)	9.5 (3.8)	6.0 (2.5)	4.0 (1.9)	3.8 (2.1)
Additional glucocorticoid therapy	–	1 pulse (1 patient)	–	1 pulse (1 patient)	–

* Glucocorticoid therapy consisted of methylprednisolone at 1000 mg/d for 3 days, tapering over 2 weeks to prednisone, 20 mg/d. One patient required a second liver transplant on day 19 and received a repeated course of the initial immunosuppressive transplantation regimen (see Methods).

Figure 1. Study flow diagram.



Patients were recruited from 2 liver transplantation centers in Australia over a 3-year period. The total number of adult patients having liver transplantations during this time was 110 in Sydney and 78 in Melbourne. The timing and number of infusions of either zoledronic acid or saline (placebo) are shown. The number of bone mineral density (BMD) measurements that were analyzed included data collection on 6 of the 9 patients who withdrew from the study and some isolated missing data points (for BMD at baseline and at 3, 6, and 12 months after liver transplantation, the numbers of patients were 32, 32, 26, and 29, respectively, for the zoledronic acid group and 30, 27, 28, and 25, respectively, for the placebo group).

after transplantation. For BMD at the lumbar spine, there was an absolute improvement at 6 months for the zoledronic acid group ($P = 0.017$). The placebo group had a decrease in BMD at the lumbar spine from baseline at 3 months ($P = 0.005$), partial recovery at 6 months, and improvement by 12 months ($P = 0.006$). There was no evidence of significant absolute change in BMD at the femoral neck or total hip over time for the zoledronic acid

group. The placebo group had a decrease in BMD at the femoral neck and total hip from baseline at 3 months ($P = 0.001$) and 6 months, with partial recovery by 12 months.

Results of Blood and Urine Tests

Levels of serum PTH were increased at 1 and 3 months after transplantation in the zoledronic acid group ($P = 0.020$ and 0.032 , respectively) but not in the placebo

group. Levels of serum 1,25-hydroxyvitamin D also increased at 1 month in the zoledronic acid group only ($P = 0.001$). There was no statistically significant between-group change in levels of 25-hydroxyvitamin D (Appendix Figure 1, available at www.annals.org); however, at 12 months, levels of 25-hydroxyvitamin D were significantly higher (66 nmol/L [SD, 32]) than pretransplantation levels in both groups ($P < 0.001$). In the placebo group, a transient, nonsignificant increase in levels of deoxypyridinoline was seen at 1 month after transplantation; this did not occur in the zoledronic acid group. Subsequently, however, levels of deoxypyridinoline decreased to equivalent levels within the normal range by 12 months in both groups ($P < 0.001$) (Appendix Figure 2, available at www.annals.org). For bone-specific ALP, there was a decrease after transplantation in both groups followed by further suppression in the zoledronic acid group at 3 months ($P = 0.022$), which was maintained for the duration of the study.

There were no statistically significant differences between treatment groups for serum levels of insulin-like growth factor I, creatinine, and testosterone in men during the study, although statistically significant within-group changes were seen in both groups over time. Levels of insulin-like growth factor I were low at baseline (Table 1), increased steeply by 1 month after transplantation (34 nmol/L [SD, 17] in the zoledronic acid group vs. 35 nmol/L [SD, 13] in the placebo group; $P < 0.001$), and subsequently were maintained within the normal range with no significant difference between groups. Serum creatinine levels remained within the normal range throughout the study, but there was a significant increase after liver transplantation ($P = 0.031$) that did not differ between the zoledronic acid and placebo groups. Baseline creatinine

levels were 74 $\mu\text{mol/L}$ (SD, 20) versus 76 $\mu\text{mol/L}$ (SD, 28) (0.84 mg/dL [SD, 0.23] vs. 0.86 mg/dL [SD, 0.32]), respectively; 12-month creatinine levels were 98 $\mu\text{mol/L}$ (SD, 20) versus 94 $\mu\text{mol/L}$ (SD, 17) (1.11 mg/dL [SD, 0.23] vs. 1.06 mg/dL [SD, 0.19]), respectively. In men, testosterone levels decreased nonsignificantly in the zoledronic acid group versus the placebo group 1 month after transplantation: 8.1 nmol/L (SD, 5.2) versus 10.9 nmol/L (SD, 6.1) (233 ng/dL [SD, 150] vs. 314 ng/dL [SD, 176]) ($P = 0.090$). However, this was followed by a gradual increase by 12 months to 13.0 nmol/L (SD, 5.9) versus 14.1 nmol/L (SD, 9.9) (375 ng/dL [SD, 170] vs. 406 ng/dL [SD, 285]) ($P < 0.001$). These data are not shown.

DISCUSSION

This study suggests that bisphosphonate therapy inhibits bone loss after liver transplantation, a finding that may have implications for other forms of solid organ transplantation. The positive effect of zoledronic acid on bone density was seen despite more glucocorticoid therapy being required for treatment of acute rejection in this group. Evidence of the antiresorptive effect of zoledronic acid was seen in the development of secondary hyperparathyroidism, presumably as a response to the inhibition of calcium release from bone into the circulation by zoledronic acid. In addition, patients treated with zoledronic acid maintained a higher degree of suppression of bone-specific ALP than those treated with placebo.

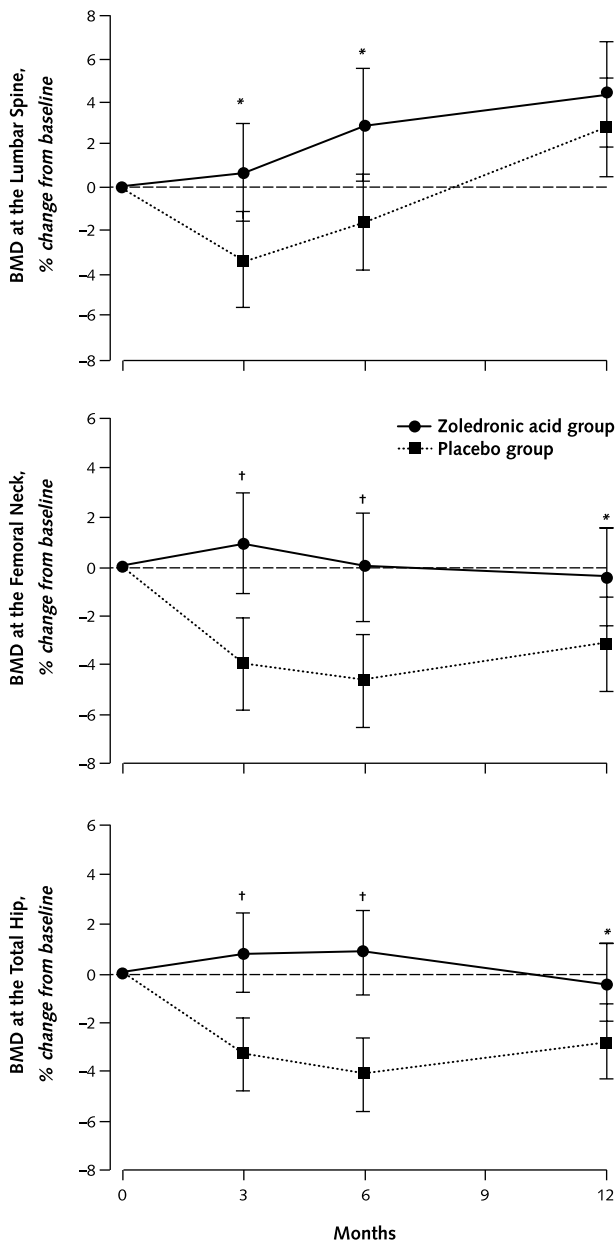
Another notable finding, with relevance for the interpretation of previous studies, is the recovery of the average BMD at the lumbar spine to near-baseline levels by 6 months after transplantation in the placebo group. By 12

Table 3. Estimated Difference between the Zoledronic Acid and Placebo Groups for the Absolute Change and Percentage Change from Baseline Bone Mineral Density*

Site of BMD Measurement	Month	Unadjusted (n = 62)				Adjusted for Baseline PTH Level and Weight (n = 52)			
		Change in BMD in the Zoledronic Acid Group Minus Change in the Placebo Group (95% CI), g/cm ²	P Value	Percentage Change in BMD in the Zoledronic Acid Group Minus Percentage Change in the Placebo Group (95% CI), %	P Value	Change in BMD in the Zoledronic Acid Group Minus Change in the Placebo Group (95% CI), g/cm ²	P Value	Percentage Change in BMD in the Zoledronic Acid Group Minus Percentage Change in the Placebo Group (95% CI), %	P Value
Lumbar spine	3	0.033 (0.003 to 0.063)	0.033	3.4 (0.5 to 6.3)	0.024	0.040 (0.010 to 0.071)	0.012	4.0 (1.1 to 7.0)	0.009
	6	0.035 (0.004 to 0.065)	0.029	3.3 (0.3 to 6.3)	0.032	0.045 (0.011 to 0.079)	0.012	4.2 (0.9 to 7.7)	0.015
	12	0.011 (-0.20 to 0.042)	0.50	1.0 (-1.9 to 4.0)	0.496	0.011 (-0.23 to 0.045)	0.540	1.1 (-2.1 to 4.4)	0.51
Femoral neck	3	0.039 (0.018 to 0.059)	<0.001	4.9 (2.3 to 7.6)	<0.001	0.037 (0.014 to 0.060)	0.002	4.7 (1.9 to 7.6)	0.002
	6	0.037 (0.017 to 0.058)	<0.001	4.5 (2.1 to 7.0)	<0.001	0.037 (0.014 to 0.061)	0.002	4.6 (1.8 to 7.4)	0.002
	12	0.025 (0.005 to 0.046)	0.018	2.8 (0.4 to 5.3)	0.023	0.023 (0.000 to 0.047)	0.052	2.7 (0.0 to 5.4)	0.056
Total hip	3	0.035 (0.019 to 0.051)	<0.001	4.1 (2.2 to 6.0)	<0.001	0.033 (0.015 to 0.050)	<0.001	3.8 (1.7 to 6.0)	<0.001
	6	0.047 (0.029 to 0.065)	<0.001	5.3 (3.2 to 7.3)	<0.001	0.045 (0.024 to 0.066)	<0.001	5.1 (2.8 to 7.5)	<0.001
	12	0.023 (0.005 to 0.041)	0.017	2.3 (0.3 to 4.3)	0.026	0.023 (0.002 to 0.044)	0.036	2.4 (0.1 to 4.7)	0.048

* BMD = bone mineral density; PTH = parathyroid hormone.

Figure 2. Bone density change.



The percentage change from baseline (means and 95% CIs) in bone mineral density (BMD) at the lumbar spine, femoral neck, and total hip for the zoledronic acid and the placebo groups ($P = 0.032, 0.002, 0.001$, respectively, for treatment effect; unadjusted analyses) is shown. The zero time point represents baseline data collected before liver transplantation; the subsequent time points are the number of months after transplantation. At 0, 3, 6, and 12 months, respectively, the numbers of patients were 32, 32, 26, and 29, respectively, for the zoledronic acid group and 30, 27, 28, and 25 for the placebo group. Estimated effects are based on models fitted to log-transformed BMD data. * $P < 0.05$. † $P < 0.001$ for individual time points, zoledronic acid versus placebo.

months, this measure had increased above baseline levels in both the placebo and zoledronic acid groups. This spontaneous improvement in BMD and the decrease in bone turnover markers, particularly deoxyypyridinoline, in the

placebo group may be related to improved general health, mobility, muscle mass, and nutrition as a consequence of improved liver function (12, 17, 20), which is reflected in the increase in levels of serum insulin-like growth factor I, creatinine, 25-hydroxyvitamin D, and testosterone in men. More bone loss was seen in the hip than in the lumbar spine in the placebo group, reaching a nadir at 6 months after transplantation, with only partial recovery seen at 12 months. No hip bone loss was seen in the zoledronic acid-treated group. Although earlier transplantation studies predominantly showed bone loss in the lumbar spine and vertebral fractures (17), several recent reports support our finding of substantial and sustained bone loss in the hip (17, 18, 21, 22). Recovery of bone density may continue for many years after liver transplantation (10, 23); however, during this period patients may be at increased long-term risk for peripheral and long bone fractures.

The natural improvement in BMD in the placebo group observed in this study highlights the importance of ensuring adequately controlled studies, without which an observed increase in bone density may be incorrectly attributed to a given treatment. Previous studies of osteoporosis therapies for transplant recipients have generally lacked randomized controls and featured suboptimal designs, resulting in conflicting and unconvincing findings. An unrandomized study reported a positive effect of pamidronate after a reduction in fractures in 13 patients who had liver transplantation (14), as did a combined study of liver and heart transplant recipients ($n = 34$) in whom treatment with pamidronate was initiated, on average, 2 years after transplantation (24). In a randomized study of 99 liver transplant recipients, a surprising finding was a lack of bone loss in the lumbar spine after transplantation in untreated patients, which possibly was related to missing data points or to a policy of early steroid withdrawal. Loss of hip bone was not decreased in patients who received a single 60-mg dose of pamidronate before liver transplantation (18). However, our findings suggest that a more potent bisphosphonate administered as soon as is feasible after surgery and at more frequent intervals can prevent the active resorptive process initiated in the hip after liver transplantation. It is difficult to draw conclusions about the effects of other therapies, including calcitonin, cyclical etidronate, calcitriol, or calcium, on transplant-related bone loss because of the lack of a contemporaneous control group (25–30), small numbers (16, 25–30), and biased patient selection with inadequate baseline BMD data (31). A large, randomized study in cardiac transplant recipients showed potential benefits with alendronate or calcitriol therapy (32); however, the reference population consisted of nonrandomized patients who declined to participate in the study.

Despite a total of 20 mg of zoledronic acid being administered over 12 months to patients with major comorbid conditions, there was no increase in adverse events other than temporary, induced secondary hypoparathy-

roidism and hypocalcemia. In particular, zoledronic acid did not adversely affect renal function. A brief report suggesting that zoledronic acid may contribute to renal impairment was based on uncontrolled evidence from a group of patients with cancer who had other risk factors for renal failure (33). A randomized, placebo-controlled study of men with prostate cancer who were treated with a zoledronic acid regimen similar to that used in our study also did not find any effect on renal function (34). Hypocalcemia in patients treated with zoledronic acid is well-recognized (35, 36) as a consequence of the potent antiresorptive effect and highlights the need for adequate calcium and vitamin D replacement before zoledronic acid therapy is started. Although our patients were prescribed maintenance doses of calcium and vitamin D, many had vitamin D deficiency at baseline, which was not adequately corrected by the time of transplantation. Although tetany and seizures have been reported after bisphosphonate-induced hypocalcemia (36, 37), our patients had frequent biochemical monitoring and intravenous calcium was used to correct transient abnormalities in severe-grade hypocalcemia without any adverse clinical effect.

The limitations of this study include a relatively small sample size and inadequate power to assess fractures. Also, multivariable analyses adjusted for weight and serum PTH levels excluded 10 of 62 (16%) patients because of missing data. A future study to test a single 4-mg dose of zoledronic acid, given as soon as is feasible after transplantation, would be worthwhile; a single dose of zoledronic acid has been shown to result in improvements in bone density over 12 months in postmenopausal women (38). Combining liver, lung, and heart transplant recipients in such a study would not only provide sufficient power to assess fractures but would also broaden the applicability to other forms of solid organ transplantation with the use of alternative immunosuppressive regimens.

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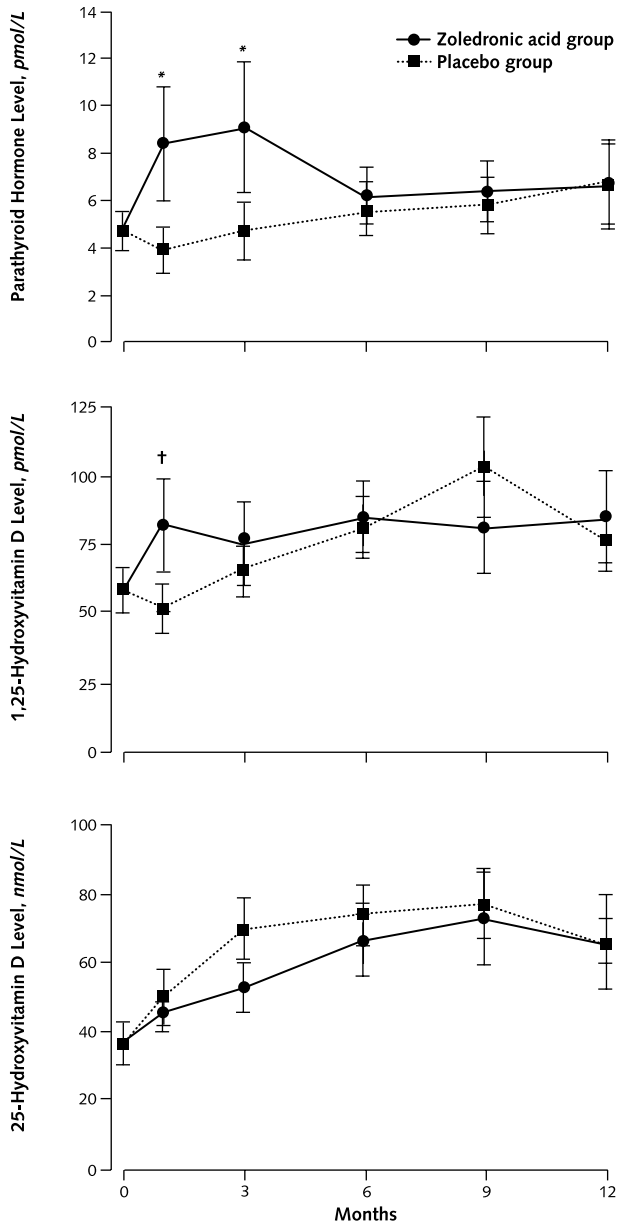
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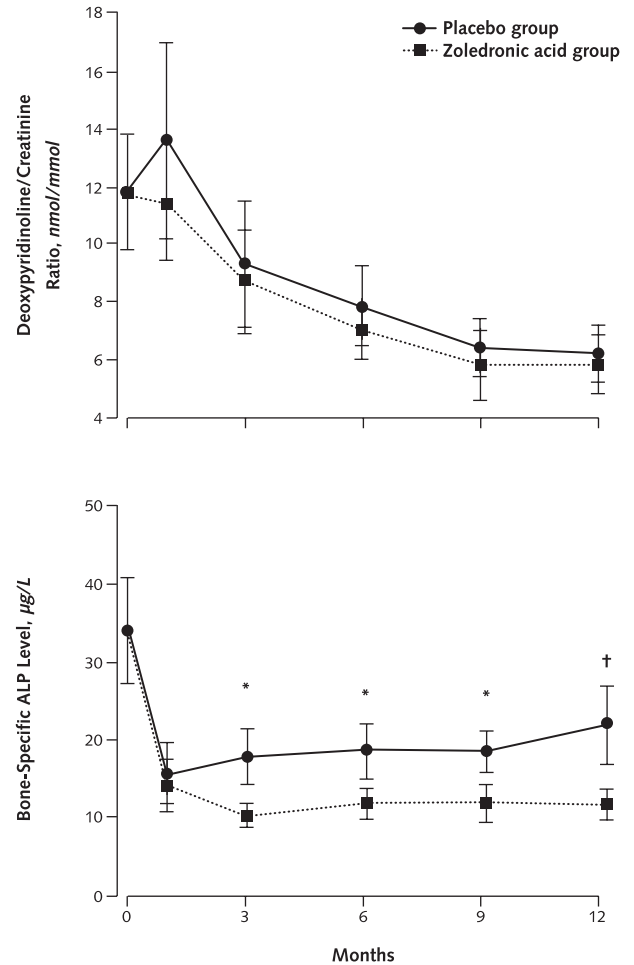
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Appendix Figure 1. Levels of vitamin D and parathyroid hormone.



Serum parathyroid hormone, 1,25-hydroxyvitamin D, and 25-hydroxyvitamin D (means and 95% CIs) after liver transplantation are shown for the zoledronic acid and placebo groups. The normal reference ranges are 2.5 to 6.5 pmol/L for parathyroid hormone; 36 to 120 pmol/L for 1,25-hydroxyvitamin D; and 39 to 120 nmol/L for 25-hydroxyvitamin D. At 0, 1, 3, 6, 9, and 12 months, respectively, the numbers of patients were 30, 32, 31, 30, 30, and 30 for the zoledronic acid group and 28, 24, 27, 28, 26, and 24 for the placebo group. The zero time point represents baseline data collected before liver transplantation; the subsequent time points are the number of months after transplantation. * $P < 0.05$. † $P < 0.001$ for individual time points, zoledronic acid versus placebo.

Appendix Figure 2. Bone turnover markers.



Urinary deoxyypyridinoline/creatinine ratio and serum bone-specific alkaline phosphatase (ALP) levels (means and 95% CIs) after liver transplantation are shown for the zoledronic acid and the placebo groups. The normal reference ranges are 3.0 to 7.4 nmol/mmol for deoxyypyridinoline and 4 to 21 µg/L for bone-specific ALP. At 0, 1, 3, 6, 9, and 12 months, respectively, the numbers of patients were 25, 30, 30, 28, 30, and 27 for the zoledronic acid group and 23, 24, 25, 25, 25, and 21 for the placebo group for urinary deoxyypyridinoline and 25, 28, 28, 26, 28, and 29 for the zoledronic acid group and 23, 23, 22, 26, 21, and 16 for the placebo group for bone-specific ALP. The zero time point represents baseline data collected before liver transplantation; the subsequent time points are the number of months after transplantation. * $P < 0.01$. † $P < 0.05$ for individual time points, zoledronic acid versus placebo.