High incidence of vertebral osteoporotic fracture within the first year after liver transplantation

S. Butin[1], I. Griffoul[1], F. Espitalier[2], E. Salamé[3,4], D. Mulleman[1,4], P. Goupille[1,4]

[1]Department of Rheumatology, [2]Department of Anesthesia and Intensive Care, [3]Department of Hepatobiliary Surgery and Transplantation, CHRU de Tours, France; [4]Université François-Rabelais de Tours, France.

Abstract

Objective

Bone loss is a complication for patients with liver diseases and after transplantation, which results in increased fracture risk. The aim of this study was to determine the incidence of osteoporotic vertebral fractures following liver transplantation.

Methods

We performed a prospective study of patients who were awaiting liver transplantation. Patients were seen at baseline (visit 1) and one year after transplantation (visit 2). At each visit, risk factors of osteoporosis were collected, biochemical tests were performed and bone mineral density with Vertebral Fracture Assessment was assessed.

Results

One hundred and fifteen patients were in the pre-transplant group and 33 patients were in the post-transplant group. In the pre-transplant group, the prevalence of vertebral fractures was 23.5%. The prevalence of densitometric osteoporosis was higher at the lumbar spine than at the femoral neck. In the post-transplant group, the prevalence of vertebral fractures at visit 1 and visit 2 was 33.3% and 60.6%, respectively with an incidence of 23.1 fractures per 100 patient-years.

Conclusion

Bone fragility was highly prevalent before transplantation and worsens one year after transplantation. Bone status should be evaluated in patients with liver diseases before transplantation to identify patients at high risk of fracture and help clinicians to prescribe appropriate preventive care.

Key words

osteoporosis, bone mineral density, spinal fracture, liver transplantation, end stage chronic liver disease

Sarah Butin, MD
Isabelle Griffoul, MD
Fabien Espitalier, MD
Ephrem Salamé, MD, PhD
Denis Mulleman, MD, PhD
Philippe Goupille, MD, PhD

Please address correspondence to:
Dr Isabelle Griffoul,
Department of Rheumatology,
Centre Hospitalier Universitaire de Tours,
37044 Tours cedex 9, France.
E-mail: i.griffoul@chu-tours.fr

Received on September 9, 2016; accepted in revised form on March 7, 2017.

Competing interests: none declared.

Introduction

Bone loss is a well-known complication of organ transplantation particularly in patients awaiting liver transplantation and during the first year after transplantation (1-3). This bone fragility increases the risk of severe fractures, which themselves increase morbidity and mortality (4-6).

The mechanisms of this bone fragility are complex and poorly understood. Hepatic osteodystrophy, a term that encompass osteomalacia and osteoporosis is used to describe bone diseases in patients with chronic liver diseases (7). Chronic liver diseases such as cholestatic liver disease, cholangitis primary sclerosing, alcoholic cirrhosis, hepatitis C infection and hereditary haemochromatosis are associated with bone loss. Thus, the incidence of osteoporotic fractures in transplant patients depends on the severity and duration of the underlying liver disease on the one hand, and on bone status before transplantation, on the other hand. The prevalence of osteoporotic fracture in the year following transplantation varies from 22 to 65% of patients (8, 9).

The aim of the present study was to determine the incidence of osteoporotic fractures one year after liver transplantation. The secondary objective was to describe bone turnover and bone mineral density (BMD) in liver transplant patients.

Methods

Patients and study design

We included prospectively all patients awaiting liver transplantation in the department of liver surgery at the University hospital of Tours, France, between September 2011 and July 2014. Candidate for multiple transplantations (liver and kidney or lung) were excluded. This study was registered with the national medicines agency: 2013-A00654-41. The protocol and the clinical data collection were approved by our ethics committee and institutional review board.

Data collection

The following characteristics were collected at baseline (visit 1): age, sex, weight (kg), height (cm), relevant medical history (*i.e.* diabetes mellitus, thyroid disease, neoplasia and HIV infection) and underlying liver disease (alcoholic cirrhosis, viral hepatitis, metabolic diseases (haemochromatosis, glycogen storage disease), autoimmune hepatitis, drug-induced liver-injury, hepatocellular carcinoma). Risk factors for bone fragility such as personal history of fracture, history of fracture of the proximal femur in a first degree relative, corticosteroids use ≥7.5 mg /d of prednisone for ≥3 months, drug inducing bone fragility, past or current smoking, past or current alcoholism, endocrinopathy, daily intake of calcium estimate (normal, ≥850 mg/d) and Fardellone questionnaire (normal ≥1000 mg/d) (10), age at menopause and hormone replacement therapy, were collected.

Blood tests were systematically performed at each visit including calcium (normal, 2.2–2.6 mmol/L), phosphate (normal, 0.8–1.45 mmol/L), creatinine (normal <100 μmol/L), creatinine clearance (mL/min) according to MDRD or Cockroft (renal function was classified as "impaired" if clearance <60 mL/min), alanine aminotransferase (ALT) (normal, 5–35 UI/L) and aspartate aminotransferase (AST) (normal, 10 - 35 UI/L), albumin (normal, 37–47 g/L), proteins (normal, 65–80 g/L), osteocalcin assay by ELISA kit (IDS-isys) (normal, 10–46 ng/mL), CTX assay by ELISA kit (eclia-cobas Roche) (normal, 0.33–0.78 μg/L for postmenopausal women and 0.16–0.44 μg/L for premenopausal women and men), 25-OH Vitamin D3 (normal, >75 nmol/L), parathyroid hormone (PTH) (normal, 11.5–78.4 pg/mL), thyroid stimulating hormone (TSH) (normal, 0.2–3.4 mUI/L).

BMD was measured at each visit using dual-energy x-ray absorptiometry (DXA) at the lumbar spine (vertebrae L2–L4) and at the left femoral neck, expressed in g/cm^2 (Lunar iDXA SN 200486). World Health Organisation (WHO) criteria were used to define osteoporosis (T-score of -2.5 SD or less) (11). A vertebral fracture assessment (VFA) aiming at the detection of vertebral fracture was performed except for patients with dorsal and lumbar spine x-rays performed in the last 3 months.

Follow-up of transplant patients

Patients who underwent liver transplantation were seen at visit 2, one year after transplantation, between July 2013 and September 2014. Clinical data, blood tests and DXA were performed as for visit 1. One day after transplantation, patients with a weight between 35 and 54 kg received corticosteroids dose of 240 mg, those between 55 and 74 kg received 350 mg, those between 75 and 94 kg received 450 mg, those between 95 and 114 kg received 500 mg. Then the dose was gradually decreased each day depending on patient weight. Eight days after transplantation, each patient, whatever its weight, received corticosteroids dose of 20 mg. All patients received tacrolimus or cyclosporine.

End points

The primary assessment criterion was the number of incident vertebral fractures after one year following transplantation. The event was defined by the occurrence of a fracture on x-ray or on VFA.

Statistical analysis

Continuous variables were expressed as median and interquartile range [IQR: 25%–75%] or mean, standard deviation (± SD) and minimum - maximum. Categorial variables were expressed as number (percentage). The incidence of vertebral factures between transplantation and visit 2 were expressed as the number of new fractures per 100 patient-years. After description of patient's characteristics, transplant patients were analysed by comparing data at visit 1 and visit 2. R 2.12.1 software (http://www.R-project.org, the R Foundation for Statistical Computing, Vienna, Austria) was used for analysis. Continuous variables were compared using the Wilcoxon test. Categorial variables were compared using the Fisher exact test. Continuous matched variables were compared using the Wilcoxon paired-test. Categorial matched variables were compared using the McNemar test.

Results

Patients

One hundred and twenty-one patients were seen at visit 1. Six patients were excluded (2 with multiple transplantations, and 4 with missing blood tests). Thus, 115 pre-transplant patients were included in this study. Twenty-three patients died before being transplanted and 59 patients were still waiting for transplantation. Thirty-three transplant patients were seen at visit 2 between July 2013 and September 2014. The median time interval between visit 1 and liver transplantation was 9 months [IQR: 5; 10] and 13 months [IQR: 12; 16] between transplantation and visit 2.

Pre-transplant patients group (n=115)

The main characteristics of the 115 pre-transplant patients group are presented in Table I. Among the 115 patients, 43 (37.4%) had alcoholic cirrhosis, 15 (13.1%) had viral liver disease, 24 (20.9%) had alcoholic and viral cirrhosis, 16 (13.9%) had alcoholic cirrhosis and non-alcoholic steato-hepatitis (NASH), 2 (1.7%) had autoimmune hepatitis, 12 (10.4%) had other liver diseases, 3 (2.6%) had primitive hepatocellular carcinoma. Twenty-two patients (19.1%) reported a personal history of severe osteoporotic fracture, 2 (1.7%) with a vertebral fracture, 20 (17.4%) with a peripheral fracture. Forty-eight (41.7%) patients had received one or more drugs that could affect bone strength (mainly proton pump inhibitors). Blood test results are presented in Table I. Twenty-six patients (22.6%) were considered as in the normal range for calcium intake and 10 patients (8.7%) had sufficient vitamin D3 level. Three patients (2.6%) received bisphosphonate before visit 1. Eight patients (7%) were prescribed with bisphosphonate after visit 1.

The prevalence of vertebral fractures (on VFA) was 23.5% (27/115). Bone parameters are presented in Table II. Five patients (4.3%) had a high level of bone turnover. Four patients (3.5%) had an isolated increase of bone resorption markers and 5 (4.3%) had an isolated increase of bone formation markers. The number of osteoporotic patients in total, as assessed by BMD, was 17/115 (14.8%). The number of osteoporotic patients was 10/115 (8.9%) at femoral neck and 15/115 (13%) at lumbar spine.

Table I. General characteristics of pre-transplant patients at visit 1.

	n=115
Demographic characteristics [a]	
Female sex	25 (21.7)
Age (year)	59 [53; 63]
Weight (kg)	77 [67; 86]
BMI (kg/m²)	27 [23; 30]
Medical history [a]	
Diabete mellitus	29 (25)
Neoplasia other than HCC	6 (5)
HIV infection	1 (0.9)
Risk factors for osteoporosis [a]	
Personal history of severe fractures	22 (19.1)
Familial history of upper femur fracture	9 (8.9)
Use of alcohol	84 (73.0)
Glucocorticoids >7.5 mg/d for over 3 months	12 (10.5)
Use of tobacco	73 (63)
Dysthyroidism	4 (3.7)
Menopause <40 years old	2 (1.7)
HRT	2 (1.7)
Calcium intake <1000 mg/d	89 (77.4)
Blood tests [a]	
Calcium (mmol/L) [b]	2.3 [2.3; 2.4]
Phosphate (mmol/L)	1.1 [1.0; 1.3]
AST (IU/L)	54 [38; 81]
ALT (IU/L)	33 [23; 57]
Clearance <60 ml/min	10 (8.7)
Proteins (g/L)	71 [66; 77]
Albumin (g/L)	35 [31; 40]
25-OH vitamin D (nmol/L)	29 [17; 45]
PTH (pg/L)	31 [23; 48]
TSH (mUI/L)	2.0 [1.3; 2.8]

[a] results are expressed as number (%) or median [interquartile range].
[b] albumin-corrected.
BMI: body mass index; HCC: hepatocellular carcinoma; HRT: hormone replacement therapy; ALT: alanine aminotransferase; AST: aspartate aminotransferase; PTH: parathyroid hormone; TSH: thyroid stimulating hormone.

Transplant patients group (n=33)

The main characteristics of the 33 transplant patients are summarised in Table III. Median age at transplantation was 59 years [IQR: 51; 63]. Among the 33 patients, 13 (39.4%) had alcoholic cirrhosis, 4 (12.1%) had viral liver disease, 4 (12.1%) had alcoholic and viral cirrhosis, 6 (18.2%) had alcoholic cirrhosis and non-alcoholic steato-hepatitis (NASH), 1 (3%) had autoimmune hepatitis, 3 (9.1%) had other liver diseases, 2 (6.1%) had primitive hepatocellular carcinoma. There was no dif-

Table II. Bone status comparison between patients with and without vertebral fracture in pre-transplant patients.

	All patients, n=115	Patients with VF, n=18	Patients without VF, n=97
T-score LS	-0.8 [-1.8 ; -0.1]	-1.1 [-2.3; -0.6]	-0.7 [-1.8; 0.1]
BMD LS	1.098 [0.989; 1.202]	1.049 [0.901; 1.12]	1.103 [0.991; 1.223]
T-score FN	-1.0 [-1.7; 0.1]	-1.6 [-2.4; -1.1]*	-1.0 [-1.6; -0.1]*
BMD FN	0.896 [0.786; 0.999]	0.778 [0.710; 0.870]*	0.909 [0.815; 1.012]*
CTX (µg/L) [a]	0.39 [0.26; 0.54]	0.41 [0.33; 0.60]	0.39 [0.24; 0.53]
Osteocalcin (ng/mL) [b]	17 [12; 23]	18.05 [15.5; 26]	17 [11.9; 22]

Results are expressed as median [interquartile range].
[a] normal values 0.33-0.78 for postmenopausal women and 0.16-0.44 for premenopausal women and men. [b] normal values 10-46.
*p<0.05. VF: vertebral fracture; LS: lumbar spine; FN: femoral neck; BMD: bone mineral density (g/cm^2).

Table III. Comparison between visit 1 and visit 2 of transplant patients.

n=33	Visit 1	Visit 2
Demographic characteristics [a]		
Weight (kg)	79 [73 ; 88]	78 [67 ; 87]
BMI (kg/m^2)	28 [24 ; 32]	28 [24 ; 31]
Risks factors of osteoporosis [a]		
Glucocorticoids >7.5 mg/d for over 3 months	4 (12)	23 (69.7)
Calcium intake <1000 mg/d	26 (79)	23 (70)
Blood tests [a]		
Calcium (mmol/L) [b]	2.3 [2.2; 2.4]	2.3 [2.2; 2.4]
Phosphate (mmol/L)	1.1 [1.0; 1.3]	1.1 [0.9; 1.2]
AST (UI/L)	52 [39; 71]*	23 [20; 52]*
ALT (UI/L)	29 [21; 53]	25 [19; 52]
Clearance < 60 ml/min	4 (12)	9 (27)
Proteins (g/L)	71 [64; 77]	71 [68; 73]
Albumin (g/L)	35 [30; 40]*	41 [40; 45]*
25-OH vit D3 (nmol/l)	29 [17; 48]	36 [28; 50]
PTH (pg/l)	29 [21; 37]*	46 [39; 69]*
TSH (UI/l)	2.1 [1.5; 3.1]	1.7 [1.1; 2.4]

[a] results are expressed as number (%) or median [interquartile range]. [b] albumin-corrected. *p<0.05.
BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; PTH: parathyroid hormone; TSH: thyroid stimulating hormone.

ference in terms of comorbidity and risk factor for osteoporosis, between transplant and pre-transplant patients groups. At visit 1, 11 (33.3%) patients had a personal history of severe fracture, 1 (3%) with a vertebral fracture, 10 (30.3%) with non vertebral fracture. At visit 1, among the 33 patients, 14 (42.4%) received at least one treatment that could affect bone strength (mainly corticosteroids, 12/14), and at visit 2, 23 (69.7%) received immunosuppressive drugs. The mean cumulative dose of corticosteroid per patient during the first seven days post-transplant was 890 mg. Blood test results are presented in Table III. At visit 2, 10 patients (30.3%) were considered as in the normal range for calcium intake and one patient (3%) had sufficient vitamin D3 level. None of the patients received bisphosphonate after visit 1. Thirty patients (90.9%) received a supplement of vitamin D.

The prevalence of vertebral fractures (on VFA) at visit 1 and visit 2 were 33.3% (11/33) and 60.6% (20/33), respectively. Bone parameters are presented in Table IV. At visit 1, one patient had an isolated increase of bone resorption markers and one had an isolated increase of bone formation markers. At visit 2, 7 patients (21.2%) had an increase of bone turnover markers, 9 (27.2%) had an isolated increase of bone resorption markers and 11 (33.3%) had an isolated increase of bone formation markers. At visit 1, the number of osteoporotic patients in total, as assessed by BMD, was 4/33 (12.1%). The number of osteoporotic patients was 3/33 (9.1%) at the femoral neck and 4/33 (12.1%) at the lumbar spine. At visit 2, the number of osteoporotic patients in total, as assessed by BMD, was 6/33 (18.2%). The number of osteoporotic patients, as assessed by BMD, was 5/33 (15.1%) at the femoral neck and 4/33 (12.1%) at the lumbar spine.

Fracture incidence in transplant patients (n=33)

At visit 2, on VFA, 7 patients (21.2%) had at least one incident vertebral fracture (5 had 1 and 2 had 2). All were asymptomatic and none of the patients had lombar x-ray during the two visits. Among them, 3 already had a prevalent vertebral fracture. Thus, vertebral fracture incidence was 23.1 per 100 patient-years. No peripheral fracture occurred during the study period.

Discussion

In this monocentric prospective study, the incidence of vertebral fracture after liver transplantation was 23.1 per 100 patient-years. The prevalence of vertebral fractures before and after transplantation was 33.3% and 60.6%, respectively, so 21.2% of our patients had an incident fracture within the 13 months after transplantation. By contrast, no patient had an incident non-vertebral fracture. Our results are in agreement with previous studies reporting that patients with severe liver diseases have bone fragility and an increased risk of fracture within the first months after transplantation (4, 6, 12). In a monocentric retrospective study, Krol *et al*., found a fracture incidence of 34% one year after transplantation (13). Leidig *et al*. studied the bone status of 235 liver or heart transplant patients and found a fracture incidence of 25.4% one year after transplantation (4). In a multivariate analysis, prevalent vertebral fractures before transplantation were highly predictive of incident fractures after transplantation. Guichelaar *et al*. found a vertebral fracture rate of 10%, 18.4% and 22.3% respectively at 4 months, 1 year and 2 years after transplantation (12).

The causes of bone loss in transplant patients are numerous. Immunosuppressants, including glucocorticoids, play a major role (6). For some authors, many risk factors such as age, female sex, malnutrition, low body mass in-

Table IV. Bone status comparison between transplant patients at visit 1 (before transplantation) and visit 2 (after transplantation).

n=33	Visit 1	Visit 2
T-score LS	-0.5 [-1.3; -0.1]	-0.75 [-1.5; -0.17]
BMD LS	1.127 [1.022; 1.181]	1.124 [1.081; 1.168]
T-score FN	-0.6 [-1.45; 0.3]*	-1.4 [-2.1; -0.6]*
BMD FN	0.942 [0.828; 1.031]*	0.867 [0.779; 0.974]*
CTX serum (µg/L)[a]	0.34 [0.20; 0.50]*	0.49 [0.32; 0.88]*
Osteocalcin (ng/mL)[b]	15.3 [11.3; 21.7]*	30 [23; 47]*

Results are expressed as median [interquartile ranges]. *$p<0.05$.
[a] normal values 0.33-0.78 for postmenopausal women and 0.16-0.44 for premenopausal women and men. [b] normal values 10-46.
LS: lumbar spine; FN: femoral neck; BMD: bone mineral density (g/cm^2); VF: vertebral fracture.

dex (BMI), past history of fractures and cumulative dose of corticosteroids, lead to bone fragility in liver transplant patients. Nearly a quarter of transplant patients in our study had a high level of bone turnover, while none had before transplantation. Cumulative glucocorticoid dose was responsible for part of bone loss by a decoupling of bone remodeling, with a decrease in bone formation and an increase in bone resorption, early after transplantation. Guichelaar *et al*. have studied the mechanisms of bone loss by performing bone biopsies in 33 liver transplant patients (14). Bone loss was observed from the time of transplantation to 4 months after transplantation with a decrease in BMD at lumbar spine and histomorphometric parameters of bone volume. Markers of bone formation were low during pre-transplantation period and returned to normal after transplantation whilst markers of bone resorption were increased before and after transplantation. The authors have shown an overall improvement in bone metabolism from the fourth month after transplantation with a return to a balanced bone remodelling. In our study, there was an increase in bone formation markers and bone resorption one year after transplantation. The increase in bone resorption has also been attributed to secondary hyperparathyroidism but this mechanism appears to be now questionable (15). We observed a significant increase in PTH concentrations after liver transplantation while 32 of our patients were receiving vitamin D, explaining the increase tendency in 25-OH vitamin D3 concentrations, although below the normal values, after transplantation. Two hypotheses may be raised to explain these finding. First, calcineurin inhibitors such as tacrolimus and cyclosporine, elicit a negative calcium balance and therefore an increase in PTH (16). Indeed, these molecules have an effect on calcium reabsorption in the distal convoluted tubule of the kidney, by acting on the transient receptor potential-vanilloid-5 (TRPV5) that regulates urinary calcium excretion (17). In addition, glucocorticoids cause inhibition of intestinal absorption and inhibit tubular reabsorption of calcium. Intestinal action of glucocorticoids is antagonistic of vitamin D and there is a reduction of the expression of specific calcium channels in the duodenum. Second, a worsening of renal fonction, possibly ascribable to immunosuppressant drugs, may have resulted in a mild increase in PTH (16).

In our study, a decrease of femoral neck BMD of 8% was found one year after transplantation, while BMD at the lumbar spine remained stable. In a monocentric retrospective study, Krol *et al*. found a decrease of BMD at both sites (-2.5% at the lumbar spine and -6.5% at the femoral neck) 6 months after liver transplantation with a stabilisation until one year after transplantation. These results are related to the difference in proportion of cortical bone and trabecular bone between these two sites. The femoral neck is mainly composed of cortical bone, whereas the lumbar spine is mainly composed of trabecular bone. Bone status was assessed one year after transplantation which may explain the stability of the BMD at the lumbar spine. Trabecular bone is more susceptible to change than cortical bone either before or after transplantation. After transplantation, the restoration of liver function with a reversal of cholestasis, vitamin D deficiency and hypogonadism lead to an improvement of the BMD at the lumbar spine.

Our study has several strengths that we would like to emphasise here. Our study on pre-transplant patients is one of the few that have analysed BMD and vertebral fractures, as assessed by VFA, before and after liver transplantation. Among transplant patients, no symptomatic fracture occurred. VFA allowed us to detect a significant number of asymptomatic vertebral fractures. Given our results we believe that all physicians should be aware of bone fragility in the context of liver transplantation, evaluate bone status and provide lifestyle advice or appropriate medication in order to prevent further fractures.

For all patients awaiting liver transplantation and in the 6 first months post transplantation we recommend a bone status assessment: risk factors for bone fragility, BMD with a VFA, biological parameters (calcium and phosphate, PTH, 25 OH vitamin D, renal function, thyroid function and testosterone in men; bone parameters are not necessary). Thus, reversible causes of bone loss may be identifiable and treated. Before liver transplantation, we propose to start an anti-osteoporotic drug if a severe fracture or a vertebral fracture on VFA is found or if T-score <-3. Teitelbaum suggests that bone loss following glucocorticoid treatment is most robust within the first 3 to 6 months (18). Therefore early after liver transplantation, in practice we should start an anti-osteoporotic drug if corticosteroids are introduced over 3 months.

This study had some limitations. First, the number of post-transplant patients was rather low, which limited the interpretation of statistical results. Second, the median time between visit 1 and transplantation was an average of 9 months, which may cause a bias in the results. Indeed, an asymptomatic vertebral fracture, identified by VFA after liver transplantation, may have occurred during this period and not after transplantation. Third, the use of VFA

has its own limitations since vertebral fractures are less well detected than with x-rays, on thoracic spine and in the case of scoliosis (19). Finally, the use of biological markers of bone remodelling in this population can be discussed for two reasons: i) blood samples must be done when fasting and in the same laboratory for comparison. Although all visits were scheduled some patients may be not be fasting at the time of blood sampling; ii) in patients with chronic liver disease, markers of bone turnover are less reliable, due to liver collagen metabolism changes induced by fibrogenesis, than in post-menopausal women.

In conclusion, patients with chronic liver disease are at risk of fragility fracture and this fragility worsens significantly and rapidly after transplantation, resulting in subsequent incident vertebral fractures. It seems essential to investigate bone status extensively and, if necessary, correct risk factors or/and start anti-osteoporotic treatment. Moreover, a close follow-up after transplantation seems essential to improve bone health in this context.

Acknowledgments

We would like to thank Nelly Jaccaz-Vallée, Fabienne Chalier for blood sampling. We thank the outpatient liver transplant and liver transplant department and particularly Sandra Duarte and Agnès Robert for giving us the list of patients awaiting transplantation. We thank Dr Virginie Martaillé, Dr Francine Lauféron and Dr Mathilde Marot for their contribution in collecting clinical data and performing BMD and VFA. We are grateful to Prof. Gérard Chalès and Dr Salliot for offering their advice.

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