ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DOTTORATO DI RICERCA IN

Scienze Mediche Generali e Scienze dei Servizi

Ciclo 36

Settore Concorsuale: 06/D2

Settore Scientifico Disciplinare: MED/13

Retrospective assessment of fracture risk through opportunistic radiological screening in a large modern cohort of liver transplant recipients

Presentata da: *Guido Zavatta*

Coordinatore Dottorato **Supervisore**

Prof.ssa Susi Pelotti **Prof. Uberto Pagotto**

Esame finale anno 2024

Abstract:

Objective: Liver transplantation has been associated with a high prevalence of osteoporosis, although most data rely on single-center studies with limited sample size, with most of them dating back to late 1990s and early 2000s. The present thesis aims to assess the prevalence of fragility fractures and contributing factors in a large modern cohort of liver transplant recipients managed in a referral Italian Liver Transplant Center.

Design and Methods: Paper and electronic medical records of 429 consecutive patients receiving liver transplantation from 1/1/2010 to 31/12/2015 were reviewed, and 366 patients were selected. Clinically obtained electronic radiological images within 6 months from the date of liver transplant surgery, such as lateral views of spine X-rays or CT abdominal scans, were opportunistically reviewed in a blinded fashion to screen for morphometric vertebral fractures. Clinical fragility fractures reported in the medical records, along with information on etiology of cirrhosis and biochemistries at the time of liver surgery were also recorded.

Results: Prevalence of fragility fractures in the whole cohort was 155/366 (42.3%), with no significant differences between sexes. Of patients with fractures, most sustained vertebral fractures (145/155, 93.5%), the majority of which were mild or moderate wedges. Multiple vertebral fractures were common (41.3%). Fracture rates were similar across different etiologies of cirrhosis and were also comparable in patients with diabetes or exposed to glucocorticoids. Kidney function was significantly worse in women with fractures. Independent of age, sex, alcohol use, eGFR, etiology of liver disease, lower BMI was the only independent risk factor for fractures (adjusted OR 1,058, 95%CI 1,001-1,118, P=0.046) in this study population.

Conclusions: A considerable fracture burden was shown in a large and modern cohort of liver transplant recipients. Given the remarkably high prevalence of fractures, a metabolic bone disease screening should be implemented in every patient awaiting liver transplantation.

Key words: liver transplantation, cirrhosis, liver failure, vertebral fractures, morphometric,

osteoporosis, mortality, secondary osteoporosis.

Abbreviations:

- NAFLD: non-alcoholic fatty liver disease
- NASH: non-alcoholic steatohepatitis
- ALD: alcoholic liver disease
- PPI: Proton-pump inhibitors
- PBC: primary biliary cholangitis
- PSC: primary sclerosing cholangitis

Table of contents: Page

Introduction 4

Epidemiology 4

Etiology 4

General overview 5

Pathophysiology of osteoporosis in cirrhosis 10

Aims of the present thesis 16

Patients and Methods 16

Statistical analysis 20

Results 21

Discussion 34

Strengths and limitations 37

Conclusions 38
References 40
Supplementary material 45

Introduction

Liver cirrhosis results from chronic inflammation to the liver. It is characterized by fibrosis and regenerative nodules which completely subvert the normal hepatic architecture and can lead to liver failure **(1)**. Chronic inflammation of the liver does not cause cirrhosis in every patient and timing of progression varies from weeks to decades depending on the etiological mechanism.

Epidemiology

In 2017, the number of people in the world with chronic liver disease was estimated to be 1.5 billion and the main etiologies of chronic liver disease were NAFLD (60%), HBV (29%), HCV (9%) and ALD (2%) **(2)**. Based on data from the Global Burden of Disease study, the age-standardized incidence rate of cirrhosis and chronic liver disease was 20.7 per 100,000 people in 2015, increased by 13% compared to 2000. The estimated incidence of cirrhosis in Europe is 26 per 100,000 people while the incidence in Asia reaches 16.5 per 100,000 people in the eastern regions and 23.6 per 100,000 people in the south-east. Chronic liver disease, cirrhosis and liver cancer are responsible for 3.5% of all deaths worldwide **(2)**.

The most frequent causes of chronic liver disease are hepatitis B virus, hepatitis C virus, alcohol (ALD) and non-alcoholic fatty liver disease (NAFLD). Over the years, the epidemiology of chronic liver disease and cirrhosis has changed: the main factors are the vaccination campaigns against HBV, the improvement of treatments against HCV, the obesity epidemic and the increase in measures against addiction from alcohol **(2)**.

Globally, the incidence of HBV infection and its complications have decreased thanks to widespread vaccination and antiviral treatments. On the contrary, although many patients with chronic HCV infection have been successfully treated by new direct antiviral drugs (DAAs), the enormous diffusion of opioid drugs and their intravenous use has led to an increase in acute HCV infections. NAFLD is

also increasing worldwide, in relation to the increase in the incidence of obesity and metabolic syndrome **(2)**. Finally, over 75 million people in the world abuse alcohol and are at risk of developing chronic alcohol-related liver disease **(1)**. Alcohol consumption is responsible for 27% of deaths worldwide from chronic liver failure, with the highest mortality rate in Europe **(2)**. A further increase in the incidence of ALD and NAFLD **(1)** is expected in the coming decades.

Etiology

The underlying causes of cirrhosis can be grouped into **(1)**:

- Viral: hepatitis B, hepatitis C, hepatitis D.

- Alcohol-related: ALD.

- Metabolic and genetic: NAFLD, hemochromatosis, Wilson's disease, α-1 antitrypsin deficiency, cystic fibrosis, lysosomal acid lipase deficiency, progressive familial intrahepatic cholestasis, type I tyrosinemia, type IV glycogen storage disease.

- Autoimmune: autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis.

- Biliary: biliary atresia, biliary stenosis.

- Vascular: Budd-Chiari syndrome, venous-occlusive disease, cardiac cirrhosis.

- From drugs: methotrexate, amiodarone, methyldopa, vitamin A.

- Cryptogenic.

General overview

Osteopenia, osteoporosis, and fragility fractures are complications frequently observed in patients with chronic liver disease and cirrhosis. The prevalence of bone disease in patients with cirrhosis is

approximately 12-55% and is therefore higher than that of the general population **(3,4).** Furthermore, over 40% of patients with chronic liver disease may experience fragility fractures **(5,6).** This is particularly true in patients with cholestatic diseases and hemochromatosis **(7)** and the patients being at most risk of fragility fractures are often candidate for transplantation. In fact, numerous studies have shown that the prevalence of osteoporosis is related to the severity of liver disease. In a 1997 study conducted on 58 cirrhotic patients undergoing transplantation, 43% had osteoporosis diagnosed by at least one of the following criteria: vertebral fracture and/or BMD of the lumbar vertebrae < 2 SD compared to the mean values of healthy subjects of the same age; in this study alcohol-abusers and patients with more severe liver disease (patients in class C according to the Child-Pugh classification) had the lowest BMD values **(8)**.

In a 2010 study of 64 patients with chronic liver disease, including 48 females and 16 males, the prevalence of osteoporosis was 45.5%, significantly higher than the prevalence of osteoporosis in healthy controls of the same age and sex; furthermore, 5.3% of patients had fractures of the dorsal or lumbar vertebrae. It was shown that cholestasis, female sex and low body weight were important risk factors for osteoporosis **(9)**.

The diagnosis of osteoporosis in patients with chronic liver disease often requires instrumental tests because it is well known that up to one third of vertebral fractures can be asymptomatic **(10)**. Vertebrae appear to be the most common fracture site in patients with chronic liver disease while fractures of the femoral neck seem relatively uncommon, most likely because femur fractures usually occur later in life, but life expectancy of the majority of patients with cirrhosis is not as long as that of the general population **(10)**.

Osteoporosis in cholestatic liver disease (PBC and PSC)

Studies regarding the association between bone disease and chronic liver disease have focused more on cholestatic diseases, which is primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) **(11)**.

The prevalence of osteoporosis in patients with PBC ranges between 20-44% **(12)**. It is not clear whether osteoporosis occurs already in the early stages of liver disease, which are characterized by cholestasis in the absence of significant liver fibrosis. Of note, it has been shown that BMD reduction is related to the degree of severity of liver disease **(13)**.

In a study conducted on 25 women with PBC and low BMD, it was observed that the BMD of the lumbar spine decreased by more than 3.5% in just 6 months **(14)**. In another study conducted on 210 women with PBC, BMD of the patients' lumbar vertebrae was 7% lower than that of healthy patients of the same age; furthermore, the average BMD loss was estimated to be up to 2% per year **(15)**.

Other factors that have been associated with increased osteoporosis risk in PBC include the duration of liver disease and the degree of cholestasis, which reflects the severity of liver disease **(13)**. Furthermore, PBC is more common in post-menopausal women, who already are at increased risk of osteoporosis **(12)**. In fact, Mounach A. et al., highlighted that the prevalence of osteoporosis and bone fractures are increased in women with PBC compared to the general population and that, low BMI, menopause, duration of the disease and vitamin D deficiency were the main risk factors **(16)**.

Individuals with PSC have multiple risk factors for osteoporosis: they may be cirrhotic, they may have cholestasis, and furthermore, they may have been taking corticosteroid therapy for many years to treat an inflammatory bowel disease such as ulcerative colitis (IBD occurs in over 90 % of patients with CSP **(12)**). In a prospective study conducted on 81 patients with CSP followed for 5 years, lumbar spine BMD was lower than that of healthy controls of the same age and sex. Of these patients, 17% of them had osteoporosis and 3% bone fragility fractures. Moreover, patients with PSC who experienced fractures were older and had long-standing inflammatory bowel disease and more advanced liver disease **(17)**.

Osteoporosis in Wilson's disease

In Wilson's disease, copper accumulates both within the liver, causing chronic liver disease, and in the bone. Copper accumulation in the bone is detrimental to the bone, with osteoporosis being documented in 43% of adults and 68% of children with Wilson's disease **(18,19)**. Treatment with copper chelating drugs such as penicillamines, to date, appears to have no positive effect on bone metabolism **(5,18)**.

Osteoporosis in viral liver diseases

The prevalence of osteoporosis in patients with cirrhosis due to viral etiology is similar to that of patients with cirrhosis due to other causes and ranges between 20 and 53% **(12).** In a study conducted on 32 male patients with cirrhosis of viral etiology, it was observed that over 50% had osteoporosis with a T-score of the lumbar vertebrae or femoral neck < -2.5 **(20)**.

In another study conducted on 74 males with hepatitis B or C with cirrhosis, 20% of patients had osteoporosis in the lumbar spine and 6.7% had vertebral fractures, along with a significantly lower BMD compared to healthy controls **(21)**. Numerous studies have investigated the effect of antiviral treatment on bone metabolism. It has been observed that ribavirin and interferon used in antiviral therapy induce bone loss but, by contrast, increase BMD in patients exhibiting a significant viral response **(22)**.

Tenofovir disoproxil fumarate (TDF) is a nucleotide analogue, approved in 2001 for the treatment of HIV and more recently for the treatment of chronic HBV infection. BMD loss, particularly in the femur and spine, is a major side effect. The relationship between metabolic bone disease and TDF has been documented in several studies conducted on patients infected with HBV and HIV and treated

with HBV therapy. TDF has also been identified as an independent risk factor along with age, smoking and body weight **(22)**.

Osteoporosis in alcoholic liver disease

Alcohol abuse is an independent risk factor for osteoporosis, so that BMD is low even in patients affected with alcohol abuse who do not still have cirrhosis. In particular, alcoholism is associated with an increased risk of hip fracture of 2.8 **(13)**. In a study of 76 men who had been drinking 27 or more units of alcohol per day for more than 24 years, only 22% of them had liver abnormalities on histological examination, although the majority of them had lower lumbar vertebral BMD than controls of the same age. Importantly, 30% had vertebral fractures, of which only 4% symptomatic **(23)**.

Osteoporosis in non-alcoholic fatty liver disease - NAFLD

NAFLD is one of the complications of metabolic syndrome and is also considered a risk factor for low BMD, as well as a risk factor for osteoporosis **(12)**. In a retrospective study including more than 7000 patients, NAFLD was associated with a 2.5-fold increased risk of osteoporotic fractures regardless of gender **(24)**. Another study demonstrated that simple steatosis did not increase the risk of fractures while non-alcoholic steatohepatitis (i.e. characterized by high blood levels of ALT and C-reactive protein) was associated with reduced bone mass **(25)**.

First Author and Year of Publication	Number of patients	Type/Etiology	Findings

Monegal A., et al., 1997 [8]	58	Patients with cirrhosis of variable etiology	43% of patients had osteoporosis and among these, alcohol abusers and patients with more advanced liver disease had lower BMD
Wariaghli G., et al., 2010 [9]	64	Patients with chronic liver disease	45.5% had osteoporosis and 5.3% had dorsal or lumbar vertebral fractures
Camisaca M., et al., 1994 [14]	25	Women with PBC and reduced BMD	Lumbar spine BMD dropped by more than 3.5% in 6 months
Eastell R., et al., 1991 [15]	210	Women with PBC	Lumbar spine BMD was 7% lower than lumbar spine BMD from healthy women of the same age, and these women experienced an average BMD loss up to 2% per year
Angulo P., et al., 1998 [17]	81	Patients with PSC of which 25% had cirrhosis	Lumbar spine BMD was lower than that of healthy controls of the same age and sex, 17% of patients had osteoporosis and 3% had fragility fractures
Hegedus D., et al., 2002 [19]	21	Wilson's disease	Osteoporosis in 43% of cases
Gallego-Rojo F. J., et al., 1998 [20]	32	Patients with cirrhosis of viral etiology	>50% of patients had osteoporosis
Chen C. C., et al., 1996 [21]	74	Patients with cirrhosis of viral etiology	20% had osteoporosis and 6.7% had fragility fractures

Pathophysiology of osteoporosis in cirrhosis

In patients with chronic liver disease, the balance in bone remodeling activities between osteoclasts and osteoblasts is profoundly altered by the liver disease. Guidelines provided by most osteoporosis societies describe the causes of osteoporotic fractures in patients with chronic liver disease as a result of nutritional deficiencies due to the underlying organ disease **(7, 26)**. However, several studies have suggested that osteoporosis in cirrhotic patients is a multifactorial disease in which different mechanisms act to deteriorate bone mass, thus determining bone fragility **(27)**. Several etiologies may explain chronic liver disease, with different pathogenetic mechanisms **(5)**. For example, while hemochromatosis and cholestatic diseases are respectively characterized by significant increase in

iron and bilirubin, which cause osteoblast inhibition **(28)**, by contrast, viral hepatitis is associated with an activation of the immune response and cytokine release which in turn stimulate bone resorption **(10,29,30)**. Overall, two main pathophysiological mechanisms underlying osteoporosis in patients with chronic liver disease have been thus far recognized, similar to primary osteoporosis: decreased bone formation or increased bone resorption.

Decreased bone formation

Reduced bone formation is due to damage to osteoblasts, which compromise their survival and differentiation **(31)**. This phenomenon has been observed in patients with cholestatic diseases, in fact, in patients with PBC and PSC, high levels of bilirubin determine detrimental effects on osteoblasts **(32)**. A similar toxic effect on osteoblasts can be due to high iron levels in patients with hemochromatosis with low BMD **(33)**. A study conducted on patients with hemochromatosis showed that 25% of them had osteoporosis and 41% had osteopenia **(34)**. Therefore, although the proliferation, differentiation and apoptosis of osteoblasts have not been studied as extensively as for osteoclasts, there is consensus that chronic liver disease exerts a negative effect on the differentiation and proliferation of osteoblasts, thereby causing a general decrease in bone formation **(7)**.

Sclerostin is a soluble protein secreted by osteocytes, whose role is to prevent the binding between *Wnt* and low-density lipoprotein receptor-related proteins 5 (LRP5) and 6 (LRP6) **(35)**. The net effect is blocking the differentiation of osteoblasts and consequently new bone formation **(35).** In a recent study, elevated levels of circulating sclerostin were observed in patients with advanced liver cirrhosis compared to healthy controls or patients with early liver cirrhosis **(36)**.

Osteoblast dysfunction could also be a consequence of decreased levels of trophic factors such as insulin-like growth factor-1 (IGF-1**) (21,37,38)**. Bone is a major target organ for IGF-1, an anabolic hormone produced mostly by the liver under the stimulation of growth hormone (GH) **(39)**. IGF-1

has a crucial function by ensuring the normal longitudinal growth of the bone during the postnatal period and therefore has a key role in the process of bone growth and development **(40)**. IGF-1 reduces osteoblast apoptosis and promotes osteoblast formation by stabilizing β-catenin and enhancing Wnt action **(40,41)**. In advanced liver cirrhosis, serum IGF-1 levels decrease because of decreased hepatocellular biosynthetic function and progressive loss of GH receptors on hepatocytes **(38,42)**.

Hypogonadism and early menopause also play a fundamental role in this context. Low levels of sex hormones (estrogen or testosterone) increase the osteoclast survival and decrease that of osteoblasts, thus leading to relative increased bone resorption rather than bone formation **(40,43,44)**. Chronic liver disease is often associated to changes in estrogen metabolism; as a consequence, there is a reduction in the levels of active estrogen metabolites, which are unable to compensate for the lack of estrogens associated with menopause, and eventually to preserve bone health **(5)**.

Increased bone resorption

Parathyroid hormone (PTH) is a main regulator of calcium homeostasis **(45)** and, in the bone stimulates osteoblasts to form bone and osteoclasts to reabsorb it **(46)**. Osteoclasts do not express the PTH receptor; therefore, bone resorption is associated with molecules produced in response to the action of PTH on osteoblasts, such as RANKL and OPG. RANKL is a soluble protein secreted by osteoblasts which binds to a receptor present on the surface of osteoclasts determining their activation **(46)**. RANKL is also produced by activated immune system cells **(47)**, in fact, it has been observed that T lymphocytes act as mediators of bone loss in ovariectomized mice **(48)**; furthermore, in a series of studies, mice with T cell deficiency were protected from bone loss **(47,48)**. This evidence has demonstrated, indirectly, the effect of chronic inflammation on bone and that the cytokines produced from the liver in patients with chronic liver disease, contribute to the activation of osteoclasts **(48,49,50).** RANK is a homotrimeric transmembrane protein that belongs to the TNF receptor

superfamily **(51)**. Macrophage colony-stimulating factor (M-CSF) has a significant role in osteoclastogenesis as it induces the expression of RANK in osteoclast precursors and stimulates their proliferation; it also stimulates the binding of RANKL to RANK, thus promoting RANK polymerization and signal transduction **(46)**.

Osteoprotegerin (OPG) is produced by osteoblasts and blocks RANKL-mediated osteoclast activation **(51)**. In addition to osteoblasts, OPG is produced by cardiomyocytes, hepatocytes and renal cells, B cells and dendritic cells. Cytokines also play a significant role in bone resorption, in particular IL-6, IL1-β, TNF-α and adipokines. IL-6 is a proinflammatory cytokine produced by osteoblasts and has the role of activating osteoclasts directly or indirectly by stimulating the production of RANKL **(52-54)**. In the liver, IL-6 is up-regulated following liver damage, which stimulates the acute phase response and induces liver regeneration, therefore being elevated in theoretically all patients with chronic liver disease **(55-57).**

IL1-β is a proinflammatory cytokine that stimulates bone resorption: it induces RANKL production and RANKL-mediated osteoclastogenesis. Furthermore, it increases the synthesis of prostaglandins within the bone, which further stimulate bone resorption **(58)**.

TNF-α, an important inflammatory cytokine, also plays a key role in bone resorption by stimulating the expression of RANKL by osteoblasts and stromal cells, by inducing the differentiation and activity of osteoclasts **(59)**. During the early phase of osteoclastogenesis TNF-α increases the pool of osteoclast precursors by stimulating the expression of the genes coding for the colony-stimulating factor 1 receptor; osteoclast precursors then differentiate into mature osteoclasts in the presence of RANKL. This process is sustained and accelerated by TNF **(60)**.

Cytokines derived from the adipose tissue (adipokines) might also play a key role in the pathogenesis of osteoporosis, by inhibiting osteoclastogenesis and reducing RANKL synthesis and by increasing OPG levels. Leptin stimulates the synthesis of proinflammatory cytokines such as IL-1 and TNF-α **(12)**.

Vitamin D and vitamin K

The reduced hepatic hydroxylation of vitamin D plays a significant role in the pathogenesis of metabolic bone disease in patients with chronic liver disease. Vitamin D is synthesized in the skin and subsequently undergoes hydroxylation in the liver, turning into 25-hydroxy-vitamin D (25-OH vitamin D); this process is deeply compromised in severe chronic liver disease. Numerous studies have shown low levels of 25-OH vitamin D in patients with chronic liver disease, and even lower levels in patients with cirrhosis **(13)**. Often the lack of liver hydroxylation is accompanied by a deficient dietary intake of vitamin D. Impaired cutaneous synthesis of vitamin D is further aggravated by jaundice.

Vitamin K also participates in bone metabolism through osteocalcin, a marker of bone resorption that contains three residues of carboxyglutamic acid, a vitamin K-dependent aminoacid. Numerous studies have shown that an increase in decarboxylated osteocalcin levels is associated with BMD reduction and an increased risk of fractures, while the administration of vitamin K might be associated with an improvement in BMD **(22)**.

Vitamin K levels may be reduced in patients with severe cholestasis; therefore, vitamin K deficiency should be considered as a further contributing factor to the multifactorial metabolic bone disease in in advanced liver disease **(22)**.

Role of fibronectin

Fibronectin is a high molecular weight glycoprotein that is produced by various cells of the human body. Osteoblasts produce fibronectin and collagen during the bone formation process **(61)**. Several studies have shown that fibronectin can infiltrate the bone matrix, where it helps enhance the mineralization of the bone matrix and its resistance, thereby affecting bone properties **(61).** Fibronectin is also produced in the liver, and it is then released into the bloodstream at a concentration

of 300 mg/l. Fibronectin production is decreased in chronic liver disease and malnutrition **(62)**. Furthermore, in patients with cholestatic diseases, chronic hepatitis C and other chronic liver diseases, the production of oncofetal fibronectin, an isoform of fibronectin, by liver stellate cells increases **(62)**. Oncofetal fibronectin can directly inhibit osteoblasts; for example, its administration in mice causes a decrease in BMD of approximately 20% over 2 weeks **(62)**.

Intestine-liver-bone axis

Alterations of the gut microbiota have been recognized as a possible mechanism underlying the typical complications of patients with liver cirrhosis **(63,64)**. The environment, diet, drugs and comorbidities influence the composition of the microbiota and lead to dysbiosis **(65)**. Dysbiosis is associated with an increase in intestinal permeability ("leaky gut syndrome") **(66)** and is characterized by an alteration of commensal bacteria with consequent increase in colonization by pathogenic bacteria, which determine persistent low-grade inflammation by activating the pathways of toll-like receptors (TLR) signaling **(67)**. Short-chain fatty acids (SCFAs), indole derivatives and polyamines are the most investigated metabolites produced by the intestinal microbiota and have an important immunomodulation function **(68)**. Some studies have shown that the gut microbiota can regulate bone formation by altering the production of IGF-1**(69)**. Maintaining adequate intestinal wall thickness is important for proper bone metabolism since osteoclastogenic cytokines are produced by immune cells residing in the intestinal subepithelial compartment **(70)**. Any change in intestinal permeability (so called "leaky gut syndrome") has been associated with high blood levels of osteoclastogenic cytokines, thereby affecting BMD **(70)**. To date, the net effects of the gut microbiota on the bone should be confirmed by further studies, although various putative mechanisms have been proposed including immune, hormonal and nutritional pathways **(22,71).**

Aims of the present thesis

Considering the fairly dated and small sample-sized literature on the topic, as well as the lack of definitive clinical consensus on patients with advanced liver disease before liver transplantation as regards evaluation and management of bone fragility, the primary objective was to describe the prevalence, type and site of fragility fractures in a large single-center (Transplant Center of IRCCS Policlinico di S. Orsola) cohort of patients with advanced liver disease undergoing liver transplantation, with full characterization of etiologies, biochemistry and radiology.

Given the large sample size, an exploratory objective was to provide sex-specific information on bone fragility in liver transplant recipients.

To the best of my knowledge this is the largest chart-review study investigating prevalence and risk factors for clinical and morphometric fractures in patients undergoing liver transplantation. The data obtained will be relevant for the evaluation and management of these patients, especially to target resources and pharmacologic therapies to certain patients at exceedingly high fracture risk.

Patients and Methods

This study was conducted in line with STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) recommendations. The Ethical Committee of our Hospital approved this study (protocol code: *16/2020/Oss/AOUBo*). Because of the retrospective nature of this study, written informed consent was waived.

Patients

The present study was made possible thanks to a collaboration and data sharing between the Unit of Endocrinology and Diabetes Prevention and Care and the Transplant Center - Unit of Internal

Medicine for the Treatment of Severe Organ Failure of the IRCCS Azienda Ospedaliero-Universitaria Policlinico Sant'Orsola of Bologna, Italy.

This was a retrospective study that included 429 consecutive patients who underwent liver transplantation from January 1, 2010, to December 31, 2015. All patients were evaluated and managed within the same hospital and received a standardized clinical, laboratory and radiological evaluation at the Unit of Internal Medicine for the Treatment of Severe Organ Failure, including imaging of thoracic and abdominal organs. The Internal Medicine protocol was in line with local and national guidelines at the time of evaluation. All patients had an abdominal CT scan performed within 6 months from the liver transplant. Biochemistries, including PTH, minerals, and bone turnover markers (CTX, bone-specific alkaline phosphatase) were recorded, when available. All recorded biochemistries were taken in a fasting state, between 8.00 and 9.00 a.m. All samples were analyzed at the Unified Metropolitan Laboratory of Bologna (LUM) **(72).**

Medical records reviewed were as follows: the local AIRT (*Association Inter Regionale Trapianti*) chart and the chart describing liver transplant surgery, which included all previous relevant clinical history of the patient, as well as concomitant pharmacologic treatments.

Briefly, all the following data were collected during chart review:

*From the AIRT chart: Primary liver disease and its onset date, previous surgical interventions, possible complications of liver disease, such as: portal thrombosis, ascites, encephalopathy, gastrointestinal (G.I.) hemorrhages and hepatocellular carcinoma; associated comorbidities: pneumopathies, infections, arterial hypertension, diabetes; physical examination: body weight, height, chest and abdominal circumference; biochemistries: liver enzymes, total bilirubin and direct bilirubin, GGT, alkaline phosphatase, urea nitrogen, creatinine, blood glucose, total proteins, albumin, triglycerides, cholesterol, INR, fibrinogen, platelets, white blood cells, red blood cells, hemoglobin, hematocrit, α-fetoprotein, CA 19-9; virologic profile; final evaluation and degree of severity of the liver disease: CHILD TUTCOT-PUGH score and MELD score.

*From the evaluation of the medical history sheets in the medical records: previous use or abuse of alcohol, previous or current smoking habit; clinical history: kidney stones and osteopenia, osteoporosis and pathological bone fractures, early menarche; pharmacological history: administration of pre-OLT corticosteroids was investigated; any anti-osteoporotic medications: bisphosphonates, denosumab, teriparatide, calcium supplements, cholecalciferol, calcifediol or calcitriol; concomitant PPIs; in patients with HBV liver disease, any previous or ongoing treatment with nucleotide analogues.

*Bone metabolism biochemistries within 6 months prior to the transplant: PTH, serum calcium, serum phosphate, serum magnesium, urinary calcium, urinary phosphorus, 25-OH vitamin D, cross-laps or CTX, bone alkaline phosphatase (BSAP).

*From PACS: DXA (only when performed within 2 years before the transplant): date, machine; diagnosis: normal bone density for age, osteopenia or osteoporosis; BMD, T-score and Z-score of the spine; BMD, T-score and Z-score of the femoral neck; BMD, T-score and Z-score of the total hip; Chest x-rays in two projections (on the days immediately before the transplant date or on the day of transplant); CT of the abdomen including lateral Scout-CT imaging of the spine (within 6 months before transplant).

After chart review, we excluded 63 patients with transplant surgery due to acute liver failure in patients with no previous history of chronic liver failure, patients not undergoing their first liver transplantation (i.e. reoperation), and any combined transplantation (concomitant kidney or heart transplantations). When high-trauma fractures (motor vehicle accidents, *etc*) were reported, this kind of bone fracture was not counted as a fragility fracture. Twenty-six patients (6.0% of the total cohort) had missing or inaccessible radiological imaging and were also excluded (**Figure 1**).

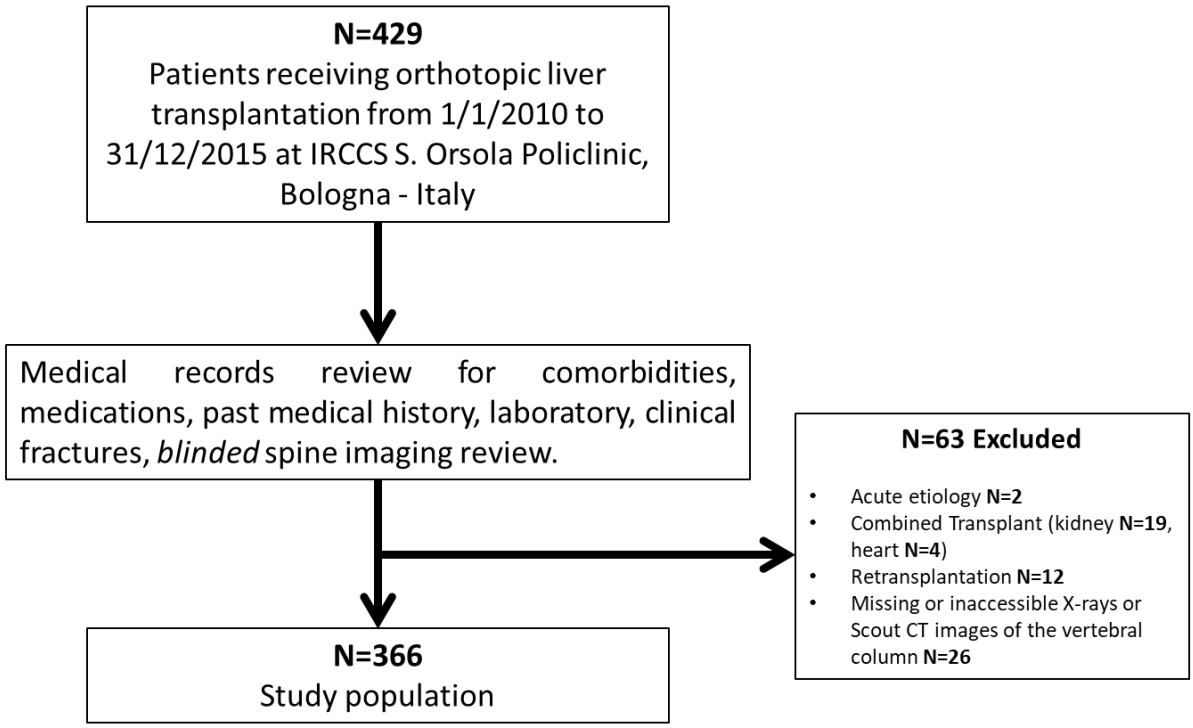

Figure 1. Flowchart of the patient selection process.

A total of 366 patients were identified. For each patient, the centralized radiological imaging data center (PACS) was searched. The PACS archive holds all clinically obtained electronic radiological/nuclear medicine images in DICOM® format of each registered patient. For each patient, we selected either a lateral view of a conventional spine X-rays or a thoraco-abdominal CT scan (Scout-Scan) performed within 6 months from the date of liver transplant. Two experts blinded to clinical data of the patients (except for sex and birth date), chronic liver disease etiology or severity re-reviewed all acquired images of the spine to screen for morphometric vertebral fractures. Inconsistent findings were solved by reaching consensus after several measurements of the vertebra. Semiquantitative visual assessment according to *Genant's* criteria **(73)** was performed to ascertain and assess severity of vertebral deformities. Percentage reductions of either anterior, middle, or posterior vertebral heights were calculated and used to define mild (20-25%), moderate (26-40%) and severe (>40%) vertebral fractures on lateral projections of spine imaging. Previous kyphoplasty or

vertebroplasty were also documented and counted as one or multiple vertebral fractures according to their extension. Date and anatomic site of clinical fragility fractures (i.e. all fractures that would cause a patient to seek medical care, including clinical spine, due to absent or low trauma) were also recorded from the medical records and AIRT charts. DXA scans performed within 2 years of liver transplant were reviewed, and the lowest value of either femur neck, total hip or lumbar spine BMD T-score was considered to classify patients into WHO bone mineral density (BMD) categories (normal BMD, osteopenia, or osteoporosis). Both T-scores and Z-scores were reported. Fractured vertebrae (L1-L4) were excluded from the analysis of lumbar BMD T-scores or Z-scores.

Statistical analysis

Absolute numbers and percentages were calculated for categorical data. The results for continuous variables were expressed as means and standard deviation (SD). Comparison of general characteristics of liver transplant recipients was performed by Mann-Whitney U test, by comparing fractured and non-fractured patients and general characteristics between sex, and within each sex. χ^2 test was used to detect associations between fragility fractures, sex and other clinical data such as diabetes or use of corticosteroids, in both sexes. Multinomial logistic regression with stepwise backward elimination was used to identify risk factors for fragility fractures across the entire population, by adjusting for potential confounders. Covariates were chosen among expected major risk factors for fragility fractures, and among significant or near-significant ($P<0.10$) parameters in simple correlations. Statistical analyses were performed using SPSS (version 26.0). P Values less than 0.05 were considered as statistically significant.

Results

Characteristics of liver transplant recipients: whole population and gender differences.

Of 366 liver transplant recipients included in the study, 144 (39.3%) had viral cirrhosis, 94 (25.7%) multifactorial disease, 50 (13.7%) cryptogenic or rare diseases, 42 (11.5%) alcoholic cirrhosis, 22 (6.0%) cholestatic disease, 12 (3.3%) autoimmune hepatitis, and only 2 (0.5%) patients non-alcoholic steatohepatitis (**Table 1**). The overall cohort was composed of 107 (29.3%) women and 259 (70.7%) men, with significant differences of sex prevalence among the etiology categories, with autoimmune and cholestatic disease being more prevalent in women, while alcoholic disease and multifactorial disease being more common in men. Clinical and anthropometric characteristics of the whole cohort are shown in **Table 1.**

Table 1 (a). Clinical characteristics of the whole population, according to sex: continuous variables.

	Women N=107		**Men N=259**		**Whole population N=366**		**P Value**
	N	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	
Age (years)	107	50,7±11,6	259	53,4±9,9	366	52,6±10,5	0,065
Weight (kg)	103	66,7±13,6	258	78,0±14,4	361	74,7±15,0	**<0.001**
BMI (kg/m^2)	96	25,5±4,5	246	25,5±3,7	342	25,5±4,0	0,347
MELD score	104	30,38±13,01	258	29,13±10,32	362	29,49±11,16	0,442
Laboratory							
GOT (U/L)	99	94±163	246	79±60	345	83±101	0,297
GPT (U/L)	100	72±211	246	64±116	346	66±150	**0,010**
Total Bilirubin (mg/dL)	100	5,6±7,1	248	6,4±14,1	348	6,2±12,5	0,590
Direct Bilirubin (mg/dL)	100	3,9±5,9	241	4,1±8,1	341	4,1±7,5	0,521
GGT (U/L)	99	61,3±57,4	245	86,2±91,4	344	79,0±83,8	**0,004**
ALP (U/L)	98	239±169	246	219±154	344	225±158	0,310

Urea Nitrogen (mg/dL)	96	26,6±18,0	243	36,3±33,1	339	33,5±29,9	**0,020**
Glucose (mg/dL)	95	90±33	245	106±40	340	101±39	**<0.001**
Total Protein (g/dL)	97	6,98±0,91	238	6,87±0,90	335	6,90±0,90	0,514
Albumin (g/dL)	98	3,49±0,62	242	3,51±0,67	340	3,50±0,66	0,708
Triglycerides (mg/dL)	98	95±61	232	88±47	330	90±51	0,611
Cholesterol (mg/dL)	97	129±52	233	120±46	330	123±48	0,203
INR	99	1,77±1,06	246	1,56±0,43	345	1,62±0,67	0,326
Fibrinogen (mg/dL)	90	225±123	224	209±100	314	213±107	0,494
Platelet count (10^9/L)	100	117,5±120,4	248	92,0±75,4	348	99,3±91,2	0,430
White blood cell (10^9/L)	99	5,07±3,60	246	5,18±3,31	345	5,15±3,39	0,261
Red blood cell (10^12/L)	99	3,60±0,71	240	3,74±0,86	339	3,70±0,82	0,165
Hemoglobin (g/dL)	100	10,89±1,76	246	11,62±2,13	346	11,41±2,05	**0,004**
Hematocrit (%)	100	32,85±5,26	243	34,80±6,16	343	34,23±5,97	**0,006**
Bone metabolism							
Lumbar BMD (g/cm^2)	13	0,83±0,15	27	0,89±0,12	40	0,87±0,13	0,305
Lumbar T-score	13	-2,15±1,25	27	-1,86±1,08	40	-1,95±1,13	0,479
Lumbar Z-score	12	-1,23±1,21	27	-1,43±1,08	39	-1,37±1,11	0,761
Femur Neck BMD (g/cm^2)	12	0,67±0,12	28	0,75±0,13	40	0,72±0,13	0,087
Femur Neck T-score	12	-1,71±1,05	28	-1,37±0,91	40	-1,47±0,96	0,301
Femur Neck Z-score	12	-0,86±0,89	28	-0,55±0,95	40	-0,65±0,93	0,400
Total Hip BMD (g/cm^2)	11	0,81±0,17	29	0,93±0,16	40	0,90±0,17	0,058
Total Hip T-score	11	-1,39±1,17	29	-0,72±1,01	40	-0,91±1,08	**0,033**
Total Hip Z-score	11	-0,75±1,14	29	-0,36±1,01	40	-0,47±1,05	0,154
PTH (pg/mL)	8	49±43	19	61±86	27	57±75	0,559
Calcium (mg/dl)	98	8,89±0,66	241	8,82±0,62	339	8,84±0,63	0,513

Phosphate (mg/dL)	82	3,10±0,76	199	3,12±0,71	281	3,11±0,72	0,965
Magnesium (mg/dl)	76	1,95±0,30	189	1,95±0,30	265	1,95±0,30	0,920
Urinary Calcium (mg/24h)	9	9,02±10,53	23	6,91±6,69	32	7,51±7,83	0,950
Urinary Phosphate (g/24h)	9	1,08±1,58	19	0,64±0,27	28	0,78±0,91	0,806
25OH Vitamin D (ng/mL)	6	13±8	30	15±7	36	14±7	0,445
Bone specific alkaline phosphatase (BSAP) (microg/L)	2	44,8±27,5	7	29,8±9,9	9	33,1±14,6	0,380
Estimated GFR (mL/min)	99	80,27±25,03	244	86,41±28,57	343	84,64±27,70	**0,010**
Serum Creatinine (mg/dL)	98	0,91±0,73	244	1,11±0,84	342	1,06±0,82	**<0.001**
Abbreviations: ALP, alkaline phosphatase; GGT, Gamma-glutamyl transferase.							

Table 1 (b). Clinical characteristics of the whole population, according to sex: categorical variables.

		Women	**Men**	**Overall population**	**P Value**
		N=107	**N=259**	**N=366**	
Fragility fractures		40/107	115/259	155/366	0,216
Ethnicity	White Caucasian	103	254	357	0,799
	Other	1	4	5	
	Total	104	258	362	
Arterial Hypertension		18/100	55/245	73/345	0,359
Child-Pugh	Missing	18	27	45	0,064
	A	18	55	73	
	B	38	70	108	
	C	33	107	140	
	Total	107	259	366	
Alcohol		$17/107_a$	$101/259_b$	118/366	**<0.001**
Smoking		$11/107_a$	$69/259_b$	80/366	**0,001**
Vitamin D3 supplements		1/107	13/259	14/366	0,064
PPI		$9/107_a$	$54/259_b$	63/366	**0,004**

Calcium carbonate supplements		1/107	6/259	7/366	0,380
Diabetes		12/107$_a$	76/259$_b$	88/366	**<0.001**
Corticosteroid use		14/107$_a$	16/259$_b$	30/366	**0,028**
Ascites		59/107	142/259	201/366	0,956
Encephalopathy		42/107	97/259	139/366	0,747
Hepatocellular carcinoma		31/107$_a$	118/259$_b$	149/366	**0,003**
GI Hemorrhage		10/107	28/259	38/366	0,676
Portal thrombosis		12/107	29/259	41/366	0,996
Cirrhosis etiology	Autoimmune Hepatitis	10$_a$	2$_b$	12	**<0.001**
	Cholestatic disease	11$_a$	11$_b$	22	
	Viral	39	105	144	
	NASH	0	2	2	
	Alcoholic	6$_a$	36$_b$	42	
	Cryptogenic/Rare disease	21$_a$	29$_b$	50	
	Multifactorial	20$_a$	74$_b$	94	
	Total	107	259	366	
Vertebral fractures		36/107	109/259	145/366	0,133
Genant's vertebral fracture grade	Mild	24	66	90	**0,031**
	Moderate	6$_a$	37$_b$	43	
	Severe	6$_a$	6$_b$	12	
	Total	36	109	145	
DXA WHO classification	Normal BMD	2	5	7	0,838
	Low BMD/osteopenia	8	12	20	
	Osteoporosis	5	10	15	
	Total	15	27	42	

Number, total numbers.
Subscript $_a$ and $_b$ within the same variable express P<0.05.

Mean age was 52 years with no significant difference between women and men. A positive smoking history was more frequent in men than women (26.6% vs. 10.3%, P=0.001). Diabetes and hepatocellular carcinoma were more frequent in men, while women were more frequently exposed to glucocorticoids. Mean BMI was 25.6 kg/m^2, with no differences between sexes. Hip BMD and eGFR were lower in women than in men, while hypertension was equally distributed between sexes.

As regards bone metabolism, fragility fractures prevalence was 155/366 (42.3%) in the overall population, with no significant differences between sexes (fracture prevalence in women at 37.4%, in men at 44.4%).

Calcium and vitamin D3 supplements were equally distributed among both sexes, while a small proportion of patients were taking bisphosphonates (13/366, 3.5%).

Laboratory parameters of mineral metabolism were tested in very few patients, and no significant differences could be observed between sexes.

Regarding vertebral fractures (n=145), mild vertebral fractures (Genant grade 1) were the most frequently observed (90/145, 62.1%), with similar prevalence between sexes. Moderate vertebral fractures (Genant grade 2) were more common in males (33.9%) compared to women (16.7%). Severe (Genant grade 3) vertebral fractures occurred more frequently in women compared to men (16.7% vs. 5.5%). The overall number of patients with clinical fragility fractures (i.e. symptomatic) was 50/366 (13.7%). Of these the majority (n=43, 86%) were vertebral fractures. Other clinical fractures were at humerus (n=1), ribs (n=7), femur (n=1), clavicle (n=1). The median period of time between clinical fracture occurrence and liver transplantation was 2 months, although the exact dates of clinical fractures were retrieved in only 3 patients. All the remaining fractures were morphometric vertebral fractures. The prevalence of fractures among transplant recipients was stable across each year of the study period (**Figure 2**).

Women with fractures, compared to women without fractures, had worsen kidney function, lower urinary calcium, lower BMD and more commonly used alcohol (Supplementary Table a. and b.).

Men with fractures, compared to men without fractures had lower 25-OH vitamin D levels, with no other obvious significant differences in terms of laboratory or clinical data (Supplementary Table c. and d.).

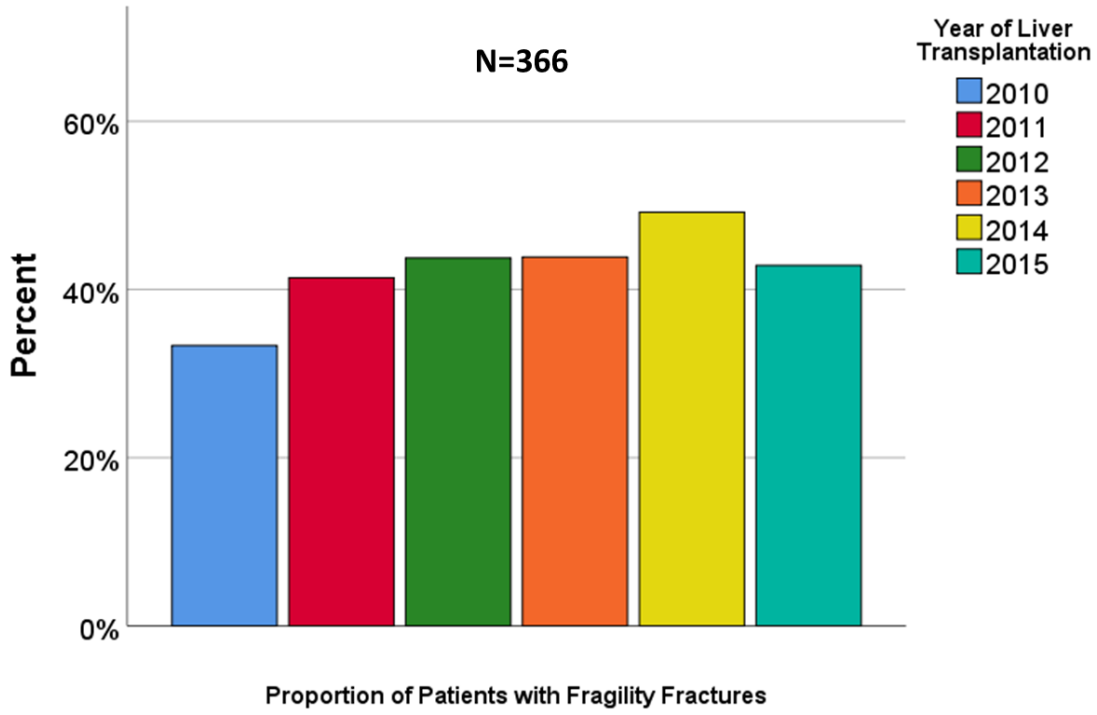

Figure 2. Percentage of fractured patients for each calendar year of the study period (P=0.639 for comparison).

Regarding vertebral fractures, most were single fractures, although a considerable proportion of patients (n=60, 41.3%) had two or more vertebral fractures, up to a maximum of 10 vertebral fractures per patient **(Figure 3)**.

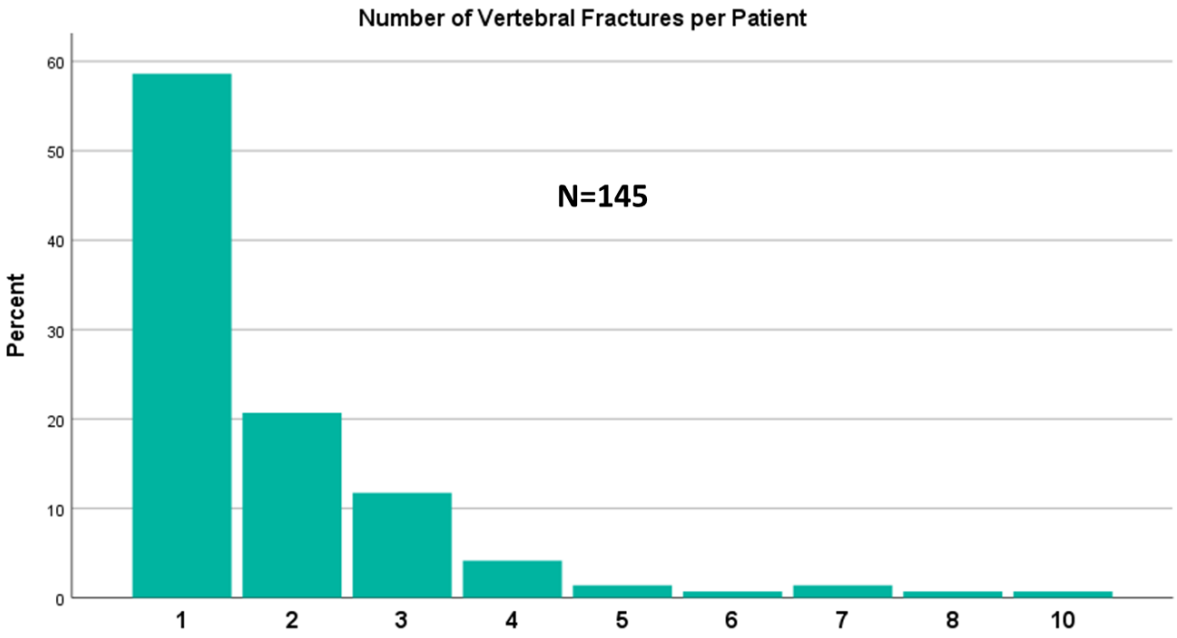

Figure 3. Distribution of single and multiple vertebral fractures across patients with vertebral fractures (N=145).

Characteristics of liver transplant recipients with fractures

Patients with fractures were no significant different as to age or BMI, while GPT was lower and serum creatinine greater than patients with no fractures, with a near-significant lower estimated GFR. The remaining parameters both from the clinical history and the laboratory were similar between groups (**Table 2 a and b**). Severity of liver disease was no different between groups. Fragility fractures showed similar rates across each liver disease etiology **(Figure 4)**.

Table 2 (a). Clinical characteristics of the patients with and without fractures: continuous variables.

	Patients without fractures		**Patients with fractures**		**Overall number**	**P Value**
	N=211		**N=155**		**N=366**	
	N	**Mean ± SD**	**N**	**Mean ± SD**		

Age (years)	211	51,8±11,4	155	53,6±8,9	366	0,350
Weight (kg)	207	73,6±14,6	154	76,3±15,5	361	0,104
BMI (kg/m^2)	194	25,2±3,8	148	26,0±4,1	342	0,053
MELD score	208	29,87±11,59	154	28,96±10,55	362	0,539
Laboratory						
GOT (U/L)	197	85±78	148	82±125	345	0,240
GPT (U/L)	196	70±137	150	62±165	346	**0,043**
Total Bilirubin (mg/dL)	198	5,3±7,1	150	7,2±17,1	348	0,624
Direct Bilirubin (mg/dL)	194	4,0±8,3	147	4,1±6,	341	0,647
GGT (U/L)	195	79±71	149	79±97	344	0,263
ALP (U/L)	195	228±154	149	221±164	344	0,271
Urea Nitrogen (mg/dL)	193	34,0±29,6	146	32,9±30,4	339	0,707
Glucose (mg/dL)	193	106±45	147	95±28	340	0,107
Total protein (g/dL)	190	6,8±0,8	145	6,9±0,9	335	0,408
Albumin (g/dL)	195	3,48±0,67	145	3,53±0,62	340	0,408
Triglycerides (mg/dL)	186	89±43	144	91±60	330	0,304
Cholesterol (mg/dL)	186	123±47	144	123±50	330	0,702
INR	196	1,61±0,58	149	1,62±0,78	345	0,913
Fibrinogen (mg/dL)	177	213±113	137	214±100	314	0,754
Platelet count (10^9/L)	198	98,78±87,69	150	100,19±95,98	348	0,626
White blood cell (10^9/L)	196	5,26±3,75	149	4,99±2,84	345	0,948
Red blood cell (10^12/L)	193	3,75±0,80	146	3,63±0,83	339	0,206
Hemoglobin (g/dL)	196	11,58±2,04	150	11,17±2,04	346	0,084
Hematocrit (%)	195	34,71±5,96	148	33,59±5,93	343	0,084
Bone metabolism						
Lumbar BMD (g/cm^2)	19	0,885±0,126	21	0,856±0,139	40	0,473

Lumbar T-score	19	-1,82±1,05	21	-2,06±1,20	40	0,424
Lumbar Z-score	19	-1,28±0,89	20	-1,44±1,29	39	0,642
Femur Neck BMD (g/cm^2)	18	0,748±0,141	22	0,704±0,127	40	0,242
Femur Neck T-score	18	-1,35±1,05	22	-1,57±0,88	40	0,414
Femur Neck Z-score	18	-0,48±0,96	22	-0,77±0,90	40	0,406
Total Hip BMD (g/cm^2)	19	0,924±0,160	21	0,879±0,182	40	0,273
Total Hip T-score	19	-0,70±1,01	21	-1,08±1,12	40	0,188
Total Hip Z-score	19	-0,28±0,95	21	-0,63±1,12	40	0,180
PTH (pg/mL)	17	72,35±92,16	10	32,60±20,17	27	0,269
Calcium (mg/dL)	190	8,80±0,58	149	8,88±0,68	339	0,325
Phosphate (mg/dL)	158	3,0±0,7	123	3,1±0,6	281	0,836
Magnesium (mg/dL)	143	1,93±0,25	122	1,96±0,33	265	0,585
Urinary Calcium (mg/24h)	11	8,72±9,14	21	6,86±7,21	32	0,427
Urinary Phosphate (g/24h)	9	1,082±1,572	19	0,644±0,294	28	0,768
25OH Vitamin D (ng/mL)	18	16±8	18	12±6	36	0,087
Bone specific alkaline phosphatase (BSAP) (microg/L)	2	22,2±4,3	7	36,3±15,1	9	0,143
Estimated GFR (mL/min)	196	86,9±28,2	147	81,6±26,7	343	0,092
Serum Creatinine (mg/dL)	195	1,02±0,84	147	1,09±0,78	342	**0,023**

Table 2 (b). Clinical characteristics of the patients with and without fractures: categorical variables.

		Without Fractures N=211	**With Fractures N=155**	**Overall number N=366**	**P Value**
Ethnicity	White Caucasian	203	154	357	0,440
	Other	5	0	1	
	Total	208	154	362	
Arterial Hypertension		43/197	30/148	73/345	0,726
Child-Pugh	Missing	30	15	45	0,508
	A	44	29	73	

	B	59	49	108	
	C	78	62	140	
	Total	211	155	366	
Alcohol use		62/211	56/155	118/366	0,173
Smoking		46/211	34/155	80/366	0,975
Vitamin D3 supplements		6/211	8/155	14/366	0,253
PPI		34/211	29/155	63/366	0,516
Calcium carbonate		5/211	2/155	7/366	0,456
Diabetes		56/211	32/155	88/366	0,192
Corticosteroid use		16/211	14/155	30/366	0,617
Ascites		112/211	89/155	201/366	0,410
Encephalopathy		83/211	56/155	139/366	0,532
Hepatocellular carcinoma		84/211	65/155	149/366	0,683
GI Hemorrhage		25/211	13/155	38/366	0,283
Portal thrombosis		27/211	14/155	41/366	0,259
Cirrhosis Etiology	Autoimmune Hepatitis	8	4	12	0,698
	Cholestatic disease	10	12	22	
	Viral	82	62	144	
	NASH	1	1	2	
	Alcoholic	21	21	42	
	Cryptogenic/Rare disease	31	19	50	
	Multifactorial	58	36	94	
	Total	211	155	366	
Vertebral fractures		0	145/155	145/366	**<0.001**
DXA WHO classification	Normal BMD	5	2	7	0,369
	Low BMD/osteopenia	9	11	20	
	Osteoporosis	6	9	15	
	Total	20	22	42	

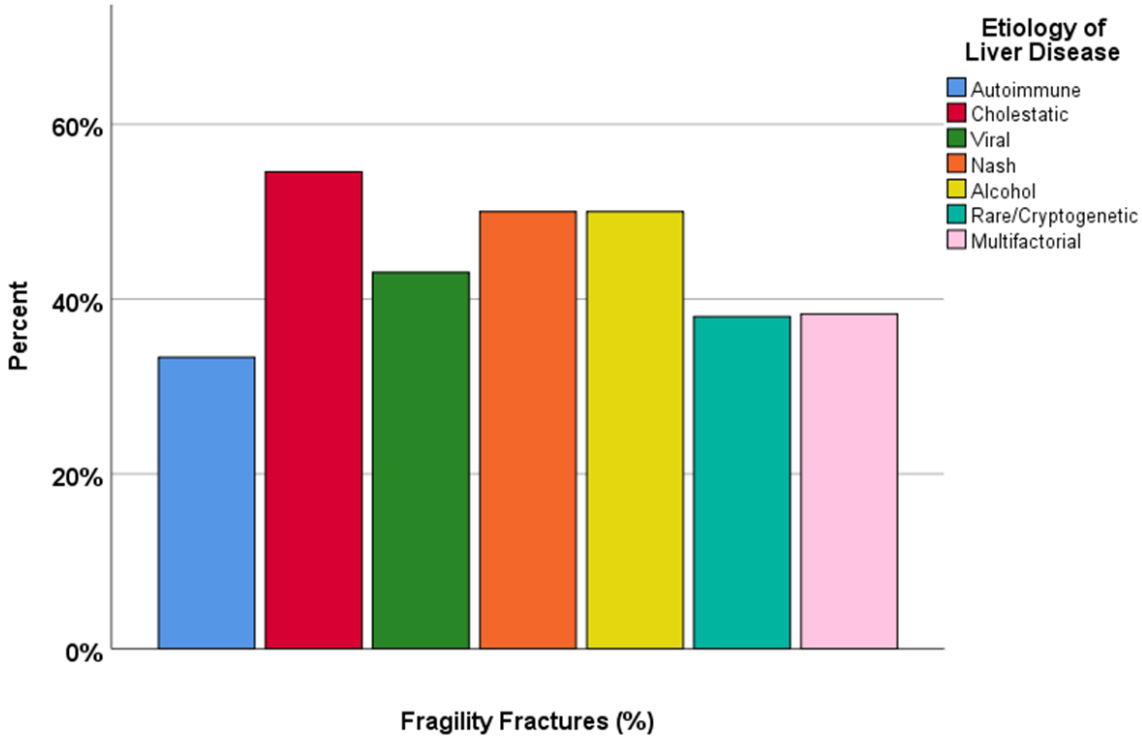

Figure 4. Percentage of fractured patients in each liver disease etiology (P=0.698).

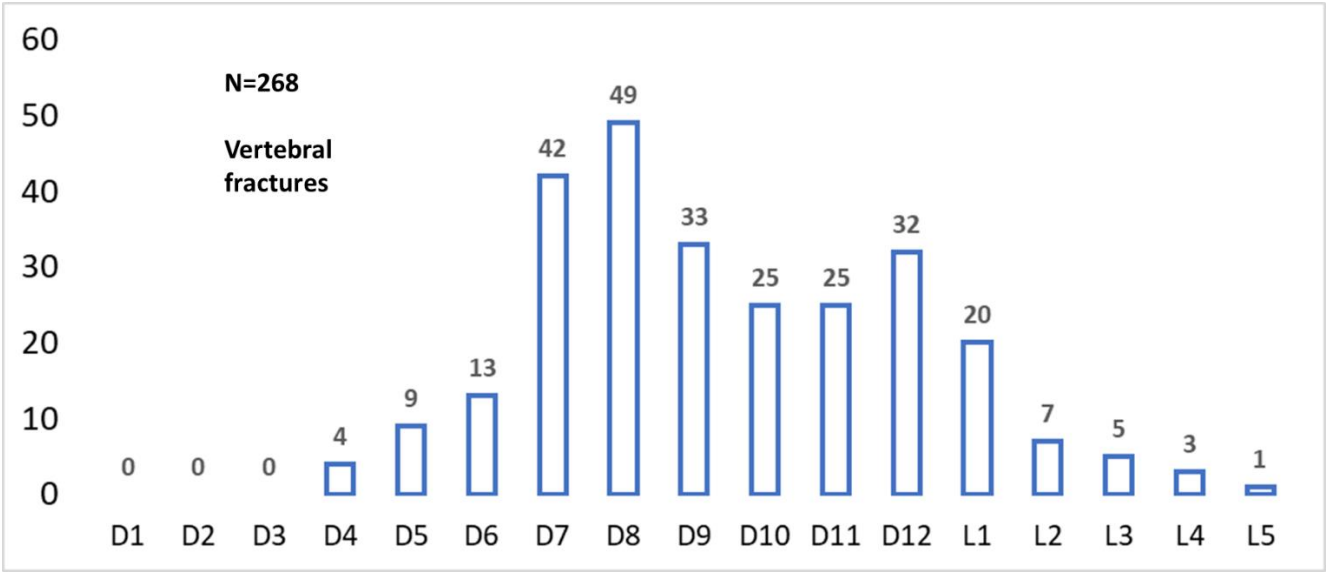

Figure 5. Distribution of vertebral fractures (absolute frequencies).

The most common vertebral fractures occurred at D7, D8 and D12 vertebrae **(Figure 5).** Lower rates of fractures were observed in the lumbar spine. The most frequently observed vertebral fracture type was wedge fractures, with a minor but considerable proportion of crush fractures **(Figure 6).**

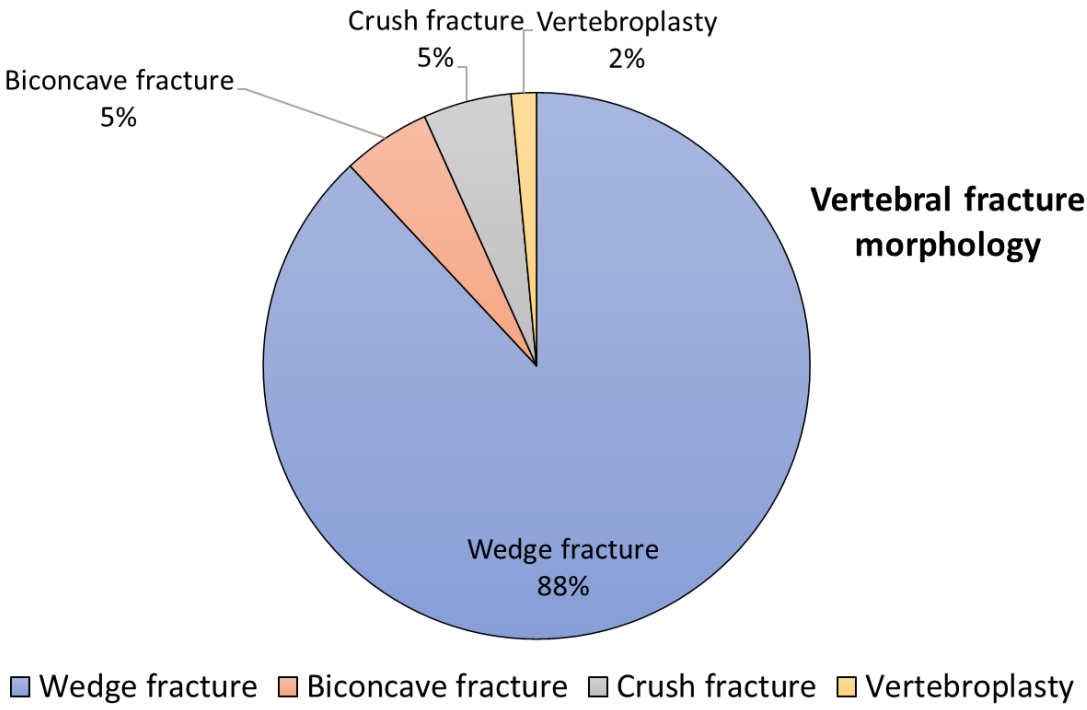

Figure 6. Types of vertebral fractures.

Characteristics of liver transplant recipients: effect of glucocorticoids on metabolic bone disease

Glucocorticoid administration was different across etiologies, although it did not seem to have an impact of fragility fractures, vertebral fractures or bone mineral density by DXA (**Table 4**).

Table 3. Effect of corticosteroid exposure.

		No Corticosteroids N=336	Corticosteroid use N=30	Total Number N=366	P Value
Fragility Fractures		141	14	155	0,617
	Total	336	30	366	

Etiology	Autoimmune Hepatitis	2_a	10_b	12	**<0.001**
	Cholestatic disease	10_a	12_b	22	
	Viral	142_a	2_b	144	
	NASH	2	0	2	
	Alcoholic	40	2	42	
	Cryptogenic/Rare disease	48	2	50	
	Multifactorial	92_a	2_b	94	
	Total	336	30	366	
Vertebral Fractures		132	13	145	0,664
	Total	336	30	366	
DXA WHO classification	Normal BMD	6	1	7	0,940
	Low BMD/osteopenia	16	4	20	
	Osteoporosis	12	3	15	
	Total	34	8	42	
Subscript a and b within the same variable express P<0.05.					

Characteristics of liver transplant recipients: effect of diabetes on metabolic bone disease.

Diabetes prevalence differed according to etiology, although it was not associated with fragility fractures (P=0.192), vertebral fractures prevalence or severity or low bone mineral density by DXA.

Table 4. Effect of diabetes on fracture risk.

		No diabetes N=278	**Diabetes N=88**	**Total N=366**	**P value**
Fragility Fractures		123	32	155	0,192
	Total	278	88	366	
Etiology	Autoimmune Hepatitis	10	2	12	**0,015**
	Cholestatic disease	21_a	1_b	22	
	Viral	101_a	43_b	144	
	NASH	2	0	2	
	Alcoholic	26_a	16_b	42	
	Cryptogenic/Rare disease	42	8	50	
	Multifactorial	76	18	94	
	Total	278	88	366	
Vertebral fractures		117	28	145	0,086
	Total	278	88	366	

Genant's vertebral fracture grade	Mild	72	18	90	0,767
	Moderate	36	7	43	
	Severe	9	3	12	
	Total	117	28	145	
DXA WHO classification	Normal BMD	5	2	7	0,666
	Low BMD/osteopenia	16	4	20	
	Osteoporosis	10	5	15	
	Total	31	11	42	
Subscript $_a$ and $_b$ within the same variable express P<0.05.					

Multivariate analysis

A logistic regression model including age, sex, BMI, alcohol use, estimated GFR, etiology (autoimmune or cholestatic disease vs. other), revealed that only BMI was negatively associated with prevalent fragility fractures (OR 1.058, 1.001-1.118, 95%CI), independent of other risk factors (Table 5). In liver transplant recipients, for each one unit decrease of BMI, risk of fragility fractures would increase by 5.8%, and vice versa.

Table 5. Multinomial logistic regression [a]

Risk factors for fragility fractures	**Beta Coefficient**	**P value**	**Adjusted Odds-Ratio**	**95%CI**	
				Lower Bound	**Upper Bound**
Constant	-1,709	0,019			
BMI	0,056	**0,046**	1,058	1,001	1,118
[a] Backward stepwise logistic regression analysis adjusted for age, sex, alcohol use, estimated GFR, etiology (autoimmune or cholestatic disease vs. other).					

Discussion

This study investigated the prevalence of fragility fractures, either clinical or morphometric, in a large cohort of patients undergoing liver transplantation, who were fully characterized in terms of radiology, biochemistries and medications. The type and the most frequent location of such fractures were also assessed, in order to generate a consistent fracture risk profile of a modern cohort of liver transplant recipients.

A cohort of 366 patients with liver disease due to different etiologies who underwent liver transplantation between January 1, 2010, and December 31, 2015, was analyzed. The prevalence of osteoporotic fractures within the cohort was 42.3%, most of which were thoracic vertebral fractures. Fractures of the femur, humerus and ribs were uncommon, despite the large sample size. Thanks to blind re-evaluation of the X-rays of the spine and Scout-CTs of the lumbar vertebral column, a substantial proportion of patients with metabolic bone disease at the time of transplantation was noted. The majority of fractures (88%) were anterior wedge fractures, of mild to moderate severity. For each one unit decrease in BMI, fragility fracture risk increased by 5.8%, based on a multinomial regression analysis, independent of age, kidney function, etiology of liver disease and alcohol consumption. Thanks to its sample size, this study appears to be the largest study investigating liver transplant candidates over the last 20 years, thus providing an updated clinical picture of modern cohorts of liver transplant recipients.

Previous studies were heterogeneous in terms of fracture prevalence in liver transplant recipients, which was usually reported somewhere between 3 and 43%. Possible selection bias in small studies or variability in the criteria used to define bone metabolic disease such as osteoporosis or low BMD also account for this heterogeneity. In a 1997 study by Monegal A., et al., conducted on 58 cirrhotic patients who were candidates for transplant, it was observed that 43% of the patients had osteoporosis diagnosed thanks to the presence of at least one vertebral fracture and/or a BMD of the lumbar vertebrae < 2 standard deviations compared to the mean values of healthy subjects of the same age **(8)**. This study therefore evaluated the presence of osteoporosis thanks to densitometry and spinal x-rays, with the limitation, however, of having been conducted on a small group of patients, although the metabolic bone disease rates were similar to those obtained in this thesis. Wariaghli G. et al., in a 2010 study conducted on 64 patients with chronic liver disease, showed that 45.5% of patients had osteoporosis and only 5.3% had vertebral fractures **(9)**. This study also came with the limitation of a

limited number of patients. Furthermore, the patients examined had only PBC or viral liver disease, thereby limiting speculations on other etiologies.

Very few studies have been published in the last ten years about risk of fractures among liver transplant candidates. A study on 128 patients found that severity of liver cirrhosis was associated with hip fractures. This study, however, examined a cohort of older patients, i.e. more than one decade older than those in the present study **(74)**. Another registry-based study carried out in Sweden assessed fracture risk in non-alcoholic fatty liver disease, showing a slightly higher rate of fractures, although with long-term risk of fractures comparable to the general population. These studies could suggest that fragility fractures might be caused by liver dysfunction alone rather than a specific chronic liver disease etiology. As the cirrhosis worsen, the bone might also worsen, reaching its worst scenario right at the time of transplantation **(75)**. This hypothesis was confirmed by a recent study which assessed 102 patients before and after liver transplantation, finding that malnutrition and low BMI were the main determinants of osteopenia/osteoporosis, independent of etiology, similarly to what was shown in the present thesis **(76)**.

In the literature, several studies have investigated metabolic bone disease in subjects with PBC and CSP. In a 1994 study by Camisaca M., et al., conducted on 25 women with PBC, it was observed a rapid BMD loss of 3.5% in only 6 months **(14)**, with no data on fracture prevalence. Eastell R., et al., in a study conducted on 210 women with PBC, described lower BMD compared to controls, although the prevalence of fractures was not assessed **(15)**. Angulo P., et al., in a study of 81 patients with CSP, demonstrated that the BMD of the lumbar vertebrae of such patients was lower than in healthy controls of the same age and sex and that 3% of patients had fragility fractures. Finally, they observed that patients with fractures had more advanced liver disease **(17)**. In this study the prevalence of fractures was presumably underestimated as the study also involved patients with CSP in the initial stages of the disease, thereby representing a limitation. The present thesis, by contrast, suggests that cholestatic disease is equally important as a risk factor for fracture as other liver disease

etiologies, because fracture prevalence was non-significantly different to other non-cholestatic disorders.

Gallego-Rojo F. J., et al., in a 1998 study conducted on 32 patients with cirrhosis of viral etiology, demonstrated the presence of osteoporosis by DXA in 50% of them. The limitations of this study were due to the small sample of patients and no reports on fracture prevalence **(20).** In the present thesis prevalence of fractures in viral cirrhosis was noted to be over 40%.

Therefore, the present study, in addition to evaluating the prevalence of fractures in a large cohort of patients, estimated the risk of fractures across various etiologies of liver disease, suggesting that etiology may not be as important as it was initially thought, based on the aforementioned previous studies. The prevalence of fractures in patients with liver disease and cirrhosis at the time of liver transplantation was remarkably high (approximately 42%) and fractures were mainly located in the thoracic vertebrae, independent of age and sex. BMI was the only single independent predictor of fracture prevalence: for every one unit decrease of BMI, risk of fractures increased proportionally.

Strengths and limitations

Among the strengths of this study is the large sample size, the consecutive enrolment of the patients, the centralized laboratory and full access to radiological imaging, with blinded review of radiological images, as well as the accuracy of a chart review study in terms of correct diagnoses. Moreover, the single-center nature of the study attenuated variability in the management of chronic liver disease before liver transplant. This study comes also with limitations, among which is the lack of a control group and the cross-sectional design. Being S. Orsola Policlinic a major referral center, this could raise the estimates of fracture prevalence, because of a possible higher incidence of more severe chronic liver disease. Unfortunately, the majority of patients did not have a complete mineral metabolism evaluation through laboratory or DXA. The finding of comparable BMD between males

and females might be affected by a limited sample size, although numerically higher BMD was found in males, as expected. This limitation also prevents speculation on underlying bone metabolism and density in these patients, and therefore on the best anti-osteoporotic medications to choose in this setting. Moreover, menopausal status was not available for all women, although a considerable proportion of women were likely premenopausal based on mean age of the population. Last, the retrospective nature of the clinical data might also hide some unintentional bias.

Conclusions

In conclusion, this study has provided substantial evidence, and confirmed previous findings from small-sized single center studies, of a considerable fracture burden in patients awaiting and undergoing liver transplantation. These data support the need for a thorough bone metabolism evaluation and management for this category of patients, and implementation of this in future guidelines.

It emerges that osteoporotic vertebral fractures are frequent complications of the underlying end-organ disease. In this large study, most patients had one or two vertebral fractures, but a considerable proportion of patients may also experience multiple vertebral fractures. The most affected sites are the thoracic vertebrae, in particular T7, T8 and T12, and the most frequent fractures were anterior wedges. Furthermore, the majority of vertebral fractures were asymptomatic, making vertebral morphometry an essential tool to screen bone fragility. Femoral fractures or other clinical fractures were uncommon possibly due to a relatively young population, based on mean age at transplantation.

Prevalence of fractures was similar across all etiologies, as opposed to previous limited-sample studies indicating cholestatic liver disorders as one of the etiologies carrying major fracture risk. Prevalence of fractures was also similar across different age groups, and comparable between males and females.

These data suggest the need to implement screening for metabolic bone diseases in all patients with chronic liver disease and cirrhosis awaiting transplantation. Osteoporotic fractures constitute, in fact, the main risk factor for subsequent bone fractures and significantly influence the quality of life of these patients before and after liver transplantation **(74)**. Screening should include laboratory tests relating to bone metabolism, a DXA to quantitate bone mass and a spine morphometry to exclude the presence of vertebral fractures.

In conclusion, even in a modern cohort of liver transplant recipients managed in a referral center, osteoporotic fractures constituted frequent complications across all ages and etiologies.

Future data on this cohort regarding fracture incidence after transplantation should be collected to estimate fracture risk in the post-transplantation period, and whether this may affect the overall survival of this population.

Acknowledgments: I am deeply thankful to Prof. Pagotto for letting me have the time to carry out several research activities, beyond the everyday clinical activities. I am also deeply thankful to Dr. Maria Cristina Morelli and Dr. Giovanni Vitale for sharing the list of the patients undergoing liver transplantation and to have had access to clinical charts, and eventually for building up the currently ongoing multidisciplinary database regarding bone metabolism in liver transplantation, which should also be fruitful in the coming months/years. Dr. Elisa Sarti helped me collect the data about clinical history of the patients. Dr. Paola Altieri helped me out check data completeness, as well as the accuracy of the radiological data.

Data availability statement: The data that support the findings of this study are available upon reasonable request.

References

1. Ginès P, Krag A, Abraldes JG, Solà E, Fabrellas N, Kamath PS. Liver cirrhosis. Lancet. 2021 Oct 9;398(10308):1359-1376. doi: 10.1016/S0140-6736(21)01374-X. Epub 2021 Sep 17. PMID: 34543610.
2. Moon AM, Singal AG, Tapper EB. Contemporary Epidemiology of Chronic Liver Disease and Cirrhosis. Clin Gastroenterol Hepatol. 2020 Nov;18(12):2650-2666. doi: 10.1016/j.cgh.2019.07.060. Epub 2019 Aug 8. PMID: 31401364; PMCID: PMC7007353.
3. Patel N, Muñoz SJ. Bone disease in cirrhosis. Clin Liver Dis (Hoboken). 2015 Oct 28;6(4):96-99. doi: 10.1002/cld.498. PMID: 31040999; PMCID: PMC6490654.
4. Guañabens N, Parés A. Liver and bone. Arch Biochem Biophys. 2010 Nov 1;503(1):84-94. doi: 10.1016/j.abb.2010.05.030. Epub 2010 Jun 9. PMID: 20537977.
5. Nakchbandi IA. Osteoporosis and fractures in liver disease: relevance, pathogenesis and therapeutic implications. World J Gastroenterol. 2014 Jul 28;20(28):9427-38. doi: 10.3748/wjg.v20.i28.9427. PMID: 25071337; PMCID: PMC4110574.
6. Compston JE, McClung MR, Leslie WD. Osteoporosis. Lancet. 2019 Jan 26;393(10169):364-376. doi: 10.1016/S0140-6736(18)32112-3. PMID: 30696576.
7. Nuti R, Brandi ML, Checchia G, Di Munno O, Dominguez L, Falaschi P, Fiore CE, Iolascon G, Maggi S, Michieli R, Migliaccio S, Minisola S, Rossini M, Sessa G, Tarantino U, Toselli A, Isaia GC. Guidelines for the management of osteoporosis and fragility fractures. Intern Emerg Med. 2019 Jan;14(1):85-102. doi: 10.1007/s11739-018-1874-2. Epub 2018 Jun 13. PMID: 29948835; PMCID: PMC6329834.
8. Monegal A, Navasa M, Guañabens N, Peris P, Pons F, Martinez de Osaba MJ, Rimola A, Rodés J, Muñoz-Gómez J. Osteoporosis and bone mineral metabolism disorders in cirrhotic patients referred for orthotopic liver transplantation. Calcif Tissue Int. 1997 Feb;60(2):148-54. doi: 10.1007/s002239900205. PMID: 9056162.
9. Wariaghli G, Mounach A, Achemlal L, Benbaghdadi I, Aouragh A, Bezza A, El Maghraoui A. Osteoporosis in chronic liver disease: a case-control study. Rheumatol Int. 2010 May;30(7):893-9. doi: 10.1007/s00296-009-1071-8. Epub 2009 Jul 28. PMID: 19636560.
10. Collier J. Bone disorders in chronic liver disease. Hepatology. 2007 Oct;46(4):1271-8. doi: 10.1002/hep.21852. PMID: 17886334.
11. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu; European Association for the Study of the Liver. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. J Hepatol. 2017 Jul;67(1):145-172. doi: 10.1016/j.jhep.2017.03.022. Epub 2017 Apr 18. PMID: 28427765.
12. Handzlik-Orlik G, Holecki M, Wilczyński K, Duława J. Osteoporosis in liver disease: pathogenesis and management. Ther Adv Endocrinol Metab. 2016 Jun;7(3):128-35. doi: 10.1177/2042018816641351. Epub 2016 Apr 6. PMID: 27293541; PMCID: PMC4892399.
13. Collier JD, Ninkovic M, Compston JE. Guidelines on the management of osteoporosis associated with chronic liver disease. Gut. 2002 Feb;50 Suppl 1(Suppl 1):i1-9. doi: 10.1136/gut.50.suppl_1.i1. PMID: 11788576; PMCID: PMC1867644.
14. Camisasca M, Crosignani A, Battezzati PM, Albisetti W, Grandinetti G, Pietrogrande L, Biffi A, Zuin M, Podda M. Parenteral calcitonin for metabolic bone disease associated with primary biliary cirrhosis. Hepatology. 1994 Sep;20(3):633-7. PMID: 8076921.
15. Eastell R, Dickson ER, Hodgson SF, Wiesner RH, Porayko MK, Wahner HW, Cedel SL, Riggs BL, Krom RA. Rates of vertebral bone loss before and after liver transplantation in women with primary biliary cirrhosis. Hepatology. 1991 Aug;14(2):296-300. PMID: 1860685.

16. Mounach A, Ouzzif Z, Wariaghli G, Achemlal L, Benbaghdadi I, Aouragh A, Bezza A, El Maghraoui A. Primary biliary cirrhosis and osteoporosis: a case-control study. J Bone Miner Metab. 2008;26(4):379-84. doi: 10.1007/s00774-007-0833-1. Epub 2008 Jul 4. PMID: 18600405.
17. Angulo P, Grandison GA, Fong DG, Keach JC, Lindor KD, Bjornsson E, Koch A. Bone disease in patients with primary sclerosing cholangitis. Gastroenterology. 2011 Jan;140(1):180-8. doi: 10.1053/j.gastro.2010.10.014. Epub 2010 Oct 16. PMID: 20955707; PMCID: PMC3043598.
18. Selimoglu MA, Ertekin V, Doneray H, Yildirim M. Bone mineral density of children with Wilson disease: efficacy of penicillamine and zinc therapy. J Clin Gastroenterol. 2008 Feb;42(2):194-8. doi: 10.1097/MCG.0b013e318032388d. PMID: 18209591.
19. Hegedus D, Ferencz V, Lakatos PL, Meszaros S, Lakatos P, Horvath C, Szalay F. Decreased bone density, elevated serum osteoprotegerin, and beta-cross-laps in Wilson disease. J Bone Miner Res. 2002 Nov;17(11):1961-7. doi: 10.1359/jbmr.2002.17.11.1961. PMID: 12412803.
20. Gallego-Rojo FJ, Gonzalez-Calvin JL, Muñoz-Torres M, Mundi JL, Fernandez-Perez R, Rodrigo-Moreno D. Bone mineral density, serum insulin-like growth factor I, and bone turnover markers in viral cirrhosis. Hepatology. 1998 Sep;28(3):695-9. doi: 10.1002/hep.510280315. PMID: 9731561.
21. Chen CC, Wang SS, Jeng FS, Lee SD. Metabolic bone disease of liver cirrhosis: is it parallel to the clinical severity of cirrhosis? J Gastroenterol Hepatol. 1996 May;11(5):417-21. doi: 10.1111/j.1440-1746.1996.tb00284.x. PMID: 8743912.
22. Guañabens N, Parés A. Osteoporosis in chronic liver disease. Liver Int. 2018 May;38(5):776-785. doi: 10.1111/liv.13730. Epub 2018 Mar 25. PMID: 29479832.
23. Jeong HM, Kim DJ. Bone Diseases in Patients with Chronic Liver Disease. Int J Mol Sci. 2019 Aug 31;20(17):4270. doi: 10.3390/ijms20174270. PMID: 31480433; PMCID: PMC6747370.
24. Li M, Xu Y, Xu M, Ma L, Wang T, Liu Y, Dai M, Chen Y, Lu J, Liu J, Bi Y, Ning G. Association between nonalcoholic fatty liver disease (NAFLD) and osteoporotic fracture in middle-aged and elderly Chinese. J Clin Endocrinol Metab. 2012 Jun;97(6):2033-8. doi: 10.1210/jc.2011-3010. Epub 2012 Mar 30. PMID: 22466338.
25. Purnak T, Beyazit Y, Ozaslan E, Efe C, Hayretci M. The evaluation of bone mineral density in patients with nonalcoholic fatty liver disease. Wien Klin Wochenschr. 2012 Aug;124(15-16):526-31. doi: 10.1007/s00508-012-0211-4. Epub 2012 Aug 1. PMID: 22850810.
26. Compston JE, Vedi S, Kaptoge S, Seeman E. Bone remodeling rate and remodeling balance are not co-regulated in adulthood: implications for the use of activation frequency as an index of remodeling rate. J Bone Miner Res. 2007 Jul;22(7):1031-6. doi: 10.1359/jbmr.070407. PMID: 17501624.
27. Eastell R, Rosen CJ, Black DM, Cheung AM, Murad MH, Shoback D. Pharmacological Management of Osteoporosis in Postmenopausal Women: An Endocrine Society* Clinical Practice Guideline. J Clin Endocrinol Metab. 2019 May 1;104(5):1595-1622. doi: 10.1210/jc.2019-00221. PMID: 30907953.
28. Guañabens N, Parés A. Management of osteoporosis in liver disease. Clin Res Hepatol Gastroenterol. 2011 Jun;35(6-7):438-45. doi: 10.1016/j.clinre.2011.03.007. Epub 2011 May 4. PMID: 21546334.
29. Wilson T, Katz JM, Gray DH. Inhibition of active bone resorption by copper. Calcif Tissue Int. 1981;33(1):35-9. doi: 10.1007/BF02409410. PMID: 6780154.
30. Schiefke I, Fach A, Wiedmann M, Aretin AV, Schenker E, Borte G, Wiese M, Moessner J. Reduced bone mineral density and altered bone turnover markers in patients with non-cirrhotic chronic hepatitis B or C infection. World J Gastroenterol. 2005 Mar 28;11(12):1843-7. doi: 10.3748/wjg.v11.i12.1843. PMID: 15793878; PMCID: PMC4305888.

31. Casado JL, Bañon S, Andrés R, Perez-Elías MJ, Moreno A, Moreno S. Prevalence of causes of secondary osteoporosis and contribution to lower bone mineral density in HIV-infected patients. Osteoporos Int. 2014 Mar;25(3):1071-9. doi: 10.1007/s00198-013-2506-3. Epub 2013 Sep 21. PMID: 24057480.
32. Marie PJ, Kassem M. Osteoblasts in osteoporosis: past, emerging, and future anabolic targets. Eur J Endocrinol. 2011 Jul;165(1):1-10. doi: 10.1530/EJE-11-0132. Epub 2011 May 4. PMID: 21543379.
33. Ruiz-Gaspà S, Martinez-Ferrer A, Guañabens N, Dubreuil M, Peris P, Enjuanes A, Martinez de Osaba MJ, Alvarez L, Monegal A, Combalia A, Parés A. Effects of bilirubin and sera from jaundiced patients on osteoblasts: contribution to the development of osteoporosis in liver diseases. Hepatology. 2011 Dec;54(6):2104-13. doi: 10.1002/hep.24605. PMID: 21837749.
34. Valenti L, Varenna M, Fracanzani AL, Rossi V, Fargion S, Sinigaglia L. Association between iron overload and osteoporosis in patients with hereditary hemochromatosis. Osteoporos Int. 2009 Apr;20(4):549-55. doi: 10.1007/s00198-008-0701-4. Epub 2008 Jul 26. PMID: 18661088.
35. Delgado-Calle J, Sato AY, Bellido T. Role and mechanism of action of sclerostin in bone. Bone. 2017 Mar;96:29-37. doi: 10.1016/j.bone.2016.10.007. Epub 2016 Oct 12. PMID: 27742498; PMCID: PMC5328835.
36. Rhee Y, Kim WJ, Han KJ, Lim SK, Kim SH. Effect of liver dysfunction on circulating sclerostin. J Bone Miner Metab. 2014 Sep;32(5):545-9. doi: 10.1007/s00774-013-0524-z. Epub 2013 Oct 15. PMID: 24126695.
37. Qiu T, Crane JL, Xie L, Xian L, Xie H, Cao X. IGF-I induced phosphorylation of PTH receptor enhances osteoblast to osteocyte transition. Bone Res. 2018 Feb 26;6:5. doi: 10.1038/s41413-017-0002-7. PMID: 29507819; PMCID: PMC5827661.
38. Kimura K, Terasaka T, Iwata N, Katsuyama T, Komatsubara M, Nagao R, Inagaki K, Otsuka F. Combined Effects of Androgen and Growth Hormone on Osteoblast Marker Expression in Mouse C2C12 and MC3T3-E1 Cells Induced by Bone Morphogenetic Protein. J Clin Med. 2017 Jan 5;6(1):6. doi: 10.3390/jcm6010006. PMID: 28067796; PMCID: PMC5294959.
39. Guerra-Menéndez L, Sádaba MC, Puche JE, Lavandera JL, de Castro LF, de Gortázar AR, Castilla-Cortázar I. IGF-I increases markers of osteoblastic activity and reduces bone resorption via osteoprotegerin and RANK-ligand. J Transl Med. 2013 Oct 25;11:271. doi: 10.1186/1479-5876-11-271. PMID: 24161214; PMCID: PMC4231608.
40. Locatelli V, Bianchi VE. Effect of GH/IGF-1 on Bone Metabolism and Osteoporsosis. Int J Endocrinol. 2014;2014:235060. doi: 10.1155/2014/235060. Epub 2014 Jul 23. PMID: 25147565; PMCID: PMC4132406.
41. Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. J Clin Invest. 2006 May;116(5):1202-9. doi: 10.1172/JCI28551. PMID: 16670761; PMCID: PMC1451219.
42. de la Garza RG, Morales-Garza LA, Martin-Estal I, Castilla-Cortazar I. Insulin-Like Growth Factor-1 Deficiency and Cirrhosis Establishment. J Clin Med Res. 2017 Apr;9(4):233-247. doi: 10.14740/jocmr2761w. Epub 2017 Feb 21. PMID: 28270882; PMCID: PMC5330765.
43. Golds G, Houdek D, Arnason T. Male Hypogonadism and Osteoporosis: The Effects, Clinical Consequences, and Treatment of Testosterone Deficiency in Bone Health. Int J Endocrinol. 2017;2017:4602129. doi: 10.1155/2017/4602129. Epub 2017 Mar 16. PMID: 28408926; PMCID: PMC5376477.
44. Seeman E. The structural and biomechanical basis of the gain and loss of bone strength in women and men. Endocrinol Metab Clin North Am. 2003 Mar;32(1):25-38. doi: 10.1016/s0889-8529(02)00078-6. PMID: 12699291.
45. Moe SM. Calcium Homeostasis in Health and in Kidney Disease. Compr Physiol. 2016 Sep 15;6(4):1781-1800. doi: 10.1002/cphy.c150052. PMID: 27783859.

46. Cao X. RANKL-RANK signaling regulates osteoblast differentiation and bone formation. Bone Res. 2018 Nov 27;6:35. doi: 10.1038/s41413-018-0040-9. PMID: 30510840; PMCID: PMC6255775.
47. Guerrini MM, Takayanagi H. The immune system, bone and RANKL. Arch Biochem Biophys. 2014 Nov 1;561:118-23. doi: 10.1016/j.abb.2014.06.003. Epub 2014 Jun 12. PMID: 24929185.
48. Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, Pacifici R. Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-alpha. J Clin Invest. 2000 Nov;106(10):1229-37. doi: 10.1172/JCI11066. PMID: 11086024; PMCID: PMC381439.
49. Tilg H, Moschen AR, Kaser A, Pines A, Dotan I. Gut, inflammation and osteoporosis: basic and clinical concepts. Gut. 2008 May;57(5):684-94. doi: 10.1136/gut.2006.117382. PMID: 18408105.
50. Walsh MC, Choi Y. Biology of the RANKL-RANK-OPG System in Immunity, Bone, and Beyond. Front Immunol. 2014 Oct 20;5:511. doi: 10.3389/fimmu.2014.00511. PMID: 25368616; PMCID: PMC4202272.
51. Lorenzo J, Horowitz M, Choi Y. Osteoimmunology: interactions of the bone and immune system. Endocr Rev. 2008 Jun;29(4):403-40. doi: 10.1210/er.2007-0038. Epub 2008 May 1. PMID: 18451259; PMCID: PMC2528852.
52. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Arch Biochem Biophys. 2008 May 15;473(2):139-46. doi: 10.1016/j.abb.2008.03.018. Epub 2008 Mar 25. PMID: 18395508; PMCID: PMC2413418.
53. Wu Q, Zhou X, Huang D, Ji Y, Kang F. IL-6 Enhances Osteocyte-Mediated Osteoclastogenesis by Promoting JAK2 and RANKL Activity In Vitro. Cell Physiol Biochem. 2017;41(4):1360-1369. doi: 10.1159/000465455. Epub 2017 Mar 9. PMID: 28278513.
54. Nakchbandi IA, Mitnick MA, Lang R, Gundberg C, Kinder B, Insogna K. Circulating levels of interleukin-6 soluble receptor predict rates of bone loss in patients with primary hyperparathyroidism. J Clin Endocrinol Metab. 2002 Nov;87(11):4946-51. doi: 10.1210/jc.2001-011814. PMID: 12414855.
55. Blaschke M, Koepp R, Cortis J, Komrakova M, Schieker M, Hempel U, Siggelkow H. IL-6, IL-1β, and TNF-α only in combination influence the osteoporotic phenotype in Crohn's patients via bone formation and bone resorption. Adv Clin Exp Med. 2018 Jan;27(1):45-56. doi: 10.17219/acem/67561. PMID: 29521042.
56. Norris CA, He M, Kang LI, Ding MQ, Radder JE, Haynes MM, Yang Y, Paranjpe S, Bowen WC, Orr A, Michalopoulos GK, Stolz DB, Mars WM. Synthesis of IL-6 by hepatocytes is a normal response to common hepatic stimuli. PLoS One. 2014 Apr 24;9(4):e96053. doi: 10.1371/journal.pone.0096053. Erratum in: PLoS One. 2019 Oct 23;14(10):e0224498. PMID: 24763697; PMCID: PMC3999098.
57. Shimada M, Matsumata T, Taketomi A, Shirabe K, Yamamoto K, Takenaka K, Sugimachi K. The role of interleukin-6, interleukin-16, tumor necrosis factor-alpha and endotoxin in hepatic resection. Hepatogastroenterology. 1995 Sep-Oct;42(5):691-7. PMID: 8751236.
58. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol Rev. 2018 Jan;281(1):8-27. doi: 10.1111/imr.12621. PMID: 29247995; PMCID: PMC5756628.
59. Zhao B, Grimes SN, Li S, Hu X, Ivashkiv LB. TNF-induced osteoclastogenesis and inflammatory bone resorption are inhibited by transcription factor RBP-J. J Exp Med. 2012 Feb 13;209(2):319-34. doi: 10.1084/jem.20111566. Epub 2012 Jan 16. PMID: 22249448; PMCID: PMC3280875.
60. Boyce BF, Li P, Yao Z, Zhang Q, Badell IR, Schwarz EM, O'Keefe RJ, Xing L. TNF-alpha and pathologic bone resorption. Keio J Med. 2005 Sep;54(3):127-31. doi: 10.2302/kjm.54.127. PMID: 16237274.

61. Bentmann A, Kawelke N, Moss D, Zentgraf H, Bala Y, Berger I, Gasser JA, Nakchbandi IA. Circulating fibronectin affects bone matrix, whereas osteoblast fibronectin modulates osteoblast function. J Bone Miner Res. 2010 Apr;25(4):706-15. doi: 10.1359/jbmr.091011. PMID: 19821765.
62. Hackl NJ, Bersch C, Feick P, Antoni C, Franke A, Singer MV, Nakchbandi IA. Circulating fibronectin isoforms predict the degree of fibrosis in chronic hepatitis C. Scand J Gastroenterol. 2010 Mar;45(3):349-56. doi: 10.3109/00365520903490606. PMID: 20017652.
63. Tilg H, Cani PD, Mayer EA. Gut microbiome and liver diseases. Gut. 2016 Dec;65(12):2035-2044. doi: 10.1136/gutjnl-2016-312729. Epub 2016 Oct 8. PMID: 27802157.
64. Tripathi A, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, Knight R. The gut-liver axis and the intersection with the microbiome. Nat Rev Gastroenterol Hepatol. 2018 Jul;15(7):397-411. doi: 10.1038/s41575-018-0011-z. Erratum in: Nat Rev Gastroenterol Hepatol. 2018 May 21; PMID: 29748586; PMCID: PMC6319369.
65. Spor A, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut microbiome. Nat Rev Microbiol. 2011 Apr;9(4):279-90. doi: 10.1038/nrmicro2540. PMID: 21407244.
66. Chen YC, Greenbaum J, Shen H, Deng HW. Association Between Gut Microbiota and Bone Health: Potential Mechanisms and Prospective. J Clin Endocrinol Metab. 2017 Oct 1;102(10):3635-3646. doi: 10.1210/jc.2017-00513. PMID: 28973392; PMCID: PMC5630250.
67. Young VB. The role of the microbiome in human health and disease: an introduction for clinicians. BMJ. 2017 Mar 15;356:j831. doi: 10.1136/bmj.j831. PMID: 28298355.
68. D'Amelio P, Sassi F. Gut Microbiota, Immune System, and Bone. Calcif Tissue Int. 2018 Apr;102(4):415-425. doi: 10.1007/s00223-017-0331-y. Epub 2017 Sep 30. PMID: 28965190.
69. Novince CM, Whittow CR, Aartun JD, Hathaway JD, Poulides N, Chavez MB, Steinkamp HM, Kirkwood KA, Huang E, Westwater C, Kirkwood KL. Commensal Gut Microbiota Immunomodulatory Actions in Bone Marrow and Liver have Catabolic Effects on Skeletal Homeostasis in Health. Sci Rep. 2017 Jul 18;7(1):5747. doi: 10.1038/s41598-017-06126-x. PMID: 28720797; PMCID: PMC5515851.
70. Hsu E, Pacifici R. From Osteoimmunology to Osteomicrobiology: How the Microbiota and the Immune System Regulate Bone. Calcif Tissue Int. 2018 May;102(5):512-521. doi: 10.1007/s00223-017-0321-0. Epub 2017 Oct 10. PMID: 29018933; PMCID: PMC5893441.
71. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu; European Association for the Study of the Liver. EASL Clinical Practice Guidelines on nutrition in chronic liver disease. J Hepatol. 2019 Jan;70(1):172-193. doi: 10.1016/j.jhep.2018.06.024. Epub 2018 Aug 23. PMID: 30144956; PMCID: PMC6657019.
72. https://*www.ausl.bologna.it/cit/urc/le-carte-dei-servizi-delle-unita-operative/laboratorio-unico-metropolitano-lum/laboratorio-unico-metropolitano-lum?searchterm=referti (last access 10/24/2023).*
73. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res. 1993 Sep;8(9):1137-48.
74. Hundersmarck D, Groot OQ, Schuijt HJ, Hietbrink F, Leenen LPH, Heng M. Hip Fractures in Patients With Liver Cirrhosis: Worsening Liver Function Is Associated with Increased Mortality. Clin Orthop Relat Res. 2022 Jun 1;480(6):1077-1088. doi: 10.1097/CORR.0000000000002088. Epub 2021 Dec 31. PMID: 34978539; PMCID: PMC9263483.
75. Wester A, Hagström H. Risk of fractures and subsequent mortality in non-alcoholic fatty liver disease: A nationwide population-based cohort study. J Intern Med. 2022 Sep;292(3):492-500. doi: 10.1111/joim.13497. Epub 2022 Apr 19. PMID: 35373876; PMCID: PMC9545244.

76. Huldén E, Castedal M, Karlsson MK, Kalaitzakis E, Swärd P. Osteoporosis in cirrhotics before and after liver transplantation: relation with malnutrition and inflammatory status. Scand J Gastroenterol. 2020 Mar;55(3):354-361. doi: 10.1080/00365521.2020.1735507. Epub 2020 Mar 17. PMID: 32180479.

77. Zavatta G, Clarke BL. Glucocorticoid- and Transplantation-Induced Osteoporosis. Endocrinol Metab Clin North Am. 2021 Jun;50(2):251-273. doi: 10.1016/j.ecl.2021.03.002. PMID: 34023042.

Supplementary material:

Supplementary Table (a). Women with and without fractures: continuous variables.

	Without Fractures			**With Fractures**			**P Value**
	N	**Mean**	**Std. Deviation**	**N**	**Mean**	**Std. Deviation**	
Age	67	49,58	12,24	40	52,60	10,34	0,298
Body Weight (kg)	63	65,62	13,81	40	68,40	13,46	0,277
MELD score	64	31,55	13,51	40	28,50	12,10	0,198
GOT	60	89,08	109,12	39	103,77	223,65	0,895
GPT	60	62,45	104,62	40	88,33	311,26	0,683
Total Bilirubin	60	5,08	6,84	40	6,51	7,63	0,125
Direct Bilirubin	60	3,36	5,32	40	4,69	6,69	0,132
GGT	59	63,90	49,48	40	57,50	68,06	0,188
ALP	59	230,37	144,50	39	253,92	202,20	0,890
Urea	58	27,26	17,67	38	25,64	18,86	0,589
Creatinine	60	0,78	0,23	40	1,29	2,34	0,493
Blood glucose	57	95,91	41,15	38	82,55	15,50	0,199
Total protein	58	6,92	0,86	39	7,07	0,99	0,431
Albumin	59	3,52	0,63	39	3,44	0,61	0,443
Triglycerides	59	92,54	47,73	39	100,64	78,72	0,564
Cholesterol	58	127,81	54,06	39	132,95	51,11	0,906
INR	59	1,74	0,81	40	1,80	1,35	0,620
Fibrinogen	56	230,25	132,72	34	216,41	107,40	0,800

Platelet Count	60	109,12	98,26	40	130,24	148,15	0,335
White blood cell	60	4,75	3,26	39	5,55	4,07	0,176
Red blood cell	60	3,65	0,67	39	3,52	0,76	0,375
Hemoglobin	60	10,99	1,80	40	10,74	1,71	0,497
Hematocrit	60	33,15	5,45	40	32,41	5,01	0,437
Lumbar BMD	4	0,73	0,11	9	0,88	0,15	0,089
Lumbar T-score	4	-2,98	1,03	9	-1,78	1,20	0,122
Lumbar Z-score	4	-1,93	0,48	8	-0,88	1,33	0,172
Femur Neck BMD	4	0,61	0,14	8	0,70	0,11	0,308
Femur Neck T-score	4	-2,18	1,28	8	-1,48	0,93	0,396
Femur Neck Z-score	4	-1,20	0,85	8	-0,69	0,91	0,393
Total Hip BMD	4	0,76	0,14	7	0,85	0,19	0,571
Total Hip T-score	4	-1,78	0,62	7	-1,17	1,39	0,506
Total Hip Z-score	4	-1,03	0,67	7	-0,60	1,37	0,850
PTH [pg/mL]	5	63,60	51,09	3	26,00	5,20	0,050
Calcium [mg/dl]	59	8,82	0,53	39	9,01	0,81	0,295
Phosphate [mg/dL]	51	3,01	0,81	31	3,25	0,65	0,185
Magnesium [mg/dl]	46	1,99	0,25	30	1,90	0,36	0,158
Urinary Calcium [mg/24h]	3	19,30	11,89	6	3,88	5,09	**0,039**
Urinary phosphate [g/24h]	3	2,06	2,75	6	0,59	0,33	0,796
25OH Vitamin D [ng/mL]	3	13,77	13,22	3	12,57	3,65	0,513
Bone specific alkaline phosphatase (BSAP) [microg/L]	1	25,30		1	64,30		0,317

Estimated GFR	61	84,59	22,32	38	73,35	27,77	**0,038**
Serum Creatinine	60	0,78	0,27	38	1,11	1,10	0,156
BMI	57	25,23	4,50	39	26,03	4,67	0,330
Number, total number. Abbreviations: ALP, alkaline phosphatase; GGT, Gamma-glutamyl transferase							

Supplementary Table (b). Women with and without fractures: categorical variables.					
Variable	**Category**	**Without Fractures**	**With Fractures**	**Total Number**	**P Value**
Ethnicity	White Caucasian	64	39	103	0,436
	Other	1	0	1	
	Total	65	39	104	
Arterial Hypertension		9	9	18	0,339
	Total	60	40	100	
Child-Pugh	Missing	14	4	18	0,294
	A	13	5	18	
	B	21	17	38	
	C	19	14	33	
	Total	67	40	107	
Alcohol use		7_a	10_b	17	**0,046**
	Total	67	40	107	
Smoking		5	6	11	0,214
	Total	67	40	107	
Vitamin D3 intake		0	1	1	0,193
	Total	67	40	107	
PPI		3	6	9	0,058
	Total	67	40	107	
Calcium carbonate supplements		0	1	1	0,193
	Total	67	40	107	
Diabetes		9	3	12	0,347
	Total	67	40	107	
Corticosteroid use		7	7	14	0,295
	Total	67	40	107	
Ascites		35	24	59	0,435
	Total	67	40	107	
Encephalopathy		26	16	42	0,903
	Total	67	40	107	
Hepatocellular carcinoma		21	10	31	0,484
	Total	67	40	107	
GI Hemorrhage		5	5	10	0,386
	Total	67	40	107	
Portal thrombosis		8	4	12	0,758

	Total	67	40	107	
Etiology	Autoimmune	6	4	10	0,591
	Cholestatic	6	5	11	
	Viral	24	15	39	
	Alcohol	2	4	6	
	Cryptogenic/rare	15	6	21	
	Multifactorial	14	6	20	
	Total	67	40	107	
Vertebral fractures		0_a	36_b	36	**<0.001**
	Total	67	40	107	
Genant's grade	Mild	n.a.	24	24	
	Moderate	n.a.	6	6	
	Severe	n.a.	6	6	
	Total	n.a.	36	36	
DXA WHO classification	Normal BMD	2	0	2	**0,042**
	Low BMD/osteopenia	1_a	7_b	8	
	Osteoporosis	3	2	5	
	Total	6	9	15	

Supplementary Table (c). Men with and without fractures: continuous variables.

	Without Fractures			**With Fractures**			**P Value**
	N	**Mean**	**Std. Deviation**	**N**	**Mean**	**Std. Deviation**	
Age	144	52,92	11,02	115	53,99	8,43	0,767
Body Weight (kg)	144	77,13	13,62	114	79,11	15,31	0,294
MELD score	144	29,13	10,60	114	29,13	10,01	0,876
GOT	137	83,45	60,80	109	74,47	59,96	0,166
GPT	136	73,76	150,23	110	52,82	48,49	**0,027**
Total Bilirubin	138	5,53	7,37	110	7,52	19,56	0,615
Direct Bilirubin	134	4,37	9,46	107	3,94	6,28	0,525
GGT	136	85,56	78,52	109	87,08	105,84	0,578
ALP	136	227,09	159,33	110	210,51	148,28	0,263
Urea	135	36,95	33,20	108	35,50	33,25	0,815
Creatinine	138	1,34	2,86	110	1,01	0,61	0,559
Blood glucose	136	111,28	47,11	109	99,51	30,28	0,142
Total protein	132	6,85	0,87	106	6,89	0,94	0,612
Albumin	136	3,47	0,70	106	3,57	0,63	0,171
Triglycerides	127	88,37	42,02	105	87,59	52,75	0,427
Cholesterol	128	121,88	44,33	105	119,58	49,96	0,582
INR	137	1,56	0,43	109	1,55	0,41	0,684

Fibrinogen	121	205,39	102,34	103	213,51	99,26	0,562
Platelet Count	138	94,30	82,67	110	89,26	65,49	0,975
White blood cell	136	5,49	3,94	110	4,80	2,25	0,323
Red blood cell	133	3,80	0,86	107	3,67	0,86	0,300
Hemoglobin	136	11,85	2,10	110	11,34	2,14	0,094
Hematocrit	135	35,42	6,07	108	34,03	6,21	0,095
Alpha fetoprotein	130	19,01	47,46	107	57,65	469,54	**0,018**
CA 19-9	128	50,00	63,54	104	58,81	80,98	0,891
Lumbar BMD	15	0,93	0,09	12	0,84	0,13	0,054
Lumbar T-score	15	-1,51	0,85	12	-2,28	1,22	0,053
Lumbar Z-score	15	-1,12	0,92	12	-1,82	1,18	0,071
Femur Neck BMD	14	0,79	0,12	14	0,71	0,14	0,089
Femur Neck T-score	14	-1,11	0,89	14	-1,63	0,89	0,160
Femur Neck Z-score	14	-0,29	0,91	14	-0,82	0,93	0,135
Total Hip BMD	15	0,97	0,14	14	0,89	0,18	0,097
Total Hip T-score	15	-0,42	0,92	14	-1,04	1,03	0,066
Total Hip Z-score	15	-0,09	0,94	14	-0,65	1,03	0,084
PTH [pg/mL]	12	76,00	106,57	7	35,43	23,89	0,612
Calcium [mg/dl]	131	8,79	0,61	110	8,85	0,64	0,567
Phosphate [mg/dL]	107	3,14	0,73	92	3,09	0,68	0,544
Magnesium [mg/dl]	97	1,91	0,26	92	1,99	0,33	0,142
Urinary Calcium [mg/24h]	8	4,76	3,63	15	8,06	7,73	0,478
Urinary phosphate [g/24h]	6	0,59	0,25	13	0,67	0,28	0,599
25OH Vitamin D [ng/mL]	15	17,49	7,79	15	12,61	6,59	**0,042**
Bone specific alkaline phosphatase (BSAP) [microg/L]	1	19,10		6	31,65	9,62	0,134
Albumin [g/dl]	113	3,45	0,67	93	3,65	0,75	0,159
Estimated GFR	135	87,95	30,57	109	84,50	25,88	0,310
Serum Creatinine	135	1,14	0,98	109	1,09	0,65	0,202
BMI	137	25,25	3,55	109	25,99	4,02	0,113
Abbreviations: ALP, alkaline phosphatase; GGT, Gamma-glutamyl transferase							

Supplementary Table (d). Men with and without fractures: categorical variables.					
Variable		**Without Fractures**	**With Fractures**	**Total**	**P Value**
Ethnicity	White Caucasian	139	115	254	0,514
	Other	4	0	4	
	Total	143	115	258	
Arterial Hypertension		34	21	55	0,317
	Total	137	108	245	
Child-Pugh	Missing	16	11	27	0,975
	A	31	24	55	
	B	38	32	70	
	C	59	48	107	
	Total	144	115	259	
Alcohol use		55	46	101	0,767
		144	115	259	
Smoking		41	28	69	0,456
	Total	144	115	259	
Vitamin D3 intake		6	7	13	0,482
	Total	144	115	259	
PPI		31	23	54	0,764
	Total	144	115	259	
Calcium carbonate supplements		5	1	6	0,167
	Total	144	115	259	
Diabetes		47	29	76	0,192
	Total	144	115	259	
Corticosteroids		9	7	16	0,957
	Total	144	115	259	
Ascites		77	65	142	0,624
	Total	144	115	259	
Encephalopathy		57	40	97	0,428
	Total	144	115	259	
Hepatocellular carcinoma		63	55	118	0,513
	Total	144	115	259	
GI Hemorrhage		20	8	28	0,074
	Total	144	115	259	
Portal thrombosis		19	10	29	0,254
	Total	144	115	259	
Etiology	Autoimmune Hepatitis	2	0	2	0,698
	Cholestatic disease	4	7	11	
	Viral	58	47	105	
	NASH	1	1	2	
	Alcoholic	19	17	36	
	Cryptogenic/Rare disease	16	13	29	
	Multifactorial	44	30	74	

Vertebral fractures		0_a	109_b	109	**<0.001**
	Total	144	115	259	
Genant's grade	Mild	n.a.	66	66	n.a.
	Moderate	n.a.	37	37	
	Severe	n.a.	6	6	
	Total		109	109	
DXA WHO classification	Normal BMD	3_a	2_a	5	0,212
	Low BMD/osteopenia	8_a	4_a	12	
	Osteoporosis	3_a	7_a	10	
	Total	14	13	27	

N, total number

Abbreviation: GI, gastrointestinal.