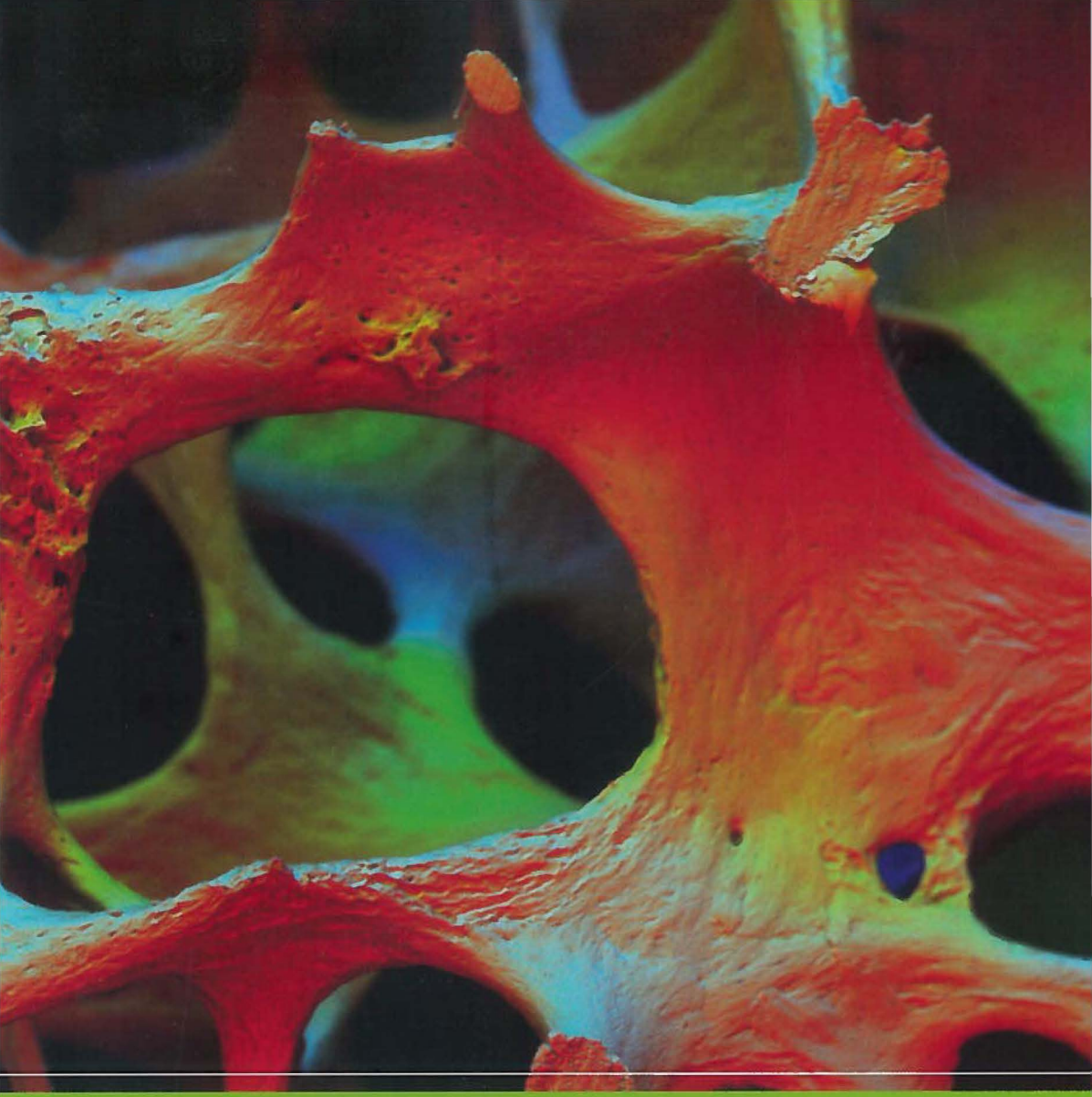
Bone disease before and after liver transplantation : clinical studies in cholestatic liver disease

Guichelaar, Maureen Maike Johanna



Bone Disease Before and After Liver Transplantation

Maureen M.J. Guichelaar

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Clinical studies in cholestatic liver disease

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Cover by Professor Alan Boyde, School of Medicine and Dentistry, Queen Mary, University of London. *Low power scanning electron microscopy image, showing osteoporotic architecture of the fourth lumbar vertebra. The bone is heavily eroded and consists mainly of thin, fragile struts.*

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Clinical studies in cholestatic liver disease

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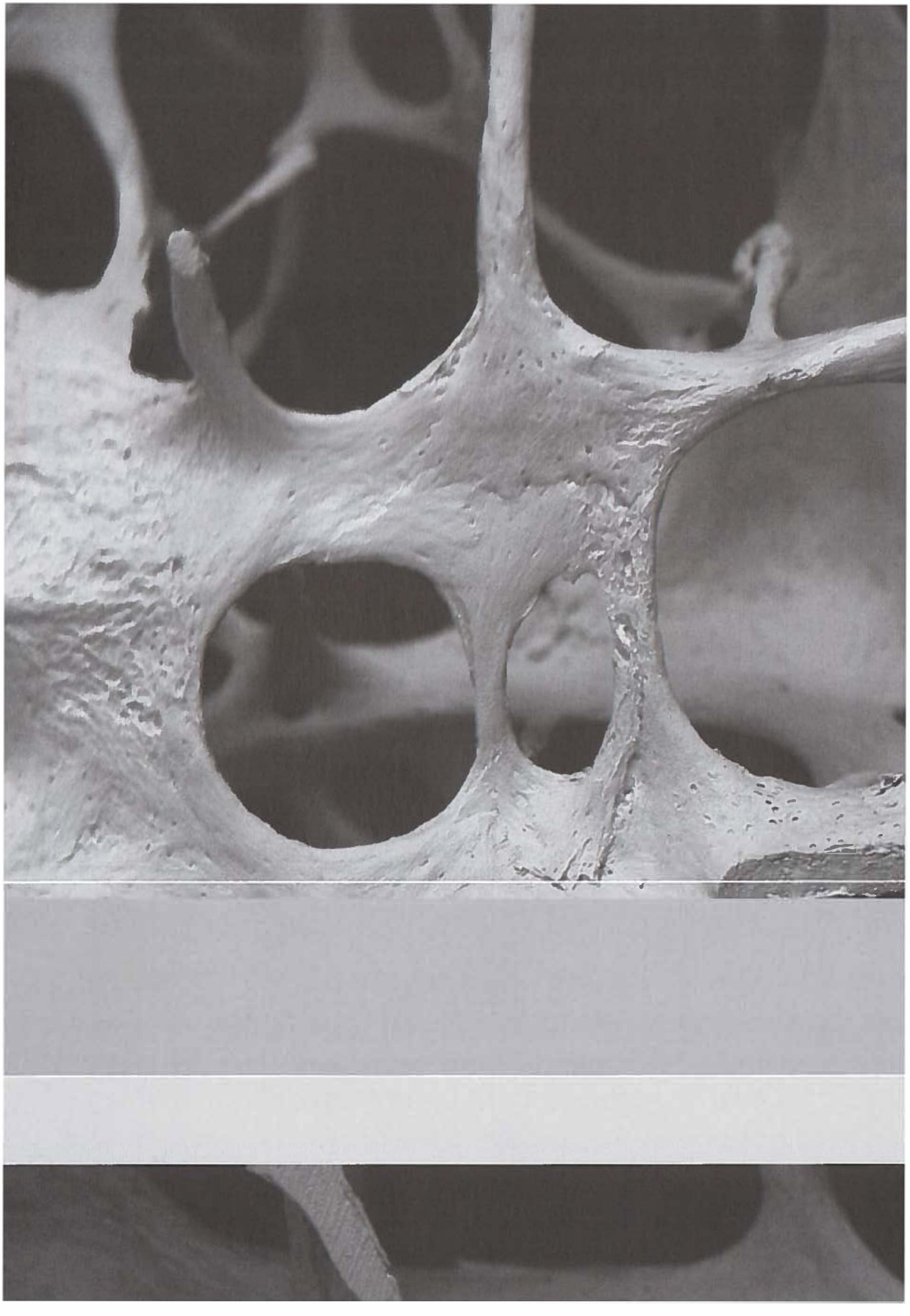
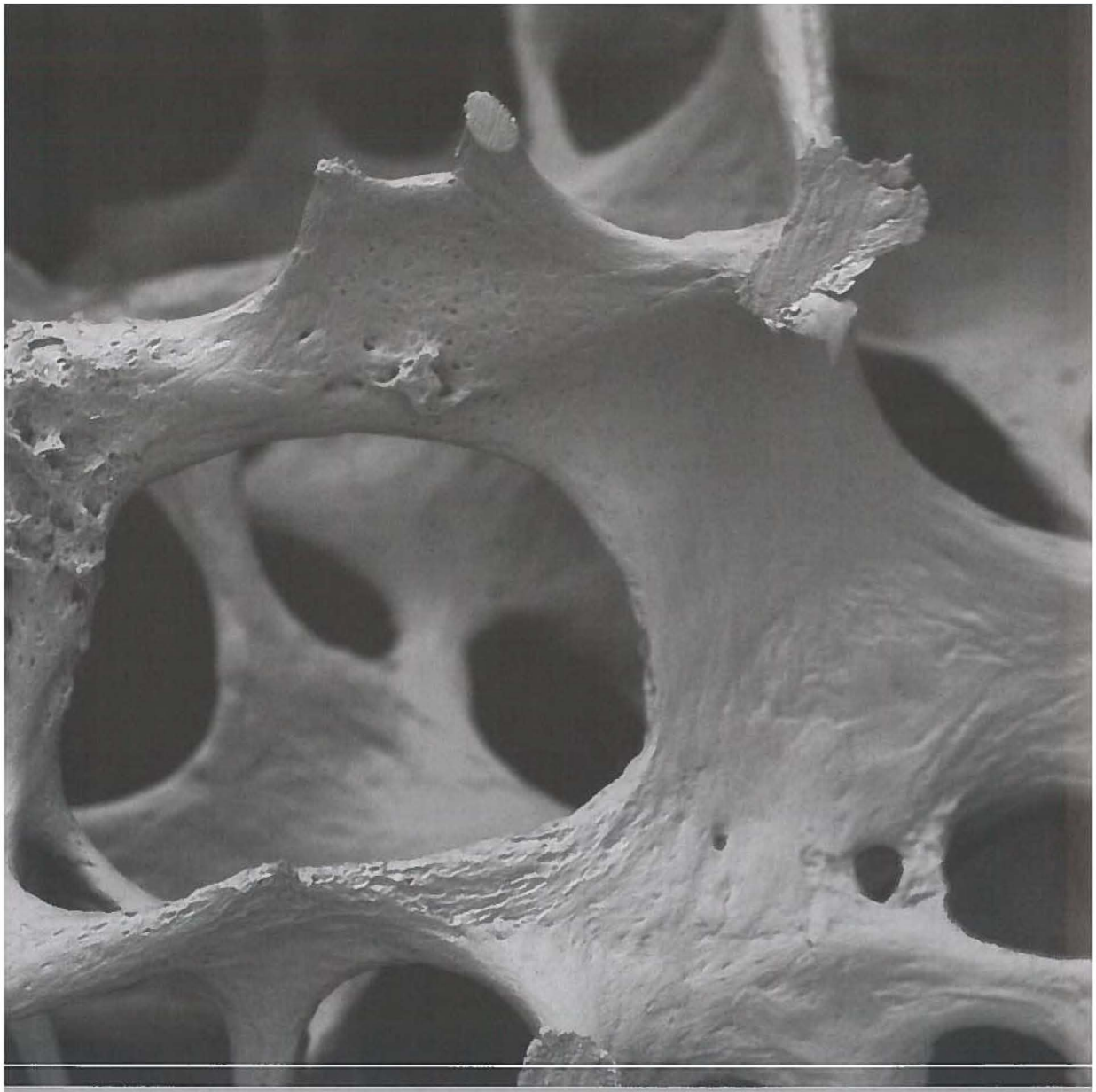
"Principles for the Development of a Complete Mind: Study the science of art. Study the art of science. Develop your senses - especially learn how to see. Realise that everything connects to everything else."

Leonardo da Vinci (1452-1519)





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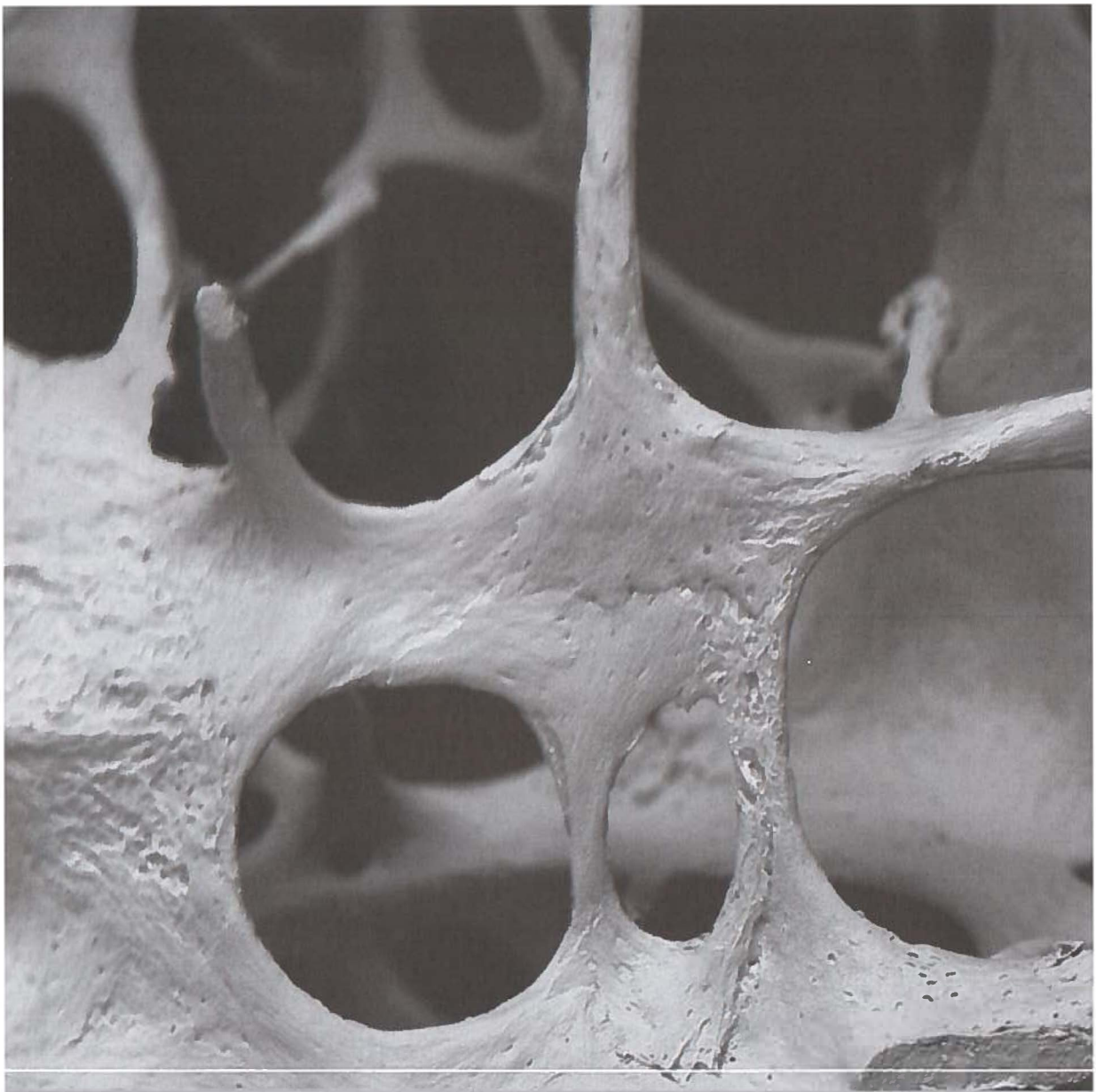
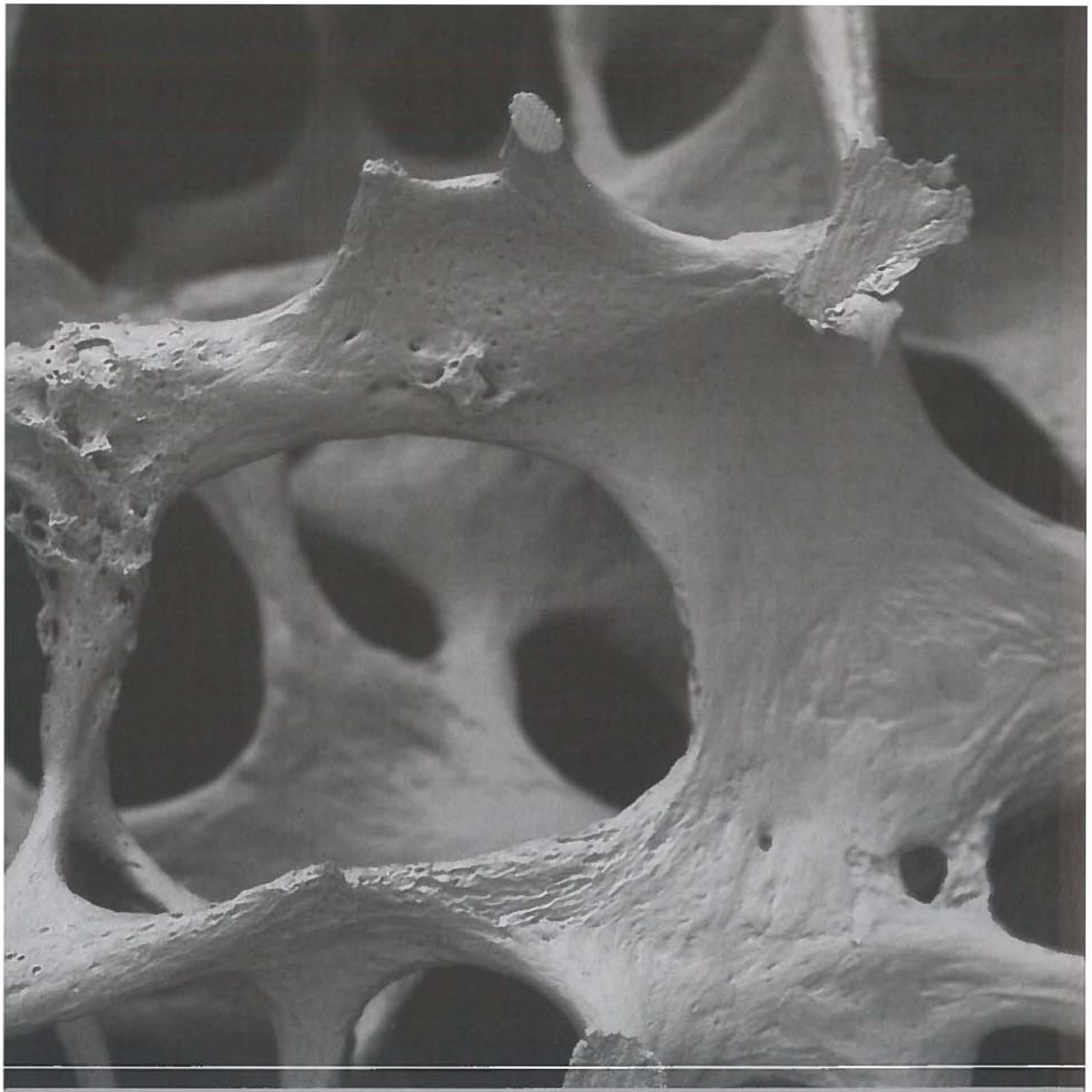
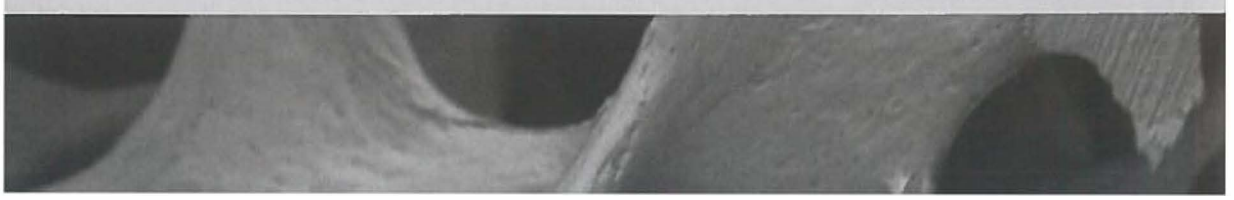


Chapter 1



Introduction and outline of the thesis



Introduction

Bone is a metabolically active organ which undergoes life-long remodeling to maintain skeletal mass and strength. In humans, 25% trabecular bone and 3% cortical bone is turned over each year. The remodeling cycle starts with bone resorption by osteoclasts to create a resorption pit. These osteoclasts undergo apoptosis and osteoblasts then deposit uncalcified bone (osteoid) which requires mineralization. Bone remodeling is controlled by many factors including genetic profile, hormonal and nutritional status, weight-bearing activities, and is regulated by several hormones and cytokines. In normal situations bone resorption, formation and mineralization work in equilibrium to maintain normal bone mineral density (BMD). BMD is maximal at the beginning of the third decade in both men and women (so-called peak BMD). It then remains stable until about 40-45 years of age and gradually declines afterwards. Decreased BMD occurs when bone resorption outstrips bone formation (i.e. osteopenia, osteoporosis) or when mineralization is defective (i.e. osteomalacia) and either condition results in reduced bone strength and increased risk of fractures.

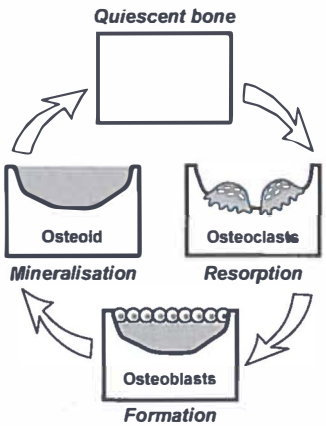


Figure 1.
Normal bone remodelling: balance of bone resorption, formation and mineralisation.

Bone mass or BMD can be measured directly by dual energy x-ray absorptiometry (DEXA), a noninvasive method which measures BMD with high accuracy and low radiation exposure. This method has widespread use for screening populations at risk of osteoporosis and can be used serially in individual patients. Osteoporosis is defined as 2.5 standard deviations below peak BMD of the reference population (T – scores < -2.5), while osteopenia is classified as BMD T-scores of -1 to -2.5. While BMD measurements allow an assessment of bone mass, no assessment of bone resorption, formation or mineralization can be made without histomorphometric analysis of bone biopsy specimens. At present no biochemical marker accurately reflects bone parameters.

An increased incidence of osteoporosis and atraumatic fracturing has long been recognized in patients with chronic liver diseases. Initially it was thought that increased fragility of bone was due to mineralization defects of newly formed bone (osteomalacia) as a result of malabsorption of vitamin D and calcium. In the seventies, the first

histomorphometric analysis of bone biopsy specimens showed that not osteomalacia, but osteoporosis was the primary bone abnormality in patients with advanced liver disease. These findings also implied that factors other than calcium and vitamin D metabolism are of etiologic importance. Under normal circumstances the liver is involved in many complex metabolic processes. Subsequently, failing liver function may result into several disturbed metabolic processes negatively affecting bone metabolism, including reduced protein metabolism, reduction of cytokines and growth hormones, poor nutritional status and muscle wasting, hypogonadism, and malabsorption of calcium and vitamin D.

Although more than thirty years has passed since the first histomorphometric studies, the etiologic mechanisms of bone loss in chronic liver disease have not been well established. This lack of knowledge of the underlying mechanisms of bone loss has led to a corresponding lack of specific therapies for these patients. Cirrhotic-stage liver disease can only be reversed by liver transplantation and it was hoped that normalization of hepatic function would also reverse the osteopenic bone disease. Unfortunately, orthotopic liver transplantation (OLT) requires immunosuppressive treatment which has been associated with a further reduction of BMD and an increase in fracturing. However, little is known about the predictive factors for posttransplant bone loss and subsequent bone gain, or about the longterm risk of fracturing.

Aim of the thesis

The aim of this thesis is to investigate the etiologic mechanisms of bone loss in a large population of patients with advanced chronic cholestatic liver disease (primary biliary cirrhosis, [PBC], and primary sclerosing cholangitis, [PSC]) and to study their skeletal complications before and after OLT.

The study is divided into:

1. A prospective study of 50 consecutive patients with PBC or PSC investigated by histomorphometric analysis of bone biopsy specimens, taken at time of OLT and at 4 months posttransplant. (Chapters 2, 3 and 4).

2. A retrospective study of 360 consecutive patients with PBC or PSC who were followed by protocol to assess BMD and fractures before and, in the long-term, after OLT (Chapters 5 and 6).

Outline of the thesis

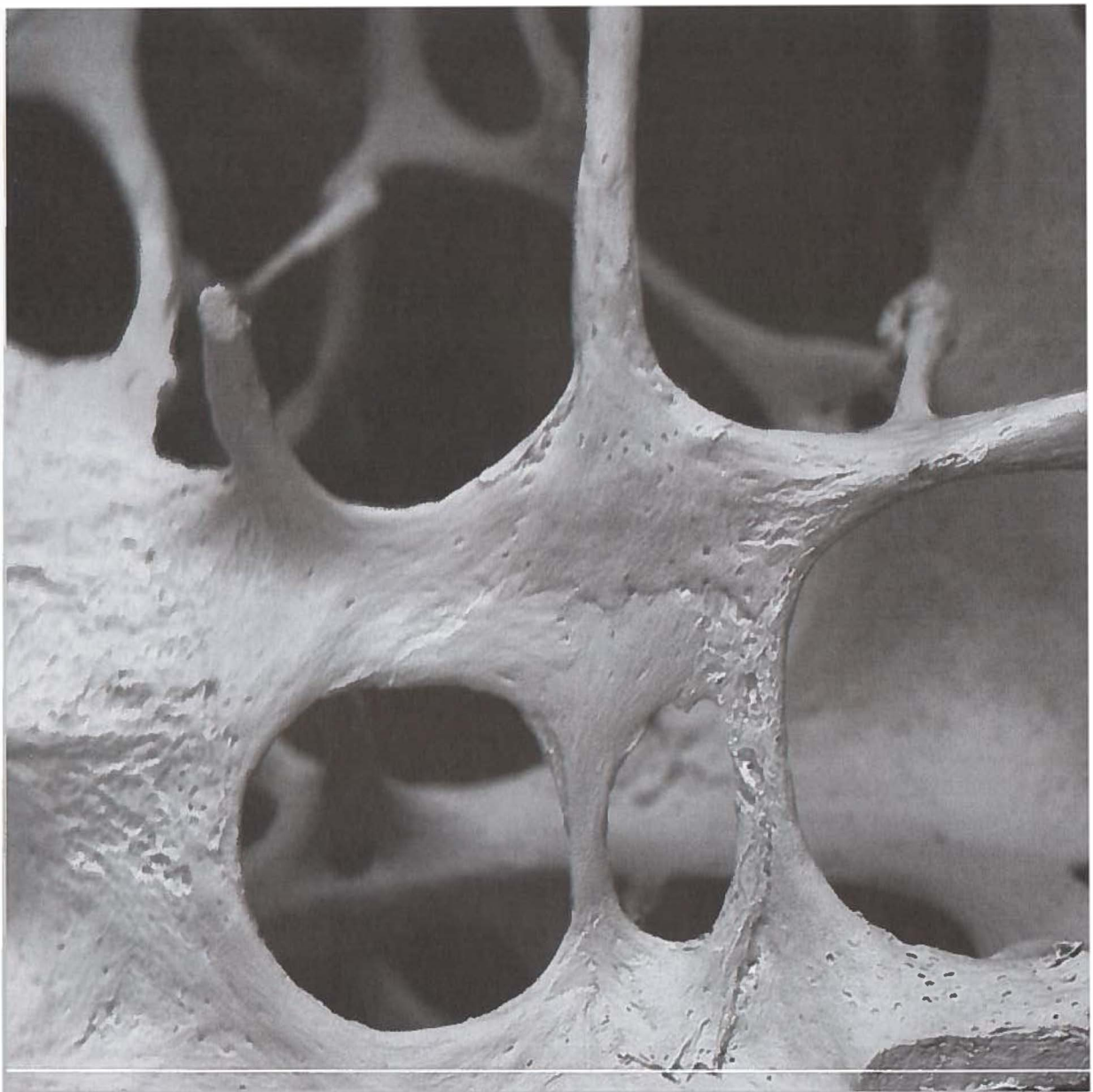
Fifty patients with advanced PBC and PSC were prospectively studied in order to investigate bone remodeling abnormalities (**chapter 2**). Patients had iliac crest bone biopsies taken during OLT after tetracycline labeling which is necessary to determine dynamic bone formation and mineralization rates. Other parameters studied included static parameters of bone volume, formation, resorption, and trabecular architecture indices (also serving as indirect bone resorption parameters). In addition, bone biochemical parameters of bone formation (osteocalcin and bone alkaline phosphatase) and bone resorption (urinary hydroxyproline) were assessed for their usefulness in predicting abnormalities in bone formation and resorption. By bone histomorphometric analysis of bone biopsy specimens, bone remodeling abnormalities in end-stage cholestatic liver disease patients were assessed by disease and gender.

Thirty-three of these cholestatic patients who underwent successful OLT had bone biopsies taken four months after OLT. In **chapter 3** we report posttransplant bone metabolism changes of these paired bone biopsy specimens. In addition, the effects of gender, disease and biochemical parameters on posttransplant bone metabolism were studied. Hierarchical cluster analysis, a statistical method grouping variables according to their functional similarity, was used to assess the functional relationship among histomorphometric parameters before and after OLT.

In **chapter 4,** clinical factors were evaluated in order to identify possible etiologic mechanisms of bone remodeling abnormalities before and after OLT. Clinical factors included demographic data, biochemical changes, and posttransplant characteristics (including immunosuppressive agents and hospitalization days). In addition, bone biochemical parameters of bone formation (osteocalcin, and bone alkaline phosphatase) and bone resorption (urinary hydroxyproline) were studied for their accuracy in predicting posttransplant changes in bone metabolism.
In contrast to histomorphometric assessment of bone metabolism, BMD assessment by DEXA is a non-invasive method, which is an accurate diagnostic tool to detect changes of BMD. In **chapter 5** we describe pretransplant BMD assessment with long-term follow-up after OLT in a large population of 360 patients with PBC or PSC. In addition to BMD, many pre- and posttransplant clinical variables were evaluated to assess predictive factors for early posttransplant BMD loss and later BMD gain. Since patients were transplanted over a 16-year time period, temporal changes in pre- and posttransplant BMD were also analyzed.

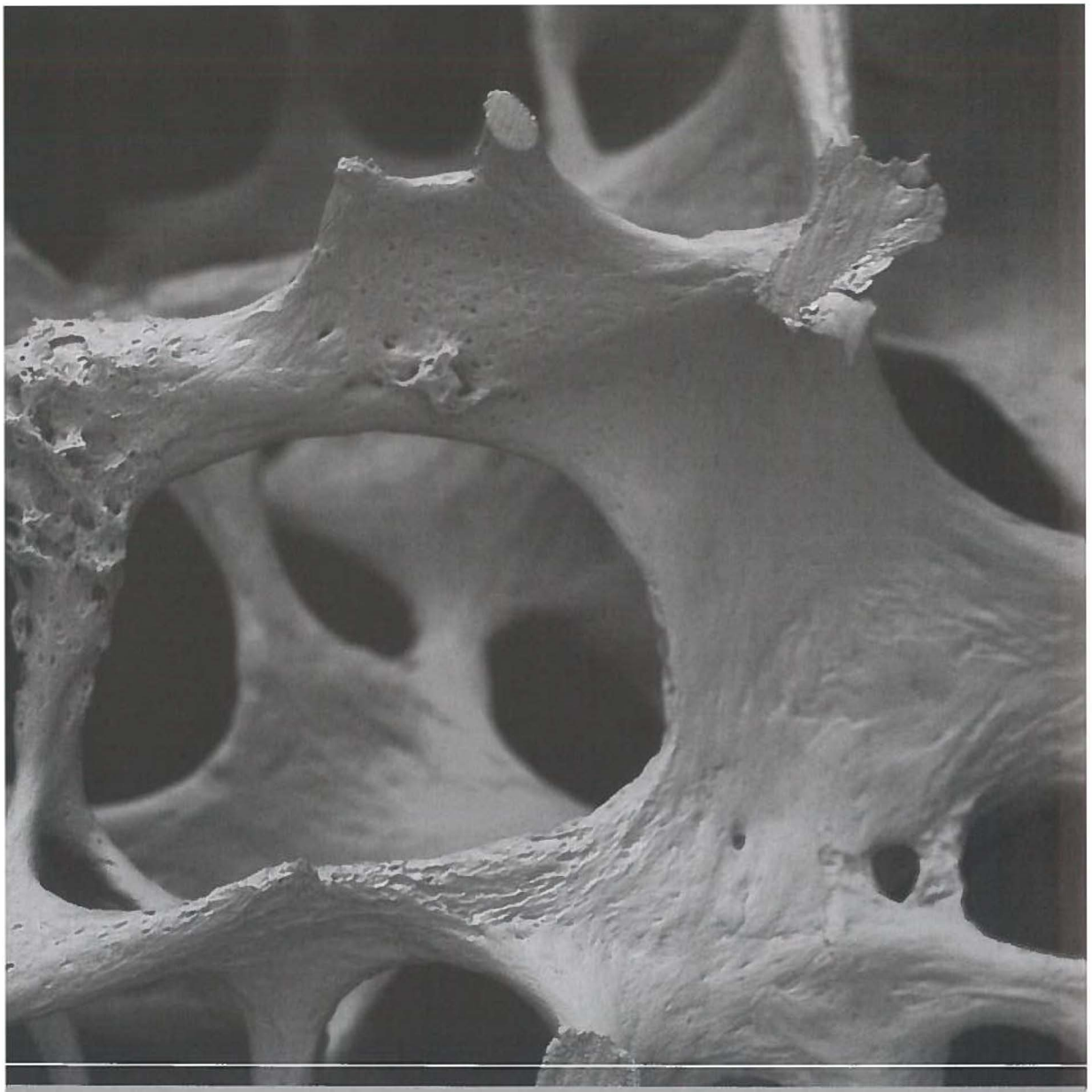
These 360 patients also underwent protocolised radiologic examination of lumbar spine, pelvis, hips and ribs before OLT with long-term follow-up after OLT. More than 7000 x-ray reports were evaluated to assess the incidence of fractures and avascular necrosis before and after OLT, and this is described in **chapter 6.** Many pre- and posttransplant clinical variables were evaluated to assess predictive factors for posttransplant fractures and avascular necrosis. In addition, we assessed whether the incidence of fractures and AVN changed over the 16-year study period.

In the last two chapters we provide reviews of the literature on hepatic bone disease and its management before OLT (**chapter 7**), and posttransplant changes in BMD and fractures with management options (**chapter 8**).



Chapter 2

Maureen M.J. Guichelaar, Michael Malinchoc, Jean D. Sibonga, Bart L. Clarke, J. Eileen Hay



Bone metabolism in advanced cholestatic liver disease: Analysis by bone histomorphometry

Hepatology 2002;36:895-903.

Abstract

Despite the clinical importance of cholestatic osteopenia, little is known about its pathophysiologic mechanism. By tetracycline-labeled histomorphometric analysis of bone biopsies taken at the time of liver transplantation, we prospectively evaluated bone resorption and formation in 50 consecutive patients with advanced primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Histomorphometric analysis confirmed low bone volume parameters, consistent with the mean T-score of the lumbar spine of –1.9 by dual energy X-ray absorptiometry. Dynamic (bone formation rates, adjusted apposition rates) and static (osteoid markers, osteoblast number) parameters of bone formation were decreased in cholestatic patients with no abnormalities in mineralization. Increased osteoclast numbers and increased eroded surface areas suggested increased bone resorption and this was supported in female patients by increased trabecular separation and decreased trabecular number. Male cholestatic patients, however, did not have significant increases in resorption parameters, although they were as osteopenic as female patients and had low bone formation markers. Bone histomorphometric changes were similar in PBC and PSC, suggesting an etiologic effect of chronic cholestasis rather than the individual diseases. Cancellous bone volume and osteoid markers correlated with bone mineral density measurements but no correlations were found between histomorphometric parameters and biochemical markers of bone metabolism. In conclusion, cholestatic osteopenia appears to result from a combination of decreased bone formation and increased resorption, especially in female patients, but the relative importance of these two abnormalities and their actual etiology remain to be elucidated.

Authors

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From the Divisions of [1]Gastroenterology and Hepatology, [2]Biostatistics, [3]Orthopedics and [4]Endocrinology, Mayo Clinic, Rochester, MN, USA; and [5]Division of Liver Transplantation, University Medical Center Groningen, the Netherlands

Abbreviations

PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; CCLD, chronic cholestatic liver disease; 25(OH)D, 25-hydroxyvitamin D; BMD, bone mineral density; OLT, orthotopic liver transplantation; BGP, bone gla-protein; DEXA, dual-energy X-ray absorptiometry.

Introduction

The majority of patients with advanced primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) have osteopenia.[1,2] Despite the potential for osteomalacia, it is now well established that this cholestatic osteopenia is caused by osteoporosis,[3-5] but little else is known about its pathophysiologic mechanism.

Bone metabolism is a complex, active process involving a balance between bone resorption and bone formation to maintain normal bone mass. In osteopenic disorders, "uncoupling" of this balance results in bone loss.[6] Although biochemical parameters of bone formation and resorption may provide a noninvasive method by which to examine bone metabolism,[7,8] no reliable biochemical marker of bone resorption or formation has been shown to be useful in patients with liver disease.[9,10] Tetracycline-labeled bone histomorphometry is a technique that allows the assessment of static parameters of bone metabolism, as well as dynamic markers of bone formation and mineralization.[11] Unfortunately, available histomorphometric data of bone metabolism in patients with chronic cholestatic liver disease (CCLD) have shown conflicting results.[12-18]

To assess the potential roles of increased resorption and/or decreased formation in the process of cholestatic bone loss, bone histomorphometric parameters were prospectively evaluated in 50 patients with advanced CCLD at time of liver transplantation. All patients had no other illnesses or medications known to influence bone metabolism. Bone status was also evaluated by bone mineral density measurements (BMD), spinal radiographs and full clinical and biochemical assessment.

Methods

Patient population

Fifty consecutive adult CCLD patients who fulfilled the following criteria were enrolled in this study: (1) advanced PBC or PSC, activated for liver transplantation; (2) absence of diseases other than PBC or PSC which affect bone metabolism; (3) no medications affecting bone metabolism in the 12 months preceding transplantation (corticosteroids, hormones, anticonvulsants, bisphosphonates, sodium fluoride); (4) normal creatinine clearance and normal thyroid function; (5) willingness to participate in the study, including consent to bone biopsy at the time of liver transplantation; and (6) completion of liver transplantation between April 1990 and July 1995. The diagnoses of PBC and PSC were made according to well-established criteria.[19-21] The study was approved by the Institutional Review Board of the Mayo Clinic.

Clinical assessment

All patients underwent a full clinical examination at the time of activation for orthotopic liver transplantation (OLT). Symptoms and signs of bone pain or fractures were sought, as well as measurements of height, weight and functional status (Karnofsky Performance Scale from 0 to 100).[22]

Biochemical testing

Blood was taken for biochemical assessment after an overnight fast and analyzed by Mayo Medical Laboratories using standard methods. Biochemical evaluation of the study patients included parameters of liver and kidney function (serum albumin, total alkaline phosphatase, total and direct bilirubin, prothrombin time, serum creatinine and iothalamate clearance), as well as parameters of bone mineral status (serum 25-hydroxyvitamin D [25(OH)D], serum calcium, ionized calcium, 24-hour urinary calcium, serum phosphorus, parathyroid hormone, magnesium), and gonadal status (in female patients, serum follicle-stimulating hormone and estradiol; in male patients, free testosterone). In addition, serum markers of bone formation (bone alkaline phosphatase, osteocalcin) and a urinary marker of bone resorption (24-hour urinary hydroxyproline) were measured. Osteocalcin (bone Gla-protein, BGP) was measured by radioimmunoassay using rabbit antibovine BGP antiserum and homogeneous bovine BGP.[23] Urinary hydroxyproline excretion was measured by the methods of Kivirikko et al.[24] and Bidlingmeyer et al.[25] Serum 25-hydroxyvitamin D was measured by the method of Kao and Heser.[26] Immunoreactive parathyroid hormone was measured by immunochemiluminometric assay.[27]

Assessment of BMD and fractures

BMD was determined by dual-energy x-ray absorptiometry (DEXA) of the lumbar spine (L1-L4) (coefficient of variation 2.2%), using a Hologic QDR 1000 densitometer (Hologic Corp., Waltham, MA). Bone mass was corrected for bone size to calculate BMD in g/cm^2. In patients with compression fractures, measurements were determined only on intact vertebrae. Large-volume paracentesis was performed, as necessary, for moderate/severe ascites prior to measurements of BMD. Radiographs of the chest were taken in all patients. Standard radiographs of the thoracolumbar spine were obtained at a tube distance of 120 cm. In addition, standard radiographs were obtained of sites of bone pain, and if negative, bone scans were performed.

Tetracycline labeling pre-OLT

All study patients received tetracycline labeling from the time of study enrollment and before OLT to allow assessment of dynamic bone parameters. Labeling was done with cycles of oxytetracycline (course A) and demeclocycline (course B). At the time of enrollment, oxytetracycline, 250 mg 4 times daily, was given for 3 days, followed by 14 days off treatment, then a further 3 days of 250 mg 4 times daily. If more than 6 weeks had elapsed from the last dose of oxytetracycline and the patient had not yet undergone OLT, then course B was started (demeclocycline, 150 mg 4 times daily for 3 days, 14 days off, then 150 mg 4 times daily for 3 days). Courses A and B were then repeated at 6-week intervals until OLT. If a patient was not able to receive oral medication during initial labeling, doxycycline 100 mg intravenously every 12 hours was given for 2 days. If a patient was called for OLT before the second label was administered (i.e. during the 14 days off drug), doxycycline 100 mg intravenously every 6 hours was given until the patient underwent OLT.

Bone biopsies

Bone biopsies were performed at time of OLT by the transplant surgeon at the standard iliac crest biopsy site using a 7.5-mm trephine.[28] Glucocorticoids were not administered

until after the bone biopsy. The bone tissue was placed into 70% ethanol then dehydrated in 95% for 1 day, and in 100% ethanol for 5 days before immersion for 4 days in polymethyl methacrylate and embedding by controlled temperature polymerization. Four pairs of consecutive 5-micron sections were obtained at 100-micron intervals. Sections were stained with Goldner-Masson-Trichrome, hematoxylin-eosin, and toluidine blue. Unstained sections were used for fluorescent microscopy analysis. Quantification of bone turnover parameters was carried out by Bioquant System IV image analysis that uses a microscope and digitizing tablet (R and M Biometrics, Nashville, TN). Bone biopsies were read stepwise from corner to corner with the use of a Zeiss microscope and fields with more than 30% distortion were discarded. Primary and derived data are generated by the software in accordance with standardized nomenclature and formulae.[29]

To reduce intraobserver variation, a mean of 4 readings was used for all measured histomorphometric parameters. Also to reduce interobserver variation, the 3 technicians responsible for quantifying all biopsies measured a reference bone biopsy to within 1 SD as per monthly quality control. Using normal female and male Mayo Clinic bone histomorphometric values as reference values (see below), bone histomorphometric parameters were expressed both as raw data and as Z-scores (sex-adjusted histomorphometric values).

Histomorphometric parameters

The following static parameters were analyzed:

Cancellous Bone Volume. Cancellous bone volume was measured as percentage of the total medullary bone volume from an unstained slide. Trabecular bone thickness, trabecular number, and trabecular separation are derived data from fractional cancellous bone volume and bone perimeter.[29]

Osteoid Markers. Osteoid thickness was measured by dividing each seam into 4 equal measurements in 50 fields or more and expressed as mean thickness of osteoid in micrometers. Osteoid volume is expressed as percentage of total bone volume and osteoid surface is expressed as the percentage of cancellous surfaces covered with osteoid.

Number of Osteoclasts Per 100 mm of Trabecular Surface Length. Osteoclasts were identified as large amorphous cells that interface with a bone-resorbing surface, with characteristically dense and somewhat granular cytoplasm (Goldner stain), and containing one or more irregularly shaped nuclei with prominent nucleoli.

Eroded Surface. Eroded surface was a scalloped surface eroded to a depth of one lamella or more and expressed as a percentage of cancellous surface showing resorption cavities.

Osteoblast-Osteoid Interface. Osteoblast-osteoid interface is the percentage of osteoid surface covered by osteoblasts, defined as cuboidal pyronine-staining cells.

Cortical Thickness. Cortical thickness is the mean thickness of cortical seams in micrometers (average of 12 measurements).

Mean Wall Thickness. Mean wall thickness is the mean thickness of the total bone structural unit in micrometers measured as the distance between the cement line and mineralized bone surface.

Dynamic data

The tetracycline double- and single-label lengths are measured using the unstained slide, which was scanned until 50 measurements were measured. Mineral apposition rate was calculated as the mean of 4 equally spaced interlabel thickness measurements (obtained from all available double labels on cancellous surfaces), divided by the time of the labeling periods, expressed as micrometers per day. The following dynamic markers are derived data using standardized formulae based on previous mentioned variables[29].

Bone Formation Rate Per Unit Bone Surface. Bone formation rate per unit bone surface is the amount of new bone mineralized per micrometer of cancellous bone surface area per day, expressed as $mm^3/mm^2/yr$.

Bone Formation Rate Per Total Bone Volume. Bone formation rate per total bone volume is the percent of new mineralized bone made per total volume of cancellous bone, expressed as $mm^3/mm^2/yr$

Adjusted Rate of Bone Apposition. Adjusted rate of bone apposition is the product of mineralization rate and mineralizing surface divided by the osteoid surface, expressed as $mm^3/mm^2/yr$.

Mineralization Lag Time. Mineralization lag time is the average lag time in days between apposition of osteoid and its mineralization.

Reference population

The female and male bone histomorphometric reference populations were established by analyzing healthy volunteers with (1) no prior history of medical disease or drug therapy known to affect bone metabolism, (2) no evidence of vertebral fractures as assessed by a lumbar and thoracic spine x-ray and no history of any hip or distal forearm (Colles') fractures, (3) lumbar spine BMD within the age- and sex-adjusted normal range, (4) no laboratory abnormalities affecting bone metabolism.[30] Reference and study bone biopsies were analyzed by the same bone histomorphometric technicians, using the same quantification and analysis procedures.

Statistical analysis

Biochemical and histomorphometric variables are expressed as means ± SEM. Transformation calculations were applied to convert raw bone histomorphometric values to Z-scores for men and women. Z-scores of histomorphometric raw measurements were obtained by subtracting the histomorphometric measurements from the mean values of sex-matched controls, and then dividing the difference by the SD of the normal population. Associations between serum and urine biochemical parameters, BMD measurements, and histomorphometric parameters were assessed using the Pearson correlation coefficient. Correction for multiple comparisons was done by resampling based multiple testing (by Proc Multtest in SAS data analysis system) applied on the comparisons between the subpopulations. Univariate *t* tests of mean differences for all bone histomorphometric parameters were performed for sex and disease diagnosis (PBC/PSC). Univariate parameters that were significant were included in multiple regression models using the backward elimination procedure to select the most significant independent predictors of BMD. All analyses were performed using the SAS data analysis system (SAS Institute, Cary, NC).[31]

Results

Demographics of the study population

Demographics of the study population are shown in Table 1. The 50 CCLD patients had a mean age of 49.24 ± 1.27 years at time of OLT, with a mean Child-Pugh score of 9.45 ± 0.25, a mean body mass index of 24.01 ± 0.58 and a mean BMD of the lumbar spine of 0.87 ± 0.02 g/cm^2. The mean T-score of the lumbar spine was −1.94 ± 0.19; 16 patients (32%) had osteoporosis (T-score < -2.5) and 19 patients (38%) had osteopenia (T-score between −1.0 and −2.5). Thirteen patients (26%) suffered from atraumatic fractures before transplantation: 7 vertebral fractures, 9 rib fractures, and 2 other fractures. Twenty-three (46%) patients had mild to moderate ascites at the time of OLT. Karnofsky Performance scoring at the time of OLT showed that 18 (36%) patients had near-normal activity (score 80-100), thirty (60%) patients required care but were ambulatory (score 40-70); 2 (4%) patients were hospitalized 2 days before OLT with a variceal bleed and renal failure. There were no significant differences between patients with PBC or PSC, or between female or male patients. Patients with fractures had significantly lower BMD values than patients without pretransplantation fractures (0.76 ± 0.14 g/cm^2 vs 0.91 ± 0.14 g/cm^2, $p < 0.05$). However, there were no differences in bone histomorphometric parameters between patients with or without pretransplantation fractures.

Table 1. Demographic data of end-stage PBC and PSC patients undergoing bone histomorphometric analysis.

Patient	N	Age (yrs)	Child-Pugh	BMI	BMD (g/cm^2)	BMD T-scores	Fractures N (%)
Total	50	49.24 ± 1.27	9.45 ± 0.25	24.01 ± 0.58	0.87 ± 0.02	-1.94 ± 0.19	13 (26%)
PBC	22	51.36 ± 1.71	10.00 ± 0.42	23.82 ± 0.86	0.86 ± 0.04	-1.92 ± 0.36	6 (27%)
PSC	28	47.57 ± 1.70	9.04 ± 0.35	24.02 ± 0.77	0.88 ± 0.02	-1.96 ± 0.20	7 (25%)
Female	33	48.64 ± 1.22	9.36 ± 0.32	24.10 ± 0.80	0.85 ± 0.03	-1.92 ± 0.25	9 (27%)
Male	17	50.41 ± 2.96	9.59 ± 0.51	23.70 ± 0.65	0.90 ± 0.03	-1.97 ± 0.30	4 (24%)

NOTE. There were no differences between patients undergoing OLT for CCLD by disease or gender, values are expressed in means ± SEM. Abbrevations: BMI, body mass index; BMD, bone mineral density.

Biochemical variables

Biochemical variables are shown in Table 2. Liver function tests were consistent with advanced CCLD. Mean serum 25(OH)D was at the lower end of the normal range. Mean serum calcium was normal when corrected for low serum albumin; ionized calcium was at the lower end of the normal range. Mean serum bone alkaline phosphatase values were mildly increased, whereas mean serum osteocalcin was within the normal range. Serum free testosterone was below normal in 70% of men. The only difference in biochemical variables at time of liver transplantation relating to disease or gender was lower values of iothalamate clearance in PBC ($p < 0.05$) and higher parathyroid hormone levels in female patients ($p < 0.05$), although all were within the normal range.

Table 2. Biochemical parameters in PBC and PSC patients undergoing bone histomorphometric analysis.

Biochemical Variables (normal range)	Mean ± SEM
Liver function tests	
Albumin (3.5-5.0 g/dL)	2.75 ± 0.08
Total alkaline phosphatase (U/L)[A]	1,348.17 ± 182.83
Total bilirubin (0.1-1.1 mg/dL)	8.39 ± 0.78
Direct bilirubin (0.0-0.3 mg/dL)	5.20 ± 0.52
Prothrombin time (8.4-10.0 sec)	13.25 ± 1.18
Calcium / vitamin D	
25-hydroxyvitamin D (ng/mL)[B]	15.61 ± 1.30
Serum calcium (8.9 - 10.1 mg/dL)	8.61 ± 0.08
Ionized calcium (4.8 - 5.3 mg/dL)	4.94 ± 0.03
Urinary calcium (25 - 300 mg/24 hr)	116.58 ± 14.71
Phosphorus (2.5 - 4.5 mg/dL)	3.04 ± 0.10
Parathyroid hormone (1.0 - 5.2 pmol/L)	2.63 ± 0.44
Bone turnover markers	
Bone alkaline phosphatase (24 - 146 U/L)	159.43 ± 25.38
Osteocalcin (µg/L)[C]	15.17 ± 2.54
Urinary hydroxyproline (15 - 45 mg/24 hr)	39.61 ± 2.30
Hormonal status	
FSH (IU/L)[D]	Pre (n=20): 19.4 ± 7.0
	Post (n=13): 44.9 ± 13.4
Estrogen (pg/mL)[E]	Pre (n=20): 64.7 ± 16.8
	Post (n=13): 28.7 ± 7.0
Free testosterone (9 - 30 ng/dL)	6.71 ± 1.25
Other	
Serum creatinine (mg/dL)[F]	0.88 ± 0.05
Iothalamate clearance (>70 mL/min/SA)	93.98 ± 3.90
Magnesium (1.7 - 2.1 ng/mL)	1.87 ± 0.03

[A] Total alkaline phosphatase = M > 19 yr: 98 - 251 U/L; F 24 - 45 yr: 81 - 231 U/L; F 46 - 60 yr: 84 - 257 U/L; F > 60 yr: 108 - 309 U/L. [B] 25(OH) vitamin D = summer: 15 - 80 ng/mL, winter: 14 - 42 ng/mL.

[C] Osteocalcin = normal ranges for men and F 20 - 50 yr: 2 - 15 ng/L, F 50 - 80: 6 - 22 ng/L.

[D] FSH = premenopausal; FSH < 36 IU/L, postmenopausal; FSH 30 - 120 IU/L.

[E] Estrogen = premenopausal; estrogen < 400 pg/mL, postmenopausal; estrogen < 35 pg/mL.

[F] Serum creatinine = F: 0.6 - 0.9 mg/dL, M: 0.8 - 1.2 mg/dL.

Bone histomorphometry

The age, sex, and menopausal status comparisons of study patients to reference population for histomorphometric analysis are given in Table 3. Histomorphometric data are given in Fig. 1 and Table 4. Cancellous bone volume, and mean wall thickness were significantly decreased in the cholestatic study population. Bone resorption parameters (eroded surface areas, and osteoclast number) were increased; in addition, analysis of cancellous bone architecture showed that trabecular number was decreased and trabecular separation increased, suggesting increased resorption.

Dynamic formation parameters (bone formation rates, adjusted apposition rates), and static formation parameters (osteoid markers, osteoblast number) were significantly decreased. There were no abnormalities in mineralization parameters (mineralization rate, mineralization lag time). Due to uncertainty of timing of OLT, compliance, and clinical problems related to the liver disease, double tetracycline-labeling could be done in 16 study patients (6 PBC, 10 PSC, 10 women, 6 men), for the assessment of dynamic markers.

Table 3. Comparison of study population to reference population for histomorphometric analysis.

	Female populations (n, %)		Male populations (n, %)	
	Study (n = 33)	**Reference (n = 18)**	**Study (n = 17)**	**Reference (n = 43)**
20 - 30 yrs	0	0	0	7 (16.3%)
30 - 50 yrs	18 (54.5%)	10 (55.6%)	8 (47.1%)	15 (34.9%)
> 50 yrs	15 (45.5%)	8 (44.4%)	9 (52.9%)	21 (48.8%)
Mean age (yrs)	48.9 ± 1.22	49.4 ± 3.77	50.4 ± 2.76	49.4 ± 2.65
Postmenopausal, n(%)	14 (41.2%)	8 (44.4%)	-	-

NOTE. All study and reference patients are white.

Comparison of patients with PBC and PSC

There were no significant differences between PSC and PBC patients when comparing the sex-adjusted Z-scores (Fig. 2 and Table 4). Bone histomorphometric changes in the two populations reflect the changes of the total population.

Comparison of female and male patients

Both female and male patients had decreased markers of bone volume (cancellous bone volume, mean wall thickness) and normal cortical width (Fig. 3 and Table 4. Female patients showed a significant increase in osteoclasts per surface length, increased eroded surface areas, increased trabecular separation and decreased trabecular number, all indicating increased bone resorption. These abnormalities were not significant in the male population. Both female and male patients had signs of low bone formation by decreased markers of mean wall thickness and osteoid markers of newly formed bone; female patients also had low bone formation rates and male patients decreased osteoblasts at the osteoid interface and low trabecular thickness, all favoring low bone formation.

Comparison of histomorphometric Z-scores showed that female patients had significantly decreased mean wall thickness, decreased trabecular number, increased trabecular separation, and increased osteoclast numbers when compared to males. Premenopausal women had decreased bone formation rates when compared to postmenopausal women (premenopausal 0.04 ± 0.02 $mm^3/mm^2/yr$, vs. postmenopausal 0.14 ± 0.07 $mm^3/mm^2/yr$, $p < 0.05$), but no other differences were observed.

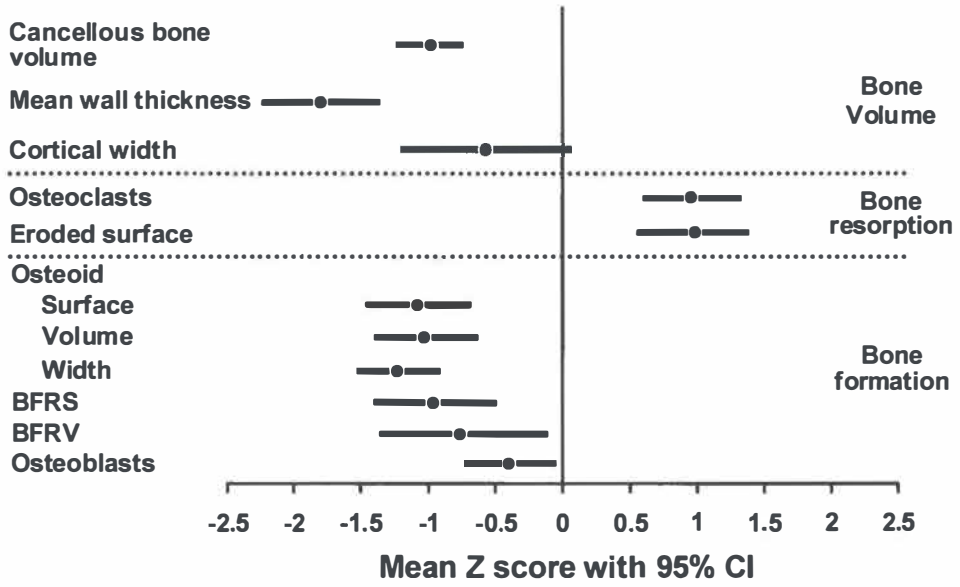

Figure 1. Histomorphometric parameters of bone biopsies taken at time of liver transplantation from 50 patients with chronic cholestatic liver disease; results expressed as Z-scores (with 95% confidence intervals) in comparison to sex-matched normal controls.

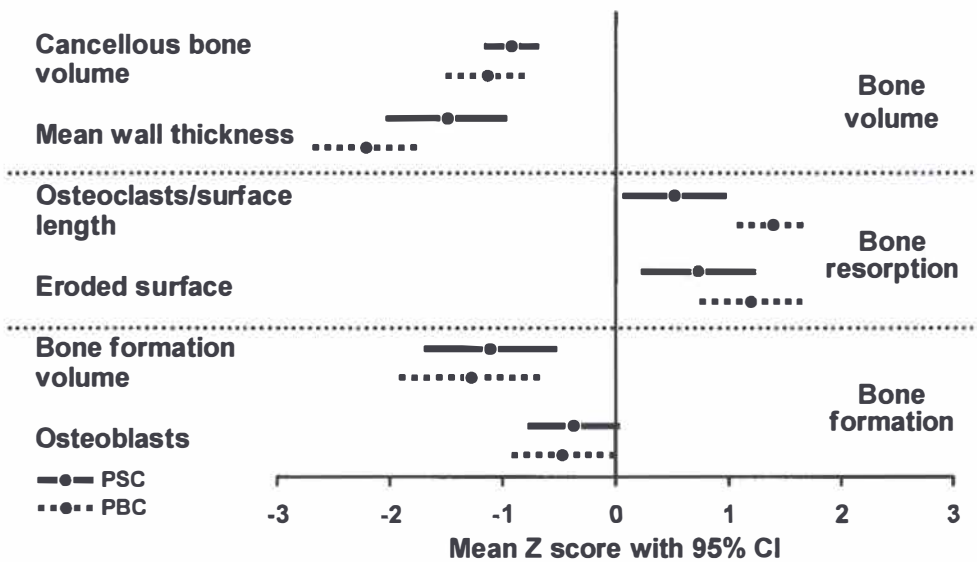

Figure 2. Histomorphometric parameters of bone biopsies taken at time of liver transplantation in patients with PBC (22 patients) and PSC (28 patients); results expressed as Z-scores (with 95% confidence intervals) in comparison to sex-matched normal controls.

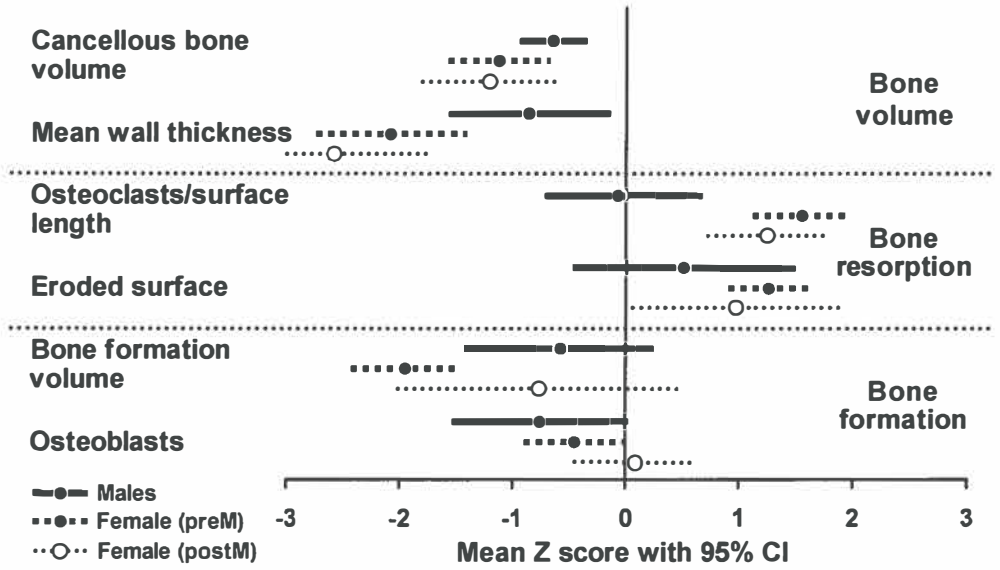

Figure 3. Histomorphometric parameters of bone biopsies taken at time of liver transplantation from 17 men, 19 premenopausal women, and 14 postmenopausal women with chronic cholestatic liver disease; results expressed as Z-scores (with 95% confidence intervals) in comparison to sex-matched normal controls.

Correlations

Histomorphometric and biochemical parameters

No correlations were found between histomorphometric parameters and biochemical markers of bone metabolism (bone alkaline phosphatase, osteocalcin, and 24-hour urinary hydroxyproline). In univariate analysis, eroded surface and number of osteoclasts did not correlate with any biochemical parameter. However, the number of osteoblasts per osteoid surface correlated with the levels of 25(OH)D ($r = 0.32$, $p < 0.05$), serum albumin ($r = 0.32$, $p < 0.05$), and serum magnesium ($r = 0.34$, $p < 0.05$), while bone formation rates correlated with serum phosphorus ($r = 0.49$, $p < 0.05$). Cancellous bone volume and mean wall thickness did not correlate with any biochemical variable, whereas cortical width correlated with serum follicle-stimulating hormone ($r = -0.44$, $p < 0.05$), estrogen ($r = 0.36$, $p < 0.05$), albumin ($r = 0.28$, $p < 0.05$), prothrombin time ($r = -0.32$, $p < 0.05$), and urinary calcium ($r = -0.37$, $p < 0.05$).

Histomorphometric parameters versus lumbar spine BMD and fractures

By univariate analysis, BMD of lumbar spine correlated with cancellous bone volume ($r = 0.37$, $p < 0.01$), osteoid volume ($r = -0.33$, $p < 0.05$) and osteoid surface ($r = -0.30$, $p < 0.05$). There were no correlations identified between histomorphometric bone markers and fractures. From the biochemical markers of bone metabolism, only osteocalcin correlated to BMD of lumbar spine ($r = -0.44$, $p < 0.01$) and rib fractures ($r = 0.60$, $p < 0.0001$). By multiple regression analysis of lumbar spine BMD against all clinical, biochemical and histomorphometric indices, increased osteocalcin and

decreased cancellous bone volume were the two independent variables related to low BMD of lumbar spine in cholestatic patients.

Table 4. Histomorphometric data in end-stage chronic cholestatic liver patients (mean ± SEM)

Histomorphometric parameters (unit of measurement)	Total CCLD population (n=50)	PBC patients (n=22)	PSC patients (n = 28)	Female patients (n=33)	Female reference (n=18)	Male patients (n=17)	Male reference (n=43)
Bone volume							
Cancellous bone volume (%)	17.99 ± 0.82****	18.56 ± 1.49****	17.54 ± 0.10***	18.83 ± 1.10****	25.60 ± 1.50	16.35 ± 1.08****	20.33 ± 1.12
Mean wall thickness (µm)	29.41 ± 1.03****	29.05 ± 1.45***	29.71 ± 1.47***	29.44 ± 1.22****	47.13 ± 1.49	29.39 ± 1.95*	34.63 ± 1.07
Cortical thickness (µm)	910.84 ± 48.82	801.14 ± 70.52	997.04 ± 63.30	903.45 ± 53.97	945.70 ± 67.86	925.18 ± 96.59	963.98 ± 41.16
Trabecular thickness (µm)	121.02 ± 4.30****	120.67 ± 6.43****	121.20 ± 5.90**	124.20 ± 5.55	131.23 ± 9.18	114.87 ± 6.59*	146.67 ± 1.11
Trabecular number (mm^{-1})	1.48 ± 0.04****	1.50 ± 0.07****	1.47 ± 0.04*	1.51 ± 0.05***	2.13 ± 0.24	1.42 ± 0.05	1.40 ± 0.05
Trabecular separation (µm)	576.71 ± 19.68****	578.12 ± 38.75**	575.32 ± 18.36**	564.18 ± 27.19***	399.44 ± 34.38	601.03 ± 23.72	612.15 ± 35.08
Bone resorption							
Osteoclasts (n/100 mm)	12.41 ± 1.28****	13.76 ± 1.08****	11.34 ± 1.89	13.53 ± 1.51****	5.92 ± 1.28	10.22 ± 2.34	8.89 ± 0.97
Eroded surface areas (%)	11.14 ± 1.04****	12.35 ± 1.29***	10.19 ± 1.07*	11.43 ± 1.39****	5.71 ± 0.73	10.58 ± 1.46	6.25 ± 0.47
Bone formation							
Osteoid thickness (µm)	7.83 ± 0.38****	8.00 ± 0.39****	7.69 ± 0.48****	8.02 ± 0.49****	11.66 ± 0.56	7.43 ± 0.55***	9.47 ± 0.45
surface (%)	7.44 ± 1.22****	8.20 ± 1.22**	6.83 ± 1.61***	7.78 ± 1.24****	16.44 ± 2.41	6.74 ± 2.69**	11.36 ± 0.72
volume (%)	0.99 ± 0.18****	0.97 ± 0.20***	1.01 ± 0.28**	1.00 ± 0.14****	1.93 ± 0.25	0.99 ± 0.46*	1.48 ± 0.12
Osteoblast-osteoid interface (%)	10.15 ± 1.98*	10.50 ± 3.86	9.72 ± 1.56	11.18 ± 2.70	15.48 ± 3.19	7.51 ± 1.80*	11.32 ± 0.98
Bone formation/sur based ($mm^3/mm^2/yr$)	0.01 ± 0.0001***	0.01 ± 0.0004**	0.01 ± 0.003*	0.01 ± 0.001**	0.02 ± 0.004	0.00 ± 0.02	0.01 ± 0.002
Bone formation/vol based ($mm^3/mm^2/yr$)	0.07 ± 0.02****	0.08 ± 0.03*	0.07 ± 0.019*	0.08 ± 0.02***	0.27 ± 0.03	0.07 ± 0.02	0.12 ± 0.02
Mineralization rate (µm/day)	0.53 ± 0.04	0.48 ± 0.05	0.55 ± 0.04	0.53 ± 0.05	0.61 ± 0.02	0.53 ± 0.04	0.61 ± 0.03
Mineralization lag time (days)	16.91 ± 1.77	16.68 ± 1.75	17.04 ± 2.71	18.08 ± 2.51	19.30 ± 0.63	14.95 ± 2.20	17.97 ± 0.78
Double labelled osteoid (%)	26.22 ± 3.11	30.10 ± 2.41	23.90 ± 2.15	24.42 ± 7.72	35.09 ± 3.83	20.90 ± 6.51	24.67 ± 2.32
Single labelled osteoid (%)	22.32 ± 2.55	24.06 ± 2.19	30.76 ± 3.44	23.52 ± 6.48	32.13 ± 5.44	20.10 ± 4.78	21.50 ± 1.95
Adjusted apposition time ($mm^3/mm^2/yr$)	0.06 ± 0.02*	0.06 ± 0.02*	0.07 ± 0.02	0.07 ± 0.01*	0.14 ± 0.02	0.06 ± 0.02	0.08 ± 0.01

*Comparing sex-adjusted Z scores of bone histomorphometry to normal population showed:
*: p value <0.05; 2: **p value <0.01; 3: ***p value <0.001; 4: ****p value <0.0001

Discussion

Although osteopenia occurs in different types of liver disease,[18,32-34] bone loss is most severe in PBC and PSC.[1,2,20] Previous reports have indicated that one third of patients with advanced CCLD meet criteria for osteoporosis with another third having osteopenia[35,36]; about 20% of advanced CCLD patients have atraumatic fracturing.[37] Despite its clinical significance, the cause of cholestatic bone loss is not understood.

While BMD can be measured by DEXA, bone metabolism can be investigated only by histomorphometric analysis of bone biopsies.[38] Our study reports histomorphometric evluation of bone metabolism in a large population of 50 patients with advanced CCLD. Importantly, the study population is homogeneous; all patients had end-stage cholestatic liver disease with no confounding illnesses or medications. An extensive histomorphometric analysis was performed, which allowed assessment of bone volume, formation and eroded and cell-covered surfaces. Evaluation of study patients showed that measurements of bone volume, both by DEXA and by bone histomorphometry, were decreased, with osteoporosis in 32% of patients.[35] There were no signs of osteomalacia, confirming that osteoporosis is the metabolic bone abnormality in cholestatic liver disease.[18-21]

In addition to decreased bone volume, bone formation by both histomorphometric static (osteoblast number, osteoid markers, mean wall thickness) and dynamic (bone formation, volume and surface based, mineralization rates) markers was decreased in the total cholestatic population. Simultaneously, markers of bone resorption (eroded surface areas and osteoclast numbers) were increased. Increased eroded surface areas may be caused by decreased bone formation rates, due to incomplete filling of eroded areas by the low number of osteoblasts. However, increased eroded surface areas coupled with increased osteoclast numbers, as seen in our study, may also indicate increased resorption. Resorption rates, however, are difficult to assess and to get additional, indirect information, trabecular separation and number were calculated.[39] In general, in patients with increased resorption activity, osteoclasts penetrate through trabecular structures, and this leads to more widely separated, less numerous, and disconnected thick trabeculae. Analysis of these indirect measures of bone resorption showed in the total study population decreased trabecular number and increased trabecular separation suggesting increased resorption activity. This, in addition to the increased osteoclast number and eroded surfaces, strongly favors increased resorption.

Comparison of our study population to previously published reports is difficult, due to varying stages of cholestatic liver disease[12-18] and variable use of medications influencing bone metabolism in reported studies.[12,15,17] Most studies have been performed in female PBC populations,[12-17] with findings of decreased bone formation rates or decreased osteoblast surfaces.[14-18] Resorption rates have been less well established. Decreased bone formation and increased bone resorption were suggested in the study of Stellon[15] who reported decreased dynamic markers of bone formation, with an increase in eroded surface areas in 30 female PBC patients. Osteoclasts were not measured, but to support an increase in resorption they showed that mean

interstitial bone thickness was reduced, indicating that there had been increased resorption depths. Fifteen female PBC patients with less severe disease than our study patients were studied by Hodgson et al.[14] and found to have significantly decreased bone formation rates; eroded surface area and osteoclast numbers were increased, although lack of statistical significance may have been due to small patient numbers. Mitchison et al.[13] also reported increased eroded surface areas and bone turnover but did not assess other bone formation or resorption parameters.

To our knowledge, studies comparing bone histomorphometry between PBC and PSC and cholestatic female and male patients have not been reported. The histomorphometric disturbances in PBC and PSC here were the same and reflect the changes in the total population; this suggests an effect on bone metabolism integral to chronic cholestasis rather than to the individual diseases. It is tempting to conjecture that a factor(s) associated with chronic cholestasis explains these changes but present data are scanty. Janes[40] studied osteoblast proliferation *in vitro* and found it was reduced by serum from cholestatic patients; unconjugated hyperbilirubinemia decreased osteoblast proliferation in a dose-dependent fashion. Several studies have shown worsening osteopenia with advanced severity of CCLD,[1,17] but multiple studies assessing cholestatic patients *in vivo*, including ours, have not revealed a correlation between serum bilirubin and bone metabolism disturbances. In our study, all patients had end-stage disease which may have masked a statistical correlation. However, duration of cholestasis, or other factors associated with cholestasis, may be more important than severity of cholestasis as reflected by serum bilirubin levels alone.[17]

Some studies have indicated that immobility leads to increased bone resorption and changes in bone formation,[41,42] however, our study patients were all ambulatory prior to OLT. In addition, there were no abnormalities of calcium, vitamin D and parathyroid hormone measurements in our study population. Interestingly, serum 25(OH)D correlated positively with osteoblast-osteoid interface. Serum 25(OH)D levels reflect the body's vitamin D stores before conversion to 1,25-dihydroxyvitamin-D in the kidney. Gerstenfeld et al.[43] showed that 1,25-dihydroxyvitamin D stimulated osteoblastic embryo cell populations *in vitro*, which may suggest a potential role for vitamin D metabolites in osteoblast formation.

In this study, both female and male patients were osteopenic and had histomorphometric low bone volume and low bone formation markers; females also had increased bone resorption. Estrogen inhibits osteoclast activity, promotes osteoclastic apoptosis and decreases bone turnover rates.[44,45] Androgens are also thought to have anti-resorptive effects on bone by a direct regulatory effect on the proliferation of osteoblasts.[45,46] The low testosterone levels in our male cholestatic patients and the low estrogen levels in our mainly postmenopausal female patients may have contributed to the development of osteopenia; however, no correlation was seen between any histomorphometric marker of resorption or formation and sex hormone levels. In addition, premenopausal women had at least as severe histomorphometric abnormalities as postmenopausal women, none of whom were on hormone replacement therapy. These sex differences in the histomorphometric appearance of cholestatic osteopenia remain unexplained.

The utility of biochemical parameters in assessment of bone formation and resorption in liver disease has been uncertain.[9,10,.14] The reduced histomorphometric bone formation seen in our study was not reflected by decreased osteocalcin or bone alkaline phosphatase levels, the slight increase in the latter probably caused by linkage between bone and liver alkaline phosphatase. In addition, there was no correlation between biochemical and histomorphometric bone formation markers. Similarly, while histomorphometric resorption markers were increased, the biochemical parameter for resorption –urinary hydroxyproline- was within normal range, and no correlation was detected. The negative correlation between osteocalcin and BMD and positive correlation between osteocalcin and fractures suggests that the increased osteocalcin levels may result from a compensatory stimulation of bone formation, as has been seen in other situations of high bone turnover.[47]

In conclusion, histomorphometric assessment of bone biopsies in 50 patients with end-stage CCLD has shown decreased bone volume with decreased static and dynamic bone formation markers. The increase in eroded surfaces and osteoclast number with decreased trabecular number and increased trabecular separation in female cholestatic patients also suggests increased resorption, which is less evident in male patients. Biochemical parameters of bone formation and bone resorption do not appear to be useful in assessing bone metabolism in CCLD patients. In cholestatic osteopenia it would appear that abnormal bone formation and abnormal bone resorption both contribute to bone loss. The relative importance of these abnormalities of bone metabolism, the timing of their development in the course of the liver disease, their etiologic pathways, and interconnection remain to be elucidated.

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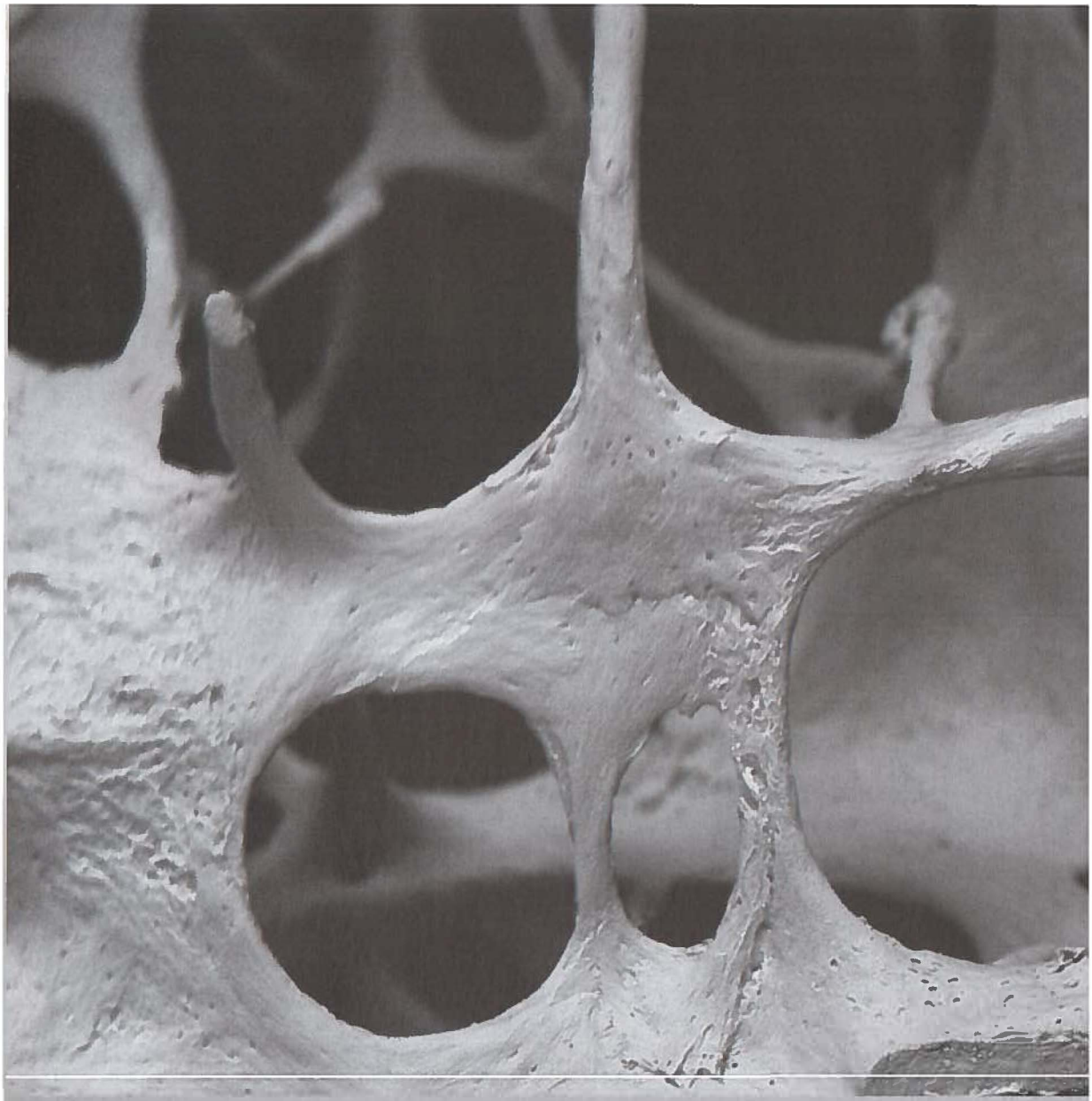

Chapter 3

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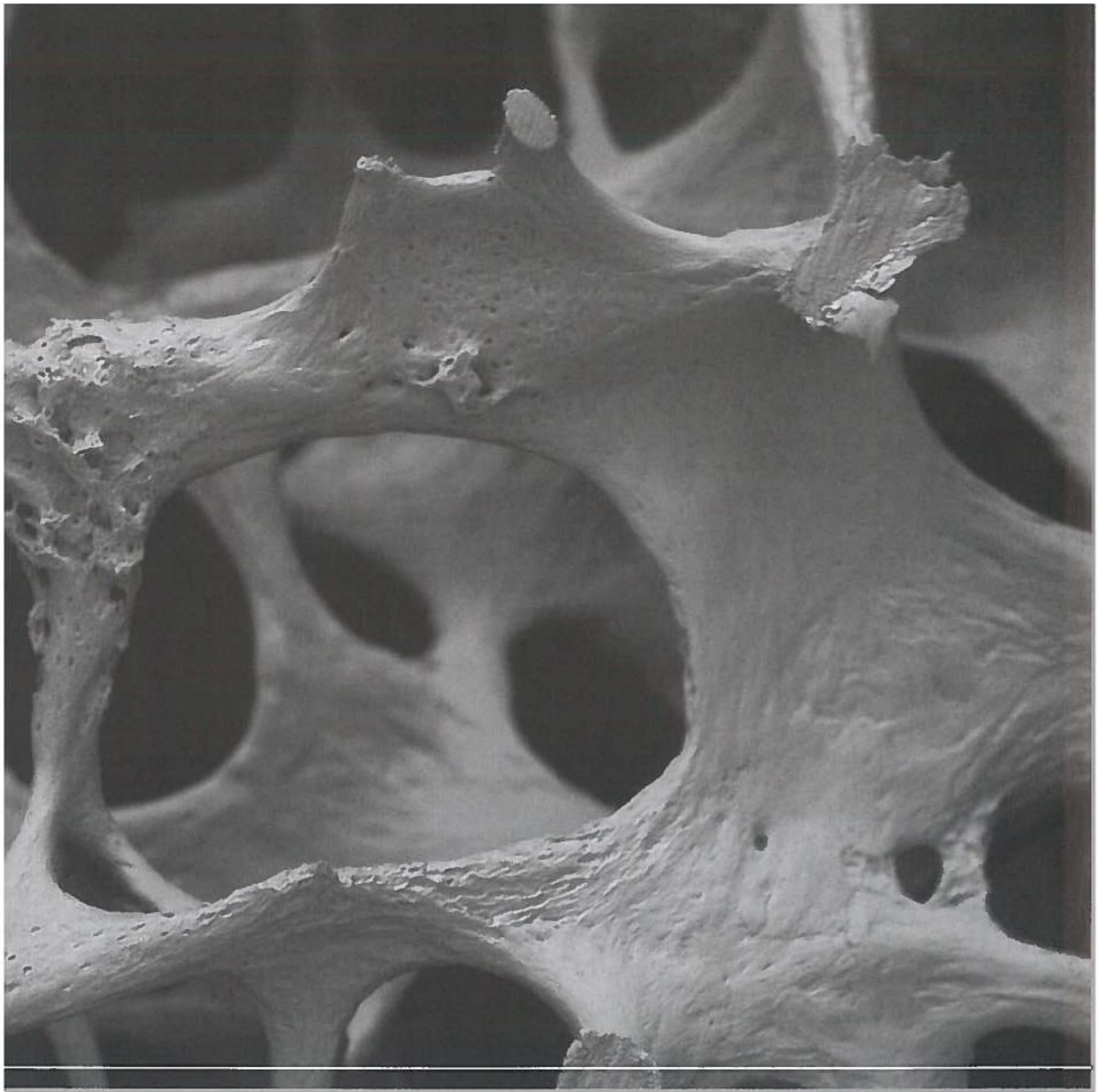

Bone histomorphometric changes after liver transplantation for chronic cholestatic liver disease

J Bone Mineral Res 2003;18:2190-2199.

Abstract

Thirty-three patients with cholestatic liver disease underwent histomorphometric assessment of paired bone biopsy specimens at time of orthotopic liver transplantation (OLT) and at 4 months thereafter. At 4 months after OLT, bone metabolism improved, with bone formation increasing to normal, and no change in bone resorption. Early posttransplant bone loss may be attributed to an additional insult to bone formation early after transplantation.

Introduction: Patients with advanced liver disease, especially chronic cholestasis, often have osteopenia, which worsens early after orthotopic liver transplantation (OLT) before starting to recover. The changes in bone metabolism leading to this rapid loss of bone after OLT, and to its recovery, are poorly defined.
Materials and Methods: In thirty-three patients with advanced chronic cholestatic liver disease, tetracycline-labeled bone biopsy specimens were analyzed prospectively at time of OLT and at 4 months after OLT, as part of a randomized trial to study the efficacy of calcitonin on posttransplant bone loss. Hierarchical cluster analysis of histomorphometric parameters was performed in an attempt to establish the functional grouping of individual histomorphometric parameters before and after OLT.
Results and Conclusions: Results showed that from the time of OLT to 4 months after OLT, bone mineral density of the lumbar spine and histomorphometric parameters of bone volume decreased, consistent with early posttransplant bone loss. Histomorphometric resorption parameters were increased before OLT, with no change after OLT. Histomorphometric formation parameters increased from low values before OLT to normal values at 4 months after OLT, with the exception of mean wall thickness values which further decreased after OLT, suggesting an additional insult to bone formation during the study period. Histomorphometric changes following OLT were similar in female and male patients, pre- and postmenopausal women, and in patients treated and not treated with calcitonin. Hierarchical cluster analysis suggested that before OLT, bone resorption was functioning independently of bone formation, but that by 4 months after OLT, their coupled relationship had improved. Therefore, despite posttransplant bone loss, by four months after OLT, bone metabolism had improved, with increased bone formation, and more coupled bone balance, as suggested by hierarchical cluster analysis.

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Abbreviations

PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; CCLD, chronic cholestatic liver disease; 25(OH)D, 25-hydroxyvitamin D; BMD, bone mineral density; OLT, orthotopic liver transplantation; BGP, bone gla-protein; DEXA, dual-energy X-ray absorptiometry.

Introduction

Osteopenia is a major complication of advanced chronic liver disease, especially in patients with chronic cholestatic liver disease (CCLD).[1-4] After orthotopic liver transplantation (OLT), bone mineral density of lumbar spine (BMD-LS) decreases further during the first 4 months, leading to posttransplant fractures in approximately 20-40% of CCLD patients[5-8]; this has been assumed to be related, in some way, to skeletal effects of high-dose immunosuppressive medications. After this early period of bone loss and with continuing normal allograft function, patients begin to gain bone mass during the subsequent posttransplant years.

Measurements of BMD identify loss or gain of bone density after OLT, but fail to show disturbances of bone resorption and formation leading to loss or gain of bone mass. Histomorphometric analysis of bone biopsy specimens provides this essential information. Although there have been conflicting histomorphometric data from patients with cholestatic osteopenia,[9-15] we have recently found that both increased resorption and decreased formation contribute to pretransplant bone loss.[16] The changes in bone metabolism that lead to the additional insult to bone mass after OLT, as well as its eventual recovery, are poorly understood. Calcitonin is a bone anti-resorptive agent, which may be of benefit in preventing bone loss after OLT; its effects on bone resorption and formation after OLT have not been studied.

To study changes in bone metabolism after OLT and the effect of calcitonin, 33 patients with CCLD underwent histomorphometric analysis of paired iliac crest bone biopsy specimens, taken at the time of OLT and at 4 months posttransplant. Hierarchical cluster analysis of histomorphometric parameters was performed in an attempt to establish the functional grouping of individual histomorphometric parameters before and after OLT.

Methods

Patient population

Sixty-three consecutive adult patients who fulfilled the following criteria were enrolled in a randomized controlled trial to test the efficacy of salmon calcitonin therapy (100 iu subcutaneously each day for the first 6 postoperative months) to prevent posttransplant bone loss[17]: (1) advanced primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC), activated for liver transplantation; (2) absence of diseases other than PBC or PSC which affect bone metabolism; (3) no medications affecting bone metabolism in the 12 months preceding transplantation (corticosteroids, hormone replacement therapy, anticonvulsants, bisphosphonates, sodium fluoride); (4) normal creatinine clearance and normal thyroid function; (5) willingness to participate in the study, including consent to bone biopsy at the time of liver transplantation; (6) completion of liver transplantation between April 1990 and July 1995. The diagnoses of PBC and PSC were made according to well-established criteria.[18-20]

In the first week after successful liver transplantation, patients were randomized (1) to receive 100 MRC units of salmon calcitonin subcutaneously once daily for 6 months starting on the seventh postoperative day; or (2) to receive no therapy. Randomization was by dynamic allocation, stratifying for gender (female versus male), diagnosis (PBC versus PSC), BMD level above or below the fracture threshold (0.98 g/cm^2), and menopausal status. Patients underwent a protocolized immunosuppressive regimen with either triple therapy with prednisone, azathioprine and cyclosporine, or dual therapy with prednisone and tacrolimus. Twenty-three patients were treated with triple therapy of cyclosporine, prednisone, and azathioprine. Cyclosporine was given to achieve the following trough levels: 250-350 ng/ml in first week, 200-300 ng/ml from week 2 to 4 months, and 100-200 ng/ml from 4 to 12 months. Prednisone/solumedrol was given as following: 1 g solumedrol at time of surgery; 100 mg BID on first 2 days, with taper to 15 mg BID by day 25; 15 mg BID on days 25 - 60: 20 mg/d from 61 days to 4 months; and 10 mg/day from 6 months onward. Azathioprine was given at 2 mg/kg per day. Ten patients received tacrolimus, with trough levels of 10-15 ng/mL first month, and 5-10 ng/mL thereafter. Prednisone was given as following; 1 g solumedrol at time of surgery; 25 mg QID on first day, tapered to 15 mg/day by day 15, and 5 mg/day by 4 months. Standard therapy for acute cellular rejection was three intravenous doses of 1 g of methylprednisone.

The study was approved by the Institutional Review Board of the Mayo Clinic. The BMD and fracture results of the randomized treatment trial with calcitonin have been previously reported[17] to show that calcitonin is ineffective in preventing posttransplant bone loss and fractures. As part of this randomized treatment trial, all patients were asked to consent to two bone biopsies, the first at the time of OLT (n=50) and the second at 4 months after OLT (n=35); paired bone biopsies were obtained in 33 patients, who form the study population for histomorphometric analysis. All patients had extensive clinical, biochemical and radiologic examination before OLT, and at 4 months and 12 months posttransplant.

Assessment of BMD and fractures

BMD was determined by DEXA of the L1-L4 lumbar spine region, using a Hologic QDR 1000 densitometer (CV 2.2%) before OLT and at 4 and 12 months after OLT. Bone mass was corrected for bone size to calculate BMD (g/cm^2). BMD measurements were compared to age- and sex-matched reference populations (Z-scores), and to young adult sex-matched reference populations at peak bone mass (T-scores). In patients with lumbar compression fractures, measurements were determined only on intact vertebrae. Large-volume paracentesis was performed, as necessary, for moderate/severe ascites before measurements of BMD to minimize the effects of ascites on BMD measurements.

Protocol-based chest X-rays and standard radiographs of the thoracolumbar spine at a tube distance of 120 cm were obtained to determine fractures before OLT, and at 4 and 12 months after OLT. Additional radiographs were taken as clinically indicated at the sites of bone pain, and if X-rays were negative, bone scans were performed to evaluate possible fractures.

Tetracycline labeling

Study patients received tetracycline labeling before bone biopsy specimens to allow assessment of dynamic bone parameters. Labeling was done with cycles of oxytetracycline (course A) and demeclocycline (course B). At the time of enrollment, oxytetracycline, 250 mg QID, was given for 3 days, followed by 14 days off label, and then followed by 3 days of 250 mg QID. If more than 6 weeks had elapsed from the last dose of oxytetracycline and the patient had not yet undergone OLT, then course B was started (demeclocycline 150 mg QID for 3 days, 14 days off, then 150 mg QID for 3 days). Courses A and B were then repeated at 6-week intervals until OLT. If a patient was not able to receive oral medication during initial labeling, doxycycline 100 mg was given intravenously every 12 h for 2 days. If a patient was called for OLT before the second label was administered (i.e., during the 14 days off-drug), doxycycline 100 mg was given intravenously every 6 h until the patient underwent OLT.

Before the 4-month posttransplant bone biopsy, oxytetracycline and demeclocycline labeling was repeated as above with bone biopsy specimens taken on days 23-27 of the labeling schedule. Because of uncertainty of timing of OLT and clinical problems related to liver disease, double tetracycline-labeling could be accurately done in 13 pre-transplant and 23 post-transplant patients, resulting in 7 patients with paired dynamic data. All patients had assessment of all static histomorphometric parameters.

Bone biopsy specimens

The initial bone biopsy specimens were performed at time of OLT by the transplant surgeon, at the standard iliac crest bone biopsy site, using a 7.5 mm trephine.[21] Glucocorticoids were not administered until after the bone biopsy. Four-month bone biopsy specimens were done as an outpatient procedure from the contralateral iliac crest under local anaesthetic. After the bone biopsy was taken, bone tissue was placed into 70% ethanol, dehydrated in 95% ethanol for 1 day, and dehydrated in 100% ethanol for 5 days, before immersion for 4 days in polymethyl methacrylate and embedding by controlled temperature polymerization. Four pairs of consecutive 5-mm sections were obtained at 100-mm intervals. Sections were stained with Goldner-Masson-Trichrome, Hematoxylin-Eosin, and Toluidine blue. Unstained sections were analyzed for fluorescent microscopy. Quantification of bone histomorphometric parameters was carried out by Bioquant System IV image analysis, using a Zeiss microscope and digitizing tablet (R and M Biometrics, Nashville, TN, USA). Bone biopsy specimens were read stepwise from corner to corner, and fields with more than 30% distortion were discarded. Primary and derived data were generated by the Bioquant IV software in accordance with standardized nomenclature and formulae,[22] for comparison with histomorphometric data for normal adult male and female references.

All bone biopsies were read nonblinded by the three trained technicians in the Mayo Bone Histomorphometric Laboratory. To reduce intraobserver variation, a mean of four readings was used for all measured histomorphometric parameters. To reduce interobserver variation, the three technicians responsible for quantifying the bone biopsies were required to measure bone histomorphometric parameters on a reference bone biopsy within 1 SD of the mean for these parameters each month to ascertain quality control. The study bone biopsies were measured by the same three technicians as the normal control bone biopsies, using the same methods. Bone histomorphometric

parameters were expressed as Z-scores (sex-adjusted histomorphometric values), using normal female and male histomorphometric reference values of the Mayo Clinic Bone Histomorphometry Laboratory (see Table 1).

Table 1. Age and sex distribution of reference and study populations for bone histomorphometry

	Female populations (n, %)		Male populations (n, %)	
	Study (n=21)	Reference (n=18)	Study (n=12)	Reference (n=43)
< 20 yrs	0	0	0	0
20 - 50 yrs	14 (66.7%)	10 (55.6%)	7 (58.3%)	22 (51.1%)
> 50 yrs	7 (33.3%)	8 (44.4%)	5 (41.7%)	21 (48.8%)
Mean age (yrs)	46.6 ± 1.6	49.4 ± 3.8	48.7 ± 3.2	49.4 ± 2.7
Postmenopausal, n(%)[A]	9 (42.9%)	8 (44.4%)	-	-

All study and reference patients are white

[A] postmenopausal status: estradiol < 35 pg/mL, FSH > 30 IU/L

Static histomorphometric parameters

The following static parameters were analyzed.

1. Cancellous bone volume (BV/TV): measured as percentage of the total medullary bone volume from an unstained slide.
2. Cancellous bone architectural parameters: trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp) are derived data from fractional cancellous bone volume and bone perimeter. Trabecular number and separation serve as indirect measures of bone resorption activity, because they reflect the effects of increased bone resorption activity on trabecular bone structure; patients with increased bone resorption activity have more widely separated, less numerous, and disconnected trabeculae.[23]
3. Osteoid parameters: osteoid thickness (O.Th) was measured by dividing each seam into four equal measurements in 50 fields or more and expressed as mean osteoid thickness of osteoid in micrometers. Osteoid volume (OV/BV) is expressed as percentage of total bone volume and osteoid surface (OS/BS) as percentage of cancellous surfaces covered with osteoid.
4. Number of osteoclasts per 100 mm of trabecular surface length (N.Oc): osteoclasts were identified as large amorphous cells that interface with a bone-resorbing surface within a resorption pit, display characteristically dense and somewhat granular cytoplasm (Goldner stain), and contain one or more irregularly shaped nuclei with prominent nucleoli.
5. Eroded surface (ES/BS): identified as a scalloped surface eroded to a depth of one lamella or more, and expressed as a percentage of cancellous surface showing resorption cavities.
6. Osteoblast-osteoid interface (N.Ob): percentage of osteoid surface covered by osteoblasts, defined as cuboidal pyronine-staining cells.

7. Cortical thickness (Ct.Th): mean thickness of cortical seams in micrometers (average of 12 measurements).
8. Mean wall thickness (W.Th): mean thickness of the total bone structural unit in micrometers, measured as the distance between the cement line and quiescent, mineralized bone surface.

Dynamic histomorphometric parameters

The tetracycline double- and single-label lengths were measured using the unstained slide, which was scanned until 50 measurements were obtained. Mineral apposition rate (MAR) was calculated as the mean of four equally spaced interlabel thickness measurements (obtained from all available double labels on cancellous surfaces), divided by the time of the labeling periods (μm/day). The following dynamic parameters were derived using standardized formulae based on previously described variables.

1. Bone formation rate per unit bone surface (BFR/BS): amount of new bone mineralized per micrometer of cancellous bone surface area per day (mm^3/mm^2/year).
2. Bone formation rate per total bone volume (BFR/BV): amount of newly mineralized bone per total volume of cancellous bone (mm^3/mm^2/year).
3. Adjusted rate of bone apposition (Aj.AR): the product of mineralization rate and mineralizing surface divided by the osteoid surface (mm^3/mm^2/year).
4. Mineralization lag time (Mlt): the average lag time in days between apposition of osteoid and its mineralization.
5. Activation frequency (Ac.f): the activation frequency, or the rate at which new remodeling cycles are initiated, was calculated by determining the reciprocal of the total period (which is calculated by summing the duration of the formation period, quiescent period, and erosion period; days $^{-1}$).

Histomorphometric adult female and male reference populations

Table 1 summarizes the age and sex distribution of the bone histomorphometric reference and study populations. The normal adult female and male bone histomorphometric reference parameters were established by analyzing iliac bone biopsies from healthy volunteers with (1) no prior history of medical disease or drug therapy known to affect bone metabolism; (2) no evidence of vertebral fractures as assessed by lumbar and thoracic spine X-rays, and no history of any hip or distal forearm (Colles') fractures; (3) lumbar spine BMD within the age- and sex-adjusted normal range, and (4) no laboratory abnormalities affecting bone metabolism. Normal adult reference and study bone biopsies were analyzed by the same Mayo Clinic Bone Histomorphometry Laboratory technicians, using the same quantification and analysis procedures. Characteristics of the normal adult reference populations have been previously published.[16,24]

Statistical analysis

All parameters (biochemical, clinical, histomorphometric) were reported as mean ± SE. Raw bone histomorphometric values were converted to Z- scores by taking the difference between the mean study histomorphometric measurements from the mean values of sex-matched normals, and then dividing this result by the SD of the sex-matched normals. Paired *t*-tests of the biochemical and histomorphometric parameters

were used to assess the within patient changes following transplantation, and independent *t*-tests were used to identify differences between female and male patients, PBC and PSC patients, and pre- and postmenopausal women. Associations between BMD and histomorphometric parameters were assessed using the Pearson correlation coefficient. Data analyses were performed using the SAS data analysis system (SAS Institute, Cary, NC, USA).[25]

Hierarchical cluster analysis is a well established, multivariate statistical method[26,27] used to organize a large number of related parameters into a single system (a hierarchical tree or dendrogram). The positioning of individual parameters within the tree is such that each parameter is closest to the other parameters with which it shares the most functional similarity and furthest away from those most dissimilar. Individual histomorphometric parameters were first converted to Z-scores and then cluster analyses were performed using the Splus statistical program (Insightful Corp., Seattle, WA, USA) to identify the degree of similarity among all the histomorphometric parameters within patients. These parameters were then organized into discrete functional groups (clusters) with maximum intracluster similarity. The degree of similarity among the different clusters was then assessed and the process proceeded sequentially until all variables were "clustered", and formed a single hierarchical tree (dendrogram), the branch length of the dendrogram corresponding to dissimilarity between/among clusters.

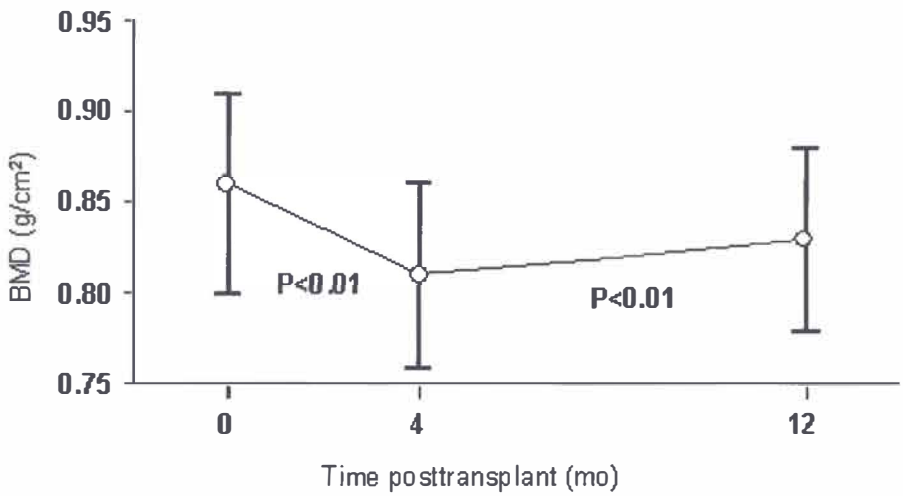

Figure 1. Changes in BMD of the lumber spine after OLT in 33 patients with CCLD.

Results

Demographics, BMD and fracture assessment of total study population

Baseline demographic data of the study patients (11 PBC, 22 PSC; 12 males, 21 females; 6 postmenopausal) and posttransplant immunosuppression and hospitalisation days are shown in table 2. Pretransplant BMD of the lumbar spine (BMD-LS) was low, with a mean BMD-LS T- score of -2.1 ± 0.23. Thirty-nine percent (n = 13) of the patients had osteoporosis (T-scores < -2.5), and 36% (n = 12) had osteopenia (T-scores between –1 and –2.5). Six patients (18%) had fractures before OLT; one patient had a single rib fracture, two patients had vertebral fractures only (two thoracic, and two lumbar), and three patients had both rib and vertebral fractures (five rib fractures, three lumbar and four thoracic fractures).

Mean BMD T-score decreased to –2.5 ± 0.2 at 4 months posttransplant ($p < 0.001$) before partially recovering to –2.3 ± 0.2 at one year ($p < 0.01$). This is similar to the changes in absolute BMD values as shown in Fig 1. Twelve patients (36%) sustained fractures during the first posttransplant year; 2 patients had rib fractures only (both multiple), 6 patients had vertebral fractures only (3 lumbar and 8 thoracic fractures), and 4 patients had both rib fractures (all multiple) and vertebral fractures (12 thoracic and 6 lumbar). One patient sustained a femoral neck fracture during the first year.

Table 2. Clinical data for 33 patients with chronic cholestatic liver disease before and after orthotopic liver transplantation.

Clinical parameters (unit of measurement)	Total patients (n=33)	Calcitonin-treated patients (n=14)	Control patients (n=19)
Pretransplant parameters			
Gender, female/male (n, %)	21 (64%) / 12 (36%)	9 (64%) / 5 (36%)	12 (63%) / 7 (37%)
Pre-existing disease, PBC/PSC[A], n,%	22 (66%) / 11 (34%)	11 (78%) / 3 (22%)	11 (58%) / 8 (42%)
Age at OLT (yrs)	47.4 ± 1.5	47.3 ± 1.8	47.4 ± 2.2
Child-Turcotte-Pugh score	9.2 ± 0.3	9.4 ± 0.6	9.1 ± 0.3
Posttransplant parameters[B]			
Hospitalization stay (days)	17.0 ± 1.0	17.5 ± 1.8	16.6 ± 1.1
Intensive care unit stay (days)	2.9 ± 0.2	2.9 ± 0.4	2.8 ± 0.3
IV glucocorticoid dosage (mg)	3619.8 ± 259.4	3315.3 ± 365.3	3844.2 ± 319.5
Oral glucocorticoid dosage (mg)	2365.6 ± 111.8	2268.6 ± 178.7	2437.0 ± 124.0
Treatment, cyclosporine/ tacrolimus, n,%	23 (69%) / 10 (31%)	10 (71%) / 4 (29%)	13 (68%) / 6 (32%)

n; number of patients; %; percentage of patients, [A] PBC/PSC; Primary biliary cirrhosis, Primary sclerosing cholangitis.
[B] Cumulative amount first 4 months following OLT.

Bone histomorphometric parameters of total population

Cancellous bone volume was decreased at the time of OLT; cortical thickness was normal, but showed a significant decrease after OLT (Fig. 2). Both direct resorption parameters (eroded surface and osteoclasts per surface length) and indirect

parameters (eroded surface and osteoclasts per surface length) and indirect resorption parameters (trabecular number and separation) showed increased bone resorption before OLT, with no significant change after OLT (Table 3).

Table 3. Bone histomorphometric parameters before and after liver transplantation in 33 patients with chronic cholestatic liver disease.

Histomorphometric parameters	Bone histomorphometry at OLT[A]		Bone histomorphometry at 4 mo PostOLT[A]		Within patient change
	Absolute value	Z-score	Absolute value	Z-score	p-values
Bone volume					
Cancellous bone volume (%)	17.7 ± 1.0	-1.0 ± 0.1[F]	17.4 ±1.04	-1.1 ± 0.2[F]	0.83
Mean wall thickness (µm)	29.0 ± 1.5	-1.9 ± 0.3[F]	26.5 ± 1.3	-2.5 ± 0.4[F]	< 0.05
Cortical thickness (µm)	965.1 ± 59.6	0.0 ± 0.2	739.6 ± 47.7	-0.8 ± 0.2[D]	< 0.01
Trabecular thickness (µm)	120.0 ± 6.0	-0.5 ± 0.2[C]	114.8 ± 4.9	-0.6 ± 0.8[C]	0.41
Trabecular number (mm^{-1})	1.5 ± 0.05	-0.5 ± 0.1[D]	1.5 ± 0.05	-0.6 ± 0.7[D]	0.67
Trabecular separation (µm)	580.8 ± 25.2	0.8 ± 0.2[E]	594.1 ± 27.2	0.8 ± 0.9[E]	0.93
Bone resorption					
Osteoclast (n/100 mm)	11.6 ± 1.4	0.9 ± 0.2[F]	15.3 ± 1.9	1.1 ± 0.2[F]	0.18
Eroded surface (%)	10.2 ± 0.9	0.9 ± 0.2[F]	10.6 ± 1.1	1.0 ± 0.2[F]	0.80
Bone formation					
Osteoid thickness (µm)	7.7 ± 0.4	-1.3 ± 0.2[F]	8.9 ± 0.5	-0.8 ± 0.2[D]	< 0.05
surface (%)	7.3 ± 1.4	-1.1 ± 0.2[F]	16.2 ± 2.1	0.2 ± 0.2	< 0.001
volume (%)	1.1 ± 0.2	-1.0 ± 0.2[F]	2.6 ± 0.4	0.5 ± 0.3	< 0.01
Osteoblast-osteoid interface (%)	9.0 ± 1.4	-1.1 ± 0.2[C]	16.4 ± 2.1	0.5 ± 0.3	< 0.05
Bone formation/sur based ($mm^3/mm^2/yr$)[B]	0.006 ± 0.002	-0.7 ± 0.2[E]	0.02 ± 0.01	0.5 ± 0.2	< 0.05
Bone formation/vol based ($mm^3/mm^2/yr$)[B]	0.09 ± 0.02	-0.8 0.2[F]	0.39 ± 0.12	1.2 ± 0.4	< 0.05
Mineralization rate (µm/d)[B]	0.5 ± 0.05	-0.4 ± 0.2	0.7 ± 0.05	0.6 ± 0.1	< 0.05
Mineralization lag time (d)[B]	17.5 ± 1.5	-0.2 ± 0.1	15.9 ± 3.1	-0.8 ± 0.2	0.27
Adjusted apposition rate ($mm^3/mm^2/yr$)[B]	0.08 ± 0.02	-0.4 ± 0.2[C]	0.11 ± 0.03	0.1 ± 0.2	0.15
Activation frequency ($days^{-1}$)[B]	0.0005 ± 0.0001	-1.1 ± 1.0[D]	0.0018 ± 0.0006	0.3 ± 1.7	<0.05

[A] Paired data are reported in the table; 13 patients had [B]dynamic measurements before OLT, and 23 after OLT, with paired dynamic data in 7 patients.

The remaining static parameters were available as paired data in all study patients. Comparing age-adjusted Z-scores of bone histomorphometry to normal population showed:

[C] p-value < 0.05; [D] p-value < 0.01; [E] p-value < 0.001; [F] p-value < 0.0001

The static bone formation parameters (number of osteoblasts and osteoid parameters) as well as the dynamic bone parameters (bone formation rates and adjusted appositional rate) increased significantly after OLT from low to normal values, with the exception of mean wall thickness, which further decreased after OLT. Activation frequency and mineralization rate also increased from low to normal values by 4 months posttransplant; mineralization lag time remained unchanged. There were no histomorphometric signs of osteomalacia before or after OLT. With the exception of mineralization lag time ($p<0.05$), which increased in male patients and decreased in female patients after OLT, there were no significant differences in histomorphometric changes after OLT between patients with PBC compared to those with PSC, between female and male patients, and between pre- and postmenopausal women (data not shown).

Effect of calcitonin therapy on bone histomorphometric parameters

Fourteen patients (9 females, 5 males) were randomized to undergo calcitonin therapy, and 19 patients (12 females, 7 males) served as control patients. With regard to any identified pre- or posttransplant variables which could have influenced the effect of calcitonin, no significant differences were found between calcitonin-treated and control patients in any clinical, biochemical, radiological or bone mineral density parameter (Tables 1 and 4).

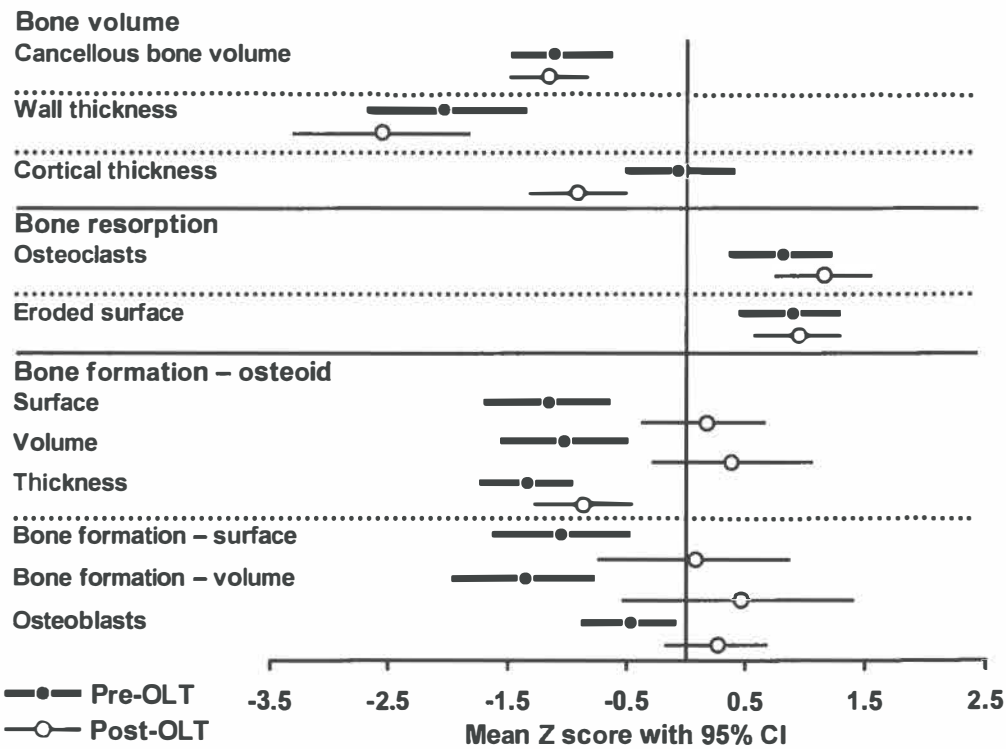

Figure 2. Changes in bone histomorphometric parameters after OLT in 33 patients with CCLD.

Table 4. Biochemical, BMD and histomorphometric parameters in calcitonin-treated patients and controls.

	Calcitonin patients (n=14)[A]		Control patients (n=19)[A]		p-values
Biochemical and BMD variables	**PreOLT[D]**	**PostOLT (4 mo)[D]**	**PreOLT[D]**	**PostOLT (4 mo)[D]**	**Pre- post OLT[B]**
Total bilirubin (0.1-1.1 mg/dL)	7.7 ± 4.4	1.0 ± 0.7	10.1 ± 6.6	2.3 ± 4.0	0.68
Albumin (3.5-5.0 g/dL)	2.8 ± 0.5	3.8 ± 0.6	2.8 ± 0.5	3.5 ± 0.5	0.17
25-hydroxyvitamin D (ng/mL)[C]	18.1 ± 10.6	33.1 ± 6.2	16.5 ± 8.8	31.7 ± 8.5	0.99
Ionized calcium (4.8-5.3 mg/dL)	5.0 ± 0.1	5.0 ± 0.1	4.9 ± 0.2	5.1 ± 0.3	0.09
BMD (g/cm^2)	0.82 ± 0.10	0.79 ± 0.10	0.88 ± 0.19	0.83 ± 0.18	0.58
BMD T-score	-2.3 ± 0.2	-2.7 ± 0.2	-2.1 ± 0.4	-2.6 ± 0.4	0.37
Bone volume					
Cancellous bone volume (%)	16.1 ± 1.0	16.8 ± 1.2	18.8 ± 1.6	17.8 ± 1.6	0.53
Mean wall thickness (µm)	26.7 ± 1.8	25.5 ± 2.1	30.8 ± 2.1	27.3 ± 1.6	0.64
Cortical thickness (µm)	874.9 ± 75.0	770.4 ± 65.7	1031.3 ± 84.5	717.9 ± 67.0	0.20
Trabecular thickness (µm)	112.7 ± 7.4	110.9 ± 6.3	125.3 ± 8.6	117.6 ± 7.1	0.65
Trabecular number (mm^{-1})	1.5 ± 0.06	1.4 ± 0.05	1.5 ± 0.07	1.5 ± 0.08	0.90
Trabecular separation (µm)	583.2 ± 25.0	579.0 ± 39.4	600.1 ± 30.0	589.7 ± 41.6	0.71
Bone resorption					
Osteoclast (n/100 mm)	10.3 ± 1.7	14.4 ± 2.4	12.6 ± 2.1	15.9 ± 2.7	0.82
Eroded surface (%)	10.5 ± 1.4	9.9 ± 1.0	10.1 ± 1.3	11.0 ± 1.8	0.65
Bone formation					
Osteoid thickness (µm)	7.3 ± 0.6	8.8 ± 0.6	8.0 ± 0.6	9.1 ± 0.7	0.59
surface (%)	9.4 ± 2.9	15.2 ± 2.4	5.6 ± 1.0	17.0 ± 3.2	0.25
volume (%)	1.3 ± 0.5	1.8 ± 0.3	0.8 ± 0.2	3.1 ± 0.7	0.17
Osteoblast-osteoid interface, %	10.6 ± 2.4	16.9 ± 2.8	8.0 ± 1.7	16.1 ± 3.0	0.90
Bone formation/sur based ($mm^3/mm^2/yr$)[E]	0.006 ± 0.002	0.013 ± 0.006	0.007 ± 0.003	0.03 ± 0.02	0.56
Bone formation/vol based ($mm^3/mm^2/yr$)[E]	0.09 ± 0.01	0.22 ± 0.03	0.1 ± 0.03	0.51 ± 0.19	0.32
Mineralization rate (µm/day)[E]	0.49 ± 0.04	0.65 ± 0.03	0.58 ± 0.06	0.68 ± 0.08	0.24
Minerlization lag time (days)[E]	22.2 ± 2.3	18.3 ± 1.13	15.6 ± 2.3	15.8 ± 1.7	0.33
Adjusted apposition rate ($mm^3/mm^2/yr$)[E]	0.03 ± 0.005	0.07 ± 0.01	0.11 ± 0.03	0.13 ± 0.04	0.36
Activation frequency ($days^{-1}$)[E]	0.0005 ± 0.002	0.0011 ± 0.0004	0.0006 ± 0.0002	0.0024 ± 0.0009	0.78

Values are listed as mean ± SE (normal range, unit of measurement). [A]There were no differences between patients treated with calcitonin and controls at preOLT and at 4 months. [B] Compares changes from baseline to 4 months posttransplant in calcitonin and control patients; p-values showed no differences in change following OLT. [C] Vitamin D = summer: 15 - 80 ng/mL, winter: 14 - 42 ng/mL. [D] Paired data (before and after OLT) are reported in the table; 3 patients treated with calcitonin and 4 control patients had paired measurements of [E]dynamic markers. The remaining static parameters were available as paired data in all study patients.

Histomorphometric comparison of pretransplant bone biopsies of the two study populations showed that there were no differences in pretransplant bone histomorphometric findings. There were no differences in histomorphometric parameters at 4 months posttransplant between patients treated and not treated with calcitonin, and histomorphometric changes after OLT were identical in calcitonin patients and controls (Table 4).

Hierarchical cluster analyses of bone histomorphometric parameters

The outcome of hierarchical cluster analyses using all histomorphometric parameters is shown in the dendrograms, in which the parameters having the most functional similarity are joined by the shortest branches into clusters; there is increasing dissimilarity between the clusters, and between parameters as branches go upwards and join towards the top of the dendrogram (Fig. 3A). Hierarchical cluster analysis of bone histomorphometric parameters at time of OLT showed that the resorption parameters (osteoclast number, eroded surface, and trabecular separation) organized into one cluster. This cluster had the greatest dissimilarity from the remaining bone metabolism markers. After OLT, the dendrogram (Fig. 3B) showed a different pattern of similarity of the various bone histomorphometric markers; bone resorption markers had more similarity with the remaining markers and were part of a more integrated system of bone histomorphometric parameters.

Correlations of BMD changes with histomorphometric changes

Bone loss, which occurred within the first 4 months following OLT, correlated with the decrease in trabecular thickness after OLT ($r = 0.47$, $p < 0.01$). The bone gain thereafter (4-12 months) correlated positively with the increase in osteoid markers from OLT to 4 months post-OLT [osteoid volume ($r = 0.35$, $p = 0.05$) and osteoid thickness ($r = 0.53$, $p < 0.01$)]. No other correlations were observed between BMD and histomorphometric markers.

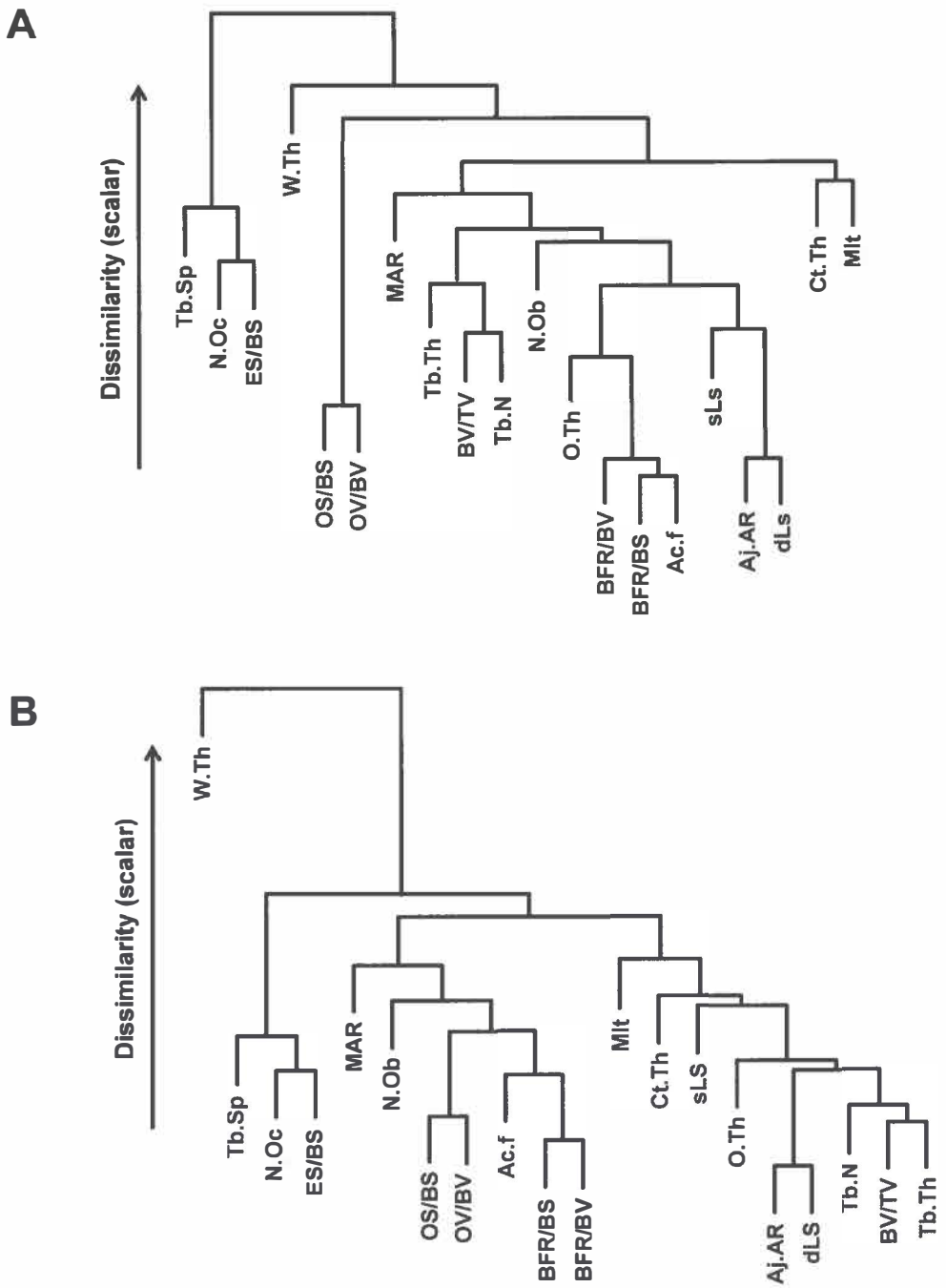

Figure 3. Dendrograms by hierarchical cluster analysis of bone histomorphometric variables at time of **(A)** OLT and **(B)** at 4 months posttransplant.

Discussion

Histomorphometric analysis of paired iliac crest bone biopsies in 33 cholestatic patients provides static and dynamic bone parameters by which to assess the changes in bone volume, resorption and formation after OLT. The severity of disturbances in bone metabolism in our study population was reflected by low pretransplant BMD of the study patients, with 39% of patients meeting criteria for osteoporosis (T scores < -2.5) at the time of OLT. In addition, histomorphometric analysis of iliac crest bone biopsy specimens immediately pretransplant showed a profound negative bone balance with decreased bone formation (decreased osteoblast number, osteoid markers and bone formation rates), and increased bone resorption (increased osteoclast number, eroded surface areas, and trabecular separation; decreased trabecular number). After OLT, BMD decreased, leading to lower BMD values at 4 months posttransplant, after which an increase of BMD occurred; this is consistent with previous publications describing changes in cholestatic patients after OLT.[1-8]

Despite the decrease in BMD at 4 months posttransplant, the iliac crest bone biopsy specimens taken at that time suggested histomorphometric improvement. All static and dynamic parameters of bone formation had significantly increased from below normal values at the time of OLT to values within the normal range by 4 months after OLT, with the exception of mean wall thickness. At the same time, 4-month measurements of direct (eroded surface, osteoclast number) and indirect (trabecular separation, trabecular number) parameters of bone resorption showed increased bone resorption, similar to the values at time of OLT. Activation frequency increased from low to normal values following OLT. Although activation frequency may directly reflect the increases in bone formation, it probably also indicates a return towards normalization of bone turnover following OLT. The histomorphometric findings of our study suggest that compensatory mechanisms resulting in increased bone formation are active by 4 months, although postoperative bone loss has not yet been reversed.

A few studies have investigated histomorphometric changes following OLT in smaller patient populations and with variable etiology of underlying chronic liver disease.[28-30] These studies also found evidence of significant increases in bone formation following OLT, and no significant change in bone resorption parameters. It has been difficult to know if the results of these previous studies could be extrapolated to a purely cholestatic population, which has never been extensively studied, but it would seem that the results of the previous studies are consistent with our findings in cholestatic patients.

Despite apparently improved bone formation parameters at 4 months after OLT, bone loss between OLT and 4 months was reflected by reductions in histomorphometric bone volume and densitometry measurements over this time period. Interestingly, mean wall thickness decreased significantly after OLT to even lower values at 4 months (mean Z-scores decreased from -1.9 to –2.5). The change in mean wall thickness between OLT and 4 months later reflects changes in mean thickness of completed bone remodeling periods preceding the 4-month bone biopsy. In normal bone, the bone formation period lasts for about 3-4 months[31]; however this time is considerably

shortened by high-dose glucocorticoids to about 1 - 2 months.[32,33] It is possible that some bone formation periods have started before OLT and finished after OLT, leading to an underestimation of the reported change in mean wall thickness. Despite this, mean wall thickness decreased by >10% during this time period, indicating an additional insult to bone formation during the study period. This insult to bone formation and mean wall thickness values is consistent with the known effect of high-dose glucocorticoids on bone formation, and suggests the important negative effects of glucocorticoids after OLT. Because, by 4 months bone formation had improved back to normal (as shown by analysis of the remaining bone formation parameters), the additional insult to bone formation had occurred early after OLT.

This additional insult to bone formation may be the key component of early posttransplant bone loss, although it has always been assumed that early posttransplant bone loss is related to increased bone turnover. Whether bone resorption, which is increased at time of OLT and at 4 months, further increases early after OLT is not evident from our study findings. Because no change in trabecular structure (trabecular number and separation) occurred during the study period, one can speculate that no clinically important increase in osteoclastic activity had occurred. In addition, previous histomorphometric studies[28,30] do not support a further increase in bone resorption by 4 months after OLT. Nor have biochemical parameters of bone metabolism provided a clear and consistent answer. However, parathyroid hormone (PTH), which increases bone resorption, has been shown to increase within the first month after OLT[34], but within normal ranges. Biochemical resorption markers have been studied by Crosbie et al.[35] in 12 patients after OLT, who showed an increase of bone resorption indices (free pyridinoline and deoxypyridinoline crosslinks) by 2 months. Interestingly, the bone formation indices (osteocalcin and procollagen type 1 carboxy propeptide) first decreased early after OLT and then started to progressively increase after 2-3 months posttransplant. As in the present study, this suggests an additional insult to bone formation early after OLT. Despite uncertainties about a temporary further increase in bone resorption early after OLT, the ongoing high bone resorption from before OLT to 4 months after OLT undoubtedly also promoted bone loss by contributing to the negative bone remodeling balance.

The normalization of bone formation parameters by 4 months after OLT most likely reflects the early recovery of bone metabolism, leading to increased BMD by 1 year. In favor of this are the correlations in our study of the increased osteoid parameters at 4 months with the gain in BMD thereafter. Improvement in bone metabolism at 4 months posttransplant was also suggested by hierarchical cluster analysis. Bone histomorphometric analysis provides information on individual bone turnover parameters when compared to normal, but does not provide any information on the functional groupings of these parameters. Hierarchical cluster analysis is a statistical technique extensively used in gene studies to organize genes into functional populations.[36,37] It is also used in clinical studies[38,39] to identify clusters of variables with highest similarity, and can be applied on histomorphometric parameters. Pretransplant cluster analysis of histomorphometric parameters in our study population showed that bone resorption parameters were organized together into one cluster, functioning separately from the bone formation and mineralization parameters. This "uncoupling" of

bone resorption and formation, which has not been previously demonstrated in cholestatic patients, may explain the unusual finding of increased resorption and decreased formation in these patients.[16] Interestingly, following OLT, hierarchical cluster analysis of the histomorphometric parameters of bone metabolism showed improvements toward a more integrated and "coupled" balance. Therefore, both bone formation and functional status (coupling) of bone metabolism markers seem to have improved after OLT. The change in position of mean wall thickness in the dendrograms is also of interest. At 4 months, all resorption and formation parameters exhibit greater similarity than before OLT, with the exception of mean wall thickness, which is isolated from all other histomorphometric parameters as completely dissimilar. Mean wall thickness at 4 months still reflects the early posttransplant period when bone formation was inhibited by factors integral to the posttransplant course. On the other hand, the other markers of bone resorption and formation represent more closely the present state of bone metabolism at 4 months after OLT with improved bone formation and more coupling of bone resorption and formation. The dissimilarity between mean wall thickness (reflecting bone loss) and the other bone formation markers (reflecting improvement) provides further support of an overall improvement in bone metabolism by 4 month posttransplant. Moreover, it further illustrates the usefulness of hierarchical cluster analyses in detecting functional (dis)similarities among individual histomorphometric bone markers.

The use of calcitonin, an inhibitor of bone resorption, was thought to be potentially beneficial in preventing osteopenia in cholestatic patients who had increased bone resorption at time of OLT. However, analysis showed that calcitonin had no effect on either direct (osteoclast number, eroded surface areas), or indirect (trabecular thickness, number, separation) parameters of bone resorption; both treated and untreated patients had increased bone resorption before and after OLT, without any change after OLT. In addition, no other histomorphometric effects of calcitonin were noted, including no effects on bone volume, formation or mineralization indices. These findings are consistent with the lack of efficacy of calcitonin on BMD and fractures in the posttransplant period.[17] The small patient numbers and short duration of therapy may have obscured a small effect of calcitonin. It is likely that the relatively weak action of calcitonin is overwhelmed by other factors operative early in the posttransplant course. Moreover, because our study suggests that an additional insult to bone formation may be a key component of early posttransplant bone loss, the mild antiresorptive effect of calcitonin may be of little benefit in this clinical setting. With the dual insult of decreased bone formation and persistently increased bone resorption following OLT, prevention of early posttransplant bone loss may require combination therapy of a potent antiresorptive agent such as a biphosphonate with a bone forming agent such as human recombinant PTH.

In conclusion, bone histomorphometric analysis of paired iliac crest bone biopsy specimens from 33 cholestatic patients showed that bone formation parameters significantly increased from low to normal values at 4 months posttransplant, whereas bone resorption remained persistently increased with no change from baseline. The increase in bone formation by 4 months posttransplant most likely reflects an early recovery of bone remodeling balance, which was also supported by hierarchical cluster

analysis showing a more coupled and integrated bone turnover status at 4 months posttransplant. Although histomorphometric mechanisms of posttransplant bone loss are not fully elucidated by this study, the decrease in mean wall thickness after OLT suggests that an additional insult to bone formation early after OLT may be the key component of posttransplant bone loss. The changes in bone metabolism following OLT were similar in all study populations, indicating no effect of gender, disease, or calcitonin treatment. Correlations of histomorphometric changes with clinical, biochemical and immunosuppressive variables during this critical time period may identify the main causes of early posttransplant bone loss and more effective prevention strategies.

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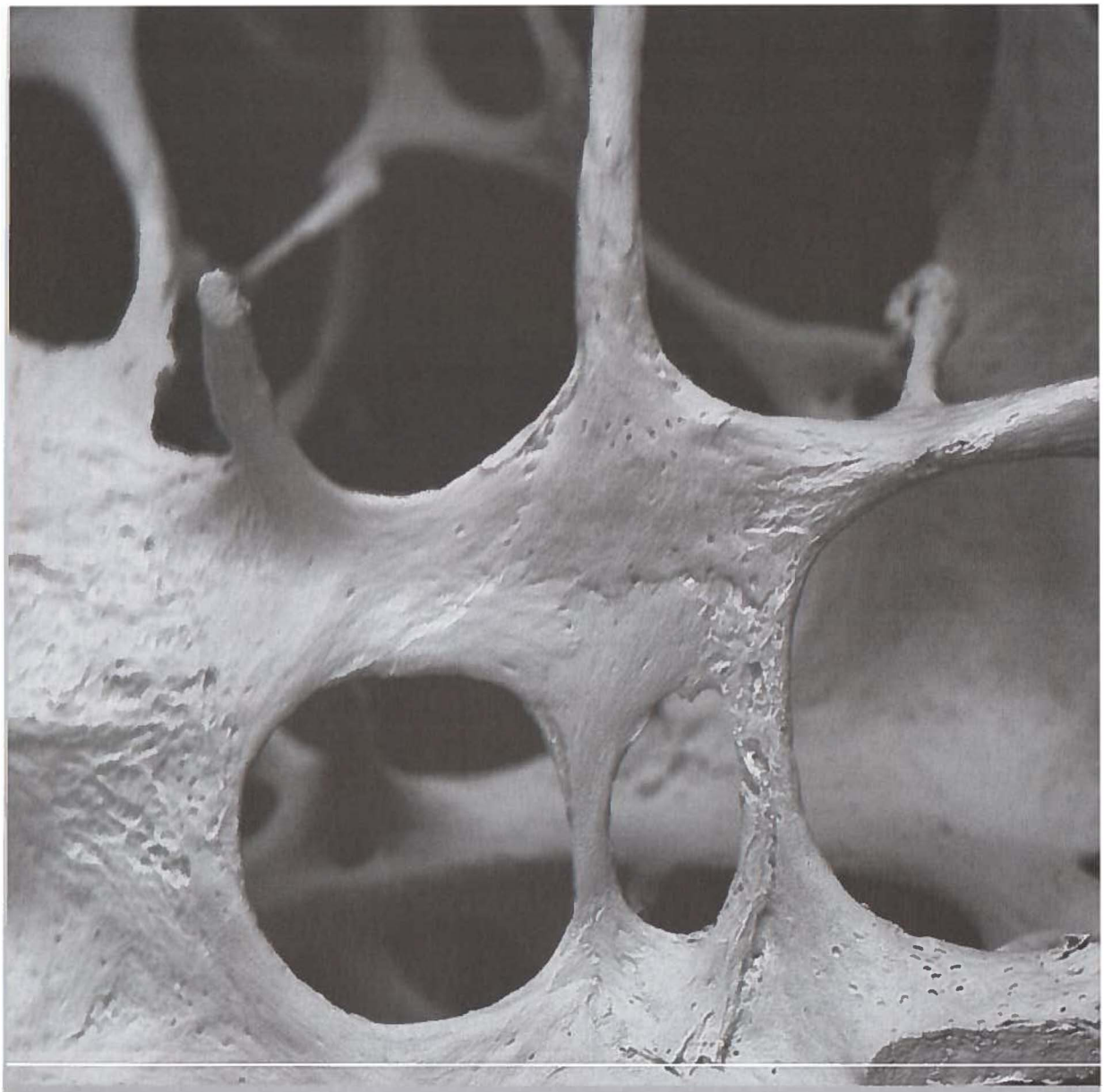
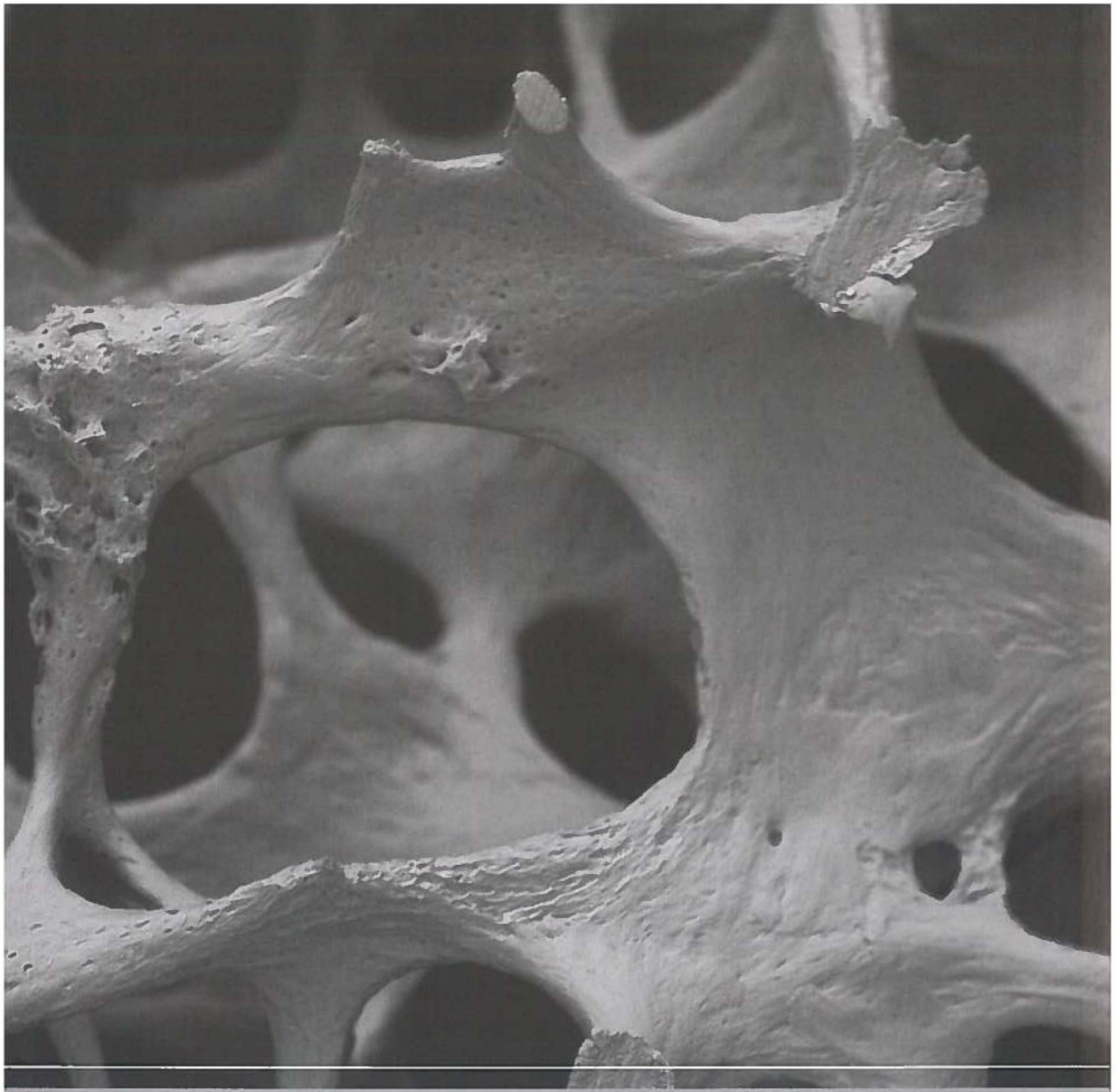


Chapter 4

Maureen M.J. Guichelaar, Michael Malinchoc, Jean D. Sibonga, Bart L. Clarke, J. Eileen Hay



Immunosuppressive and postoperative effects of orthotopic liver transplantation on bone metabolism

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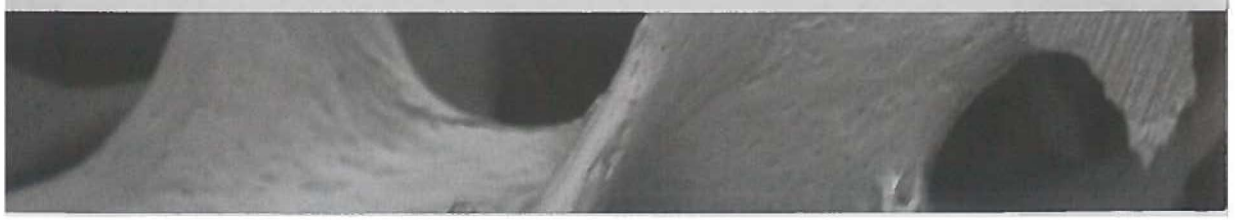

Abstract

Bone loss occurs early after orthotopic liver transplantation (OLT) in all liver transplant recipients and leads to postoperative fractures, especially in cholestatic patients with the lowest bone mass. Little is known about the underlying changes in bone metabolism after OLT or about the etiology of these changes. Histomorphometric analysis of bone biopsies, a method which allows assessment of bone volume, resorption and formation, has shown improved bone metabolism at 4 months after OLT. It has further suggested that accelerated posttransplant bone loss occurs in the first 1-2 months after OLT, probably by an additional insult to bone formation. This study attempts to correlate the histomorphometric bone changes in paired bone biopsies (OLT and 4 months after OLT) of 33 patients undergoing OLT for chronic cholestatic liver disease, with the many clinical and biochemical changes in these patients over the same time period. Cumulative steroid dosage early after OLT is shown to be important, presumably by decreasing bone formation rates. The actual effect of calcineurin inhibitors on this early phase of bone loss is less clear, although posttransplant histomorphometric findings suggest that tacrolimus-treated patients have an earlier recovery of bone metabolism and trabecular structure, when compared to cyclosporine patients. Other factors important in the recovery of bone metabolism after the early phase of bone loss are recovery of liver and gonadal function and better calcium balance.

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Abbreviations

25(OH)D, serum 25-hydroxyvitamin D; Aj.Ar, adjusted rate of bone apposition; BFR/BS, bone formation rate per trabecular bone surface area; BMF/BV, bone formation rate per osteoid-covered surface area; BGP, bone Gla-protein; BMD, bone mineral density; BV/TV, cancellous bone volume; Ct.Th, cortical thickness; DEXA, dual-energy X-ray absorptiomery; ES/BS, eroded surface; FSH, follicle-stimulating hormone; MAR, mineralization rate; MLT, mineralization lag time; N.Oc, number of osteoclasts per 100 mm trabecular surface length; OLT, orthotopic liver transplantation; Ob.S/OS, osteoblast-osteoid interface; OS/BS, osteoid surface; OS/BV, osteoid volume; O.Th, osteoid thickness; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; PTH, parathyroid hormone; SE, standard error of the means; W.Th, wall thickness; OKT3, anti-CD3 monoclonal antibody therapy.

Introduction

Bone loss with subsequent fracturing is a major complication in the early months after orthotopic liver transplantation (OLT) and has been most extensively studied in osteopenic patients with chronic cholestatic liver disease.[1-3] Many factors integral to the early posttransplant course are potentially deleterious to bone. Cyclosporine, tacrolimus, and glucocorticoids all have known in vitro effects on bone metabolism, but clinical studies have failed to elucidate the actual contribution of immunosuppressive drugs to posttransplant bone loss. The etiologic mechanisms involved in this accelerated bone loss after OLT, as well as its eventual recovery, remain undefined.

To gain insight into the pathogenetic mechanisms of bone loss and gain after OLT, detailed histomorphometric analysis of paired bone biopsies, performed before and after OLT in patients with primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC), has recently been reported.[4] This study shows improved bone metabolism by 4 months, suggesting that the major etiologic cause of the accelerated posttransplant bone loss is present very early after OLT and is probably related to an additional insult to bone formation.

By analysis of correlations of histomorphometric parameters with many clinical, biochemical, and radiological parameters both before OLT and during the first posttransplant year, this study tries to identify factors associated with postoperative bone loss and gain. Biochemical markers of bone formation (bone alkaline phosphatase, osteocalcin), and bone resorption (urinary hydroxyproline) also were studied to assess their usefulness in predicting changes in bone metabolism and bone turnover status.

Methods

Patient population

Thirty-three adult patients undergoing OLT for PBC or PSC, and without any other complicating illnesses or medications before OLT were included in this study. The study was approved by the Institutional Review Board of the Mayo Clinic. Diagnoses of PBC and PSC were made according to well-established criteria.[5-7] The 33 patients were part of a treatment trial of calcitonin, which showed that posttransplant calcitonin had no effect on posttransplant bone loss, fracture development or histomorphometric changes after OLT.[4,8] Patients consented to undergo two bone biopsies (at the time of OLT and at 4 months later), and had clinical, biochemical, radiological, and bone density measurements at time of activation for OLT, and at 4 and 12 months after OLT.

Clinical course

Symptoms and signs of bone pain or fractures were sought, as well as measurements of height, weight and functional status (Karnofsky Performance Scale rated from 0 to 100).[9] From time of activation for OLT until the end of the first posttransplant year, oral calcium supplements of 1.5 g/day was prescribed for all patients. Vitamin D therapy

(400 IU/day) was given if (1) serum 25-hydroxyvitamin D was <30 ng/ml, (2) patients had hypocalcemia, (3) parathyroid hormone (PTH) level was >50 pmol/l, or (4) urinary calcium was <75 mg/24 h. Following OLT, patients received either a two-drug immunosuppressive regimen with tacrolimus and prednisone, or triple therapy with cyclosporine A, prednisone and azathioprine. Cyclosporine and tacrolimus doses were adjusted to maintain whole-blood trough levels within the desired therapeutic range. Acute cellular rejection of the allograft was treated with intravenous Solu-Medrol on days 1, 3 and 5 after the diagnosis of rejection. Steroid-resistant rejection was treated with monoclonal therapy with OKT3. Karnofsky score, hospitalization days, and posttransplant complications were recorded in all patients.

Biochemical testing

Blood was taken for biochemical assessment after an overnight fast and tested by Mayo Medical Laboratories, using standard methods. Biochemical testing included parameters of liver and kidney function (serum albumin, total alkaline phosphatase, total and direct bilirubin, prothrombin time, serum creatinine and iothalamate clearance), as well as parameters of bone mineral metabolism (serum 25-hydroxyvitamin D [25(OH)D]), total calcium, ionized calcium, 24-hour urinary calcium, serum phosphorus, parathyroid hormone (PTH). Gonadal status was assessed in all female (serum FSH and estradiol) and male patients (free testosterone). In addition, serum markers of bone formation (bone alkaline phosphatase, osteocalcin) and urinary marker of bone resorption (urinary hydroxyproline) were measured. Osteocalcin (bone Gla-protein, BGP) was measured by radioimmunoassay using rabbit antibovine BGP antiserum and homogeneous bovine BGP.[10] Urinary hydroxyproline excretion was measured by the methods of Kivirikko et al[11] and Bidlingmeyer et al.[12] Serum 25-hydroxyvitamin D was measured by the method of Kao and Heser.[13] Immunoreactive PTH was measured by immunochemiluminometric assay (ICMA).[14]

Assessment of bone disease

Measurements of bone mineral density (BMD) of the lumbar spine (L1-L4) were performed by dual-energy x-ray absorptiometry (DEXA) (coefficient of variation 2.2%), using a Hologic QDR 1000 densitometer before OLT, and at 4 and 12 months after OLT. Results were expressed as T-scores (sex-adjusted to peak bone mass of normal population) and Z-scores (age- and sex-adjusted).

All patients had bone biopsies taken just before OLT and again at 4 months after OLT. To assess dynamic bone parameters, tetracycline bone labeling was done with oxytetracycline and demeclocycline as previously described.[4] The initial bone biopsies were performed just before OLT by the transplant surgeon at the standard iliac crest bone biopsy site, using a 7.5 mm trephine.[15] Glucocorticoids were not administered until after the bone biopsy. Bone biopsies 4 months after OLT were taken during an outpatient procedure from the contralateral iliac crest under local anaesthetic. After the bone biopsies were taken, bone tissue was prepared for quantification of bone histomorphometric parameters using standardized methods. Quantification of bone histomorphometric parameters was carried out by Bioquant System IV image analysis, using a microscope and digitizing tablet (R and M Biometrics, Nashville, TN, USA). Primary and derived data were generated by the software in accordance with standardized nomenclature and formulae.[16] Using bone histomorphometric values

obtained at the Mayo Clinic in normal females and males as reference values,[4,17] bone histomorphometric parameters of the study population were expressed both as raw data and as Z-scores (sex adjusted histomorphometric values).

Histomorphometric parameters

The following static parameters were analyzed: Cancellous bone volume (BV/TV, trabecular bone as a percentage of the total medullary volume bone), osteoid volume (OS/BV, osteoid volume as a percentage of total bone volume), osteoid surface (OS/BS, percentage of trabecular surfaces covered with osteoid), osteoid thickness (O.Th, mean thickness of osteoid in μm), number of osteoclasts per 100 mm trabecular surface length (N.Oc), eroded surface (ES/BS, percentage of trabecular surface showing resorption cavities), osteoblast-osteoid interface (Ob.S/OS, percentage of osteoid surface covered by osteoblasts), cortical thickness (Ct.Th, mean thickness of cortical seams in μm), and wall thickness (W.Th, mean thickness of the total bone structural unit in μm, measured as the distance between the cement line and the mineralized bone surface).

The following dynamic parameters were analyzed; Bone formation rate per trabecular bone surface area (BFR/BS, amount of new bone mineralized per micrometer of trabecular bone surface area per day, expressed as $mm^3/mm^2/yr$), bone formation rate per osteoid-covered surface area (BFR/BV, the average amount of new mineralized bone made per day per micrometer of osteoid-covered surface, expressed as $mm^3/mm^2/yr$), adjusted rate of bone apposition (Aj.Ar, mean distance between tetracycline labels divided by the labeling interval in days, expressed as $mm^3/mm^2/yr$), mineralization rate (MAR, averaged distance between the midpoints of two consecutive tetracycline labels, divided by the time of the labeling periods, μm/day), mineralization lag time (MLT, the average lag time in days between deposition of osteoid and its mineralization).

Statistical analysis

Biochemical and histomorphometric variables are expressed as means ± standard error of the means (SE). Transformation calculations were applied to convert raw bone histomorphometric values to Z-scores; Z-scores of histomorphometric data were obtained by subtracting the actual histomorphometric measurement from the mean value of sex-matched healthy controls, and then dividing the difference by the standard deviation of the healthy control population. Associations between the biochemical parameters, BMD measurements, and histomorphometric parameters were assessed using the Pearson correlation coefficient. Univariate t-tests of mean differences for all clinical, biochemical, bone density, and bone histomorphometric parameters were performed. When assessing tacrolimus and cyclosporine effects for posttransplant histomorphometric differences, the ANCOVA test was used to adjust for differences in prednisone dosages and pretransplant histomorphometric values. All analyses were performed using the SAS data analysis system (SAS Institute, Cary, NC).[18]

Results

Clinical data before and after OLT

The thirty-three study patients (11 PBC, 22 PSC; 12 males, 21 females, 6 postmenopausal) had a mean age at OLT of 47.4 ± 1.5 yrs and a mean Child-Pugh score of 9.2 ± 0.3. As previously reported (4), the mean lumbar spine BMD was of 0.86 ± 0.03 g/cm^2 at time of OLT, and decreased in the first 4 months after OLT to 0.81 ± 0.03 g/cm^2 before partially recovering by 1 year to 0.83 ± 0.02 g/cm^2 (Table 1).

Histomorphometric changes following OLT are shown in figures 1A and 1B. As previously reported, before OLT, bone volume parameters (cancellous bone volume, mean wall thickness) and all bone formation parameters (i.e osteoblast number, osteoid markers, bone formation rates) were decreased. Both direct (osteoclast number, eroded surface area), and indirect (trabecular number, separation) parameters of bone resorption showed increased bone resorption. At 4 months after OLT, bone volume parameters (mean wall thickness, cortical thickness) had decreased further, bone resorption remained unchanged and bone formation parameters and activation frequency had increased from below normal to the normal range.
Karnofsky performance scoring at the time of OLT showed that 14 (42.4%) patients had near-normal activity (score 80 - 100), 18 (54.5%) patients required help with activities of daily living but were ambulatory (score 40-70), and 1 patient was hospitalized at time of OLT due to a variceal bleed. After OLT, patients stayed on average for 4.0 ± 2.2 days in the ICU, and for 21.9 ± 10.9 days in the hospital. All patients were ambulatory at time of hospital discharge. Immunosuppression was tacrolimus and prednisone in 10 patients, whereas 23 patients were on cyclosporine, azathioprine and prednisone. Cumulative prednisone dose during the first 4 months was 5989.3 ± 338.8 mg. Sixteen patients (6 PBC, 10 PSC; 10 females, 6 males) had biopsy-proven rejection episodes within the first 4 months following OLT; of these patients 3 patients had a second rejection episode within the first 6 months. Sixteen rejection episodes were treated with Solu-Medrol, and 3 rejection episodes with OKT3.

Laboratory data before and after OLT

Liver function improved and kidney function deteriorated after OLT (Table 1). Serum calcium, 25(OH)D, PTH, and phosphorus increased following OLT, whereas urinary calcium decreased, but all changes remained within the normal ranges. Hypogonadism occurred in 9 (75%) male patients (free testosterone < 9 ng/dL) and 11 (52%) female patients (estradiol < 35 pg/mL) before OLT. Of these 11 hypogonadal women, 4 females were postmenopausal before and after OLT (estradiol < 35 pg/mL, FSH > 30 IU/L); 4 females had low estradiol and FSH before OLT, with increases of FSH after OLT to the postmenopausal range; 3 females had low FSH and estradiol before OLT and had normalization of estradiol after transplantation. Serum FSH increased significantly in both pre- and postmenopausal women following OLT, whereas free testosterone increased after OLT in male patients. Despite histomorphometric evidence of increased bone resorption before and after OLT, urinary hydroxyproline remained within the normal range throughout the study period. Although bone alkaline phosphatase did not change significantly after OLT, osteocalcin increased significantly over the whole study period to above normal values at 1 year.

Table 1. Biochemical and bone mineral density parameters before and after orthotopic liver transplantation in 33 patients with chronic cholestatic liver disease.

	PreOLT	PostOLT (4 months)	PostOLT (1 year)
Bone mineral density parameters			
BMD (g/cm^2)	0.86 ± 0.03	0.81 ± 0.03****	0.83 ± 0.02**
T-scores	-2.1 ± 0.2	-2.5 ± 0.2****	-2.3 ± 0.2*
Z-scores	-1.6 ± 0.2	-1.9 ± 0.2****	-1.7 ± 0.2*
Liver function (normal range)			
Albumin (3.5-5.0 g/dL)	2.8 ± 0.09	3.6 ± 0.09****	3.8 ± 0.07
Total alkaline phosphatase (U/L)[A]	1531 ± 273.6	406 ± 80.3****	347.0 ± 80.5
Total bilirubin (0.1-1.1 mg/dL)	9.1 ± 1.0	1.7 ± 0.5****	1.0 ± 0.1
Direct bilirubin (0.0-0.3 mg/dL)	5.8 ± 0.7	0.7 ± 0.4****	0.2 ± 0.3
Prothrombin time (8.4-10.0 sec)	13.0 ± 0.2	11.5 ± 0.1****	11.4 ± 0.1
Kidney function			
Serum creatinine (mg/dL)[B]	0.8 ± 0.03	1.5 ± 0.09****	1.5 ± 0.07
Iothalamate clearance (>70 mL/min/SA)	97.3 ± 5.1	49.6 ± 4.0****	54.0 ± 2.7
Mineral metabolism			
25-hydroxyvitamin D (ng/mL)[C]	17.1 ± 1.6	32.3 ± 1.3****	35.0 ± 1.7
serum calcium (8.9-10.1 mg/dL)	8.7 ± 0.07	9.3 ± 0.07****	9.3 ± 0.07
ionized calcium (4.8-5.3 mg/dL)	5.0 ± 0.03	5.0 ± 0.03	5.0 ± 0.03
urinary calcium (25-300 mg/24 hr)	114 ± 16.2	101 ± 11.6	72 ± 8.5*
phosphorus (2.5-4.5 mg/dL)	3.2 ± 0.1	4.1 ± 0.1****	3.6 ± 0.1***
parathyroid hormone (1.0-5.2 pmol/L)	2.0 ± 0.2	3.4 ± 0.3****	4.0 ± 0.5
Bone metabolism			
Bone alkaline phosphatase (24-146 U/L)	146 ± 32.8	113 ± 12.6	138 ± 13.3*
Osteocalcin (µg/L)[D]	15.2± 2.8	20.2 ± 12.3*	33.6 ± 3.7***
Urinary hydroxyproline (15-45 mg/24 hr)	34.8 ± 1.8	23.0 ± 1.3*	23.4 ± 2.1
Gonadal hormones			
FSH premenopausal F; (FSH<36,)	15.1 ± 4.9	50.8 ± 11.5*	31.2 ± 57.7
Postmenopausal F; (FSH 30-120)	60.2 ± 8.4	93.7 ± 10.8**	111.7 ± 16.0*
Estradiol (pg/mL)[E]			
Premenopausal F	63.9 ± 12.3	87.1 ± 13.0	99.5 ± 13.3
Postmenopausal F	19.0 ± 3.4	17.0 ± 4.4	21.8 ± 2.7
Free testosterone (9-30 ng/dL)	6.6 ± 0.9	11.7 ± 1.1*	12.4 ± 0.9*

Results expressed as means ± SEM. Change from baseline to 4 mo postOLT, and 4 mo to 12 mo postOLT ;
*p-value < 0.05, **< 0.01, ***< 0.001, **** < 0.0001.

[A] total alkaline phosphatase; M > 19 yr: 98 - 251 U/L; F 24 - 45 yr: 81 - 231 U/L; F 46 - 60 yr: 84 - 257 U/L; F > 60 yr: 108-309 U/L. [B] serum creatinine = F: 0.6 - 0.9 mg/dL, M: 0.8 - 1.2 mg/dL.

[C] 25(OH) vitamin D = summer: 15 - 80 ng/mL, winter: 14 - 42 ng/mL.

[D] osteocalcin = normal ranges for men and F 20 - 50 yr: 2 - 15 ng/L, F 50 - 80: 6 - 22 ng/L.

[E] estradiol = premenopausal F < 400 pg/mL, postmenopausal F < 35 pg/mL.

Table 2A. Correlation factors of pretransplant biochemical parameters with histomorphometric changes (from preOLT- 4 months postOLT).

	Bone volume loss			Bone formation increase				Bone resorption increase		
	Cancellous bone volume	Cortical thickness	Mean wall thickness	Osteoblast number	Osteoid Markers[A]	Dynamic formation[B]	Minerali-zation[C]	Osteoclast number	Indirect activity[D]	Eroded surface
Liver/ kidney function										
Albumin	-0.40								-0.53[E]	
Total bilirubin		0.43								
Direct bilirubin		0.42	0.35							
Mineral metabolism										
Urinary calcium		-0.48								
Phosphorus							0.85			
PTH									-0.35[E]	
25(OH)D		-0.42								
Bone metabolism										
Osteocalcin					-0.39					
Urinary hydroxyproline									0.53[F]	
Gonadal hormones										
Estradiol				0.54						

Abbreviations: 25(OH)D, serum 25-hydroxyvitamin D; FSH, follicle-stimulating hormone; OLT, orthotopic liver transplantation; PTH, parathyroid hormone. Note: The table summarizes the correlation [r] factors, all correlations are significant ($p<0.05$). # Baseline alkaline phosphatase, prothrombin time, creatinine and iothalamate clearance, serum and ionized calcium, bone alkaline phosphatase, and FSH and free testosterone did not have any correlation with bone histomorphometric changes after OLT.

[A] Osteoid parameters (newly formed bone) are osteoid surface, volume, thickness. [B] Dynamic bone parameters are bone formation rates, apposition rates, activation frequency.

[C] Mineralization parameters are mineralization lag time, mineralization rate, [D] Indirect parameters of bone resorption are trabecular thickness, number, and separation.

[E] Correlations showed that patients with high baseline albumin and PTH had an increase of trabecular number and decrease of trabecular separation after OLT: less resorption.

[F] Correlations showed that patients with high baseline urinary hydroxyproline had a decrease of trabecular number after OLT

Table 2B. Posttransplant correlation factors of 4 month biochemical parameters with 4 month histomorphometric parameters

	Bone volume			Bone formation				Bone resorption		
	Cancellous bone volume	Cortical thickness	Mean wall thickness	Osteoblast number	Osteoid Markers[A]	Dynamic Formation[B]	Minerali Zation[C]	Osteoclast number	Indirect Activity[D]	Eroded surface
Liver/ kidney function										
Alkaline phosphatase					-0.43	–0.44	-0.49	-0.42		-0.42
Total bilirubin					-0.36					
Direct bilirubin					-0.39					
Prothrombin time				0.39	0.36	0.45				
Creatinine				-0.38						
Mineral metabolism										
Serum calcium						-0.51[E]				
Ionized calcium									-0.42[F]	-0.42
Urinary calcium				0.41						
Phosphorus	0.48*		0.46*							
PTH					0.43	0.46		0.38		
Bone metabolism										
Bone alkaline phosphatase	-0.35									
Osteocalcin				0.60**	0.71**	0.55*				
Gonadal hormones										
FSH					0.50	0.60	0.53			
Estradiol									0.59[G]	
Free testosterone									0.59[G]	

Abbreviations: 25(OH)D, serum 25-hydroxyvitamin D; FSH, follicle-stimulating hormone; PTH, parathyroid hormone. The table summarizes the correlation [r] factors, all correlations are significant ($p<0.05$, *$p<0.01$, **$p<0.001$)

Posttransplant (4 month) albumin, iothalamate clearance, 25(OH)D, urinary hydroxyproline had no correlation with 4-month histomorphometric parameters.

[A] Osteoid parameters are osteoid surface, volume, thickness. [B] Dynamic bone parameters are bone formation rates, apposition rates, activation frequency.

[C] Mineralization parameters are mineralization lag time, mineralization rate. [D] Indirect parameters of bone resorption are trabecular thickness, number, and separation.

[E] Negative correlation serum calcium with activation frequency. [F] Negative correlation ionized calcium with trabecular separation (less indirect resorption).

[G] Positive correlation estradiol and free testosterone with trabecular number (less indirect resorption).

Correlations of histomorphometric bone indices with clinical and biochemical parameters

Correlations were assessed (a) between pretransplant clinical and biochemical parameters and histomorphometric changes from pre- to posttransplant (Table 2A) and (b) between histomorphometric indices at 4 months and clinical and biochemical parameters at 4 months (Table 2B). Pretransplant Karnofsky and Child scores, posttransplant rejection episodes and hospitalization days did not correlate with any bone histomorphometric parameter.

Bone volume

The loss of cortical thickness at 4 months after OLT correlated positively with pretransplant total and direct bilirubin, and negatively with pretransplant 25(OH)D and urinary calcium but did not correlate with any 4 month clinical or biochemical parameter (Table 2A). The loss of mean wall thickness after OLT also correlated positively with baseline direct bilirubin and loss of cancellous bone volume negatively with serum baseline albumin. Cancellous bone volume at 4 months correlated negatively with 4 month bone alkaline phosphatase, and positively with phosphorus which also correlated with mean wall thickness (Table 2B). Intravenous prednisone doses during the first 1 month correlated positively with bone volume losses (cancellous bone volume, osteoid thickness and trabecular thickness). A summary of the effects of prednisone on bone histomorphometry is given in Table 3.

Bone formation

Static bone formation

The increase in osteoblast number at 4 months after OLT correlated positively with baseline estradiol. Posttransplant osteoblast number correlated negatively with posttransplant creatinine, and positively with osteocalcin, prothrombin time, and urinary calcium. Increases in osteoid markers (newly formed bone) at 4 months after OLT correlated positively with baseline osteocalcin. Osteoid markers at 4 months correlated positively with prothrombin time, PTH, FSH and osteocalcin; negative correlations were seen with alkaline phosphatase and bilirubin.

Dynamic bone formation and mineralization

Bone formation rates at 4 months correlated negatively with total alkaline phosphatase (but no correlation with bone alkaline phosphatase) and positively with PTH, osteocalcin and prothrombin time. Activation frequency at 4 months correlated positively with PTH and FSH, and negatively with serum calcium and alkaline phosphatase. The increase in mineralization rate from pretransplant to 4 months postOLT correlated highly with pretransplant serum phosphorus and the actual 4 month postOLT mineralization rate with FSH and negatively with alkaline phosphatase. Intravenous prednisone doses during the first 1 month and oral doses during the first 4 months also correlated negatively with posttransplant bone formation (osteoblast number, adjusted apposition rate, bone formation rates).

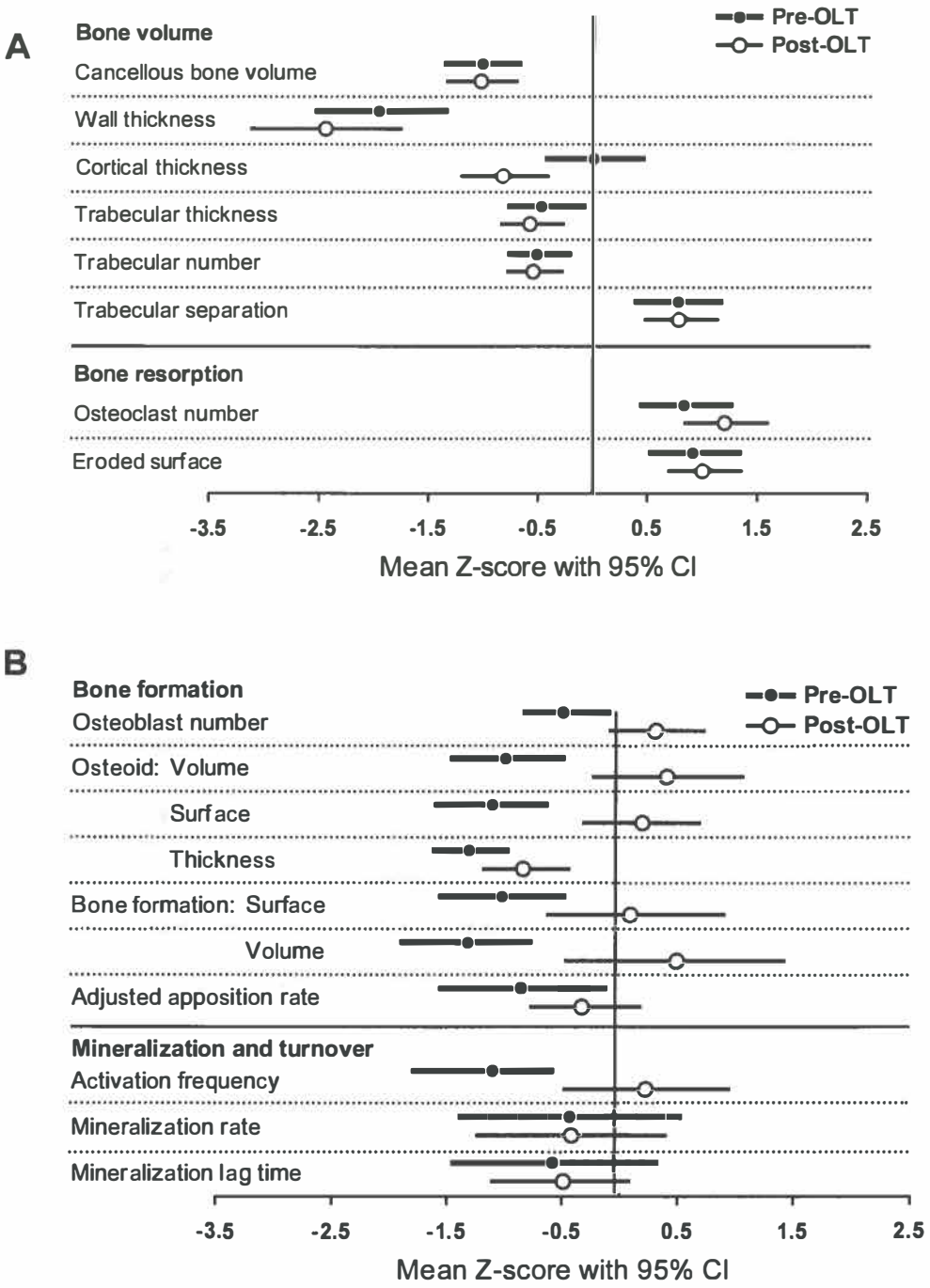

Figure 1. (A) Histomorphometric changes, expressed as Z-scores, of bone volume and resorption. **(B)** Bone formation and mineralization from pretransplant to 4 months after orthotopic liver transplantation in 33 patients with PBC and PSC

Bone resorption

An increase in trabecular number and decrease in trabecular separation was seen in patients with high baseline albumin, and PTH. In addition, a decrease in trabecular number was seen in patients with high baseline urinary hydroxyproline. Posttransplant trabecular number at 4 months correlated positively with 4 months testosterone and estradiol, whereas posttransplant trabecular separation correlated negatively with ionized calcium. At 4 months posttransplant, the number of osteoclasts and eroded surface both correlated negatively with total alkaline phosphatase; osteoclast number also correlated positively with PTH, and eroded surface negatively with ionized calcium. Oral prednisone doses during the first 4 months correlated positively with indirect bone resorption parameters (trabecular separation, and decreased trabecular number).

Histomorphometric comparison of tacrolimus and cyclosporine patients

Comparison was made of the histomorphometric changes between patients treated with cyclosporine and those treated with tacrolimus (table 4). Patients treated with tacrolimus received less prednisone ($p<0.05$) but there were no other clinical or biochemical differences between the two populations. After adjustment for different prednisone doses and baseline histomorphometry, the posttransplant histomorphometric comparison showed that tacrolimus-treated patients had an improvement of cancellous bone architecture by 4 months (increase of cancellous bone volume, trabecular thickness, decreased trabecular separation), whereas this was still diminished in cyclosporine patients. In addition, adjusted apposition rate and mean wall thickness – both bone formation markers- had increased in tacrolimus patients by 4 months posttransplant ($p<0.05$).

Table 3. Correlations of prednisone dosage with posttransplant bone histomorphometric parameters.

Prednisone	Change in bone metabolism (preOLT - 4 mo postOLT)	Bone metabolism status at 4 mo postOLT
1 mo cumulative intravenous dosage	Loss cancellous bone volume (r = 0.40) Loss trabecular thickness (r = 0.46)	Osteoblasts (r = -0.38)
4 mo cumulative oral dosage		Appositional rate (r = -0.41) Bone formation rates (r = -0.41) Trabecular number (r = -0.42) Trabecular separation (r = 0.38)
1 mo total prednisone	Loss cancellous bone volume (r =0.37) Loss osteoid thickness (r = 0.35) Loss trabecular thickness (r = 0.41)	
4 mo total prednisone	Loss cancellous bone volume (r = 0.38) Loss trabecular thickness (r = 0.39)	Activation frequency (r = -0.41)

All correlations are significant (p-value < 0.05).

Table 4. Comparison of clinical and histomorphometric parameters in patients treated with cyclosporine (CyA) or tacrolimus (FK506).

Variables	CyA patients (n = 23)		FK506 patients (n = 10)	
Gender (F/M)	15 (65%) / 8 (35%)		6 (60%) / 4 (40%)	
Disease (PBC/PSC)	6 (26%) / 17 (74%)		5 (50%) / 5 (50%)	
Age at OLT (yrs)	46.3 ± 8.6		49.8 ± 8.2	
Child scores	9 ± 1.8		10 ± 1.6	
Cum pred 4 mo postOLT (mg)	6679.4 ± 369.8		4389.3 ± 424.0***	
Pts with postOLT fractures yr 1	8 (35%)[A]		3 (33%)[B]	
Bone density parameters	**PreOLT**	**PostOLT 4 mo**	**PreOLT**	**PostOLT 4 mo**
BMD (g/cm^2)	0.86 ± 0.04	0.81 ± 0.03	0.84 ± 0.04	0.81 ± 0.04
T-scores	-2.0 ± 0.3	-2.5 ± 0.3	-2.2 ± 0.4	-2.5 ± 0.4
Z-scores	-1.6 ± 0.3	-2.0 ± 0.3	-1.6 ± 0.4	-1.8 ± 0.3
Histomorphometric parameters				
Bone volume				
Cancellous bone volume (%)	18.1 ± 1.3	15.6 ± 1.2*	16.6 ± 5.2	21.5 ± 6.7*
Mean wall thickness (µm)	30.2 ± 2.0	26.3 ± 8.2*	25.4 ± 1.4	29.1 ± 9.1*
Cortical thickness (µm)	1021.3 ± 70.7	712.2 ± 56.1	835.5 ± 261.1	802.7 ± 250.8
Trabecular thickness (µm)	125.3 ± 7.6	107.1 ± 5.8*	107.7 ± 6.3	132.3 ± 5.9*
Trabecular number (mm^{-1})	1.5 ± 0.1	1.4 ± 0.1*	1.5 ± 0.1	1.6 ± 0.1*
Trabecular separation (µm)	585.2 ± 31.6	639.3 ± 32.8	570.6 ± 35.4	490.2 ± 22.5
Bone resorption				
Osteoclasts (n/100 mm)	11.1 ± 1.6	15.2 ± 2.2	12.8 ± 4.0	15.5 ± 4.8
Eroded surface areas (%)	11.1 ± 1.2	11.5 ± 1.5	8.3 ± 2.6	8.3 ± 2.6
Bone formation				
Osteoid thickness (µm)	7.9 ± 0.6	8.4 ± 0.5	7.2 ± 2.3	10.0 ± 3.1
surface (%)	8.6 ± 1.9	14.4 ± 2.6	4.3 ± 1.4	20.3 ± 6.4
volume (%)	1.3 ± 0.3	2.2 ± 0.5	0.5 ± 0.2	3.4 ± 1.1
Osteoblast-osteoid interface (%)	8.2 ± 1.8	16.2 ± 2.8	10.8 ± 3.4	16.8 ± 5.3
Bone formation/vol based[C]	0.065 ± 0.01	0.23 ± 0.05	0.059 ± 0.02	0.69 ± 0.22
Bone formation/sur based[C]	0.0052 ± 0.001	0.012 ± 0.003	0.0033 ± 0.001	0.048 ± 0.015
Mineralization rate (µm/day)	0.53 ± 0.18	0.52 ± 0.03	0.57 ± 0.18	0.70 ± 0.21
Mineralization lag time (days)	18.5 ± 1.6	17.8 ± 0.03	13.9 ± 4.3	17.9 ± 5.6
Adjusted apposition time[C]	0.05 ± 0.01	0.071 ± 0.008*	0.09 ± 0.03	0.172 ± 0.05*
Activation frequency (days^{-1})	0.2 ± 0.03	0.4 ± 0.1	0.10 ± 0.02	1.5 ± 0.4

P-values for differences between FK506 and cyclosporine patients, adjusted for prednisone doses and pretransplant histomorphometric variables; * p<0.05, ** p<0.01, *** p<0.001.

[A] Six patients had 22 vertebral fractures (3.7 per patient), and 4 patients had rib fractures (all bilateral),

[B] Two patients had 7 vertebral fractures, and 1 patient had bilateral rib fractures.

[C] Variables are expressed as mm^3/mm^2/yr.

Discussion

Previous histomorphometric assessment of the 33 study patients has shown early improvement in bone metabolism by 4 months posttransplant, with recovery of bone formation and improved functional status of bone turnover towards a more coupled balance of resorption and formation[4]. Despite this improvement in bone metabolism by 4 months, a loss of bone volume, both by histomorphometry and DEXA, indicates that an additional insult to bone mass has occurred during this 4 month period after OLT. Detailed histomorphometric analysis suggested that this posttransplant loss of bone mass was probably mainly related to a further, transient reduction in bone formation, occurring early after OLT and recovering to normal by 4 months. It would appear therefore that the etiologic factors causing rapid early bone loss after OLT are most active very early after OLT. By analysis of histomorphometric changes in relation to the multiple clinical and biochemical variables during this period, this study seeks to identify these etiologic factors of bone loss as well as the mechanisms of recovery of bone metabolism.

Several factors have been implicated as potential etiologic factors of early posttransplant bone loss, with immunosuppressive agents assuming the greatest importance. Most posttransplant studies have not found any consistent relationship between immunosuppressive doses and bone loss, but histomorphometric markers of bone metabolism have not been studied. Our study also failed to show any relationship between glucocorticoids and bone density changes, but several interesting correlations with histomorphometric parameters were seen. Cumulative glucocorticoid doses at 1 and 4 months correlated positively with bone volume losses and inversely with bone formation parameters. Presumably this reduced bone formation by steroids leads to, or at least contributes to, the loss of bone volume. These findings are consistent with the known inhibitory effects of steroids on osteoblasts and bone formation.[19,20] In addition, as indicated above, it would appear that early posttransplant bone loss is mediated through an increased but transient inhibitory effect on bone formation. The correlation of steroids with reduced bone formation and bone losses following OLT in this study suggests an etiologic connection.

The role played by calcineurin inhibitors in posttransplant bone loss remains unknown. Previous studies comparing the effects of tacrolimus and cyclosporine on bone loss have been contradictory, with some studies indicating similar effects on bone metabolism and bone loss,[21,22] and others suggesting that tacrolimus is more potent in stimulating bone osteogenesis and bone formation.[23-25] Both cyclosporine and tacrolimus cause increased bone turnover in vitro.[21,23,26] but no increase in bone turnover and resorption was seen here. However, although similar bone density losses were seen in both cyclosporine- and tacrolimus-treated patients, the histomorphometric changes after OLT were different in the two groups. After adjusting for cumulative prednisone dose, patients treated with tacrolimus had an improvement in cancellous bone architecture and increases in adjusted apposition rate of bone and mean wall thickness by four months compared to cyclosporine patients. These findings suggest that tacrolimus-treated patients may have an earlier recovery of bone metabolism after

the initial phase of bone loss compared to cyclosporine-treated patients. The mechanism for this difference is not obvious from our study findings.

In addition to its direct effects on bone metabolism, glucocorticoids alter calcium homeostasis, causing bone loss. Glucocorticoids inhibit calcium absorption from the gastrointestinal tract and stimulate excretion of urinary calcium by interfering with renal tubular mechanisms;[27,28] this latter mechanism may cause a compensatory increase of PTH and therefore more bone turnover and resorption. PTH in our study correlated with bone turnover (both resorption and formation parameters) at 4 months after OLT, but no correlation between PTH and glucocorticoid doses was seen. An increase in PTH has been shown to occur as early as in the first month after OLT,[29] and any relation to cumulative glucocorticoid dose may therefore have been missed at the 4 month evaluation in our study. Early bone loss correlated with low pretransplant serum vitamin D level and urinary calcium, suggesting that calcium balance is somehow protective against early posttransplant bone loss. In addition, better calcium balance at 4 months had beneficial effects on bone formation (increased) and bone resorption (decreased).

Loss of bone volume after OLT was greatest in patients with high pretransplant bilirubin levels. In addition, at 4 months after OLT, increased formation of new bone (osteoid) was associated with lower bilirubin. With cholestasis as a recognised risk factor for osteopenia, it is not surprising that improved histomorphometric status at 4 months is seen in patients with lack of cholestasis. This provides further evidence for the etiologic association of cholestasis with osteopenia before OLT and is consistent with previous in vitro studies indicating that osteoblastic proliferation was diminished by serum of cholestatic patients.[30] The increased loss of bone volume at 4 months in patients with the highest pretransplant bilirubin levels suggests that the impaired bone metabolism of cholestatic osteopenia in some way renders it more susceptible to the early posttranplant influences which effect bone loss.

Changes in gonadal status may also affect bone metabolism after OLT. The importance of hypogonadism on bone mass has been studied mainly in patients with alcoholic liver disease.[31,32] Our study shows that two-thirds of male cholestatic patients were hypogonadal at time of OLT, with increasing free testosterone levels after OLT. This increased testosterone at 4 months correlated with less bone resorption (increased trabecular number), a known effect of androgenic hormones. In addition, FSH increased after OLT in both postmenopausal and premenopausal women, suggesting central suppression of the hypothalamic-pituitary function in females with advanced liver disease, a mechanism for which there is also some evidence in males with chronic liver disease.[33] Pretransplant estradiol and FSH at 4 months correlated respectively with an increase in osteoblast numbers and bone formation parameters at 4 months but had no correlation with bone loss. These findings suggest that recovery of hormonal function mainly contributes to bone metabolism after the initial phase of bone loss; in the early phase of bone loss it is likely that other factors overwhelm the minor effects of the still recovering gonadal status.

Biochemical indices of bone metabolism provide a non-invasive method to measure changes in bone metabolism. Although biochemical bone markers are unreliable in

identifying bone resorption and formation in pretransplant cholestatic patients,[34,35] their use in posttransplant patients has not been validated. The present analysis shows that osteocalcin measured after OLT correlated with all posttransplant bone formation parameters. Interestingly, osteocalcin continued to increase after the first 4 months to higher values by 1 year; this most likely reflects a continuing increase in bone formation during this period. Bone alkaline phosphatase and urinary hydroxyproline were ineffective in predicting bone formation or resorption.

In conclusion, this study confirms for the first time that cumulative steroid dosage after OLT contributes to early posttransplant bone loss. Its effect is most likely due to a further reduction in bone formation, compounding the already compromised bone formation of patients with chronic cholestatic liver disease. After this first phase of bone loss, histomorphometric assessment suggests that patients with tacrolimus therapy have an earlier recovery of bone formation and trabecular structure by 4 months when compared to cyclosporine patients; this may alter the longterm outcome for fracturing. The improvement in bone metabolism by 4 months after OLT is probably multifactorial with contributions from improvements in hepatic synthetic function, lack of cholestasis, gonadal recovery and better calcium balance. Of the bone biochemical indices, only osteocalcin seems to be a reliable tool to assess changes in posttransplant bone formation.

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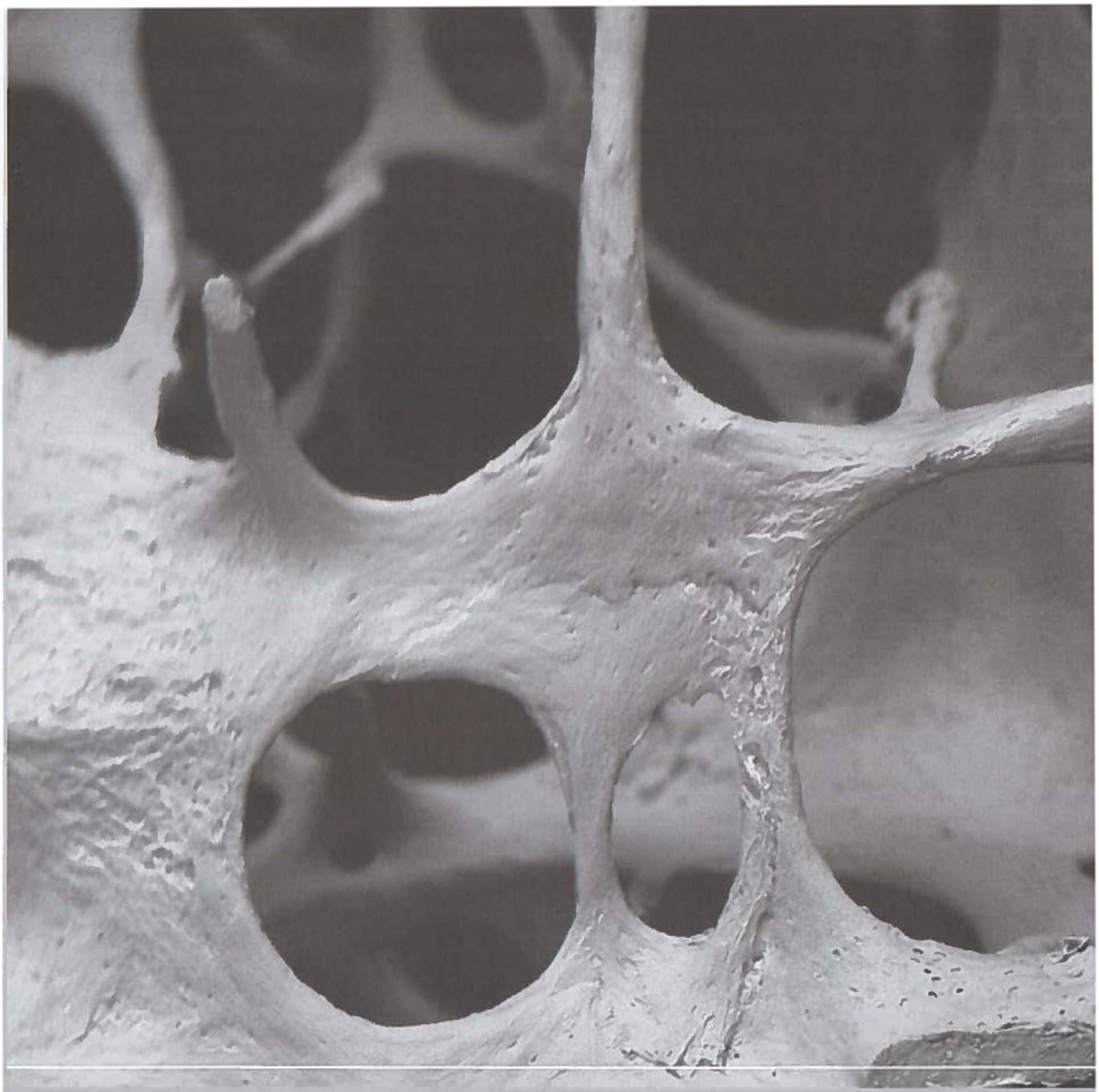
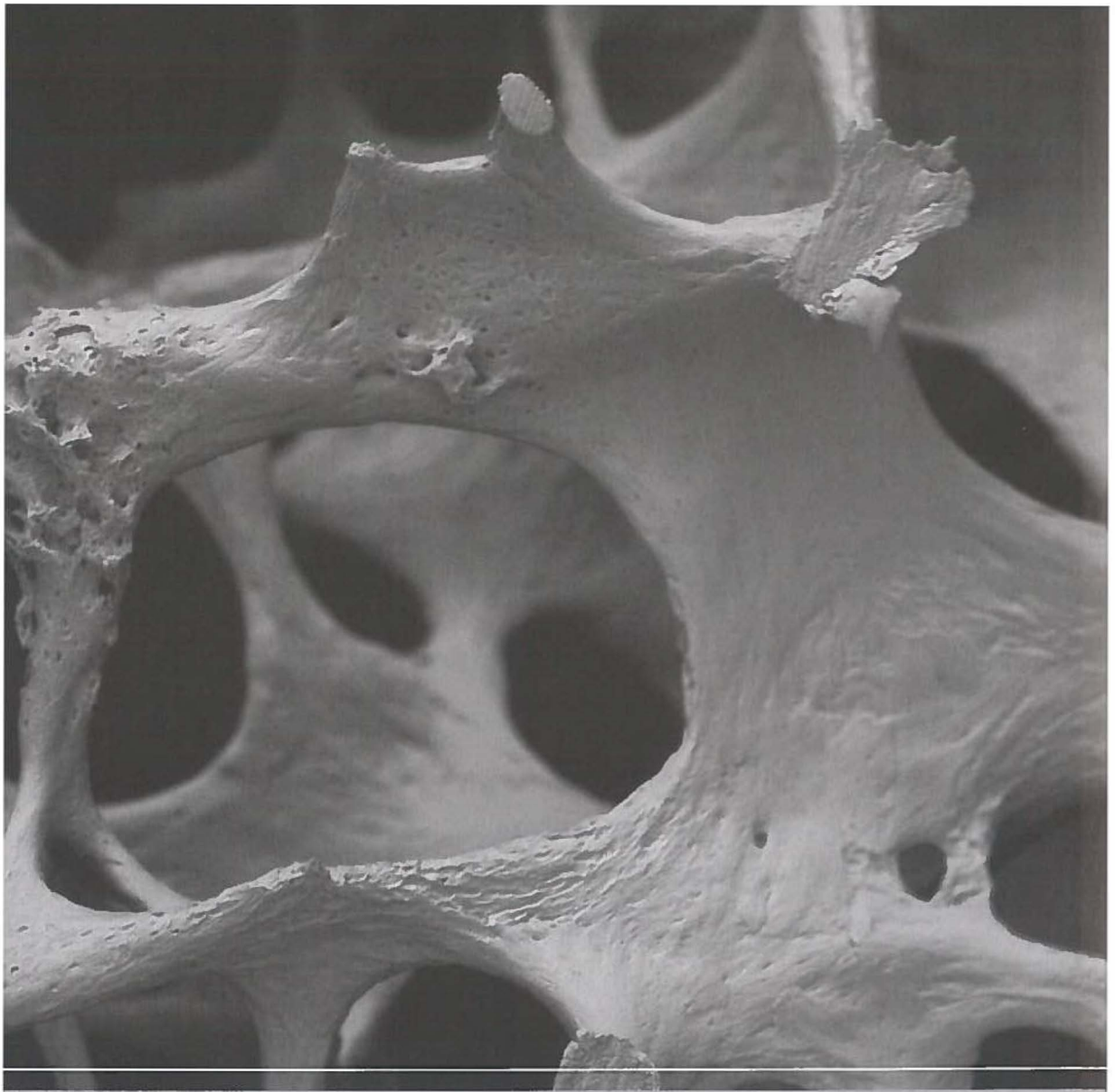


Chapter 5

Maureen M.J. Guichelaar, Rebecca Kendall, Michael Malinchoc, J. Eileen Hay



Bone mineral density before and after OLT: Long-term follow-up and predictive factors

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Abstract

Fracturing after liver transplantation (OLT) occurs due to the combination of pre-existing low bone mineral density (BMD) and early posttransplant bone loss, the risk factors for which are poorly defined. The prevalence and predictive factors for hepatic osteopenia and osteoporosis, posttransplant bone loss, and subsequent bone gain were studied by the long-term posttransplant follow-up of 360 consecutive adult patients with end-stage primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC).

Only 20% of patients with advanced PBC or PSC have normal bone mass. Risk factors for low spinal BMD are low body mass index, older age, postmenopausal status, muscle wasting, high alkaline phosphatase and low serum albumin. A high rate of spinal bone loss occurred in the first 4 posttransplant months (annual rate of 16%) especially in those with younger age, PSC, higher pretransplant bone density, no inflammatory bowel disease, shorter duration of liver disease, current smoking and ongoing cholestasis at 4 months. Factors favouring spinal bone gain from 4 to 24 months after transplantation were lower baseline and/or 4-month bone density, premenopausal status, lower cumulative glucocorticoids, no ongoing cholestasis and higher levels of vitamin D and parathyroid hormone. Bone mass therefore improves most in patients with lowest pretransplant BMD who undergo successful transplantation with normal hepatic function and improved gonadal and nutritional status. Patients transplanted most recently, have improved bone mass before OLT; although bone loss still occurs early after OLT, these patients also have a greater recovery in BMD over the years following OLT.

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Abbreviations

OLT, orthotopic liver transplantation; BMD, bone mineral density; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; MELD, model for end-stage liver disease; IBD, inflammatory bowel disease; CyA, cyclosporine A; TAC, tacrolimus; PTH, parathyroid hormone; BMD-LS, BMD at the lumbar spine; HRT, hormone replacement therapy; BMI, body mass index.

Introduction

Osteopenia and osteoporosis have long been recognised as complications of cirrhosis, especially in patients with chronic cholestatic liver disease.[1-4] It is however only in the era of successful orthotopic liver transplantation (OLT) that the full clinical significance of morbidity from fracturing has been realised. Despite the high prevalence of bone disease in chronic cholestatic liver disease, the underlying etiologic mechanism of bone loss remains obscure and its management empiric and unsatisfactory.

Preexisting low bone mineral density (BMD) of the spine at the time of OLT and pretransplant fracturing have been shown to be major risk factors for posttransplant skeletal complications.[5,6] In the early months after orthotopic liver transplantation, patients suffer from rapid bone loss which leads to a high incidence of atraumatic fracturing in the early post-operative years.[7-9] Since bone loss is seen early after all solid organ transplants,[9,10] it has been assumed that high doses of glucocorticoids play a major etiologic role and this has recently been confirmed in bone histomorphometric analysis of bone biopsy specimens after OLT.[11] Other factors integral to the early postoperative course may further contribute to bone loss, including immobility, and disturbances of mineral metabolism.[7,12-14] Fortunately for most patients this first postoperative year represents the nadir in bone density and a recovery of bone mass starts to occur after the early months of bone loss.

Although some studies have assessed bone loss and bone gain after OLT, the study samples were small and data of long-term follow-up after OLT are limited. In addition, analysis of risk factors for pretransplant hepatic osteoporosis or osteopenia and posttransplant bone loss and bone gain has not been well established, although these are important to determine preventive strategies. We aimed to determine the prevalence and predictive factors for low bone mass before OLT, posttransplant bone loss, and bone gain at the lumbar spine with long-term follow-up after OLT in a large population of 360 consecutive adult patients with end-stage primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), transplanted at a single centre.

Methods

Patients

From March 1985 to January 2001, all adult patients undergoing OLT at the Mayo Clinic Rochester with a diagnosis of either PBC or PSC were assessed clinically, biochemically, radiologically, and by measurements of bone mineral density (BMD) before OLT, at 4 and 12 months after OLT, and annually thereafter. The diagnoses of PBC and PSC were made according to well-established criteria.[15-17] The study was approved by the Institutional Review Board of the Mayo Clinic. All patients were followed till January 2002, death or retransplantation.

Clinical assessment

All patients underwent a full clinical examination at each time of evaluation: before OLT, at 4 and 12 months after OLT and annually thereafter. Liver function status was

assessed by Child-Turcotte-Pugh score and model for end-stage liver desease (MELD) score. Functional status was assessed by the Karnofsky Performance Scale from 0 (completely immobile) to 100 (normal activity).[18] Female patients were judged to be pre- or postmenopausal according to clinical symptoms and biochemical testing. Nutritional status, muscle wasting, complications of liver disease and other illnesses were noted and medications were recorded. The presence or absence of muscle wasting was a global assessment made by the transplant hepatologist. The dietary calcium intake of each patient was estimated, and general dietary instructions were given before OLT, during the early postoperative period, and annually thereafter. Oral calcium supplements (1.5 g/day) were prescribed for all patients. Vitamin D supplementation was given in response to low 25-hydroxyvitamin D levels to normalise serum levels. Inflammatory bowel disease (IBD) was diagnosed by colonoscopy and surveillance biopsies at time of activation for orthotopic liver transplantation and annually thereafter in all PSC patients.

Immunosuppression

From 1985 to 1990, all patients were treated with a standard protocol of cyclosporine A (CyA) and prednisone with or without azathioprine. From 1990 to 1993, some patients were treated with tacrolimus (TAC) and prednisone as part of the multicenter FK506 trial, the control arm of which was standard triple therapy with prednisone, CyA and azathioprine. The TAC arm of this study received only about half the total prednisone dose of the CyA arm; patients were tapered to 5 mg per day of prednisone by 4 months, whereas the CyA patient were tapered to 10 mg per day of prednisone by 6 months. In 1994, standard immunosuppression was changed to tacrolimus, prednisone, and azathioprine, though some patients were treated as part of a multicenter trial with Neoral. In 1999, azathioprine was replaced by mycophenylate mofetil 1g twice daily for the first 2-4 postoperative months. CyA and tacrolimus doses were adjusted according to serum levels, the desired levels depending on time from transplantation, presence of rejection and toxicity. During the whole study period, acute cellular rejection of the allograft was treated with 1 g of intravenous Solu-Medrol on days 1, 3, and 5 after the diagnosis of the rejection. Steroid-resistant rejection was treated with monoclonal therapy with OKT3.

Biochemical indices

Blood was taken for biochemical assessment after an overnight fast and tested by Mayo Medical Laboratories, using standard methods. Biochemical testing included parameters of liver and kidney function (serum albumin, total alkaline phosphatase, total and direct bilirubin, international normalized ratio, serum creatinine), as well as parameters of mineral metabolism (serum 25-hydroxyvitamin D, serum calcium, serum phosphorus, parathyroid hormone [PTH]). Serum 25-hydroxyvitamin D was measured by the method of Kao and Heser.[19] Immunoreactive parathyroid hormone was measured by immunochemiluminescent-metric assay[20] since June 1989. PTH values which were achieved by preceding methods were excluded from this study.

Measurements of bone mineral density at the lumbar spine (BMD-LS)

Bone mineral density measurements at the lumbar spine were taken in all patients in a protocolised fashion; at time of activation for OLT, 4 months after OLT, at 1 year and

yearly thereafter. From March 1985 till April 1988, BMD measurements were performed on lunar machines by dual photon absorptiometry. Since April 1988, dual energy X-ray absorptiometry using Hologic machines, has been used. Phantoms were used to cross-calibrate the two machines, and a formula was established to convert DPA data to dual energy X-ray absorptiometry data. Bone mass was corrected for bone size to calculate BMD in g/cm2. Measurements of BMD-LS had a reproducibility of 2.2%. In patients with compression fractures, measurements were determined on only the intact vertebrae. BMD readings (all calibrated to dual energy X-ray absorptiometry measurements) were expressed as T-scores (standard deviations from peak bone mass of a young, sex-matched reference population) and Z-scores (standard deviations from age- and sex-adjusted reference values). Osteoporosis is defined according to World Health Organization criteria[21]; a T-score of higher than -1.0 is considered normal, a T-score between –1 and –2.4 is osteopenia, and a T-score of –2.5 and less is osteoporosis.

Statistical analysis

Following liver transplantation, patients were followed till death or retransplantation. To study bone loss and bone gain, BMD values of all patients were synchronized at their OLT date (time = 0), taking the overall mean as the baseline BMD value. Posttransplant BMD values were derived by taking from every patient the change from baseline and plotting that from OLT. This method was used to limit bias caused by a changing population with time. The posttransplant BMD points were plotted and fitted using least squares regression with time as the predictor of BMD and using a restricted cubic spline with knots at 4 months and 3 years (natural spline function in Splus [Mathsoft, Seattle, WA]).

Parameters are reported as means ± standard deviation. Associations between patient characteristics and quantitative endpoints such as BMD-LS or qualitative endpoints such as osteoporosis were tested for significance using the Student t test for comparison of means or the chi-square test for comparison of proportions. Changes over time were assessed by the analysis of variance method. The Pearson correlation coefficient was used to assess the linear association between quantitative patient characteristics and BMD. The patient characteristics that were univariately significant formed a pool of potential predictors of BMD. The backwards elimination variable selection procedure was used to find the independent variables that predicted these endpoints. Such variables with a *P* value less than 0.05 were included in the final multivariate regression model to predict low BMD. All analyses were performed using the SAS data analysis system (SAS Institute, Cary, NC).[22]

Results

Demographics of total patient population

Three hundred-sixty adult patients underwent OLT for PBC (156 patients) or PSC (204 patients) and were followed after OLT; 142 were males and 218 females, of whom 148 were postmenopausal (Table 1). The PBC patients were predominantly females (135 of 156 patients), whereas the PSC patients were predominantly males (121 of 204 patients). The mean age was 49.5 ± 10.5 years with PSC patients being younger than PBC patients ($p < 0.01$), and males being younger than females ($p < 0.01$). The mean Child-Turcotte-Pugh score of 8.7 ± 1.8 and MELD score of 17.3 ± 8.8 indicated advanced cholestatic liver disease. Most patients (86.1%) had near-normal activity with minor symptoms and assistance at time of OLT (Karnofsky score, 50 - 100); 26 (7.7%) patients required considerable assistance (score, 30 - 40), and 21 (6.2%) patients were severely disabled or hospitalized (score, 0 - 20). A total of 8 (3.2%) patients smoked at time of activation for OLT and 19 (7.6%) patients had been active drinkers. There were no differences between subpopulations (PBC/PSC, and females/males) concerning Karnofsky scoring or drinking/smoking habits.

Table 1. Demographic data of patients with end-stage PBC and PSC before OLT.

	Total	PBC	PSC	Female	Male
No. of patients	360	156	204	218	142
Age at OLT (yrs)	49.5 ± 10.5	53.2 ± 8.6[B]	46.8 ± 11.0[B]	50.8 ± 9.6[B]	47.6 ± 11.6[B]
Child-Turcotte-Pugh score	8.7 ± 1.8	8.7 ± 1.7	8.7 ± 1.9	8.6 ± 1.8	8.8 ± 1.8
MELD score	17.3 ± 8.8	17.6 ± 8.8	17.0 ± 8.7	16.8 ± 8.9	17.9 ± 8.6
Time since diagnosis (yrs)[#]	7.4 ± 5.4	7.9 ± 5.3	7.1 ± 5.3	7.4 ± 5.3	7.4 ± 5.4
BMI (kg/m^2)	24.4 ± 4.6	24.5 ± 4.8	24.4 ± 4.5	24.2 ± 5.1	24.8 ± 3.9
Poor nutritional status (n,%)	28 (11.3%)	12 (12.4%)	16 (10.6%)	16 (11.3%)	12 (11.3%)
Muscle wasting (n,%)[##]	202 (81.1%)	81 (81.8%)	121 (80.7%)	108 (75.5%)[B]	94 (88.7%)[B]
N pts with BMD	342	151	191	206	136
BMD (g/cm^2)	0.86 ± 0.16	0.83 ± 0.17[C]	0.89 ± 0.16[C]	0.84 ± 0.16[D]	0.90 ± 0.16[D]
Z-score	-1.4 ± 1.4	-1.4 ± 1.4	-1.4 ± 1.4	-1.3 ± 1.4	-1.6 ± 1.4
T-scores	-2.0 ± 1.4	-2.2 ± 1.5	-1.9 ± 1.4	-2.1 ± 1.4	-1.9 ± 1.4
Osteopenia, n (%)	131 (39.3%)	50 (33.1%)	81 (42.4%)	73 (35.4%)	58 (42.6%)
Osteoporosis, n (%)	129 (37.7%)	66 (43.7%)[A]	62 (32.5%)[A]	83 (40.3%)	45 (33.1%)
Fractures, n (%)	66 (18.6%)	34 (22.4%)	32 (15.7%)	45 (20.1%)	21 (15.2%)

Differences between PBC versus PSC, females versus males [A]$p < 0.05$, [B]$p < 0.01$, [C]$p < 0.001$, [D]$p < 0.0001$

[#] Years since first diagnosis until OLT.

[##] Poor nutritional status, muscle wasting, Karnofsky scoring, smoking and drinking history was recorded in 248 of the total patients.

NOTE: Values of BMD, T and Z-scores are expressed as means ± SE; the remaining variables are expressed as mean ± SD.

The majority of patients (81.1%) had muscle wasting at time of OLT, males more often than females ($p < 0.01$). IBD was seen in 139 of 204 PSC patients (66.7%), with 95.6% having chronic ulcerative colitis; 46 patients were on glucocorticoid treatment within 5 years before OLT with a mean duration of treatment of 5.0 ± 5.6 years. At time of activation for OLT, 64 patients were on cholestyramine, 111 were on ursodeoxycholic acid, 5 were on anticonvulsants, 45 were on thyroid hormone replacement therapy (HRT), 18 females were on hormone replacement therapy (HRT), and 4 patients used bisphosphonates.

To determine any temporal changes, the 16-year study period (1985 - 2000) was divided into three transplantation periods: 1985 -1989 (n = 93), 1990 - 1995 (n = 153) and 1996 - 2001 (n = 115) (Table 2). The percentage of patients with PBC and PSC and the gender distribution remained the same over these three periods, but PBC patients became older and more postmenopausal with time. Age and menopausal status did not change in the PSC population. Increases in mean body mass index (BMI) and decreases in muscle wasting were seen over time, but no temporal differences were seen in Child-Turcotte-Pugh, MELD, or Karnofsky scores.

Assessment of baseline BMD-LS and biochemical variables

At time of activation for OLT, 38% of patients had osteoporosis, 39% osteopenia, and only 23% of patients had normal bone mass (Table 1). PBC patients had a higher prevalence of osteoporosis and lower BMD than PSC patients ($p < 0.001$) and, although female patients had lower BMD values that male patients ($p < 0.0001$), no significant differences concerning T- and Z-scores were observed between the subpopulations. The T-scores of the 64 cholestyramine-treated patients did not differ significantly from T-scores in the untreated patients (T-scores –2.1 ± 1.4 versus –2.0 ± 1.4), nor did the T-scores of the 46 glucocorticoid-treated patients differ from untreated patients (T scores –2.2 ± 1.6 versus –2.0 ± 1.4). In addition, PSC patients with and without IBD before OLT had similar T-scores at baseline (T-scores –2.0 ± 1.2 versus –1.8 ± 1.4). In the early period from 1985 - 1989, 57% patients had pretransplant osteoporosis with a mean T-score of –2.5 ± 1.6 and Z-score of –2.0 ± 1.6; in the latest period from 1996 - 2000, 26% had osteoporosis with a mean T-score of –1.7 ± 1.2 and Z-score of –1.0 ± 1.2. The increase in pretransplant bone mass with time was seen in the female, male and PSC subpopulations, but bone mass remained stable in the PBC patients (Table 2).

Biochemical indices at time of OLT reflected end-stage cholestatic liver disease with low albumin (3.0 ± 0.5 g/dL), high total and direct bilirubin (11.2 ± 11.0 mg/dL, 7.0 ± 7.1 mg/dL, respectively), and high alkaline phosphatase (1107.8 ± 855.1 U/L). Levels of serum calcium (8.8 ± 0.6 mg/dL), 25-hydroxyvitamin D (20.2 ± 17.9 ng/mL), phosphorus (3.5 ± 0.8 mg/dL), creatinine (1.2 ± 0.9 mg/dL) and PTH (2.3 ± 2.0 pmol/L) were in the normal range. Male patients had higher alkaline phosphatase levels than female patients (1177.5 ± 934.4 vs 1000.7 ± 706.5, respectively; $p < 0.01$), no other differences were observed between subpopulations (PBC/PSC, females/males). Increases in vitamin D levels and decreases in serum bilirubin and alkaline phosphatase levels were seen with time over the study period (Table 2).

Table 2. Temporal changes in clinical, biochemical and bone density parameters before OLT

Variables	Transplantation periods			
	1985-1989 (n=93)	1990-1995 (n=152)	1996-2001 (n=115)	p-values[A]
Clinical parameters[B]				
PSC / PBC (n, %PBC)	45 / 48[C] 52%)	95 / 57 (38%)	64 / 51 (44%)	NS
M / F (n, %F)	31 / 62 (67%)	62 / 90 (59%)	49 / 66 (57%)	NS
Age at OLT (yrs)– Total	45.9 ± 9.1	50.3 ± 9.9	51.4 ± 11.7	< 0.001
- PBC	48.5 ± 6.8	54.4 ± 8.4	56.2 ± 8.6	< 0.0001
- PSC	43.2 ± 10.5	47.9 ± 9.9	47.6 ± 12.4	NS
Child-Turcotte-Pugh score	8.8 ± 1.7	8.9 ± 1.9	8.5 ± 1.8	NS
MELD score	17.9 ± 10.5	17.5 ± 9.3	16.5 ± 6.5	NS
Duration of disease (yrs)	6.3 ± 4.6	7.8 ± 5.4	7.9 ± 5.9	< 0.05
BMI (kg/m^2)	23.6 ± 4.3	24.1 ± 4.3	25.6 ± 5.1	< 0.01
Muscle wasting (n,%)	-	130 (88%)	72 (71%)	< 0.001
Poor nutritional status (n,%)	-	24 (16%)	4 (4%)	< 0.01
Glucocorticoid use (n,%)	19 (20%)	20 (14%)	7 (6%)	< 0.01
Laboratory parameters[B]				
Albumin (3.5 - 5.0 g/dL)	2.9 ± 0.5	3.1 ± 0.5	3.0 ± 0.6	NS
Alkaline phosphatase (U/L)[D]	1311.4 ± 871.6	1181.9 ± 935.2	845.0 ± 648.4	< 0.0001
Total bilirubin (0.1 - 1.1 mg/dL)	12.4 ± 10.3	11.5 ± 12.1	9.9 ± 10.0	NS
Direct bilirubin (0.0 - 0.3 mg/dL)	8.2 ± 6.4	7.1 ± 7.7	5.8 ± 6.6	< 0.05
Creatinine (mg/dL)[E]	1.2 ± 1.0	1.2 ± 1.0	1.1 ± 0.6	NS
Calcium (8.9 – 10.1 mg/dL)	8.8 ± 0.5	8.7 ± 0.7	8.9 ± 0.6	NS
PTH (1.0 – 5.2 pmol/L)	1.4 ± 1.4	2.4 ± 2.2	2.2 ± 1.7	NS
25(OH)D (ng/mL)[F]	17.0 ± 13.9	18.0 ± 12.9	29.6 ± 26.9	< 0.0001
Bone disease parameters				
Osteoporosis (N, %)	52 (57%)	49 (34%)	27 (26%)	< 0.0001
Osteopenia (N, %)	24 (26%)	60 (41%)	47 (44%)	< 0.05
Normal BMD (N, %)	15 (17%)	36 (25%)	31 (30%)	< 0.05
Total T-scores[G]	-2.5 ± 1.6	-2.0 ± 1.4	-1.7 ± 1.2	< 0.0001
Total Z-scores	-2.0 ± 1.6	-1.3 ± 1.4	-1.0 ± 1.2	< 0.0001
PBC T-scores	-2.2 ± 1.7	-2.2 ± 1.5	-2.1 ± 1.2	NS
PBC Z-scores	-1.6 ± 1.6	-1.2 ± 1.4	-1.2 ± 1.1	NS
PSC T-scores	-2.8 ± 1.4	-1.8 ± 1.4	-1.3 ± 1.1	< 0.0001
PSC Z-scores	-2.5 ± 1.4	-1.4 ± 1.3	-0.8 ± 1.2	< 0.0001

Abbreviations: NS, not significant; M, male; F, female; 25(OH)D, 25-hydroxyvitamin D. [A] p-values indicate statistical changes with time. [B] Changes with time of the clinical and laboratory variables were similar in PBC and PSC patients, except age and menopausal status at OLT. [C] Of females with PBC, % postmenopausal increased over the 3 eras (65%, 78%, 85%,respectively; $p < 0.05$). [D] total alkaline phosphatase = M > 19 yr: 98 - 251 U/L; F 24 - 45 yr: 81 - 231 U/L; F 46 - 60 yr: 84 - 257 U/L; F > 60 yr: 108 - 309 U/L. E serum creatinine = F: 0.6 - 0.9 mg/dL, M: 0.8 - 1.2 mg/dL, [F] 25(OH)vitamin D = summer: 15 - 80 ng/mL, winter: 14 - 42 ng/mL. [G] Mean ± SE

Clinical characteristics after OLT

After OLT, patients spent, on average, 25.6 ± 21.0 days in the hospital during the first 4 months. Fifty-one (14.2%) patients were retransplanted after OLT at a mean of 1.3 ± 2.6 years; 78 patients (21.7%) patients died after transplantation at a mean of 5.1 ± 4.3 years; 160 patients (44.4%) had rejection episodes after OLT; and 43 patients (11.9%) sustained nonanastomotic biliary strictures. The average daily dose of prednisone during the first month was 140.0 ± 49.3 mg; this was less in patients treated with TAC compared to those receiving CyA ($p < 0.01$; 124.8 ± 42.0 vs 145.8 ± 51.5 mg/dL, respectively). Similarly, the average daily prednisone dose from month 1 to month 4 was higher in patients treated with CyA ($p < 0.01$; 54.4 ± 17.9 mg) compared to those treated with TAC (39.6 ± 13.4 mg). Mean CyA serum levels were 242.2 ± 89.0 ng/ml (215 patients) and 222.5 ± 67.6 ng/dl (201 patients) at 1 and 4 months, respectively; mean TAC levels were 9.4 ± 3.6 ng/dL (119 patients) and 9.0 ± 2.8 ng/dL (117 patients) at 1 and 4 months. There are no differences in immunosuppression between males and females or between PBC or PSC. Temporal changes in immunosuppression were seen with the more recent transplanted patients having less rejection episodes, less hospitalisation days, less treatment with CyA, more treatment with TAC, lower cumulative prednisone doses and lower CyA serum levels when compared to the earlier 2 transplantation periods (Table 3).

Hormone replacement therapy (HRT) was used in 57 of 148 postmenopausal females after OLT, started at 939.4 ± 587.3 days after OLT; only 27 started during the first 2 years after OLT. Sixteen patients received bisphosphonates after OLT: etidronate in 1 patient (in first post-OLT year), pamidronate in 2 patients (in first and second year after OLT), and alendronate in 13 patients (all started after the first 2 posttransplant years). Sixty-three patients were enrolled in a randomised trial of calcitonin therapy or no treatment after OLT; this trial showed no effect of calcitonin therapy on bone mass after OLT.[23]

Biochemical characteristics after OLT

After OLT, liver function improved ($p < 0.001$) and at 4 months, levels of total bilirubin (2.1 ± 5.2 mg/dL) and alkaline phosphatase (299.9 ± 499.0 U/L) had decreased and serum albumin (3.7 ± 0.6 g/dL) increased. Albumin further increased by 1 year posttransplant to 4.0 ± 0.4 g/dL after which it remained stable. Alkaline phosphatase showed a small but significant increase from 4 to 8 year after OLT, leading to alkaline phosphatase levels of 207.1 ± 291.1 U/L at year 8. Kidney function deteriorated with increased serum creatinine at 4 months (1.3 ± 0.6 mg/dL, $p < 0.0001$) and with a further increase by year 4 posttransplant (1.4 ± 0.5 mg/dL, $p < 0.05$), after which creatinine remained stable. Serum calcium (9.1 ± 0.8 mg/dL), 25-hydroxyvitamin D (31.6 ± 13.8 ng/mL), phosphorus (3.8 ± 0.7 mg/dL), and PTH (4.3 ± 4.5 pmol/L) all increased after OLT ($p < 0.05$). PTH further increased to 4.8 ± 3.4 pmol/L at 1 year posttransplant, whereas vitamin D continued to increase during the first 4 posttransplant years to 40.9 ± 14.9 ng/mL ($p< 0.001$). Phosphorus decreased to 3.5 ± 0.6 mg/dL ($p < 0.05$) at year 4 posttransplant, after which it remained stable. Recently transplanted patients had higher albumin and PTH levels and lower creatinine and phosphorus levels compared to earlier patients (Table 3).

Table 3. Temporal differences in clinical, immunosuppressive and biochemical parameters after OLT

	Transplantation periods			
	1985-1989 (n=93)	1990-1995 (n=152)	1996-2001 (n=115)	P-values[#]
PostOLT characteristics				
Hospitalization days[A]	25.1 ± 20.5	25.7 ± 18.4	17.4 ± 15.2	< 0.001
Rejection episodes	47 (50.5%)	75 (50%)	37 (32.2%)	< 0.01
Stricture formation	12 (12.9%)	28 (18.4%)	3 (2.6%)	< 0.01
Pts with CyA treatment (%)	86 (100%)	97 (77%)	15 (15%)	< 0.001
Immunosuppression				
Prednisone first mo (daily dose, mg)	141.5 ± 55.8	150.1 ± 48.1	124.8 ± 41.4	< 0.001
Cyclosporine first mo (ng/mL)	213.4 ± 77.9	270.1 ± 88.2	204.5 ± 89.8	< 0.0001
Tacrolimus first mo (ng/mL)	**	9.3 ± 6.0	9.5 ± 2.0	NS
Prednisone mo 1 - 4 (daily dose, mg)	55.0 ± 18.6	53.9 ± 18.6	39.5 ± 13.5	< 0.0001
Cyclosporine mo 1 - 4 (ng/mL)	198.6 ± 68.5	248.8 ± 57.2	190.3 ± 63.1	< 0.0001
Tacrolimus mo 1 - 4 (ng/mL)	**	8.4 ± 4.1	9.2 ± 2.0	NS
Laboratory values[##]				
Albumin (3.5-5.0 g/dL) – 4 mo	3.6 ± 0.6	3.7 ± 0.6	3.9 ± 0.5	< 0.05
Creatinine (mg/dL) – 4 mo	1.2 ± 0.7	1.4 ± 0.6	1.1 ± 0.3	< 0.01
Creatinine (mg/dL) – 1 yr	1.3 ± 0.4	1.4 ± 0.6	1.2 ± 0.3	< 0.001
Creatinine (mg/dL) – 2 yr	1.4 ± 0.4	1.4 ± 0.5	1.2 ± 0.3	< 0.01
PTH (1.0 – 5.2 pmol/L) – 4 mo	**	3.8 ± 3.1	5.5 ± 6.0	< 0.001
PTH (1.0 – 5.2 pmol/L) – 1 yr	**	4.3 ± 3.3	5.7 ± 3.5	< 0.01
Phosphorus (2.5-3.5 mg/dL) – 1 yr	3.9 ± 0.6	3.9 ± 0.7	3.6 ± 0.6	< 0.001
Phosphorus (2.5-3.5 mg/dL) – 2 yr	3.6 ± 0.5	3.5 ± 0.6	3.2 ± 0.6	< 0.0001

Abbreviation: NS, not significant

[#] P-values indicate statistical changes with time.

[A] Hospitalization days during first 4 months posttransplant.

* Values are listed as average daily doses of prednisone, average daily serum levels of FK and CyA

[##]Laboratory differences between the 3 transplantation periods were assessed at 4 mo, 1 y and 2 y posttransplant. There were no significant differences concerning total and direct bilirubin, alkaline phosphatase, calcium and vitamin D at all time points.

** No patients were treated with TAC from 1985-1990. PTH was measured by an earlier, less reliable assay in this era.

Table 4. Bone mineral density of the lumbar spine in 360 liver transplant recipients over 8 years

	PreOLT	4 mo post	1 yr post	4 yr post	8 yr post
BMD total patients					
N pts with BMD	342	276	262	157	74
Osteopenia, n (%)	131 (39%)	102 (37%)	102 (39%)	72 (46%)	38 (51%)
Osteoporosis n (%)	128 (38%)	141 (51%)	113 (44%)	44 (28%)	22 (30%)
T-scores	-2.01 ± 0.08	-2.43 ± 0.08	-2.15 ± 0.09	-1.79 ± 0.15	-1.81 ± 0.37
Z-scores	-1.39 ± 0.08	-1.77 ± 0.08	-1.46 ± 0.08	-0.97 ± 0.14	-0.81 ± 0.36
BMD by disease					
N pts PBC / PSC	151/191	123/153	115/147	68/89	29/45
PBC T-scores	-2.18 ± 0.08	-2.51 ± 0.08	-2.21 ± 0.09	-1.76 ± 0.13	-1.88 ± 0.74
PBC Z-scores	-1.34 ± 0.12	-1.63 ± 0.12	-1.30 ± 0.12	-0.67 ± 0.21	-0.58 ± 0.70
PSC T-scores	-1.88 ± 0.10	-2.37 ± 0.10	-2.10 ± 0.12	-1.80 ± 0.19	-1.75 ± 0.40
PSC Z-scores	-1.43 ± 0.10	-1.89 ± 0.10	-1.59 ± 0.11	-1.19 ± 0.18	-0.96 ± 0.39
BMD by gender					
N pts F/M	206/136	168/108	162/100	99/58	49/25
Female T-scores	-2.10 ± 0.10	-2.50 ± 0.10	-2.22 ± 0.11	-1.80 ± 0.18	-1.75 ± 0.41
Female Z-scores	-1.27 ± 0.10	-1.63 ± 0.10	-1.33 ± 0.10	-0.74 ± 0.17	-0.46 ± 0.39
Male T-scores	-1.89 ± 0.12	-2.33 ± 0.12	-2.03 ± 0.14	-1.78 ± 0.25	-1.99 ± 0.75
Male Z-scores	-1.56 ± 0.13	-1.99 ± 0.13	-1.67 ± 0.14	-1.34 ± 0.23	-1.46 ± 0.75
BMD by baseline BMD*					
N pts low/average/high	84/173/85	72/139/65	64/135/63	38/86/33	21/43/10
Low T-scores	-3.81 ± 0.08	-4.12 ± 0.08	-3.76 ± 0.17	-3.29 ± 0.17	-3.16 ± 0.23
Low Z-scores	-2.95 ± 0.11	-3.14 ± 0.10	-2.80 ± 0.11	-2.14 ± 0.19	-1.93 ± 0.40
Average T-scores	-2.00 ± 0.05	-2.40 ± 0.06	-2.13 ± 0.08	-1.74 ± 0.10	-1.87 ± 0.18
Average Z-scores	-1.36 ± 0.07	-1.73 ± 0.08	-1.44 ± 0.09	-0.91 ± 0.12	-0.85 ± 0.25
High T-scores	-0.24 ± 0.09	-0.80 ± 0.10	-0.55 ± 0.12	-0.21± 0.25	-0.80 ± 1.38
High Z-scores	-0.13 ± 0.09	-0.43 ± 0.12	-0.15 ± 0.12	0.24 ± 0.26	-0.17 ± 1.27
BMD by OLT period					
N pts 1985-89/ 1990-95/ 1996-2001	91/145/106	72/125/79	72/117/73	47/82/28	34/40/0
T-scores, 1985-1989	-2.51 ± 0.18	-2.87 ± 0.19	-2.68 ± 0.20	-2.30 ± 0.28	-2.28 ± 0.39
Z-scores, 1985-1989	-2.00 ± 0.17	-2.35 ± 0.18	-2.14 ± 0.19	-1.61 ± 0.27	-1.42 ± 0.38
T-scores, 1990- 1995	-2.00 ± 0.12	-2.41 ± 0.12	-2.19 ± 0.12	-1.76 ± 0.17	-1.79 ± 0.40
Z-scores, 1990-1995	-1.31 ± 0.11	-1.72 ± 0.11	- 1.46 ± 0.11	-0.90 ± 0.16	-0.74 ± 0.39
T-scores, 1996-2001	-1.69 ± 0.12	-2.12 ± 0.13	-1.65 ± 0.14	-1.36 ± 0.43	**
Z-scores, 1996-2001	-1.00 ± 0.11	-1.40 ±0.12	-0.90 ± 0.14	-0.53 ± 0.40	**

N = number of patients; % percentage of patients T- and Z-scores are recorded as mean ± SE.* Baseline low BMD < 0.75 g/cm^2, baseline average 0.75 – 0.96 g/cm^2, baseline high > 0.96 g/cm^2

** Patients in this era have not yet been followed for 8 years.

Follow-up of posttransplant BMD-LS after OLT

T- and Z-scores at the lumbar spine fell at 4 months after OLT (Table 4) (Fig. 1), with a high incidence of osteoporosis (51%) in the total patient population. Thereafter, BMD increased up to 4 years after OLT and remained above pretransplant levels in the total patient population. Z-scores continued to improve from 4 to 8 years, whereas T-scores remained stable (Fig. 1). The same pattern of changes in posttransplant BMD, with bone loss followed by bone gain, was seen when comparing PBC and PSC patients (Fig. 2) and male patients and pre- and postmenopausal female patients (Fig. 3). However, although all groups of patients lost bone mass early after OLT, recovery of bone mass differed depending on the initial severity of osteopenia or osteoporosis. Patients with the lowest baseline BMD experienced the greatest gain in bone mass after OLT, with BMD exceeding baseline levels by 1 - 2 years after OLT; on the other hand, patients with the highest baseline BMD failed to recover to baseline BMD values at any time after OLT (Fig. 4). The rates (adjusted to annual rates) of early bone loss and bone gain were analyzed in the total patient population and in the subpopulations using changes in BMD from baseline. A high rate of bone loss in the first 4 months was seen (15.9 ± 18.9 %/yr), after which bone mass started to increase from 4 to 12 months at an annual rate of 6.4 ± 14.3% and from 12 to 24 months at an annual rate of 6.7 ± 20.3%. Three hundred seventeen patients (82.2%) lost bone mass during the first 4 months after OLT, of which 100 patients (41.3%) lost 0 - 5%, 91 patients (37.6%) lost 10 - 15% and the remaining 8 patients (2.5%) lost 15 - 30% of baseline BMD. Eleven patients (4.6%) had stable BMD after OLT, and 32 (13.2%) patients gained 1 - 5% bone mass during the first 4 months. The rate of bone loss in the first 4 postoperative months was significantly more in patients with PSC compared to those with PBC (18.0 ± 20.0 %/yr versus 13.3 ± 17.8 %/yr, respectively). No other significant differences in rates of bone loss (0 - 4 months) and bone gain (4 - 24 months) were seen between PBC and PSC subpopulations, or between females and males. Postmenopausal women treated with HRT, started on average at 3 years posttransplant, have better T-scores from 4 to 8 years after OLT than postmenopausal women without HRT (mean T-scores at 2, 4 and 8 years after OLT in patients with HRT are -2.15 ± 0.17, -1.88 ± 0.22 and -1.43 ± 0.48, respectively compared to those without HRT: -2.28 ± 0.22, -2.10 ± 0.37 and -2.55 ± 0.88, respectively).

After OLT, the rate of early posttransplant bone loss during the first 4 months after OLT was not significantly different in the three transplantation periods (Fig. 5). Bone gain during the first 2 years, however, was greater in the more recently transplanted patients with 11.2% ± 19.3 gain in patients transplanted during 1996 - 2000 compared to 4.4% ± 13.8 during the 1985 - 1989 period and 3.8% ± 10.2 during 1990 - 1995.

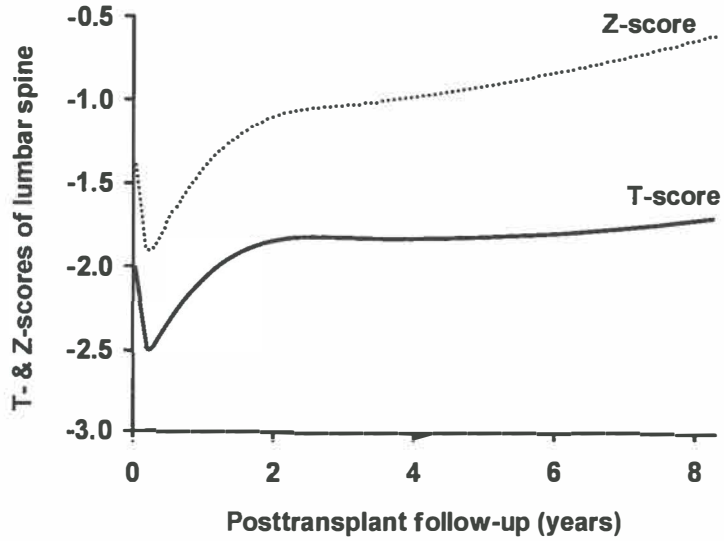

Figure 1. BMD-LS (mean T- and Z-scores) of 360 patients, before and for 8 years after OLT for PBC and PSC.

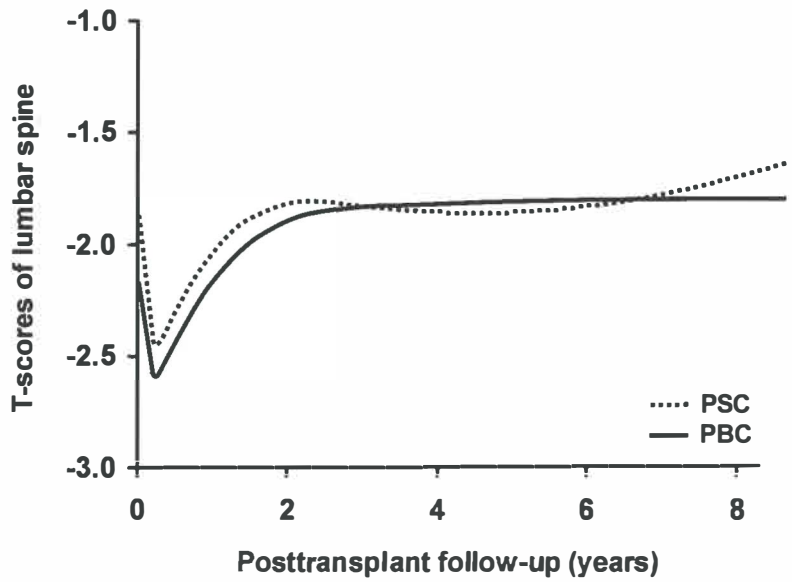

Figure 2. T-scores of BMD-LS before and for 8 years after OLT of 154 patients with PBC and 206 patients with PSC.

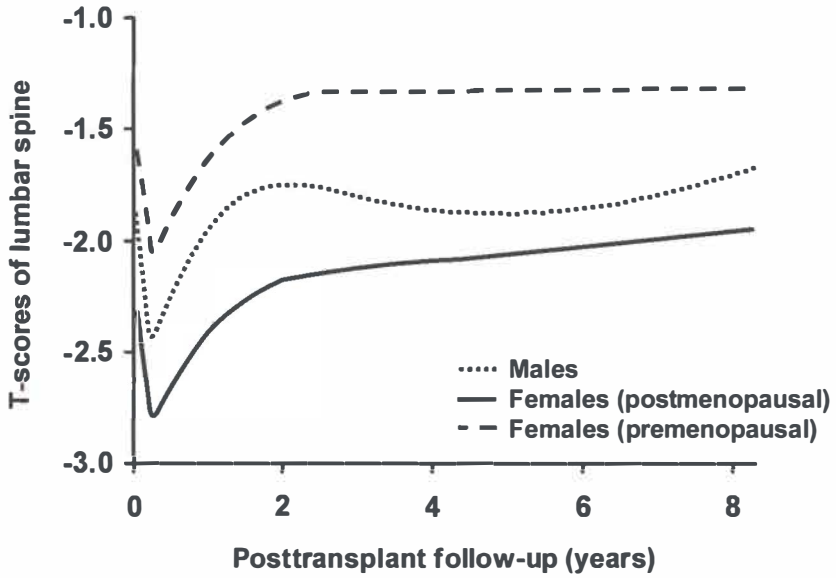

Figure 3. T-scores of BMD-LS before and for 8 years after OLT of 142 male and 218 female patients with chronic cholestatic liver disease.

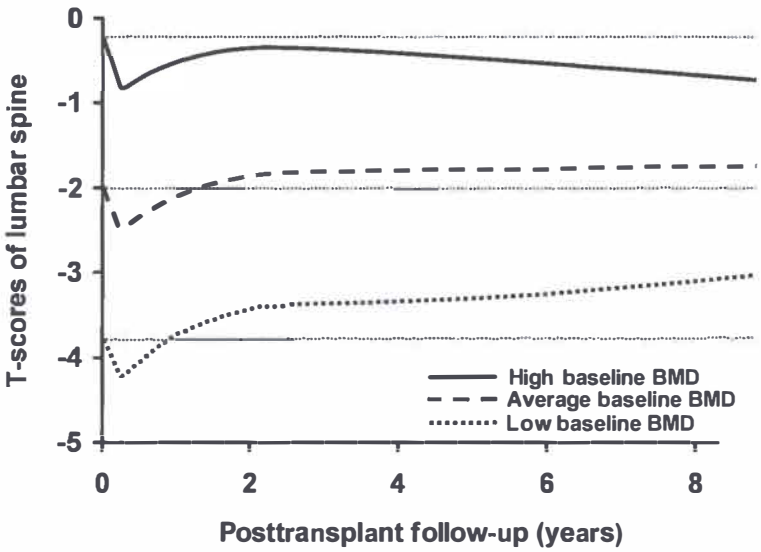

Figure 4. T-scores of BMD-LS before and for 8 years after OLT of patients starting with low (< 0.75 g/cm^2), average (0.75 < BMD < 0.96 g/cm^2) and high (> 0.96 g/cm^2) BMD levels before transplantation.

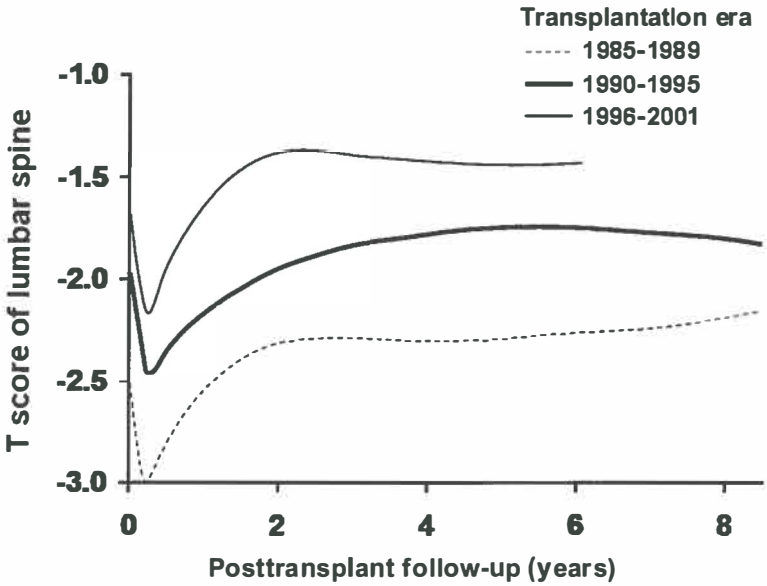

Figure 5. T-scores of BMD-LS before and for 8 postoperative years in patients undergoing OLT in 3 eras; 1985 - 1989, 1990 - 1995, 1996 - 2001.

Predictors for pretransplant bone density

Univariate analysis (Table 5) showed that pretransplant T-scores of lumbar spine correlated positively with male gender, BMI, and albumin and negatively with age, PBC disease, duration of disease (y), OLT number, postmenopausal status, muscle wasting, and alkaline phosphatase. No correlations were seen with Child-Turcotte-Pugh score, MELD score, Karnofsky scoring, smoking and alcohol use, IBD status in PSC patients, and other biochemical indices, including calcium and vitamin D levels. Multivariate analysis indicated that independent risk factors for low pretransplant BMD were decreased BMI, female gender, an older age, increased alkaline phosphatase, decreased albumin and higher OLT number.

Risk factors for posttransplant bone loss

Several pretransplant factors (Table 5) correlated with posttransplant loss at the lumbar spine bone during the first 4 months; more bone loss at 4 months was seen with PSC as the underlying liver disease, younger age at time of OLT, no presence of IBD disease before OLT, smoking at time of OLT, higher pretransplant BMD, and shorter duration of disease before OLT. The posttransplant factor that correlated positively with bone loss was an increased serum direct bilirubin level at 4 months; a trend was seen with total bilirubin and posttransplant non-anastomotic biliary strictures. Bone loss was not affected by gender, postmenopausal status, pretransplant Child-Turcotte-Pugh and MELD scores, pretransplant nutritional status, BMI and muscle wasting, Karnofsky score, alcohol intake or any pretransplant biochemical index other than bilirubin. In addition, no correlations were seen with posttransplant rejection, hospitalization days,

Table 5. Univariate correlations for pre- and posttransplant BMD in patients with end-stage cholestatic liver disease

Variables	Regression coefficient	p-value
Baseline BMD		
Age (yrs#)*	-0.029 ± 0.008	0.0005
PBC	-0.059 ± 0.025	0.03
Male gender*	0.068 ± 0.018	0.0001
Postmenopausal status	-0.083 ± 0.024	0.0006
OLT number	0.0095 ± 0.0028	0.0007
Body mass index (kg/m^2)*	0.091 ± 0.0019	< 0.0001
Muscle wasting	-0.0559 ± 0.025	0.03
Duration disease (yrs#)	-0.029 ± 0.017	0.086
Albumin (mg/dL)*	0.037 ± 0.017	0.03
Alkaline phosphatase (U/L#)*	-0.0026 ± 0.0010	0.012
Posttransplant bone loss (0 to 4 mo)		
Age (yrs#)	0.0065 ± 0.0028	0.02
PBC*	0.016 ± 0.006	0.004
Duration disease (yrs#)*	0.01 ± 0.005	0.046
Current smoking	-0.035 ± 0.017	0.045
Inflammatory bowel disease	0.031 ± 0.013	0.014
Baseline BMD (g/cm^2)	-0.073 ± 0.017	< 0.0001
Biliary strictures, non-anastomotic	-0.016 ± 0.0083	0.06
Direct bilirubin, 4 months (mg/dL)*	-0.0024 ± 0.0011	0.04
Total bilirubin, 4 months (mg/dL)*	-0.0013 ± 00058	0.07
Cyclosporine level, 4 months (mg/dL#)	-0.009 ± 0.005	0.07
Posttransplant bone gain (4 to 24 mo)		
Postmenopausal status	-0.034 ± 0.013	0.013
OLT number	0.0055 ± 0.0017	< 0.0001
Baseline BMD (g/cm^2)	-0.075 ± 0.030	0.014
BMD, 4 months (g/cm^2)	-0.011 ± 0.030	0.0006
Glucocorticoid doses 4 months (mg)	-0.0017 ± 0.001	0.09
Biliary strictures, non-anastomotic*	-0.026 ± 0.015	0.07
Direct Bilirubin 1 –2 yr (mg/dL)	-0.017 ± 0.006	0.006
Total bilirubin 1 –2 yr (mg/dL)	-0.017 ± 0.001	0.02
Alkaline phosphatase 2 yr (U/L)	-0.068 ± 0.0029	0.02
Vitamin D 1 – 2 yr (ng/mL)	0.00067 ± 0.00028	0.01
PTH 4 mo – 1 yr (pmol/L)*	0.005 ± 0.0017	0.0003
Phosphorus 2 yr (mg/dL)	-0.019 ± 0.009	0.03
Creatinine 4 mo – 1 yr (mg/dL)	-0.020 ± 0.0009	0.005

*Independent predictors for baseline BMD (model r-square: 17%), posttransplant bone loss during the first 4 months (model r-square 6%), posttransplant bone gain from 4 - 24 months (r-square 5%)

Age and duration of disease are reported in decades. Alkaline phosphatase, cyclosporine levels, and OLT numbers are reported as hundreds of units.

NOTE. No correlations before or after OLT were seen with Child-Turcotte-Pugh and MELD-score, pretransplant nutritional status, Kamofsky scoring or alcohol use. In addition, no correlations were seen with most pretransplant labs, including calcium and vitamin D markers. Following OLT, bone loss and bone gain rates did not correlate with posttransplant rejection, hospitalization days, and serum levels of cyclosporine and tacrolimus.

glucocorticoid doses or serum levels of TAC, although a trend was seen with 4-month serum CyA levels. Multivariate analysis of the pretransplant and posttransplant risk factors indicated that independent risk factors for posttransplant bone loss were PSC disease, shorter duration of disease and higher posttransplant serum direct bilirubin.

Predictive factors for posttransplant bone gain

Univariate analysis indicated that bone gain at the lumbar spine during the first 2 posttransplant years (Table 5) was increased in those who were premenopausal at time of OLT, and in those with low pretransplant BMD, low 4-month BMD and higher OLT number. Bone gain was increased with higher posttransplant levels of vitamin D and PTH. Less bone gain occurred in the presence of increased posttransplant levels of creatinine, bilirubin, alkaline phosphatase and phosphorus, by the development of non-anastomotic biliary strictures and by higher average daily doses of glucocorticoids (trend). Bone gain after OLT was not affected by posttransplant rejection, longer hospitalization stay or serum levels of calcineurin inhibitors. Multivariate analysis of the pre- and posttransplant factors indicated that the only independent predictive factor for posttransplant bone gain was OLT number.

Discussion

Osteoporosis and its milder form, osteopenia, are important complications of advanced chronic liver disease and are found with a high incidence in patients awaiting liver transplantation, especially for chronic cholestatic liver disease.[1-4] Despite its frequency, hepatic bone disease is generally overshadowed by the more urgent complications of chronic liver disease and may remain unrecognised unless the diagnosis is specifically sought. Following OLT however, this situation changes, as early aggressive bone loss occurs in almost all patients.[5-10] In patients who are already osteoporotic or osteopenic, this further bone loss results in an increase in fracture rates.[7-9] Studies have indicated that this early period of bone loss is followed by recovery of bone metabolism[11-14] and a subsequent gain of BMD, but long-term follow-up data are limited. Although immunosuppression is assumed to play a role in posttransplant bone loss, risk factors for bone loss and bone gain after OLT have not been well established, and larger studies assessing bone disease before and after OLT are lacking. Whether temporal changes have led to a reduction in pretransplant and posttransplant bone disease is unclear. We therefore studied prospectively a large cholestatic population before and after OLT with long-term follow-up after OLT, to assess predictive factors for low bone mass before OLT and for posttransplant bone loss and subsequent bone gain.

Previous studies have shown a correlation between bone disease in PBC and PSC and advanced histologic disease,[4,24,25] and this present study confirms the high prevalence of osteoporosis and osteopenia in patients with advanced PBC and PSC. Gender did not influence the degree of osteopenia or osteoporosis seen in our patient population. Just as females in general have lower BMD than males, cholestatic females had lower BMD than cholestatic males but when values were adjusted in age and sex, similar Z-scores were found in PBC and PSC patients, supportive of a common etiologic role in these two cholestatic diseases. The important etiologic role of cholestasis on pretransplant BMD has been suggested in previous studies,[24,26-28] but the actual connection between low BMD and cholestasis remains obscure. There was a direct correlation in our study between alkaline phosphatase levels and pretransplant BMD, but the relative importance of liver and bone isoenzymes to this correlation is unknown, and no correlation was seen with bilirubin levels. Low serum albumin correlated with low BMD, but it is difficult to know if this represents an effect of nutritional status or of hepatic synthetic function on bone metabolism. As in previous studies,[29-31] no link between pretransplant osteopenia and any abnormality of calcium or mineral metabolism was found. All patients received calcium supplements and vitamin D therapy to correct low serum levels, but despite this, pretransplant osteopenia was very common, suggesting no important role for abnormalities in calcium or vitamin D metabolism in the etiology of cholestatic osteopenia. Whether allelic variants of the vitamin D receptor have an effect on hepatic bone loss remains to be determined.[32-34]

Over the last two decades, changes have occurred in the management of advanced liver disease, in immunosuppressive regimens, and in the allocation and waiting time for liver transplantation. Over this time, an improvement is seen in pretransplant BMD, spinal T-scores increased from –2.5 before 1990 to –1.7 after 1996, and this may give more insights into etiology. The severity of the liver disease, as reflected by MELD and

Child-Turcotte-Pugh scores, has not changed, the duration of disease before OLT has increased, and patients have become older. On the other hand, BMI has increased, muscle wasting and nutritional status (including vitamin D levels) have improved and bilirubin is lower; these factors may all have contributed to increased BMD before OLT.

An independent determinant of pretransplant low BMD identified in this patient population was low BMI, as seen in other studies.[2,24,25] BMI reflects both lean and fat tissue mass and influences BMD by several mechanisms: lean tissue stimulating the skeleton by providing mechanical stress,[35,36] and fat tissue effecting the production of leptin[37,38] and estradiol in female patients.[39,40] The importance of lean tissue on BMD is further emphasised by the independent correlation seen in our study with muscle wasting. Muscle wasting may simply reflect less physical activity in these patients, although most study patients here were ambulatory before OLT despite their advanced liver disease. These findings further stress the importance of adequate nutritional status and physical activity to prevent pretransplant bone loss. Before OLT, male patients had significantly more muscle wasting than female patients, a difference which could not be explained on the basis of their Karnofsky performance status alone. In males, however, muscle mass is also dependent on the anabolic effect of testosterone.[41,42] Testosterone levels have been shown to be reduced in males with advanced cholestatic liver disease, with a 70% incidence of hypogonadism (free testosterone < 9 pg/dL).[11] Hypogonadism may therefore be another contributing factor to muscle wasting (and osteopenia) in our male population, as has been suggested by histomorphometric analysis in male cholestatic patients.[11] Unfortunately, free testosterone levels were not available for most of our patients. As expected, age and menopausal status also correlated with increased pretransplant bone loss here, both well-recognised risk factors for osteoporosis and osteopenia in the general population.

Between OLT and 4 months posttransplant, the incidence of osteoporosis and osteopenia increased abruptly in all patient groups due an average of 5% bone loss over only 4 months, a very high rate of bone loss rarely seen in other clinical situations. Males and females were equally affected. The rate of bone loss did not change with time, despite changes in immunosuppressive regimens. The rate of bone loss was highly variable from patient to patient but the vast majority (82%) of patients lost bone mass during the first 4 months. Since bone loss after transplantation probably occurs early in this 4-month period, perhaps by 1-2 months,[43] it is possible that some of the 18% whose bone mass at 4 months was not lower than their pretransplant level had actually lost and then gained back bone mass by 4 months. Several demographic factors were identified in this study as risk factors for this early bone loss: younger age, PSC rather than PBC, no IBD disease, shorter duration of disease, current smoking history and higher baseline BMD values. Surprisingly, the only posttransplant factors negatively associated with bone loss were bilirubin levels at 4 months and non-anastomotic strictures, and no correlations were seen with posttransplant glucocorticoids, serum levels of calcineurin inhibitors, hospitalization days and rejection episodes. Our finding that liver recipients with higher BMD at OLT (who are also the younger patients with PSC) lose more bone after OLT than those with lower BMD is in agreement with previous studies,[44,45] but the reason for this is unknown. Nor is it obvious why PSC patients without IBD but the same baseline BMD or with shorter

disease duration should lose more bone. Some of the patients with IBD were on glucocorticoids but had the same baseline BMD as those without glucocorticoid therapy; in these patients, glucocorticoids were already exerting an influence on bone mass, and it could be speculated that this may have reduced the effect of posttransplant glucocorticoid use. The fact that cholestasis, as reflected by high bilirubin levels at 4 months or non-anastomotic strictures, worsened bone loss emphasizes again that important effect of cholestasis on bone metabolism.

Although posttransplant glucocorticoids are assumed to be a major factor in early bone loss, data correlating glucocorticoids with posttransplant BMD are few.[45-47] In this study, posttransplant bone loss did not correlate with either the average daily dose of glucocorticoids or episodes of rejection which required bolus Solu-Medrol therapy. Recent histomorphometric findings[11] in cholestatic patients at time of OLT have shown a direct correlation between posttransplant bone volume losses and glucocorticoid doses. In addition, this histomorphometric study indicated that the main insult leading to early posttransplant bone loss, occurs very early after OLT, and is probably mainly related to further decreases in bone formation, a well-recognized effect of glucocorticoid therapy. All patients in this BMD study received high doses of glucocortocoids during and early after OLT, and this may have been sufficient to maximize their skeletal effect on bone metabolism, and thus obscure any effect of dose tapering on preventing bone loss. Studies using glucocorticoid-free regimens will be needed to fully appreciate their effect on bone loss in the early posttransplant period. The contribution of calcineurin inhibitors to posttransplant bone loss has not been well established, and may be overwhelmed by the profound effects of glucocorticoids on bone metabolism. Analysis of this study was not able to demonstrate any definite effect of serum levels of TAC or CyA on bone mass nor any difference between the two drugs. In this study, however, there were too few patients who received neither CyA nor TAC to allow any assessment of the effect of calcineurin inhibitor-free immunosuppression.

Data on the long-term effect of OLT on BMD are very limited but have suggested some improvement in BMD with time.[48,49] Our study with long-term follow-up of posttransplant BMD in a large cholestatic population indicated an increase in BMD during the first 2 years; after this, T-scores remained stable throughout the 8 years of the study, whereas Z-scores, which are age adjusted, continued to increase. These studies indicate that our cholestatic patients do not lose bone mass with age at the usual rate of 1 - 2% per year, presumably secondary to the concurrent ongoing beneficial effects of OLT on BMD recovery.

Although no early correlations after OLT were shown in our study, glucocorticoid doses would seem to be important for influencing the rate of bone gain. Bone gain after OLT was not significantly influenced by sex or disease, but it was reduced in postmenopausal patients and in patients with increased cholestasis or elevated serum creatinine. In addition, not only do patients with high baseline BMD lose more bone after OLT, but they also have less bone gain over the subsequent posttransplant years. Conversely, it would appear that patients with the most severe bone loss before OLT- that is, those with the most compromised bone metabolism- have the most to gain by

the improved metabolic milieu after OLT. Although similar bone losses were seen after OLT throughout the study period, bone gain during the first 2 years after OLT is greater in the more recently transplanted patients (11% per year for patients after 1996 compared to 3.8% and 4.4% in the earlier 2 transplantation periods). During this latter period from 1996-2000, there was less rejection, less hospitalization days, less biliary stricturing, less prednisone dosing, and TAC rather than CyA as the primary immunosuppressant. In addition, the patients from the last transplantation period had lower posttransplant creatinine and phosphorus levels and higher PTH levels when compared to the earlier transplantation periods.

Despite no apparent connection between vitamin D and pretransplant osteopenia, vitamin D correlated with bone gain after OLT. Vitamin D is important in osteoblastogenesis,[50] and previous studies have shown that it correlates with increased number of osteoblasts in cholestatic osteoporosis;[51] this may explain the greater bone gain with higher levels of vitamin D in the posttransplant period. The importance of adequate mineral metabolism to bone gain was further illustrated by positive correlations with posttransplant PTH and negative correlations with posttransplant phosphorus.

In summary, most patients (77%) with advanced PBC and PSC have osteopenic bone disease and only 23% of patients have normal bone mass. After adjusting for differences in age and sex, a similar severity of osteopenia was found in patients with PBC and PSC. At time of OLT, risk factors for hepatic osteopenia are low BMI, older age, postmenopausal status, the presence of muscle wasting, high alkaline phosphatase, and low serum albumin. An improvement in bone mass occurred with time over the 16-year study period (in PSC, females and males), perhaps due at least in part to better nutritional status, increased BMI, increased vitamin D, and less cholestasis. After OLT, aggressive bone loss occurs during the first 4 months, and this did not change over time despite changes in immunosuppressive regimens with less posttransplant glucocortoid doses, less rejection episodes, less hospitalization days, and less nonanastomotic biliary stricturing. Risk factors for bone loss were younger age, PSC, higher pretransplant BMD, no IBD, shorter duration of disease, current smoking, and ongoing cholestasis at 4 months. After the first 4 postoperative months, bone gain then occurs during the first 2 years and was increased in the more recently transplanted patients. Other factors favouring improvement in bone mass are lower baseline and/or 4-month BMD, premenopausal status for females, lesser glucocorticoids, no ongoing cholestasis and higher levels of vitamin D and parathyroid function. Bone mass therefore improves most in patients with the lowest BMD who undergo successful transplantation and have normal hepatic allograft function and improved gonadal and nutritional status. In addition, patients with osteoporosis or osteopenia can be expected to gain bone mass for at least 8 years, despite getting older.

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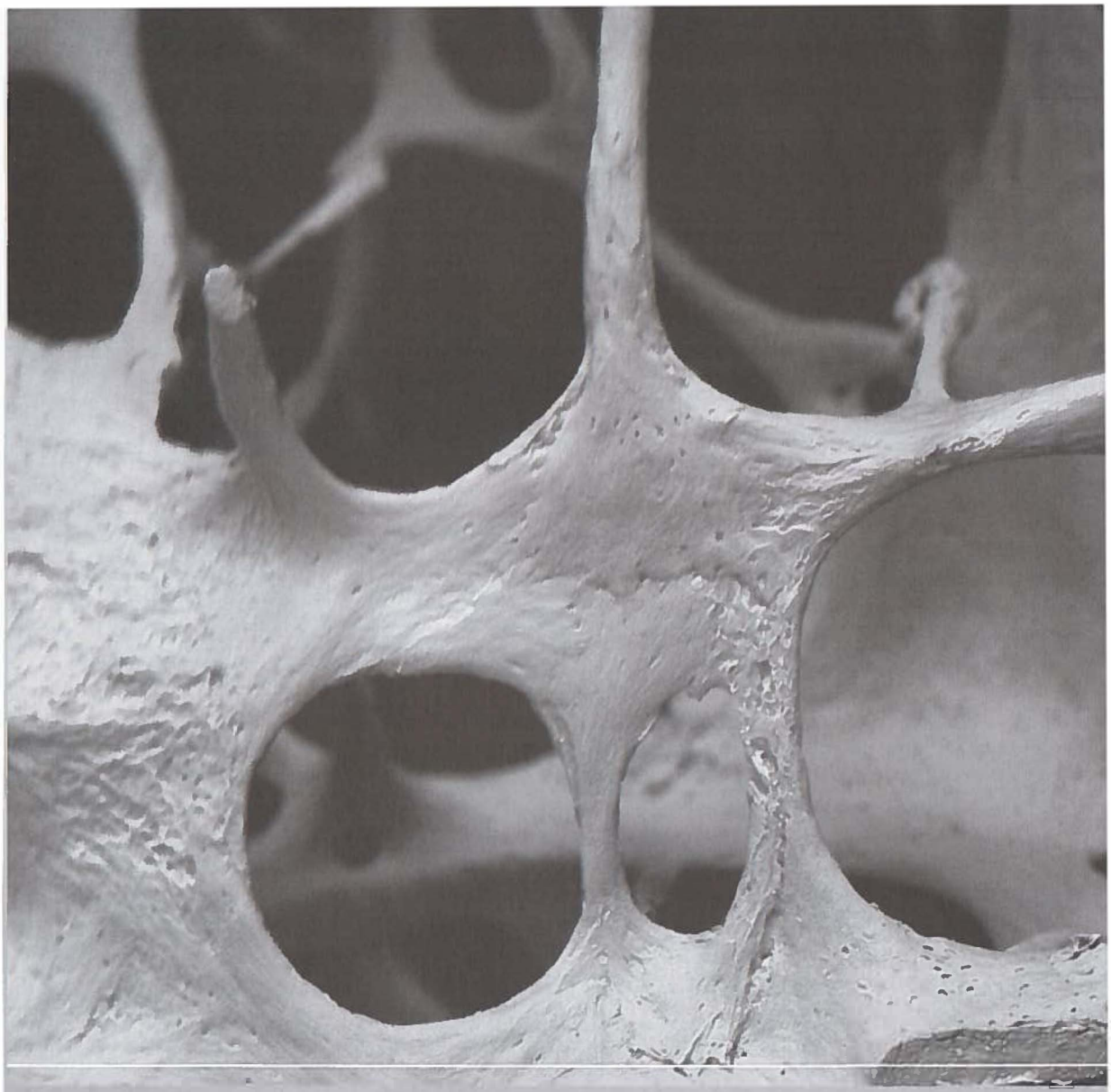
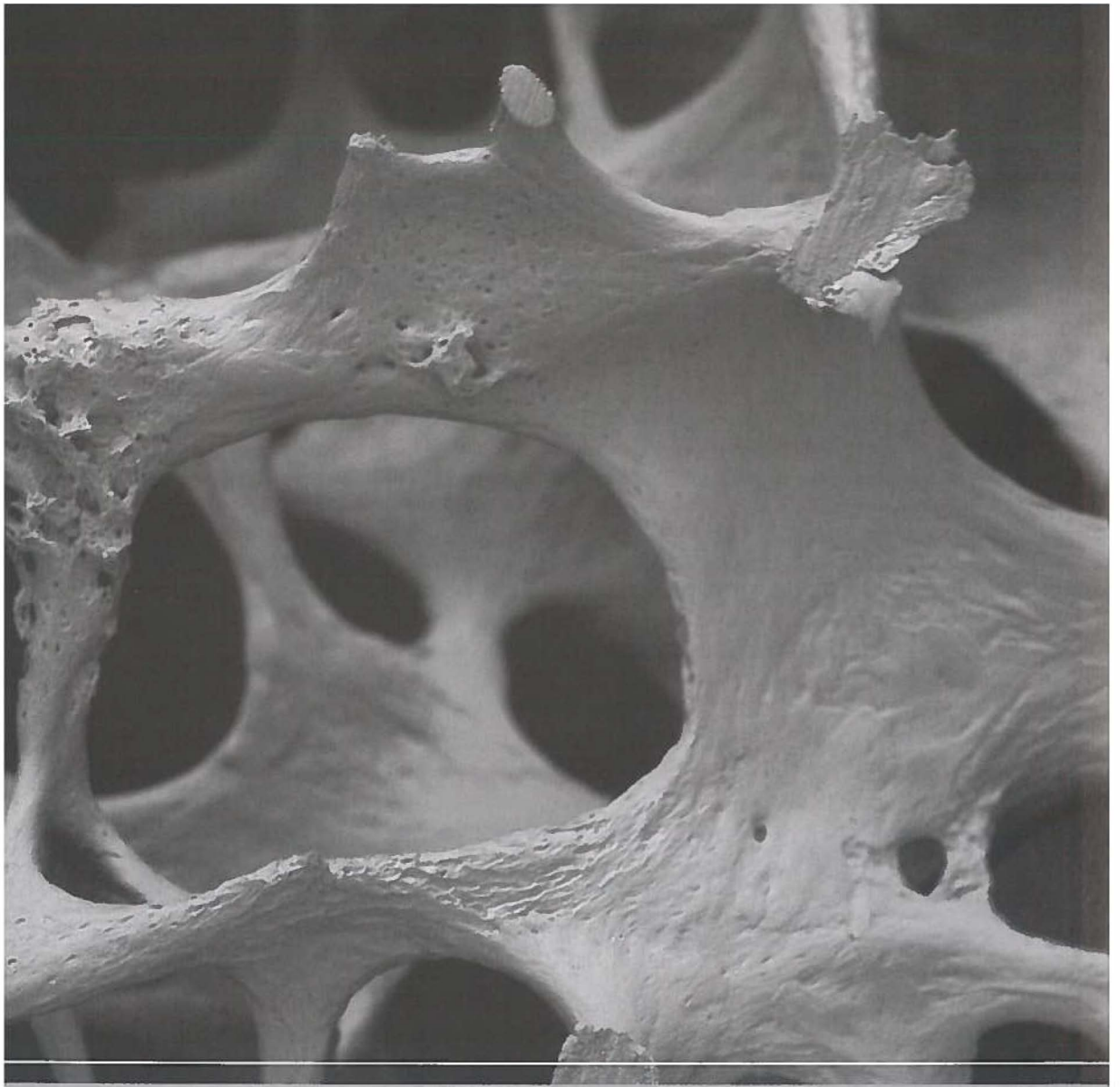


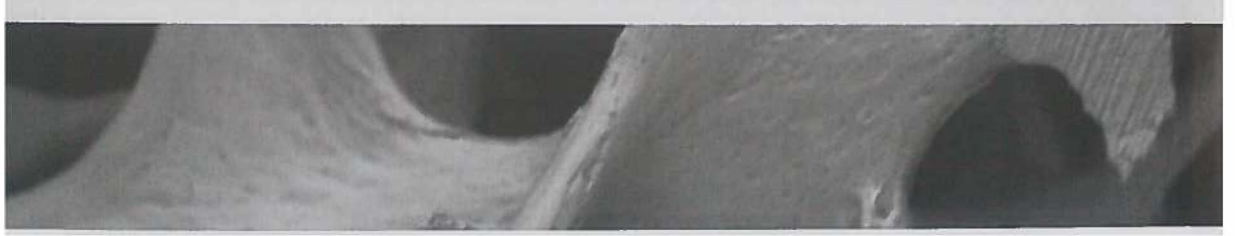
Chapter 6

Maureen M.J. Guichelaar, Jeffrey Schmoll, Michael Malinchoc, J. Eileen Hay



Fractures and avascular necrosis before and after OLT: Long-term follow-up and predictive factors

Hepatology 2007;46:1198-1207.



Abstract

With early posttransplant bone loss, orthotopic liver transplant (OLT) recipients experience a high rate of fracturing and some avascular necrosis (AVN), but little is known about the incidence of and predictive factors for these skeletal complications. We studied 360 consecutive patients who underwent transplantation for primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) and assessed both vertebral and nonvertebral (rib, pelvic, and femur) fractures in a protocolized fashion. Before OLT, 20% of patients had experienced fracturing, and 1.4% patients had experienced AVN. Following OLT, there was a sharp increase in fracturing, with a 30% cumulative incidence of fractures at 1 year and 46% at 8 years after transplantation. In contrast to previous studies, there was a similar incidence of posttransplant vertebral and nonvertebral fractures. The greatest risk factors for posttransplant fracturing were pretransplant fracturing and the severity of osteopenia and posttransplant glucocorticoids. Nine percent of liver recipients experienced AVN after OLT, and this correlated with pretransplan and posttransplant lipid metabolism, bone disease (bone mineral density and fracturing), and posttransplant glucocorticoids. A novel association between cholestasis and AVN was also identified, the mechanism for which is not known. Fortunately, recent years have seen an increase in the bone mass of liver recipients and, along with this, less fracturing and less AVN. Nonetheless, 25% of patients undergoing OLT for chronic cholestatic liver disease still develop de novo fractures after OLT; this situation demands an ongoing search for effective therapeutic agents for these patients.

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Abbreviations
25(OH)D, 25-hydroxyvitamin D; AVN, avascular necrosis; BMD, bone mineral density; BMD-LS, bone mineral density at the lumbar spine; BMI, body mass index; CCLD, chronic cholestatic liver disease; CI, confidence interval; CTP, child-turcotte-pugh; CyA, cyclosporine A; HR, hazard ratio; HRT, hormone replacement therapy; MELD, model for end-stage liver disease; OLT, orthotopic liver transplantation; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; PTH, parathyroid hormone.

Introduction

Following orthotopic liver transplantation (OLT), bone mass is rapidly lost during the early postoperative months. Although the period of bone loss is short, the extent of bone loss in already osteopenic patients leads to an increase in fracturing. Pain and immobility from skeletal complications causes morbidity in liver transplant recipients,[1-8] especially in patients with chronic cholestatic liver disease (CCLD). This results most frequently from osteopenic fracturing but also, to a lesser extent, from avascular necrosis (AVN); the latter is regarded as a different etiologic entity from osteopenic fracturing and is perhaps related to insults to the vascular supply of bone, including trauma, hyperlipidemia and glucocorticoid use.[9-14] The incidence of, and risk factors for posttransplant fractures and AVN are poorly defined.

We have recently confirmed, in a large population of liver transplant recipients undergoing OLT for CCLD, that bone mass is rapidly lost during the first 4 postoperative months but then starts to increase and may reach or surpass pretransplant levels by 2-3 years after OLT.[15] This study aims to assess the incidence and predictive variables for pretransplant and posttransplant fractures (vertebral and nonvertebral) and AVN in this same population of liver transplant recipients followed from the pretransplant period to 8 years after OLT.

Methods

Patients

From 1985 - 2001, all adult patients undergoing OLT at the Mayo Clinic (Rochester, MN) with either primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC) were studied by protocolized assessment before and after OLT (4 months, annually). Patients who underwent transplantation were sequentially assigned an OLT number. Diagnoses of PBC and PSC were made according to well-established criteria.[16-18] The study was approved by the Mayo Institutional Review Board. All patients were followed until July 2002, death or retransplantation.

Clinical assessment

Patients underwent clinical and biochemical assessment at each time of evaluation. The liver function was assessed by Child-Turcotte-Pugh (CTP) and model for end-stage live disease (MELD) scores, and the functional status was assessed with the Karnofsky performance scale.[19] The menopausal status was determined by clinical symptoms and biochemical testing. The nutritional status, muscle wasting, complications of liver disease and other illnesses were noted, and medications were recorded. Muscle wasting was assessed globally by the transplant hepatologist. General dietary instructions and oral calcium supplements (1.5 g/day) were given to all patients with vitamin D supplementation to normalize serum 25-hydroxyvitamin D [25(OH)D] levels. Inflammatory bowel disease was diagnosed by colonoscopy and surveillance biopsies at the time of activation for OLT and annually thereafter in all PSC patients.

Immunosuppression
From 1985 -1990, standard immunosuppression was cyclosporine A (CyA) and prednisone with or without azathioprine. From 1990 - 1993, patients were treated with tacrolimus and prednisone (multicenter FK506 trial) or the standard triple therapy with prednisone, CyA and azathioprine; the tacrolimus patients received only about half the total prednisone dose of CyA arm. In 1994, standard immunosuppression was changed to tacrolimus, prednisone, and azathioprine, whereas some patients were treated in the multicenter Neoral trial. In 1999, azathioprine was replaced by mycophenolate mofetil for the first 2 - 4 postoperative months. The CyA and tacrolimus doses were adjusted according to the desired serum levels. Acute cellular rejection of the allograft was treated with 1 g of intravenous Solu-Medrol on 3 alternate days.

Biochemical indices
Biochemical testing included markers of liver and kidney function, and indices of calcium metabolism and was performed by Mayo medical laboratories. Serum 25(OH)D was measured by the method of Kao and Heser.[20] Immunoreactive parathyroid hormone (PTH) has been measured by an immunochemiluminescent metric assay[21] only since June 1989.

Measurements of bone mineral density at the lumbar spine (BMD-LS)
BMD-LS measurements were taken at the time of activation for OLT, 4 months after OLT, 1 year after transplantation, and yearly thereafter. Before April 1988, the bone mineral density (BMD) was measured by dual-photon absorptometry. Since April 1988, dual-energy X-ray absorptometry using Hologic machines has been used. Phantoms were used to cross-calibrate the two machines, and conversion formula established to convert to dual-energy X-ray absorptometry data. The bone mass was corrected for the bone size to calculate BMD ($g/cm^{2)}$. Measurements of BMD-LS had a reproducibility of 2.2%. In patients with compression fractures, measurements were determined on only the intact vertebrae. BMD readings were expressed as T-scores (standard deviations from peak bone mass of a young, sex-matched reference population) and Z-scores (standard deviations from age-adjusted and sex-adjusted reference values). A T-score higher than -1.0 is considered normal, a T-score between –1 and –2.4 indicates osteopenia, and a T-score of –2.5 or lower indicates osteoporosis.[22]

Radiologic follow-up of fractures
Standard radiographs of the pelvis, chest and thoracolumbar spine at a tube distance of 120 cm were obtained at time of activation for OLT, 4 and 12 months after transplantation, and then yearly thereafter. Additional radiographs were taken as clinically indicated at the site of any bone pain, and if they were negative, a bone scan was performed. If AVN was suspected, additional magnetic resonance imaging scans were taken. All radiographs, bone scans and magnetic resonance imaging scanning were performed in the Mayo Clinic and judged for fractures by trained radiologists. All radiologic reports (n = 7710) performed in this study population were reviewed to assess new fractures and AVN.

Statistical analysis

Parameters are reported as means ± standard deviation. We aimed to describe the incidence of posttransplant fracturing and AVN while taking into account the competing risk occurrences of death or retransplantation, using competing risk analysis.[23,24] Patients who had not experienced any of the endpoints (death, retransplantation, fractures, and AVN) were censored at their last radiologic assessment date. Cox proportional hazards modeling was used to determine which patients' characteristics were associated with posttransplant fracturing. Posttransplant variables with serial measurements were used in the time-dependent analysis to assess the association between these variables and posttransplant fractures and AVN. The backwards elimination variable selection procedure was used to find the independent variables that predicted posttransplant fractures and AVN. Such variables with a p-value less than 0.05 were included in the final multivariate regression model to predict low BMD. All analyses were performed with the SAS data analysis system[25] and S-PLUS analysis system.[26]

Results

Pretransplant clinical and biochemical variables

The clinical, biochemical and BMD characteristics of the study population before and after OLT have previously been reported in detail.[15] One-hundred fifty-six PBC patients (135 females, 21 males) and 204 PSC patients (83 females, 121 males) underwent transplantation; 148 females were postmenopausal. The PBC patients were older than the PSC patients (53.2 ± 8.6 versus 46.8 ± 11.0 years), and the females were older than the males (50.8 ± 9.6 versus 47.6 ± 11.6 years). The patients had end-stage liver disease with mean CTP score of 8.7 ± 1.6, a MELD score of 17.3 ± 8.8, abnormal liver function, and significant osteoporosis and osteopenia (Table 1). Eleven patients had diabetes mellitus; 2 patients were treated with insulin. Other pretransplant medications were cholestyramine (n = 64), ursodeoxycholic acid (n = 111), anticonvulsants (n = 5), thyroid replacement therapy (n = 45), hormone replacement therapy (HRT; n = 18), and bisphosphonates (n = 4).

To assess temporal changes, the study period (1985 - 2001) was divided into three periods by OLT date; period 1, 1985 -1989 (n = 93); period 2, 1990 -1995 (n = 153); and period 3 1996 - 2001 (n = 115). As reported in a previous article[15] from period 1 to period 3, patients became older (45.9 ± 9.1 versus 50.3 ± 9.9 versus 51.4 ± 11.7 years) and more postmenopausal (66% versus 67.4% versus 85%). There were temporal increases in the pretransplant 25(OH)D (17.0 ± 13.9 versus 18.0 ± 12.9 versus 29.6 ± 26.9 ng/mL), duration of disease before OLT (6.3 ± 4.6 versus 7.8 ± 5.4 versus 7.9 ± 5.9 years), body mass index (BMI; 23.6 ± 4.3 versus 24.1 ± 4.3 versus 25.6 ± 5.1 kg/m^2), and T-scores (-2.5 ± 1.6 versus -2.0 ± 1.4 versus -1.7 ± 1.2), the last resulting in less osteoporosis (57% versus 34% versus 26%). Temporal decreases were seen in muscle wasting (87.8% versus 71.3%), poor nutritional status (16.1% versus 4.0%), alkaline phosphatase (1311.4 ± 871.6 versus 1181.9 ± 935.2 versus 845.0 ± 648.4 mg/dL) and direct bilirubin (8.2 ± 6.4 versus 7.1 ± 7.7 versus 5.8 ± 6.6 mg/dL). No significant changes were seen in the ratio of PBC to PSC, gender distribution, CTP or MELD scores, pretransplant albumin, creatinine, ionized calcium, or PTH.

Pretransplant fractures and AVN

Three hundred thirty-four (95.6%) of 360 patients had a pretransplant assessment of fractures for a mean time of 3.6 ± 4.9 years before OLT. Sixty-six (19%) patients developed pretransplant fractures (34 PBC and 32 PSC; 44 females and 22 males); 43 patients had spinal fractures (17 single fractures and 26 multiple fractures), 34 patients had rib fractures (13 single fractures and 21 multiple fractures) and 9 patients had other fractures (including femur, tibia, and calcaneus fractures). Five patients (1.4%) had AVN at the femur head (3 bilateral), only one of whom had steroid therapy before OLT.
There were no differences in rates of fracturing or AVN according to assessments by gender or disease. In the univariate analysis, only BMD was associated with pretransplant fractures ($p < 0.01$). Pretransplant fracture rates decreased with time (23% versus 21% versus 12% from period 1 to period 3); this was significant ($p < 0.05$) only in patients with PSC [9 patients (20.0%) versus 19 patients (20.0%) versus 4 patients (6.4%)] and not in patients with PBC.

Table 1. Bone mineral density and biochemical changes after OLT in 360 patients transplanted for PBC and PSC.

	Baseline	Time after OLT			
	PreOLT	**4 months**	**1 year**	**4 years**	**8 years**
BMD total patients					
Osteopenia, n (%)	131 (39%)	102 (37%)	102 (39%)	72 (46%)	38 (51%)
Osteoporosis, n (%)	128 (38%)	141 (51%)	113 (44%)	44 (28%)	22 (30%)
T-scores	-2.0 ± 0.1	-2.4 ± 0.1	-2.2 ± 0.1	-1.8 ± 0.2	-1.8 ± 0.4
Z-scores	-1.4 ± 0.1	-1.8 ± 0.1	-1.5 ± 0.1	-1.0 ± 0.1	-0.8 ± 0.4
Laboratory follow-up					
Albumin (3.5-5.0 g/dL)	3.0 ± 0.5	3.7 ± 0.6****	4.0 ± 0.4****	4.0 ± 0.4	3.9 ± 0.4****
Total bilirubin (0.1- 1.1 mg/dL)	11.2 ± 11.0	2.1 ± 5.2****	1.2 ± 3.0	1.3 ± 4.8	1.3 ± 2.8
Direct bilirubin (0.0- 0.3 mg/dL)	7.0 ± 7.1	1.0 ± 2.7****	0.5 ± 2.1	0.3 ± 0.6	0.6 ± 1.9
Alkaline phosphatase (U/L)[A]	1107.8 ± 855.1	299.9 ± 499.0****	175.6 ± 234.2***	150.5 ± 219.3	207.1 ± 291.1**
Creatinine (mg/dL)[B]	1.2 ± 0.9	1.3 ± 0.6****	1.3 ± 0.5****	1.4 ± 0.5*	1.4 ± 0.5
Calcium (8.9 – 10.1 mg/dL)	8.8 ± 0.6	9.1 ± 0.8****	9.2 ± 0.6	9.2 ± 0.5	9.3 ± 0.5
25(OH)D (ng/mL)[C]	20.2 ± 17.9	31.6 ± 13.8****	36.2 ± 17.0***	40.9 ± 14.9****	38.9 ± 14.7
PTH (1.0 – 5.2 pmol/L)	2.3 ± 2.0	4.3 ± 4.5****	4.8 ± 3.4***	3.7 ± 2.0	3.0 ± 1.3
Cholesterol (mg/dL)[D]	211.5 ± 130.3	201.7 ± 84.6	193.8 ± 63.3	197.9 ± 48.7	209.7 ± 74.7
Triglycerides (mg/dL)[D]	119.1 ± 69.1	161.1 ± 101.4	142.1 ± 70.1	148.5 ± 91.9	150.6 ± 97.3
Uric acid	4.9 ± 3.9	6.1 ± 1.7	6.6 ± 1.8	6.6 ± 1.9	7.1 ± 2.1

[A] Total alkaline phosphatase = M > 19 jr: 98 - 251 U/L; F 24 - 45 yr: 81 - 231 U/L; F 46 - 60 yr: 84 - 257 U/L

[B] Serum creatinine = F:0.6 - 0.9 mg/dL, M: 0.8 - 1.2 mg/dL. [C] 25(OH)D = summer: 15 - 80 ng/mL, winter: 14 - 42 ng/mL. [D] Desired < 200 mg/dL

Laboratory changes between 2 consecutive time points were significant with $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ (asterix at second time point)

Posttransplant clinical and biochemical variables

The average hospitalization stay (during the first 4 months after transplantation) was 25.6 ± 21.0 days. Fifty-one (14.2%) patients were retransplanted after OLT at a mean of 1.3 ± 2.6 years, and 78 patients (21.7%) died after transplantation at a mean of 5.1 ± 4.3 years. Rejection occurred in 44.4% of patients, and non-anastomotic biliary strictures occurred in 11.9%. The liver function and indices of calcium metabolism improved following OLT (Table 1). HRT was used in 57 of 148 postmenopausal females after OLT, with only 27 females starting HRT during the first 2 years. Sixteen patients received bisphosphonates after OLT: etidronate in 1 patient (in the first post-OLT year), pamidronate in 2 patients (first and second years after OLT), and alendronate in 13 patients (all started after the first 2 posttransplant years). Sixty-three patients were enrolled in a randomised trial of calcitonin therapy or no treatment after

OLT; this trial showed no effect of calcitonin therapy on BMD or fractures after OLT.[27] Posttransplant loss in BMD during the first 4 months and subsequent gain has been reported in detail[15] (Table 1). Posttransplant bone loss was greater in patients with PSC than in patients with PBC (-18.0 ± 20.0 versus -13.3 ± 17.8 %/year, $p < 0.05$), and bone gain was less in CyA-treated than in tacrolimus-treated patients (5.0% ± 17.2 versus 10.4% ± 9.6%, $p < 0.05$). In addition, early bone loss did not change over time, but bone gain from 4 - 12 months was greater in period 3 (4.6 ± 13.8 versus 5.4 ± 10.2 versus 10.0 ± 19.3%/year). As reported previously[15] temporal decreases were seen in posttransplant hospitalization days, rejection episodes, nonanastomotic biliary strictures, treatment with cyclosporine (rather than tacrolimus), and cumulative prednisone doses. Significant temporal increases were seen in serum albumin and PTH, whereas serum creatinine and phosphorus decreased with time.

Posttransplant fractures

Following OLT, the mean radiologic follow-up was 5.3 ± 4.3 years: 7.9 ± 5.1 years in period 1, 5.8 ± 3.7 years in period 2, and 2.5 ± 1.9 years in period 3. The fracture rate increased sharply after OLT (Table 2), with 25% patients having fractures between OLT and 6 months after transplantation. During the following years, fractures continue to occur but at a lower rate, leading to a cumulative incidence of 45.9% patients (n = 158) with fractures 8 years after transplantation. In a subpopulation of 63 patients, it was found that most fractures were symptomatic, resulting in bone pain. PBC patients had significantly more fractures than PSC patients ($p < 0.01$; Fig. 1A), and females had significantly more than males ($p < 0.05$; Fig. 1B). There was no difference in fracturing between postmenopausal women with HRT (n = 57) and those without HRT (n = 83; the cumulative incidence 8 years after transplantation was 60.8% versus 51%). Bisphosphonate therapy was used in too few patients (4 before transplantation and 16 after transplantation) to make any assessment of the effect. Significant differences in posttransplant fractures were seen when patients were separated by baseline BMD (Fig. 2A) or by pretransplant fracturing (Fig. 2B). Fracture rates decreased with time ($p < 0.01$; Fig. 3) in both PBC and PSC.

Patients with fractures were older than those without fractures (51.1 ± 10.3 versus 48.3 ± 10.5 years, $p < 0.01$), had more pretransplant muscle wasting (91.9% versus 74%, $p < 0.01$), lower BMI (23.5 ± 4.6 versus 25.1 ± 4.5 kg/m^2, $p < 0.01$), lower BMD (0.80 ± 0.15 versus 0.91 ± 0.15 g/cm^2, $p < 0.01$), higher total bilirubin (12.3 ± 11.2 versus 10.4 ± 10.8 mg/dL, $p < 0.05$), higher direct bilirubin (7.7 ± 7.1 versus 6.4 ± 7.0 mg/dL, $p < 0.05$), higher alkaline phosphatase (1231.5 ± 896.6 versus 1008.8 ± 809.2 mg/dL, $p < 0.01$), and lower vitamin D (12.3 ± 10.3 versus 15.3 ± 12.2 ng/mL, $p < 0.05$).

Patients with fractures had increased average daily glucocorticoid doses 1 month (147.0 ± 51.7 versus 133.8 ± 46.4 mg, $p < 0.01$), and at 4 months after transplantation (53.9 ± 19.9 versus 45.7 ± 46.4 mg, $p < 0.01$), were treated with CyA rather than tacrolimus [100 (62.3%) versus 65 (32.5%), $p < 0.01$], had more hospitalization days (25.0 ± 20.2 versus 21.3 ± 16.7, $P < 0.05$), and 4 months after transplantation had lower BMD (0.75 ± 0.15 versus 0.87 ± 0.14 g/cm^2, $p < 0.01$), lower albumin (3.7 ± 0.5

versus 3.8 ± 0.6 g/dL, p < 0.01), and higher alkaline phosphatase (309.3 ± 504.1 versus 291.2 ± 495.7 mg/dL, p < 0.05).

Table 2. Cumulative incidence of posttransplant fractures in 360 patients with end-stage PBC or PSC

		Time after OLT									
		4 months after OLT		12 months after OLT		2 years after OLT		4 years after OLT		8 years after OLT	
	N at OLT	Cumulative incidence of fractures (n)	N at risk[A]	Cumulative incidence of fractures (n)	N at risk[A]	Cumulative incidence of fractures (n)	N at risk[A]	Cumulative incidence of fractures (n)	N at risk[A]	Cumulative incidence of fractures (n)	N at risk[A]
Total patients	360	68 (18.9%)	262	108 (30.1%)	213	123 (34.3%)	189	140 (39.2%)	153	158 (45.9%)	66
PBC patients**	156	38 (24.4%)	106	59 (38.0%)	82	68 (43.8%)	68	74 (47.9%)	57	81 (53.3%)	22
PSC patients**	204	30 (14.7%)	156	49 (24%)	131	55 (27.0%)	121	66 (32.6%)	96	77 (40.2%)	44
Male patients*	142	19 (13.4%)	112	35 (24.6%)	94	36 (25.4%)	89	46 (32.9%)	69	54 (40.8%)	26
Female patients*	218	49 (22.5%)	150	73 (33.6%)	119	87 (40.1%)	100	94 (43.4%)	84	104 (49.3%)	40
Premenopausal females	70	14 (20.0%)	48	18 (25.7%)	42	20 (28.6%)	39	25 (36.1%)	31	26 (37.7%)	19
Postmenopausal females	148	35 (23.7%)	102	55 (37.4%)	77	67 (45.5%)	61	69 (47%)	53	78 (54.9%)	21
Pre-OLT fractures**** [B]	66	30 (45.5%)	32	42 (63.6%)	18	45 (68.2%)	13	48 (73.1%)	10	50 (77.3%)	5
No pre-OLT fractures****	294	37 (12.8%)	227	64 (22.2%)	193	76 (26.4%)	174	90 (31.5%)	141	106 (38.8%)	60
Cyclosporine pts [C]	198	47 (23.7%)	138	75 (37.9%)	106	83 (41.9%)	93	92 (46.6%)	83	107 (54.8%)	51
Tacrolimus pts	111	16 (14.4%)	92	24 (21.6%)	81	28 (25.2%)	73	35 (32.6%)	48	37 (35.3%)	8
Low pre-OLT BMD**** [D]	84	22 (21.7%)	71	44 (43.5%)	44	53 (51.5%)	32	55 (55%)	23	57 (58.2%)	10
Average pre-OLT BMD****	172	39 (22.7%)	121	50 (29%)	108	57 (33.2%)	99	68 (39.7%)	82	81 (49.4%)	32
High pre-OLT BMD****	86	7 (8.1%)	70	14 (16.3%)	62	15 (17.4%)	56	17 (19.9%)	47	30 (24.4%)	23
Period 1985-1989**	93	29 (31.2%)	57	38 (40.9%)	46	43 (46.2%)	38	44 (47.3%)	36	52 (56%)	24
Period 1990-1995**	152	24 (15.8%)	114	46 (30.3%	89	53 (34.9%)	81	67 (44.3%)	66	76 (51.2%)	42
Period 1996 - 2001**	115	15 (13.1%)	91	24 (21.0%)	78	27 (23.6%)	70	29 (25.4%)	51		

Differences in fractures after OLT with respect to cumulative incidence of fractures between subpopulations were *p<0.05, **p<0.01. ***p<0.001, ****p<0.0001

[A] Patients who had not died, had not undergone retransplantion, and had not sustained any fracture. [B] Five patient did not have pretransplant radiologic screening and were excluded from the analysis. [C] Patients who switched from tacrolimus to cyclosporine or vice versa were excluded from the analysis.

[D] Pre-OLT low BMD < 0.75 g/cm², pre-OLT average 0.75 – 0.96 g/cm², Pre-OLT high > 0.96 g/cm²; 342 of 360 patients had BMD measurements taken before OLT.

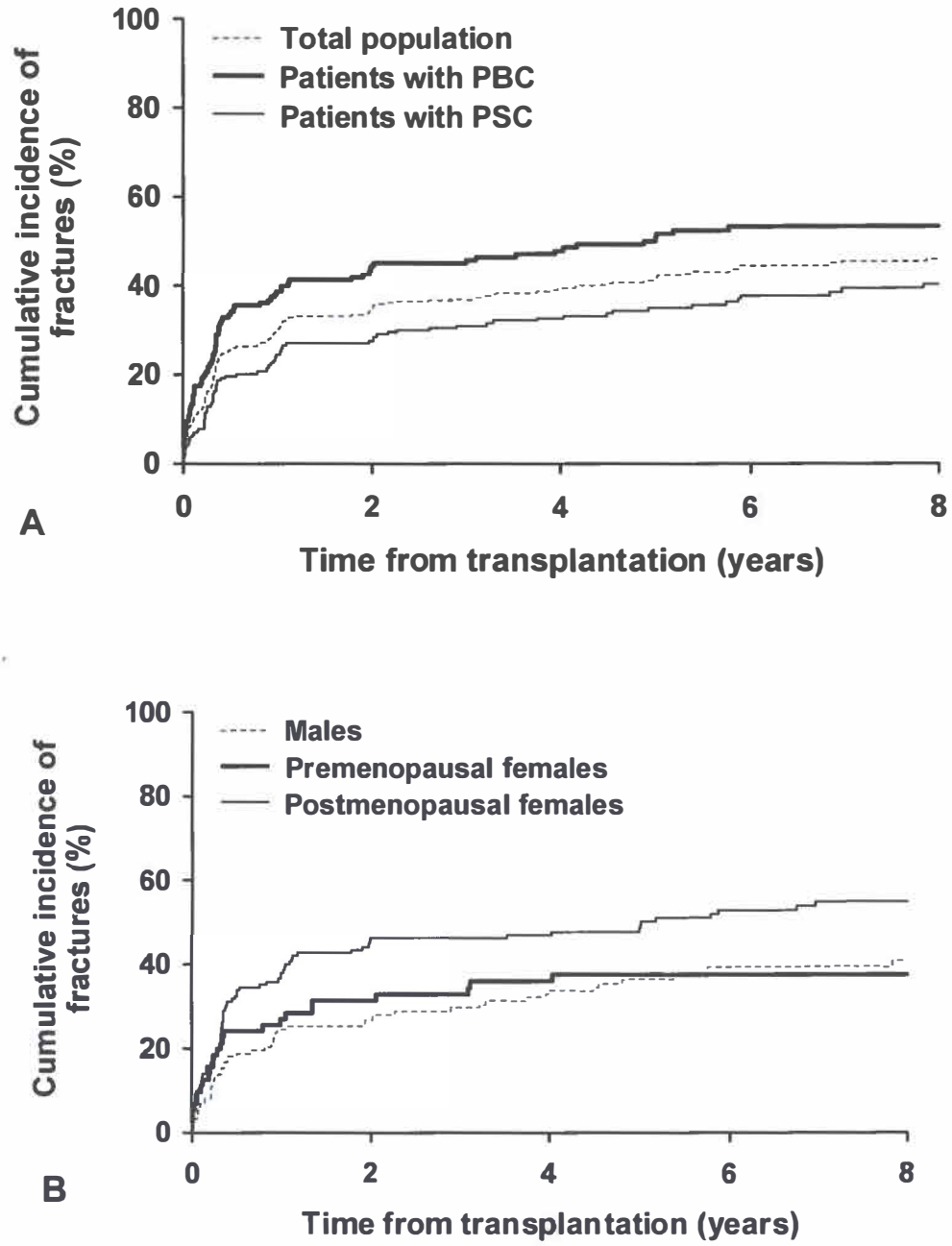

Figure 1. Cumulative incidence of posttransplant fractures in 360 cholestatic patients undergoing liver transplantation for **(A)** PBC (156 patients) and PSC (204 patients), and **(B)** males (142 patients), premenopausal females (70 patients), and postmenopausal females (148 patients).

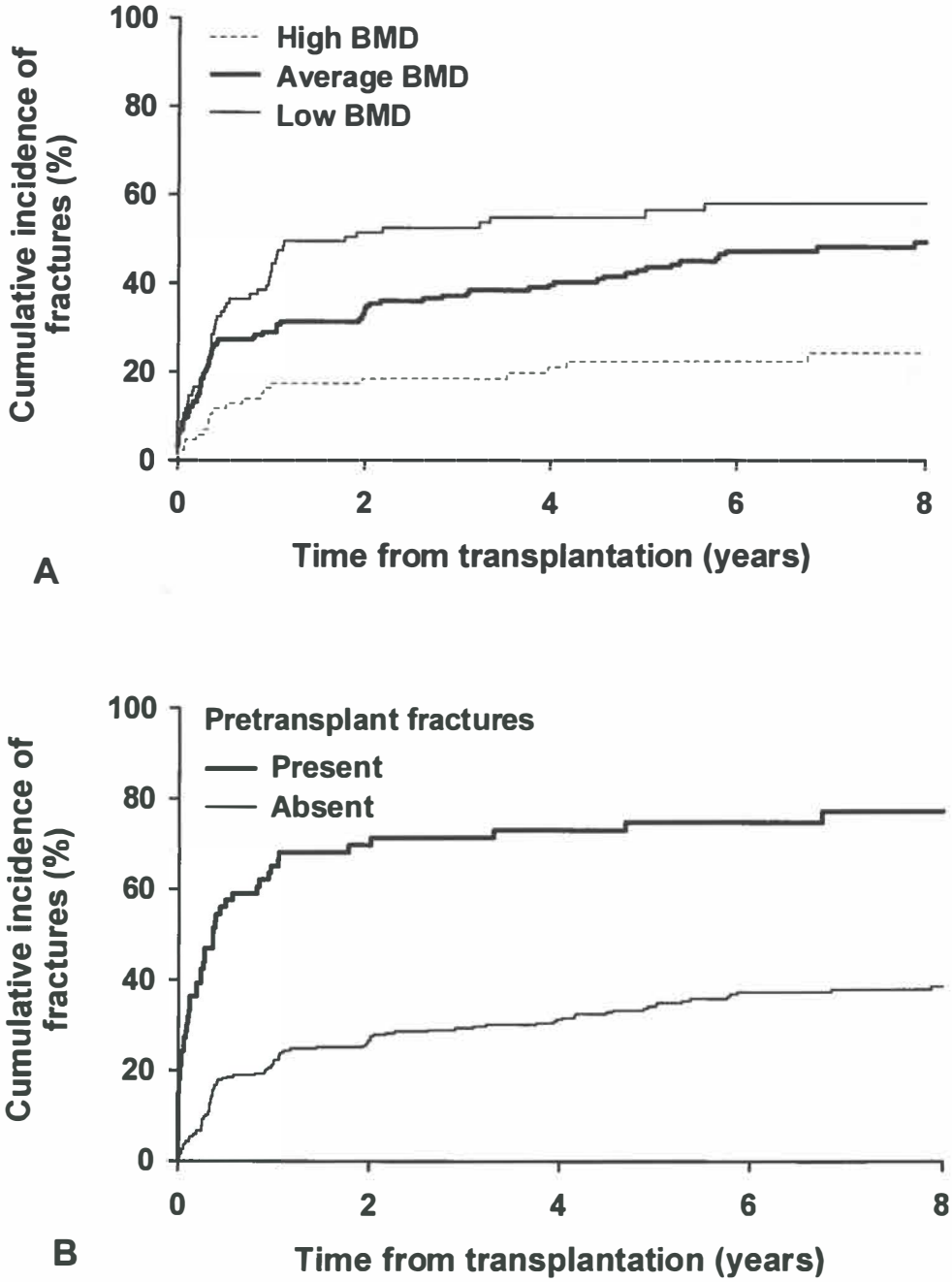

Figure 2. Cumulative incidence of posttransplant fractures in cholestatic patients undergoing liver transplantation stratified according to **(A)** pretransplant BMD and **(B)** pretransplant fracturing. Sixty-six patients had pretransplant fracture. Pre-OLT low BMD < 0.75 g/cm^2 (n = 84); pre-OLT average BMD = 0.75 – 0.96 g/cm^2 (n = 172); pre-OLT high BMD > 0.96 g/cm^2 (n = 86).

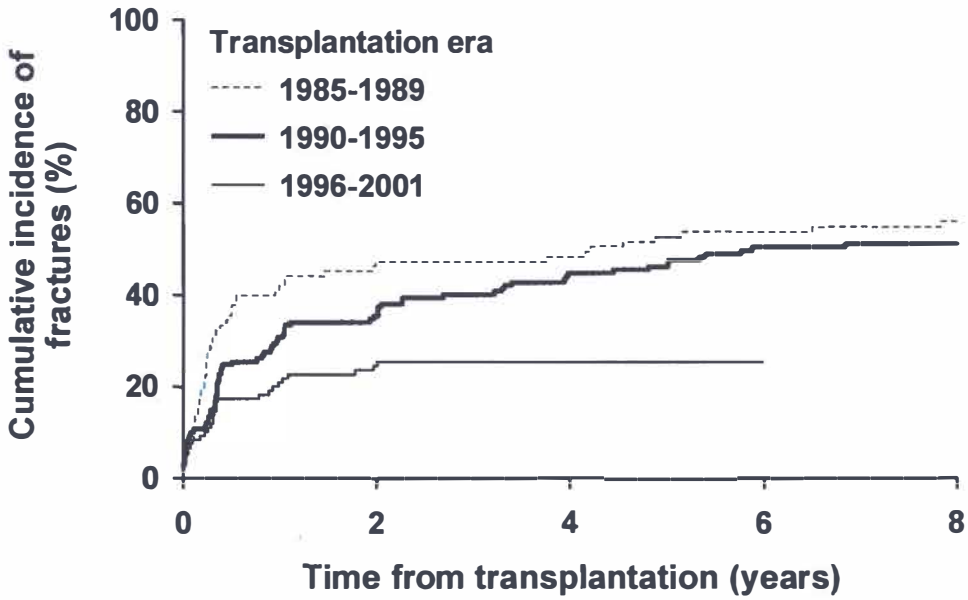

Figure 3. Cumulative incidence of posttransplant fracatures in cholestatic patients undergoing liver transplantation stratified according to the time of transplantation: 1985 - 1989 (n = 93), 1990 - 1995 (n = 152), and 1996 - 2001 (n = 115).

Posttransplant fractures by location

Fractures divided by location are shown in Fig. 4 and Table 3. Of the 158 patients with fractures during the first 8 years after OLT, 99 patients (28.5%) sustained spinal fractures, which occurred on average at 1.9 ± 2.0 years after OLT. Twenty-six patients had a single spinal fracture (7 lumbar, and 19 thoracic fractures), and 63 patients had multiple spinal fractures (6 at lumbar spine, 16 at thoracic spine, and 41 both at the thoracic and lumbar regions).

Seventy-eight patients (22.6%) had rib fractures: 23 patients had a single fracture, and 55 patients had multiple rib fractures. Rib fractures occurred on average 1.3 ± 1.7 years after transplantation. In addition, 26 (7.6%) patients had pelvic fractures, 12 patients (3.2%) had femoral neck fractures and 25 patients (7.2%) had other fractures (including humerus, clavicle, wrist, ankle, calcaneus, and metatarsal bones). Pelvic fractures occurred on average 1.8 ± 1.9 years after OLT, and other fractures (including femur) occurred on average 2.3 ± 2.2 years after OLT.

Posttransplant AVN

After OLT, AVN occurred in 27 patients (16 PSC and 11 PBC; 13 males and 14 females) with a cumulative incidence of 8.9% (Fig. 4) at a mean of 2.4 ± 3.6 years after OLT. Patients with AVN had a lower BMD both before and after OLT than those without AVN (0.77 ± 0.14 versus 0.87 ± 0.16 g/cm^2, $p < 0.01$) and more fracturing before transplantation (38.5% versus 17%, $p < 0.05$) and after transplantation (85.2% versus

41.4%, $p < 0.05$). Most patients with AVN (25 of 27) underwent transplantation during periods 1 and 2 (13 and 12 patients, respectively). The sites of posttransplant AVN were the proximal femur (22 patients), the distal femur (3 patients), the proximal humerus (1 patient), and the metatarsal bone (1 patient). Of the 22 patients with proximal femur AVN, 6 patients had unilateral AVN, and 16 had bilateral AVN; 3 had preceding femoral neck fractures.

Table 3. Cumulative incidence of fractures by location and avascular necrosis (AVN) in the total patient population.

Fractures	Time after OLT									
	4 month		1 year		2 year		4 year		8 year	
	CI #	N	CI #	N	CI #	N	CI #	N	CI #	N
Spinal	36 (10%)	292	66 (18.4%)	250	80 (22.3%)	227	87 (24.4%)	196	99 (28.5%)	102
Rib	33 (9.2%)	296	54 (15%)	263	60 (16.7%)	248	70 (19.6%)	213	78 (22.6%)	107
Pelvic	5 (1.4%)	321	14 (3.9%)	298	19 (5.3%)	282	20 (5.6%)	252	26 (7.6%)	139
Other*	12 (3.3%)	315	15 (4.2%)	298	18 (5%)	284	23 (6.5%)	249	32 (9.5%)	136
AVN	3 (0.8%)	323	13 (3.6%)	299	17 (4.7%)	285	21 (5.9%)	251	27 (8.9%)	140

* Including patients with femur fractures. Other locations were humerus, clavicle, wrist, ankle, calcaneus, and metatarsal bones. NOTE: N, number of patients at risk; CI #, cumulative incidence of fractures.

Risk factors for posttransplant fractures

Univariate analysis indicated that many factors correlated with posttransplant fractures: older age, female gender and postmenopausal status, poor nutritional status, muscle wasting, underlying disease of PBC, low BMI, low OLT number, low BMD, the presence of pretransplant fractures, and elevated serum alkaline phosphatase levels (Table 4). Posttransplant risk factors for fracturing were the average daily dose of corticosteroids at 1 month and at 4 months, cyclosporine therapy, rejection episodes, and low BMD (Table 5). No other correlations were seen after OLT, including hospitalization days, changes in BMD (early bone loss or later bone gain), serum levels of calcineurin inhibitors (cyclosporine, tacrolimus), and all biochemical indices.

Multivariate analysis indicated that the independent pretransplant predictors for posttransplant fractures were pretransplant fractures, low BMD, and underlying PBC disease. In addition, independent posttransplant predictors are glucocorticoid doses and low BMD.

Risk factors for posttransplant AVN

Univariate analysis of pretransplant parameters data indicated that low BMI, low BMD, low serum triglyceride levels, low OLT number, and pretransplant fractures correlated with posttransplant AVN (Table 4). The changes in triglyceride levels from the

pretransplant period to the posttransplant period were greater in those with AVN [baseline 93.3 ± 47.2 to 213.2 ± 181.0 mg/dL after OLT] than in patients without AVN [121.2 ± 70.3 to 156.2 ± 89.6 mg/dL, $p < 0.0001$]. This increase correlated univariately with AVN [hazard ratio (HR) = 0.99, 95% confidence interval (CI) = 0.98 – 1.00, $p < 0.05$]. Posttransplant risk factors for AVN (Table 5) were high 4-month average steroid doses, cyclosporine therapy, the presence of nonanastomotic biliary strictures, rejection episodes, posttransplant fractures, low BMD, elevated levels of alkaline phosphatase or cholesterol, and low levels of serum creatinine.

Multivariate analysis indicates that independent pretransplant predictors for posttransplant AVN are pretransplant fractures, low BMI, low OLT number, and low triglycerides. Independent posttransplant predictors for posttransplant AVN were nonanastomotic strictures and fractures.

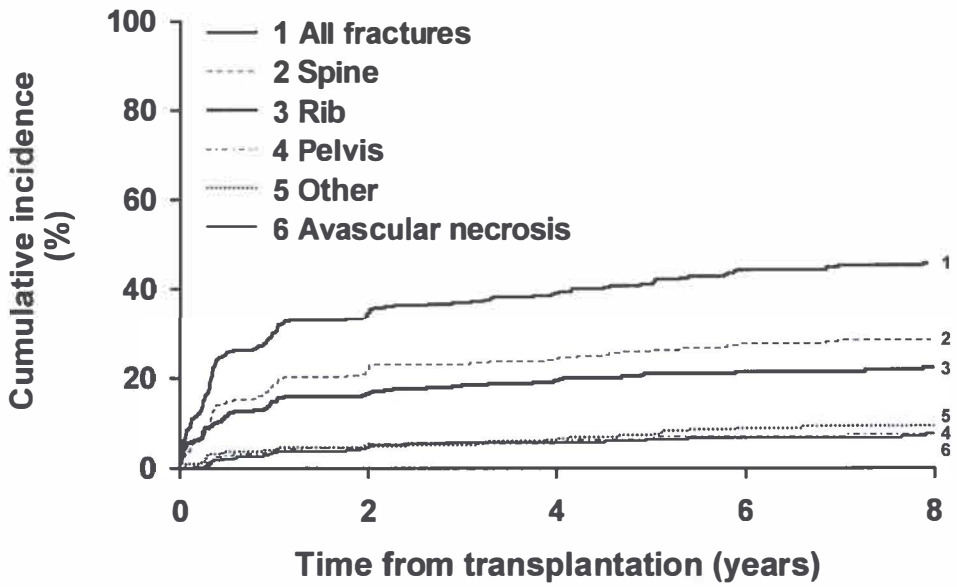

Figure 4. Cumulative incidence of posttransplant fractures (by fracture location) and AVN.

Table 4. Pretransplant risk factors for posttransplant fractures and avascular necrosis.

	Fractures			Avascular necrosis		
Variables*	**Hazard Ratio**	**95% CI**	**p-value**	**Hazard Ratio**	**95% CI**	**p-value**
Age at OLT (yrs)	1.03	(1.01 - 1.04)	< 0.01	0.97	(0.94 - 1.01)	NS
PSC disease[#]	0.70	(0.51 - 0.94)	< 0.05	1.16	(0.52 - 2.59)	NS
Male gender	0.73	(0.53 - 1.00)	0.05	1.46	(0.66 - 3.20)	NS
Menopausal status	1.59	(1.03 - 2.45)	0.05	0.72	(0.23 - 2.19)	NS
Duration of disease (yrs)	1.005	(0.98 - 1.03)	NS	0.93	(0.85 - 1.02)	NS
Poor nutritional status	1.57	(1.09 - 2.27)	0.05	1.46	(0.50 - 4.26)	NS
Muscle wasting	2.95	(1.43 - 6.08)	0.01	2.82	(0.37 - 21.53)	NS
BMI (kg/m^2)[##]	0.93	(0.89 - 0.96)	0.001	0.82	(0.72 - 0.94)	0.01
MELD score	1.001	(0.99 - 1.02)	NS	0.97	(0.92 - 1.02)	NS
Child-Turcotte-Pugh score	1.04	(0.95 - 1.13)	NS	0.95	(0.76 - 1.19)	NS
Karnofsky score	1.02	(0.92 - 1.31)	NS	1.01	(0.77 - 1.33)	NS
OLT number	0.999	(0.998 - 1.00)	0.001	0.998	(0.997 - 1.00)	0.05
PreOLT fractures[#, ##]	5.56	(4.0 - 7.8)	0.001	3.21	(1.44 - 7.15)	0.01
PreOLT BMD (g/cm^2)[#,##]	0.027	(0.01 - 0.075)	0.0001	0.02	(0.0002- 0.29)	0.01
Albumin (g/dL)	0.88	(0.67 - 1.17)	NS	1.31	(0.63 - 2.72)	NS
Prothrombin time (INR)	0.72	(0.34 - 1.55)	NS	1.16	(0.01 - 2.05)	NS
Bilirubin total (mg/dL)	1.01	(0.99 - 1.02)	NS	1.004	(0.97 - 1.04)	NS
Bilirubin direct (mg/dL)	1.01	(0.99 - 1.03)	NS	1.009	(0.96 - 1.06)	NS
Alkaline phosphatase (U/L)	1.01	(1.00 - 1.00)	0.05	1.00	(1.00 - 1.00)	NS
Creatinine (mg/dL)	0.99	(0.85 - 1.16)	NS	0.58	(0.22 - 1.51)	NS
Calcium (mg/dL)	1.11	(0.88 - 1.41)	NS	1.36	(0.74 - 2.52)	NS
25(OH)D (ng/mL)	0.99	(0.98 - 1.002)	NS	1.005	(0.99 - 1.03)	NS
PTH (pmol/L)	0.96	(0.87 - 1.07)	NS	1.007	(0.78 - 1.30)	NS
Phosphorus (mg/dL)	1.06	(0.88 - 1.28)	NS	1.11	(0.09 - 1.77)	NS
Uric acid	0.973	(0.92 - 1.03)	NS	0.988	(0.87 - 1.12)	NS
Cholesterol (mg/dL)	1.001	(1.000 -1.002)	NS	1.00	(0.99 - 1.00)	NS
Triglycerides (mg/dL)[##]	1.001	(0.999 -1.003)	NS	0.99	(0.98 - 1.00)	0.05

Multivariate analysis indicated that

[#] Independent positive predictor for posttransplant fractures is pretransplant fractures (HR 2.77, 95% CI (1.91 – 4.01) $p < 0.0001$); Independent negative predictors are PSC disease (HR 0.67, 95% CI (0.49 – 0.92), $p < 0.05$) and pretransplant BMD (HR 0.98, 95% CI (0.97 – 0.99, $p < 0.0001$).

[##] Independent positive predictor for posttransplant AVN is pretransplant fractures (HR 2.95, 95% CI (1.31 – 6.61) $p < 0.01$); independent negative predictors are BMI (HR 0.80, 95% CI (0.70 – 0.92) $p < 0.05$), OLT number (HR 0.83, 95% CI (0.69 – 0.99) $p < 0.05$) and pretransplant triglycerides (HR 0.39, 95% CI (0.20 – 0.74) $p < 0.01$).

Table 5. Posttransplant risk factors for posttransplant fractures and avascular necrosis

Variables*	Fractures			Avascular necrosis		
	Hazard Ratio	95% CI	p-value	Hazard Ratio	95% CI	p-value
1 mo glucocorticoids (mg)	1.004	(1.001 - 1.007)	< 0.01	1.005	(0.997 - 1.01)	NS
4 mo glucocorticoids (mg)#	1.01	(1.002 - 1.019)	< 0.05	1.02	(1.00 - 1.09)	< 0.05
Cyclosporine serum (ng/mL)	0.997	(0.99 - 1.00)	NS	0.99	(0.99 - 1.01)	NS
Tacrolimus serum (ng/mL)	0.94	(0.86 - 1.04)	NS	2.64	(1.10 - 6.32)	< 0.05
Cyclosporine treatment	1.85	(1.22 - 2.79)	< 0.01	2.95	(0.99 - 8.76)	< 0.05
Rejection episodes	1.23	(1.07 - 1.57)	< 0.01	1.49	(0.99 - 2.34)	0.056
Hospitalization days	1.001	(0.998 - 1.004)	NS	0.997	(0.99 - 1.01)	NS
Non-anastomotic biliary strictures##	1.16	(0.67 - 2.03)	NS	5.09	(2.24 - 11.55)	<0.0001
PostOLT BMD (g/cm^2)#	0.027	(0.008 - 0.092)	< 0.0001	0.034	(0.003 - 0.382)	< 0.01
BMD loss (PreOLT – 4mo)	0.90	(0.002 - 3.77)	NS	1.11	(0.03 - 37.85)	NS
BMD gain (4-24 mo)	0.172	(0.012 - 2.55)	NS	0.15	(0.01 - 2.36)	NS
PostOLT fractures##	-	-	-	2.64	(1.10 - 6.32)	< 0.05
Albumin (g/dL)	0.80	(0.58 - 1.10)	NS	1.20	(0.54 - 2.66)	NS
Prothrombin time (INR)	0.74	(0.27 - 1.98)	NS	0.13	(0.004 - 4.38)	NS
Bilirubin total (mg/dL)	1.007	(0.99 - 1.03)	NS	1.002	(0.94 - 1.07)	NS
Bilirubin direct (mg/dL)	1.02	(0.99 - 1.05)	NS	1.21	(0.67 - 1.68)	NS
Alkaline phosphatase U/L)	1.00	(1.00 - 1.00)	NS	1.001	(1.00 - 1.001)	< 0.01
Creatinine (mg/dL)	1.09	(0.90 - 1.32)	NS	0.20	(0.05 - 0.79)	< 0.05
Calcium (mg/dL)	1.12	(0.85 - 1.47)	NS	1.18	(0.64 - 2.19)	NS
25 (OH)D (ng/mL)	1.003	(0.99 - 1.01)	NS	0.99	(0.97 - 1.03)	NS
PTH (pmol/L)	0.99	(0.94 - 1.05)	NS	0.92	(0.73 - 1.16)	NS
Phosphorus (mg/dL)	1.06	(0.84 - 1.35)	NS	0.78	(0.42 - 1.46)	NS
Cholesterol (mg/dL)	1.001	(0.99 - 1.00)	NS	1.003	(1.00 - 1.005)	< 0.05
Triglycerides (mg/dL)	1.00	(0.99 - 1.00)	NS	1.003	(1.00 - 1.01)	NS

Multivariate analysis indicated that

#independent positive predictor for posttransplant fractures is 4 months daily steroids (HR 1.02, 95%CI (1.01 - 1.02), p < 0.001); independent negative predictor is posttransplant BMD (HR 0.98, 95% CI (0.97 – 0.99), p < 0.0001)

##Independent positive predictors for posttransplant AVN are posttransplant non-anastomotic strictures (HR 4.87, 95% CI (1.8 – 13.5), p < 0.01) and posttransplant fractures (HR 74.1, 95% CI (27.2 – 201.7), p < 0.0001)

Discussion

The majority (80%) of patients with CCLD in this study had osteoporosis or osteopenia before OLT, and 20% had radiologic evidence of fractures. A Pretransplant radiologic assessment was performed in 95% patients, but not all patients had long-term radiologic screening before OLT. The 20% prevalence of fractures may therefore be an underestimation. Pretransplant fractures were associated with low BMD, and the majority occurred in trabecular bone (spine and ribs), whose higher rate of bone turnover makes it more susceptible to changes in bone metabolism. These findings are consistent with previous studies in chronic liver disease both before and after OLT.[28,29]

After OLT, the fracture rate abruptly increases with 25% of patients sustaining a new posttransplant fracture by 6 months, with an ongoing fracture rate thereafter, affecting 46 % of patients by 8 years after OLT. There are few previous studies with which to compare our data; Leidig-Bruhner et al[1] analyzed posttransplant vertebral fractures by Kaplan-Meier analysis, also indicating a high posttransplant vertebral fracture rate (33%) but a nonvertebral fracture rate of only 7%. In our study, nonvertebral fractures are as common as vertebral fractures. Although the spine was the commonest site of posttransplant fracturing, the cumulative incidence of rib fractures is almost as great. Overall, most fractures occurred at sites of trabecular bone, with the spine and ribs accounting for more than 90% of total fractures. The difference in nonvertebral fracture rates in Leidig-Bruchner et al.'s study is probably related to methodologic differences in fracture screening.

Pretransplant BMD and pretransplant fracturing are clearly identified as major risk factors for posttransplant fracturing. The severity of osteoporosis/osteopenia at the time of OLT is very important: posttransplant fractures in the first 12 months after OLT occurred in 50% of patients with pretransplant BMD less than 0.75 g/cm^2 compared to only 18% of patients with pretransplant BMD greater than 0.96 g/cm^2. In addition, 80% of patients with pretransplant fractures sustained a new posttransplant fracture after OLT. Surprisingly, neither the rate of bone loss during the first 4 months after OLT nor rate of bone gain thereafter correlated with fracturing.

Posttransplant fractures also correlated independently with age. Advanced age is associated with osteopenia, but other factors such as muscle mass, coordination and activity may play an etiologic role in fracture occurrence. Female patients sustained more posttransplant fractures than male patients (34% versus 25% at 1 year), and this was due entirely to the high fracture rate in postmenopausal females; premenopausal female patients had fracture rates similar to those of male patients. Overall, PBC patients had more fractures after OLT than PSC patients, and this likely reflects an age and postmenopausal influence.

In parallel with improved pretransplant BMD over the 16-year study period,[15] fewer fractures were seen in more recently transplanted patients both before and after OLT. The reduction in pretransplant fracturing with time was significant only in PSC patients, whereas fracture rates remained stable in PBC patients despite an aging and more menopausal patient population. It is likely that temporal improvements in nutritional

status, BMI and vitamin D levels have contributed to less fracturing in PSC patients and to the stable fracture rates in PBC patients, in the latter situation offsetting the negative effects of increased age and postmenopausal status.

Temporal improvements of posttransplant fracture rates were seen in both patients with PBC and patients with PSC. Bone loss during the first 4 posttransplant months did not change with time, and this implied that increased BMD before OLT in the more recently transplanted patients was important in causing the temporal reduction in posttransplant fracturing. The lower cumulative doses of glucocorticoids 1 and 4 months after OLT were likely responsible at least in part for this improvement. Bone histomorphometric studies have indicated the important effect of glucocorticoids early after OLT, with the main effect being to decrease bone formation rates.[29,30] As shown by others,[31,32] patients on cyclosporine had more fracturing than patients on tacrolimus, but this effect may well reflect the differences in average steroid dose between the two groups. Both cyclosporine and tacrolimus cause increased bone turnover,[33,34] but these histomorphometric effects in liver recipients have not been demonstrated. Whether temporal improvement in fracturing will be maintained in the MELD era is unknown. No correlation was seen here between the fracturing and MELD score, the individual components of MELD, or disease duration, and this is encouraging. Nonetheless, a longer wait for OLT, increasing age, and debility may take its toll.

AVN is regarded as a different etiologic entity from osteopenic fracturing and thought to correlate with abnormalities in the vascular supply to bone, particularly at the femoral head.[9,10,13] A well-known risk factor in the general population is femoral neck fracturing. Scant data are available about AVN after OLT.[4,35,36] In this study, the cumulative incidence of AVN after OLT was 9%. The majority (80%) occurred at the femoral head, but only 3 patients had a femoral neck fracture preceding the diagnosis of AVN. Posttransplant AVN was not influenced by gender or disease, but correlated with pretransplant and posttransplant BMD and fractures. Eighty-five percent of patients with posttransplant AVN also sustained fractures after OLT. This suggests that bones prone to fracturing also have abnormalities predisposing them to AVN.

In this study, posttransplant AVN also correlated with posttransplant glucocorticoids. Glucocorticoids are a well-recognized risk factor for AVN, with several potential etiologic mechanisms; increased fat emboli in the microvasculature of bone, glucocorticoid-induced apoptosis of osteocytes,[12] or an increase in size of intraosseous adipocytes, with subsequent increase in femoral head pressure and decrease of blood supply.[10,11] It is tempting to speculate that this latter mechanism may be involved in the observed association of AVN with cholesterol levels and with the pretransplant-to-posttransplant increase in triglyceride levels. The temporal improvement in the occurrence of AVN with increased use of tacrolimus may be explained by the use of lower doses of glucocorticoids.

An unexpected association was seen between AVN and both posttransplant nonanastomotic biliary strictures and alkaline phosphatase levels, suggesting a potential etiologic role for cholestasis. Cholestasis is well known for its suppressive effect on bone metabolism, with reductions in osteoblastogenesis and osteoblastic

proliferation in vitro[37], and reduced osteoblast number and function in vivo.[29,30] Osteocytes are derived from osteoblasts and may have a role in the mechanosensory function of bone. A decrease in osteocytes, perhaps potentiated by cholestasis, may cause mechanosensory disturbances with subsequent collapse of bone, disruption of its vascular supply and consequently AVN.[12] An etiologic connection between cholestasis and AVN may also contribute to the increased incidence of AVN (1.4%) in end-stage cholestatic patients before OLT versus an incidence of less than 0.01% of the US population according to the National Institute of Health registration.

In summary, at the time of OLT, 20% of patients with CCLD have already experienced osteopenic fractures and 1.4% of patients have had AVN. The highest rate of fracturing occurs in the first 12 months after OLT (cumulative incidence of 30%); thereafter, there is a smaller but steady cumulative increase in fracturing, so that by 8 years, almost 46% of patients have sustained a fracture. Overall, the majority (> 90%) of fractures occur at sites of trabecular bone (the spine, ribs and pelvis). In contrast to previous studies, vertebral and non-vertebral fractures occur with similar incidences after OLT. The greatest risk factors for posttransplant fracturing are pretransplant fracturing and the severity of osteopenia, PBC disease, and posttransplant glucocorticoids. In addition to fractures, 9% of liver recipients experienced AVN after OLT, and this correlated with pretransplant and posttransplant lipid metabolism, bone disease (BMD and fracturing), and posttransplant glucocorticoids. A novel association between cholestasis and AVN was also identified, the mechanism for which is not known. Fortunately, recent years have seen an increase in bone mass of liver recipients and, along with this, less fracturing and less AVN. Nonetheless, 25% of patients undergoing OLT for CCLD still develop de novo fractures after OLT; this situation demands an ongoing search for effective therapeutic agents for these patients.

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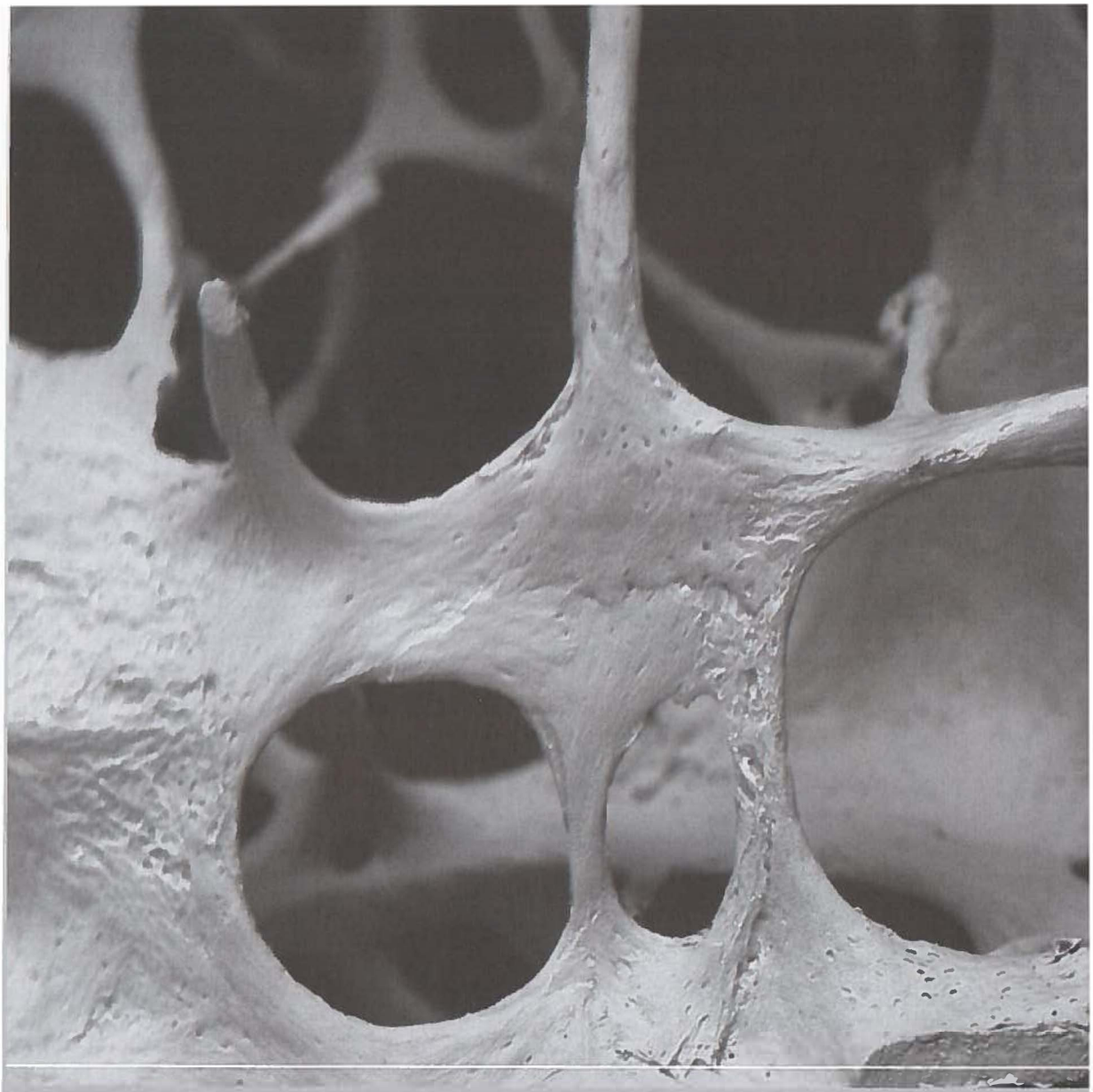

Chapter 7

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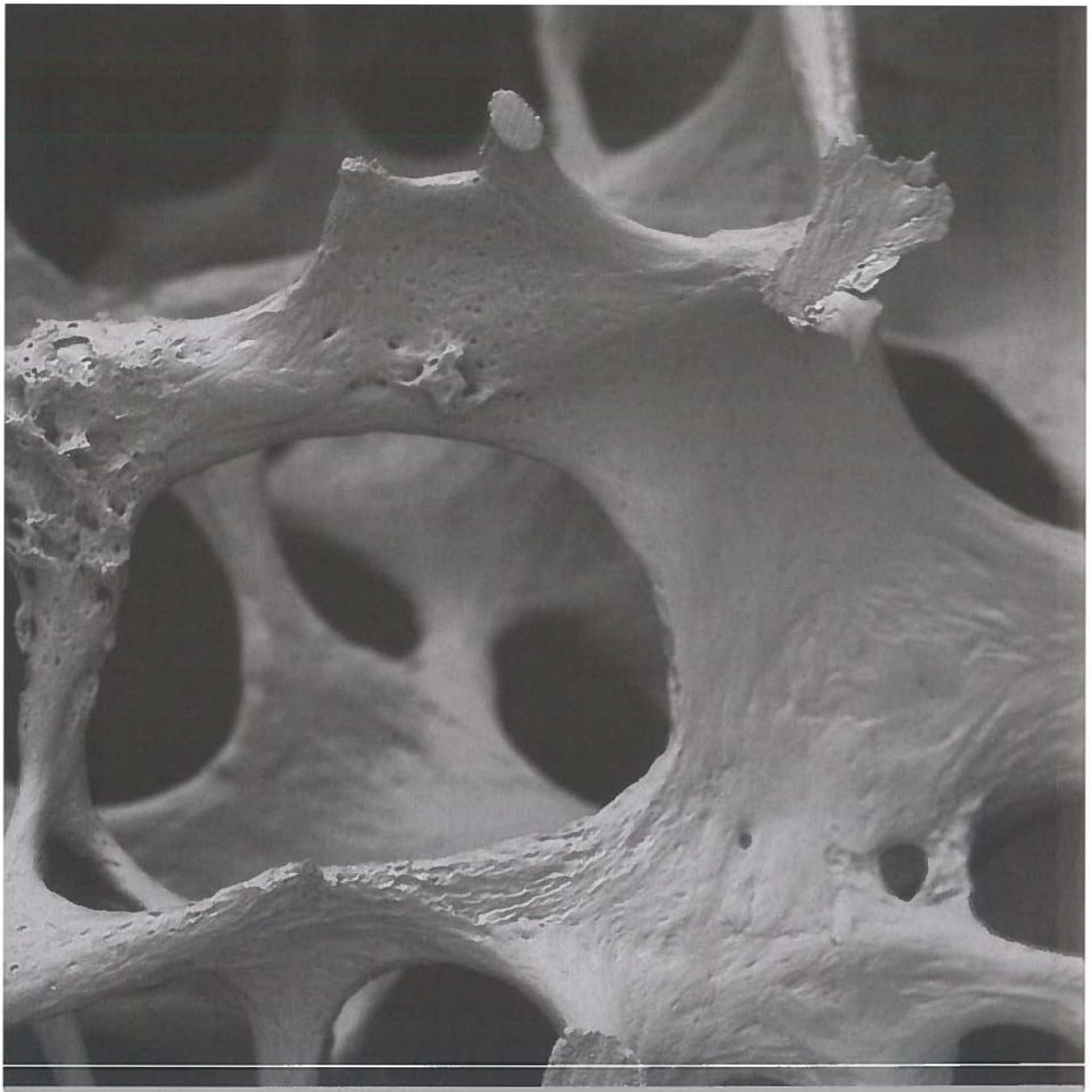

Evaluation and management of osteoporosis in liver disease

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Introduction

Osteoporosis is a well-recognized complication of chronic liver disease.[1] Although best characterized in chronic cholestatic conditions,[2,3] osteoporosis occurs in cirrhosis of all etiologies, from autoimmune hepatitis to alcoholic liver disease, hemochromatosis and viral hepatitis.[4-9] Many different etiologic factors contribute to the occurrence of osteoporosis in liver disease and these vary according to the type, severity and progression of the liver disease, as well as other contributing conditions.

The rationale for evaluation and management of osteoporosis is prevention of the clinical morbidity of pain and immobility due to fracturing. Most fracturing in liver patients occurs after liver transplantation (OLT), but pretransplant osteoporosis is the main risk factor for posttransplant fracturing and therefore its understanding and subsequent management are of prime importance.[10]

By World Health Organization (WHO) criteria, osteoporosis is the term used for bone of reduced bone mass (or density) to less than 2.5 standard deviations below normal adult peak bone mass, adjusted for male or female sex; in other words, a T-score of less than –2.5. Implicit in this definition is that the bone, albeit of small volume, is normally mineralized and has no other pathological changes. A milder degree of reduced bone density is called osteopenia (T-score of –1.0 to –2.5), which is also used as a collective term for both osteopenia and osteoporosis.

This chapter will discuss the clinical importance of hepatic osteopenia, the identification of risk factors for the individual patient and the selection of patients, timing and methods for diagnostic screening. General supportive measures to maximize bone health should be utilized in all patients at risk and, for the patient with established osteoporosis, specific therapeutic measures may be justified, despite the lack of adequate randomized trials of these agents in patients with hepatic osteopenia.

Clinical importance of osteoporosis in chronic liver disease

The incidence of osteoporosis and fracturing in different types of chronic liver disease varies widely, depending on the patient population, the underlying liver disease and its severity. It is characterized best in primary biliary cirrhosis (PBC), with an overall incidence of osteoporosis of 20 to 30% with fractures occurring in 7-14% of patients.[11-14] In end-stage disease PBC, the incidence of osteoporosis was 41% (15) with fractures in 21% of patients.[15] Most fractures occur in the spine and ribs. Generally, patients with primary sclerosing cholangitis (PSC) have fewer symptoms from osteopenia than patients who have PBC, because they are younger and predominantly male, but for their age and sex, they have just as much bone loss as patients who have PBC. In advanced PSC, 32% patients had osteoporosis and 16% fractures.[15] Osteopenia is equally prevalent in children with chronic cholestasis and this worsens with disease severity and progression.[16-18]

Significant osteoporosis is reported increasingly in patients with posthepatitic cirrhosis, especially secondary to hepatitis C. The pretransplant T score of 68 patients with hepatitis C was -1.43 with 28% having osteoporosis and only 35% having normal bone mass[6], a finding confirmed by other studies.[19] On the other hand, a large Japanese study of hepatitis C found that only women over 60 years of age had significant

osteopenia compared to the general population.[20] Osteoporosis has been confirmed in 18% to 23% of patients who have alcoholic liver disease,[6,21] hemochromatosis[8,22] and autoimmune hepatitis.[23] Several studies of patients who had end-stage liver disease of varying etiologies confirm a high but variable incidence of osteoporosis (11% to 48%) an osteopenia (18% to 35%).[4,24-27]

Despite its incidence, hepatic osteopenia often is overshadowed by more urgent complications of advanced liver disease. Most liver transplant recipients, however, lose bone mass in the first 3 to 6 months after OLT, with a high incidence of posttransplant fracturing in 30% to 40% of patients with cholestatic liver disease. Patients at highest risk of posttransplant complications are those who are already osteopenic before OLT, especially those with pretransplant fractures. The main indication for aggressive management of hepatic osteopenia is to prevent posttransplant fracturing.

Risk factors for osteopenia in chronic liver disease

Bone undergoes continuous remodeling with a balance of bone resorption and formation, a highly complex process regulated by many hormones and growth factors.[28-30] Bone mass is mainly under genetic control but also essential are mechanical stress from weight-bearing, good nutrition, adequate calcium and vitamin D and a normal hormonal environment. Multiple complications of chronic liver disease may cause bone loss: poor nutrition, inadequate calcium intake, vitamin deficiencies, immobility and muscle wasting, hypogonadism, drugs and life style factors (alcoholism, smoking). In addition, chronic cholestasis and cirrhosis itself are recognized as additional risk factors for osteopenia (Table 1). The contribution of different risk factors to the development of osteopenia in different types of liver disease will be briefly discussed.

Abnormalities of calcium and vitamin D

Many abnormalities of calcium and vitamin D occur in the osteopenia associated with PBC and PSC. These include reduced dietary intake, reduced absorption of both calcium and vitamin D, low serum levels of 25hydroxyvitamin D[31-33] and impaired cutaneous synthesis of vitamin D in the presence of jaundice. 1,25 dihydroxyvitamin D, however, is often normal[33], 25-hydroxylation of vitamin D by the liver is usually preserved[34] and no correlation has ever been identified between osteopenia and low levels of vitamin D or calcium or parathyroid dysfunction.[2,13] Histomorphometric studies have shown no evidence of osteomalacia.[35] Even more compelling against a pathogenic role for vitamin D in cholestatic osteopenia, are two trials[36,37] in which vitamin D therapy did not prevent ongoing bone loss in primary biliary cirrhosis (PBC) despite normalizing serum vitamin D levels.

In alcoholic cirrhosis, hemochromatosis and autoimmune hepatitis, osteoporosis and low vitamin D levels are common but no consistent correlation of osteopenia and indices of calcium or vitamin D metabolism has been found and most histomorphometric studies show no osteomalacia.[4,26,38-40] However, in patients with posthepatitic cirrhosis due to hepatitis B or C, bone mineral density (BMD) has correlated with vitamin D levels.

Hypogonadism

Sex hormone levels are important for regulation of bone mass, and hypogonadism leads to "high-turnover" bone loss and osteopenia.[41] Hypogonadism is a common feature of chronic liver disease in both males and females with reduced gonadotrophin release from the hypothalamus or primary gonadal dysfunction. In males with cirrhosis due to hepatitis B or hepatitis C, serum testosterone and estrogen levels were within the normal range until decompensation of cirrhosis occurred with a decrease in testosterone and increase in oestrogen levels.[42] Amenorrheic females with cirrhosis and both cirrhotic and noncirrhotic alcoholic liver disease commonly have low serum levels of FSH, LH and oestradiol but normal levels of sex-hormone binding globulin and testosterone.[43]

Despite the frequency of these hormonal abnormalities in liver disease, evidence for any correlation with osteopenia is scant. Low testosterone levels are common in alcoholic cirrhosis but correlation with osteopenia has not been established.[4,26,39] Osteopenia in hemochromatosis is associated with low testosterone levels although iron overload may also play a role here.[8,22] Patients with PBC are frequently postmenopausal; despite this, menopausal status is not an independent risk factor for osteopenia in large studies of PBC patients.[13,14] Hypogonadism therefore does not appear to be a major risk factor for osteopenia in patients with liver disease.

Body mass index and nutritional factors

Low body mass index (BMI) is a known risk factor for osteoporosis, and indeed in two large studies in PBC, this correlation has been confirmed, suggesting the importance of muscle mass and nutrition to normal bone mass.[13,14] Patients with advanced liver disease may be deficient in vitamin K. Osteocalcin, a bone matrix protein synthesized by osteoblasts, is vitamin K-dependent but the importance of vitamin K in regulating normal bone metabolism is still poorly defined.[44] There are no randomized, prospective studies with adequate numbers of patients to assess the effect of vitamin K on osteoporotic bones and no studies have linked osteopenia in humans to vitamin K deficiency. No relationship has been found between osteopenia of PBC and vitamin K levels. In a small study of 50 cirrhotic females with viral hepatitis, vitamin K therapy for 2 years prevented the bone loss seen in the controls.[45]

Chronic alcohol ingestion, even without liver disease, may be detrimental to bone metabolism, inducing osteoporosis and fracturing[38], possibly by promoting osteoclastogenesis.[26] A histologic study of 22 chronic alcoholics, 11 of whom had severe liver disease, showed osteoporosis with reduced bone formation, especially in those who drank only spirits; fracture risk increased with heavy drinking.[47]

Genetic factors: vitamin D receptor genotype

Genetic factors play the predominant role in the determination of bone mass[48] and polymorphisms for the vitamin D receptor and collagen type 1 a 1 gene are being investigated for any potential role in the regulation of bone mass. In osteoporotic women with PBC, correlation of the VDR genotype with bone mass and the risk of vertebral fracture was positive in one study[49] but negative in another.[50] In 55 men with alcoholic or posthepatitic cirrhosis, no relationship was found between lumbar BMD and VDR genotype.[51,52] The *b* alleles of the VDR gene may have no relationship to BMD or the effect of liver disease may have overwhelmed or eliminated their effect.

Table 1. Risk factors for osteoporosis

Risk factors for osteoporosis
Genetic factors
Age (after peak bone mass at about 40 years)
Female sex
Family history (e.g. maternal hip fracture younger than 60 years old)
White race
Nutritional and lifestyle factors
Low BMD (< 19)
Physical inactivity
Alcohol excess
Smoking
Low calcium intake
Hormonal factors
Hypogonadism
Premature menopause (< younger than 40 years)
Excessive thyroid replacement
Past/present history
Prolonged corticosteroids (5 mg or more for at least 3 months)
Previous atraumatic fracture
Loss of height
Other medications
Factors specific to liver disease
Cirrhosis of any etiology
Cholestasis – bilirubin greater that times ULN for 6 months
?? deficiency of vitamin K

transplant T-score of lumbar BMD in patients with PBC (mean T score -2.22) and PSC (-1.93) was significantly lower than in patients with chronic active hepatitis (-1.23) and with alcoholic cirrhosis (-0.86).[55] Many studies have shown that osteopenia increases with disease severity, suggesting a correlation between osteopenia and cholestasis. In ambulatory PBC patients,[56] the Z-score of lumbar BMD correlated inversely with the bilirubin level. Two large studies of patients with PBC with all stages of disease,[13,14] the severity of osteopenia was most severe in patients advanced histologic disease and high bilirubin levels. Patients who have early PSC have normal bones, but patients with advanced disease have significant osteopenia.[57]

There remains some debate about whether cholestatic osteopenia is a distinct entity, but most of the studies with no correlation between osteopenia and cholestatic disease are limited by small patient numbers.[58,59] A recent large retrospective British study of 272 patients with probable PBC found the mean *z* scores to be normal;[60] however, this population was neither cholestatic nor decompensated. Therefore these patients would

not be expected to be osteopenic. Present data support the entity of cholestatic osteopenia in both females and males, as not simply an age-related or menopausal phenomenon.

The actual connection between osteopenia and cholestasis remains unknown with no proven etiologic role for abnormalities of calcium or vitamin D or parathyroid dysfunction. Pretransplant bone biopsies of cholestatic osteopenia has shown low bone formation rates and evidence of increased resorption,[35] but no correlation has been found between any histomorphometric abnormality and bilirubin, hepatic synthetic function, or indices of calcium and vitamin D. Does bilirubin affect bone metabolism directly? Osteoblast proliferation in vitro was inhibited by unconjugated bilirubin and by the serum of jaundiced patients,[61] and this is consistent with a rat model for cholestatic osteopenia where less bone formation was seen.[62]

Cirrhosis

A correlation of osteoporosis with the histologic severity of chronic liver disease, independent of other risk factors, is seen, not only in cholestatic liver disease, but also in alcoholic cirrhosis, chronic active hepatitis, hemochromatosis and posthepatitic cirrhosis.[4,19,26,63] The etiologic link between cirrhosis and bone loss is understood poorly, but it may involve low levels of insulin-like growth factor (stimulates osteoblast proliferation) or abnormalities of growth factor, growth factor-binding protein or osteoprotegerin, low levels of which may led to increased bone resorption.[64] Portosystemic shunting has been implicated in some animal studies.[65]

Corticosteroids and other drugs

Corticosteroids have many effects on bone[66] all of which cause bone loss. These factors include increased resorption, decreased formation, and calcium malabsorption. This seems likely to be a major factor in osteopenia in autoimmune hepatitis, although the present studies do not allow separation of the effects of the liver disease from that of the steroids.[9]

Patients should be screened for any other drugs which may predispose to bone loss, including anticonvulsants, anticoagulants, lithium, tamoxifen, immunosuppressants, long-acting benzodiazepines, and over-replacement of thyroid hormone.

Risk factors in the individual patient

There are multiple causes of bone loss in chronic liver disease (Table 1) but major factors are advanced cirrhosis and chronic cholestasis. For the individual patient who has PBC, two large trials, one from the United States[13] and one from Europe,[14] confirm that the risk of osteoporosis correlates with patient age, histologic severity of liver disease, and BMI. In the former study, 72% of patients with stage 3/4 PBC, who were over 57 years, with a BMI of less than 24, had osteoporosis compared to none with stage 1/ 2 disease, younger than 57 years with a BMI more than 24. There was a gradation of risk between these two extremes; males have as severe disease as females, and in neither study was menopausal status a risk factor. Similarly, osteopenia in PSC correlates with more advanced disease and patient age.[3,57] Risk factors for non-cholestatic liver diseases are less well characterized, but they include include steroid therapy in autoimmune disease.

Screening for osteopenia

Diagnosis by BMD measurement

The rationale for treatment of osteopenic bone disease is prevention of fracturing. Bone density accounts for about 80% of variability in bone strength to resist fracturing and prospective studies have shown that fracture risk increases with decreasing bone density. Thus the degree of osteopenia, as measured by BMD, is used as a surrogate for bone strength to assess a patient's fracture risk. Until fractures occur, no clinical or biochemical marker of calcium or vitamin D metabolism, cholestasis, or hepatic synthetic function will identify the patient with osteopenia; therefore BMD must be measured directly. There are biochemical markers of bone metabolism: serum levels of procollagen propeptides of type 1 collagen, osteocalcin and bone alkaline phosphatase are markers of bone formation and urinary excretion of pyridinium cross-links, especially pyridinoline and deoxypyridinoline, are markers of bone resorption.[67] Unfortunately these markers are affected by the extent of liver collagen metabolism in patients with chronic liver disease and are unreliable in these patients.[53,68] In cholestatic patients undergoing bone biopsies, no correlation was seen between histomorphometric and biochemical markers.[69]

There are several sensitive, highly specific and non-invasive techniques that measure BMD with excellent precision; the most commonly used method is dual-energy x-ray absorptiometry (DEXA). BMD measurements, generally taken at the lumbar spine or femoral neck, are recorded as an absolute value and also as a T-score, a sex-adjusted value compared to peak bone mass of the normal population. In the elderly or in those with previous vertebral fractures, the lumbar spine measurement may be unreliable due to degenerative changes, compression fractures and deformities.

Which patients to screen

Based on the known risk factors for hepatic osteopenia, the following patients, both male and female, with liver disease should undergo measurement of BMD:

- All patients with chronic cholestasis (bilirubin > 3mg/dL for 6 months)
- All patients with cirrhosis
- Pre-cirrhotic patients with additional risk factors (Table 1)
- All potential liver transplant candidates.

Clinical progression is followed by serial measurements, depending on baseline level and other risk factors. Repeat screening in 2 years is undertaken in patients with normal or mildly osteopenic BMD. If treatment for osteoporosis is introduced, the efficacy of therapy can be followed by serial measurements of BMD. Patients with osteoporosis should undergo radiographs of thoracic and lumbar spine to assess for compression fractures.

Management of osteopenia in chronic liver disease

Management of the patient with hepatic osteopenia involves correction of reversible risk factors, supportive measures to maximize bone health, and consideration of specific therapies for established osteoporosis or fracturing. Unfortunately there are no randomized studies looking at efficacy of therapy to prevent fractures in patients with chronic liver disease. Available clinical studies are small, limited to patients with PBC, rely on BMD alone, and are underpowered to look at fractures.

Correction of any risk factors

All reversible causes which exacerbate bone loss, should be sought aggressively and treated. Screening for indices of calcium and vitamin D metabolism, including parathyroid hormone level, should be performed and any obvious deficiency treated. Gonadal and thyroid status should be assessed. Drug therapy should be reviewed, and any osteopenia-producing drugs reduced to minimum, especially steroids. Hypothyroidism should not be over-treated. Life-style changes to promote bone health should be considered, such as no smoking and minimal alcohol.

Supportive measures for bone health

With no proven effective prevention or therapy for hepatic osteopenia, effective supportive measures to reduce bone loss should be introduced early.

Nutrition

The regular intake of adequate amounts of protein and calories is essential to maximize bone and muscle health.[70] Soy proteins which contain isoflavones, may have beneficial effects on calcium balance in postmenopausal women without HRT.[71]

Exercise

Regular exercise, combined with adequate dietary calcium, can increase the mineral content of bone.[72] Exercise must be ongoing and tailored to the individual to avoid further musculoskeletal complications and noncompliance but the optimal exercise program is not known. The minimum desirable level of activity is full mobility with a regular walking program and exercises for the care and strengthening of the back with the goal of 30 to 60 minutes of weight-bearing exercise five times per week. A physical therapy program may be necessary for the implementation of activity in the debilitated patient.

Calcium supplementation

A diet high in calcium has been shown to be bone-protective in age-related bone loss [73] and in post-menopausal osteoporosis,[74] but in a retrospective study, no difference in BMD was seen between PBC patients with and without calcium supplements.[75] In a small cross-over study in PBC, the introduction of calcium supplementation to patients treated with calcitonin and to controls resulted in increased bone mass in both groups.[12] Although the effectiveness of calcium supplements in patients with liver disease is unproven, adequate dietary calcium to achieve positive calcium balance is essential to maintain bone turnover and repair. The National Institutes of Health (NIH) age-specific

guidelines for daily calcium are 1000 mg/d for all adults and 1500mg/d for all adults at risk of osteoporosis[76].

Table 2. Clinical studies of Vitamin D and Calcium for treatment of hepatic osteopenia

Methods			Results	
Patients	**Study design**	**Duration treatment**	**BMD**	**Study conclusions**
12 PBC females (11 pmp) [36]	100 ug/day oral 25(OH)D - Prospective, no controls	1 yr	Bone biopsy: bone volume decreased, no changes bone formation. BMD of radius decreased (0.82-0.77 g/cm^3)	Despite increases in serum vit D, oral 25(OH)D did not halt bone loss in PBC
8 PBC (7 F, 3 pmp)[37]	Oral 25(OH)D_3 (40-120 µg/d), all Ca (1 g/d) - Prospective, no controls	1 yr	Bone biopsy: changes of bone volume varied in patients BMD-LS decreased in all patients	Oral 25(OH)D did not reverse bone loss
18 male ALC [40]	Vit D_2 (50.000 IU, 2-3 times/wk), or 25(OH)D 20-50 mg/day, or no treatment. - Randomized controlled	> 6 mo (mean 11 mo)	BMD-LS: Increases in vit D_2 (0.69 – 0.82), and 25(OH)D pts (0.62 – 0.79) stable in controls patients (0.62 – 0.65 g/cm).	Hepatic osteoporosis in alcoholic cirrhosis responds to oral 25(OH)D and Vit D_2.
13 HBV, 63 HCV (26M, 50F) [78]	Calcitriol (1α25(OH)D_3, µ/d) (38 pts) or no therapy (39 pts) - Randomized controlled	> 12 mo	BMD-LS change +1.1%/yr in M and –0.5%/yr in F treated patients. Greater BMD-LS losses were seen in controls; –0.4%yr in M, –2.3%/yr in F	Calcitriol prevented bone loss in post-hepatitic cirrhosis
25 PBC females (19 pmp) [12]	Cross-over study; 6 mo placebo or calcitonin All patients received 1g per day of calcium .	12 mo	BMD increased in first treatment period in both groups of patients	Calcium may have transient effect; calcitonin ineffective
203 PBC patients[75]	Retrospective study – 96 patients on oral calcium	12 mo	No difference in BMD-LS between treated and nontreated patients	No effect of calcium supplementation

Abbreviations: ALC, Alcoholic liver cirrhosis; BMD-F, femur BMD; BMD-LS, lumbar spine BMD; Ca, calcium; HBV, hepatitis B cirrhosis; HCV, hepatitis C virus; PBC, primary biliary cirrhosis; pmp, postmenopausal patients; PSC, primary sclerosing cholangitis.

Vitamin D supplementation

Vitamin D has never been proven to be effective in treating osteopenia associated with liver disease (Table 2). In two randomized controlled trials in PBC, vitamin D did not prevent the progression of cholestatic osteopenia despite normalizing blood levels.[36,37] In a small study in alcoholic cirrhosis, vitamin D supplementation appeared beneficial.[40] Adequate oral vitamin D to normalize blood levels seems desirable; this will maximize calcium absorption and avoid any osteomalacia. Supplementation is usually satisfactorily given as 50,000 IU of vitamin D given orally three times per week; some recent reports, in PBC and post-hepatitic cirrhosis, advise the use of calcitriol, the active 1,25 dihydroxyvitamin D[77,78] but this has not been tested against regular vitamin D. Cholestyramine therapy reduces the intestinal absorption of fat-soluble vitamins, including vitamin D, and may contribute to calcium malabsorption.

Specific measures for osteoporosis

Drugs inhibiting bone resorption (antiresorptive drugs) are the predominant therapeutic agents for the treatment and prevention of osteoporosis. The most studied in postmenopausal patients[79] are bisphosphonates, estrogens, the selective estrogen receptor modulators (SERMs), and calcitonin. The more potent agents, which produce larger increases in BMD and affect both trabecular and cortical bone, are the bisphosphonates and estrogens. These reduce the risk of both vertebral and nonvertebral fractures, while calcitonin and the SERM, raloxifene, with their weaker action have lesser benefit on vertebral fracturing and little effect on sites with more cortical bone.[80,81] Some agents which stimulate bone formation are now available.

Hormone replacement therapy (HRT)

Estrogen is antiresorptive for bone and, despite the other potential risks of HRT, it is effective for the prevention and treatment of postmenopausal bone loss and fractures.[82-85] Historically, HRT was avoided in liver patients because of the potential risk of worsening cholestasis but it appears to be safe[75,86,87] and even efficacious in two small studies of 26 postmenopausal PBC patients with an increase in lumbar BMD without any hepatic side effects.[75,86] Similarly in a single case of autoimmune hepatitis with early menopause, HRT was safe and effective.[88] Transdermal estrogen is effective in postmenopausal women and is the preferred route of administration.[89] A recent British study randomized to transdermal estrogen 42 patients who had PBC and were already treated with ursodeoxycholic acid, calcium and vitamin D. Treated patients had an increase in lumbar BMD at 1 year compared to controls with a low incidence of side effects.[90]

With increasing concerns about the risks of HRT[85] consideration should be given to selective estrogen receptor modulators (SERMs). Raloxifene is US Food and Drug Administration (FDA)-approved for treating postmenopausal osteoporosis[91] although trials against placebo using 60 mg/day have shown less bone protection than expected from conjugated estrogens or alendronate. In the Multiple Outcomes of Raloxifene Evaluation (MORE) trial[92], 7700 postmenopausal women were treated over 3 years with

an increase in spine and hip BMD but no decrease in nonvertebral fractures. Phytoestrogens are plant and food products with a mild estrogen effect with some skeletal benefits in animal studies and in one small human study with 56 postmenopausal women randomized to a natural isoflavone. Little is known about the dosing, efficacy or side effects of these compounds.

Hypogonadism is a risk factor for osteoporosis in men but the amount of androgen required to maintain bone mass is unknown.[93] For therapy of osteopenia, it is important to use a testosterone preparation that is converted to estrogen. Testosterone replacement in hypogonadal men increases BMD and has been effective in 6 hypogonadal men with hemochromatosis.[22] There are several considerations in the patient with chronic liver disease, however. First, because an increase in testosterone-binding globulin may cause an overestimation of the total testosterone level, free testosterone is the recommended measurement to assess gonadal status in liver patients. Second, replacement of testosterone to normal levels in men with cirrhosis carries the theoretical increased risk of hepatocellular carcinoma. Unfortunately, there are no guidelines for the optimal treatment of men with liver disease and low testosterone. If the serum level of free testosterone is low in the presence of osteoporosis, however, the advantages and potential risks of testosterone supplementation should be discussed with the patient. If used, transdermal testosterone is the preferred route of administration, thereby avoiding hepatic exposure to oral doses and high levels with depot injections or implants. Similarly, there are no data to assess whether premenopausal females with hypothalamic amenorrhea should be treated with the contraceptive pill. In eugonadal patients with liver disease, alternative therapies for osteoporosis should be considered.

Bisphosphonates

These potent drugs are now the preferred agents for prevention and treatment of osteoporosis with reduction of both vertebral and nonvertebral fractures.[81] Efficacy is seen in postmenopausal osteoporosis[94-96] and steroid-induced osteoporosis.[97-98] Etidronate has now been superceded by the more potent agents, alendronate and risedronate. Despite earlier concerns about reducing bone formation to deleteriously low levels (adynamic bone) with too high a dose or too prolonged therapy, studies now show a wide margin of therapeutic safety with alendronate with up to 10 years of therapy.[99] Bisphosphonates should be used with caution in patients with severe renal impairment.

Unfortunately, data in liver patients are very sparse and limited mainly to studies with small numbers of PBC patients (Table 3). Early studies with etidronate showed little efficacy.[100-102] More recently 13 patients who had PBC treated with alendronate for 2 years (10 mg/day) gained more BMD than 13 patients who had PBC receiving etidronate treatment (400 mg/day for 14 days every 3 mo) with no significant adverse effects; unfortunately no fracture assessment could be made here.[103] In a small randomized, placebo-controlled study of PBC patients treated with steroids, bone loss was prevented by cyclical etidronate.[104]

Table 3. Clinical studies of bisphosphonate therapy for treatment of hepatic osteopenia in PBC.

Methods			Results		
Patients	**Study design**	**Duration Tx**	**BMD**	**Fractures (#)**	**Study conclusions**
12 PBC (9F, 3M) ; all on prednisone (10 mg/d)[104]	Etidronate (400 mg/d for 2 wks, then 11 wks of 500 mg/d Ca) or 500 mg/d Ca. Randomized, controlled	1 yr	BMD-LS stable (0.4%) in treated versus - 3% loss in controls. No difference change BMD-F (–0.1% vs –1.5% in controls)	-	Cyclical etidronate prevents bone loss in gluco-corticoid-treated PBC patients
23 PBC females [101]	Etidronate (400 mg/d) for 14 d every 3 mo or fluoride (50 mg/day). All Ca (1-1.5 g/d). Randomized, controlled	2 yr	BMD-LS and BMD-F remained stable in etidronate pts (resp. 0.53%, 0.4% change) vs bone loss in fluoride pts (BMD-LS –1.9%; BMD-F –5.8%)	Three pts with # in etidronate; Five pts with # in fluoride	Cyclical etidronate is more effective in halting bone loss and better tolerated than fluoride
26 PBC females [103]	Alendronate (10mg/d) or etidronate (400 mg/d) for 14 days every 3 months - Randomized, controlled	2 yr	Increases in BMD-LS of 5.8% and BMD-F 3.5% with alendronate (13 pts) and less changes in etidronate (13 pts) (BMD-LS: 1.9%,BMD-F: 0.4%).	One pt with # in alendronate, two pts with # in etidronate	Alendronate is more effective for increasing bone mass than cyclical etidronate
13 osteopenic, preOLT pts[106]	60 mg intravenous pamidronate every 3 mo before and after OLT – Treatment trial, no controls			No post OLT fractures (compared with historic controls)	Intravenous pamidronate prevents posttransplant fractures.

BMD-F: femur BMD; BMD-LS: lumbar spine BMD; Ca: calcium; OLT: orthotopic liver transplantation; PBC: primary biliary cirrhosis; pmp: postmenopausal; PSC: primary sclerosing cholangitis

The efficacy of bisphosphonates in treating or preventing hepatic osteopenia remains to be proven; however, preliminary results with newer agents are promising and should be considered in the patient with severe osteopenia. In patients with esophageal varices, oral bisphosphonates generally are avoided due to the risk, albeit small, of mucosal ulceration in the esophagus or stomach. Intravenous preparations every 3 - 6 months (pamidronate or zolendronic acid) may be considered in patients with esophageal varices and severe osteopenia, especially in the pretransplant period, but safety data with these agents for use in osteoporosis are limited to 12 months.[105] Preliminary studies have suggested that pretransplant therapy with intravenous bisphosphonates will reduce fracturing in the posttransplant period.[106] Hopefully studies confirming the efficacy of such therapy will soon be available.

Calcitonin

Calcitonin has some efficacy in postmenopausal and steroid-induced osteopenia by the subcutaneous or intranasal route.[107-111] A small prospective, cross-over study of 25 osteopenic patients with PBC did not show any beneficial effect from 6 months of therapy, but a small effect of calcitonin could have been missed by the small patient numbers and short treatment period.[12] Sequential administration of 1,25-dihydroxyvitamin D, calcitonin, and oral calcium carbonate to 36 osteopenic females with PBC-stabilized BMD, compared with a decrease in BMD in the 23 nonosteopenic controls, but this effect could have been from the calcium and vitamin D therapy alone.[112] Calcitonin, a relatively weak antiresorptive agent, may lack the potency necessary to treat hepatic osteopenia.

Fluoride

Sodium fluoride stimulates osteoblast proliferation and increases bone formation, but excessive doses lead to increased bone fragility despite increased bone density. In a large randomized trial of postmenopausal women, 75 mg/d of fluoride increased BMD without reducing fractures[113] but later postmenopausal studies have suggested that 50 mg/d dose of fluoride is more efficacious. Twenty-two patients with PBC were treated with fluoride (50 mg/d) or placebo for 2 years with improvement in BMD in treated group; unfortunately no patients in either group sustained new fractures[114] and thus the efficacy of this agent in PBC remains unknown. Fluoride is not FDA-approved for osteoporosis.

Parathyroid hormone

Parathyroid hormone (PTH) increases bone strength primarily by stimulating bone formation, by increasing the number and action of osteoblasts. Teriparatide is the first 34 amino acids of PTH and produces the chief biologic effects of PTH; 1637 postmenopausal women with previous fractures were randomized to 20 or 40 µg/d subcutaneously or placebo with reduction in the recurrent fracture rate of both vertebral and nonvertebral fractures and an increase in vertebral, femoral and total body BMD.[115] Other studies have confirmed this efficacy.[116,117] Recent trials in combination with alendronate showed no synergy and perhaps some lessened effect with the two agents.[118,119] The effects of recombinant PTH in hepatic osteopenia has not been studied.

Miscellaneous agents

Although ursodeoxycholic acid increases calcium absorption in PBC,[120] its use for 3 years in PBC had no effect on BMD.[121] Interferon has effects on bone metabolism in vitro and may reduce bone resorption; the effects on BMD in liver disease have not been assessed. Whether human recombinant insulin-like growth factor or growth hormone will have beneficial effects on hepatic osteopenia remains to be investigated.[122 123]

Liver transplantation

Bone loss occurs in the early months after liver transplantation in most liver recipients and exacerbates any pre-existing osteopenia with a high incidence of fracturing. Despite this, severe osteopenia, at least in cholestatic patients, improves in the long-term with normal allograft function after OLT (Guichelaar and colleagues, submitted for publication, 2005), and this must be considered the most proven efficacious therapy at this time for hepatic osteopenia.

Summary

Hepatic osteopenia is an important clinical complication of advanced chronic liver disease. Its risk factors are established best for primary biliary cirrhosis and are histologic severity of disease (stage 3/ 4), age and low body mass index. The main risk factor for noncholestatic disease is the presence of cirrhosis but other contributing factors are hypogonadism, alcohol intake, medications, nutritional factors including calcium and vitamin D and physical inactivity. Screening for osteopenia, by measurement of bone mineral density, is recommended in all patients with cirrhosis of any etiology, patients with chronic cholestasis and all potential liver transplant recipients.

In osteopenic patients, all reversible factors contributing to bone loss must be corrected. Bone health is maximized with calcium supplementation, adequate vitamin D, good nutrition and an exercise program. Despite the lack of clinical trials in hepatic osteopenia, in the osteoporotic patient, especially the potential liver transplant recipient, specific interventions can be considered. For eugonadal patients, bisphosphonates are the most potent agents to consider, either orally or intravenously, if large varices are present. These may be also the first choice for those with hypogonadism, although hormone replacement therapy remains an option in postmenopausal females, and transdermal testosterone remains an option in males. Liver transplantation remains the only proven therapy for hepatic osteopenia. Many questions relating to hepatic osteopenia and its associated fracturing remain unanswered, including the true incidence and risk factors in different types of liver disease. Trials of bisphosphonates and the newer bone-forming agents are needed to demonstrate fracture prevention in cirrhosis. Effective management of hepatic osteopenia may however await better understanding of its etiology.

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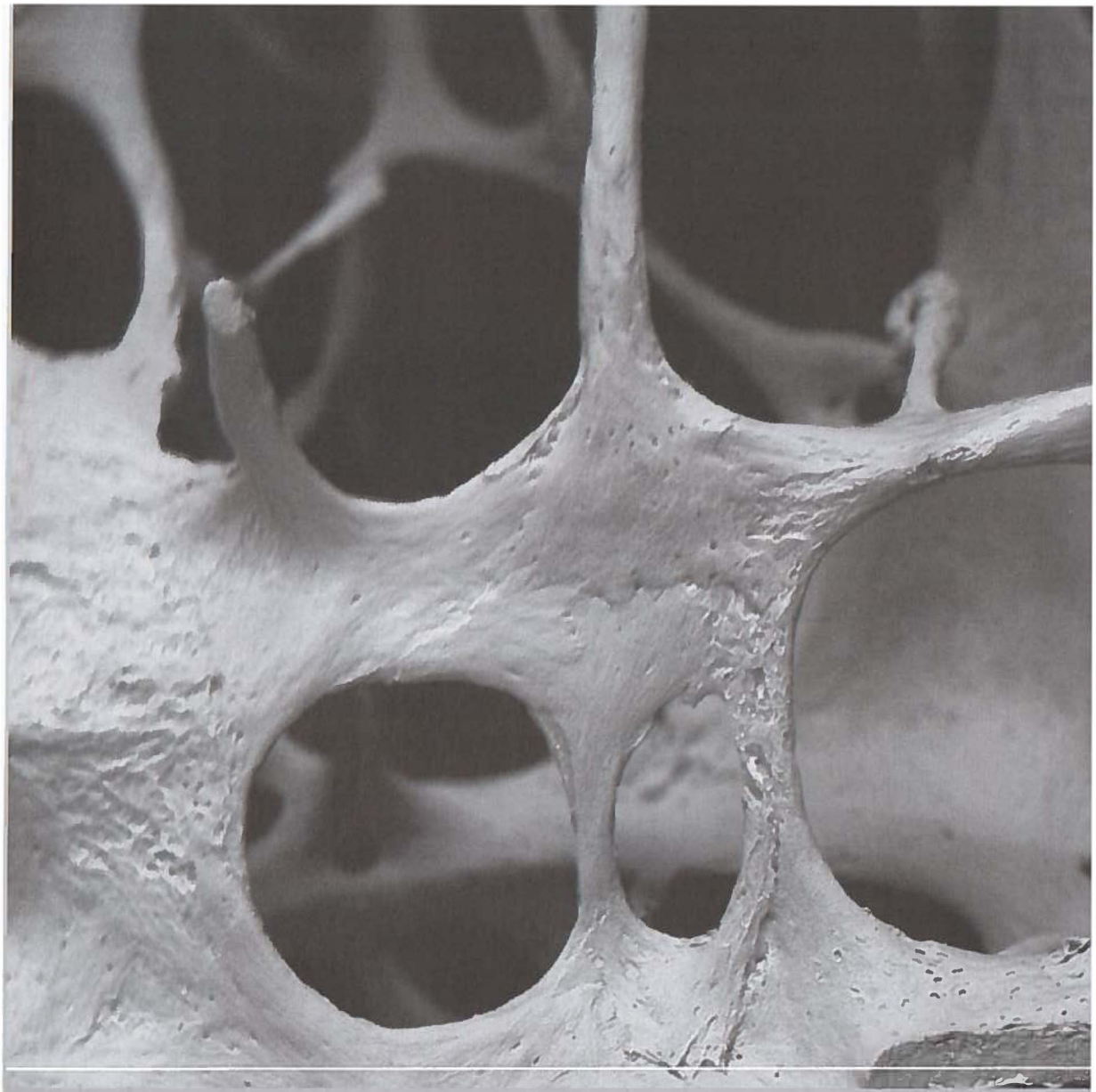

Chapter 8

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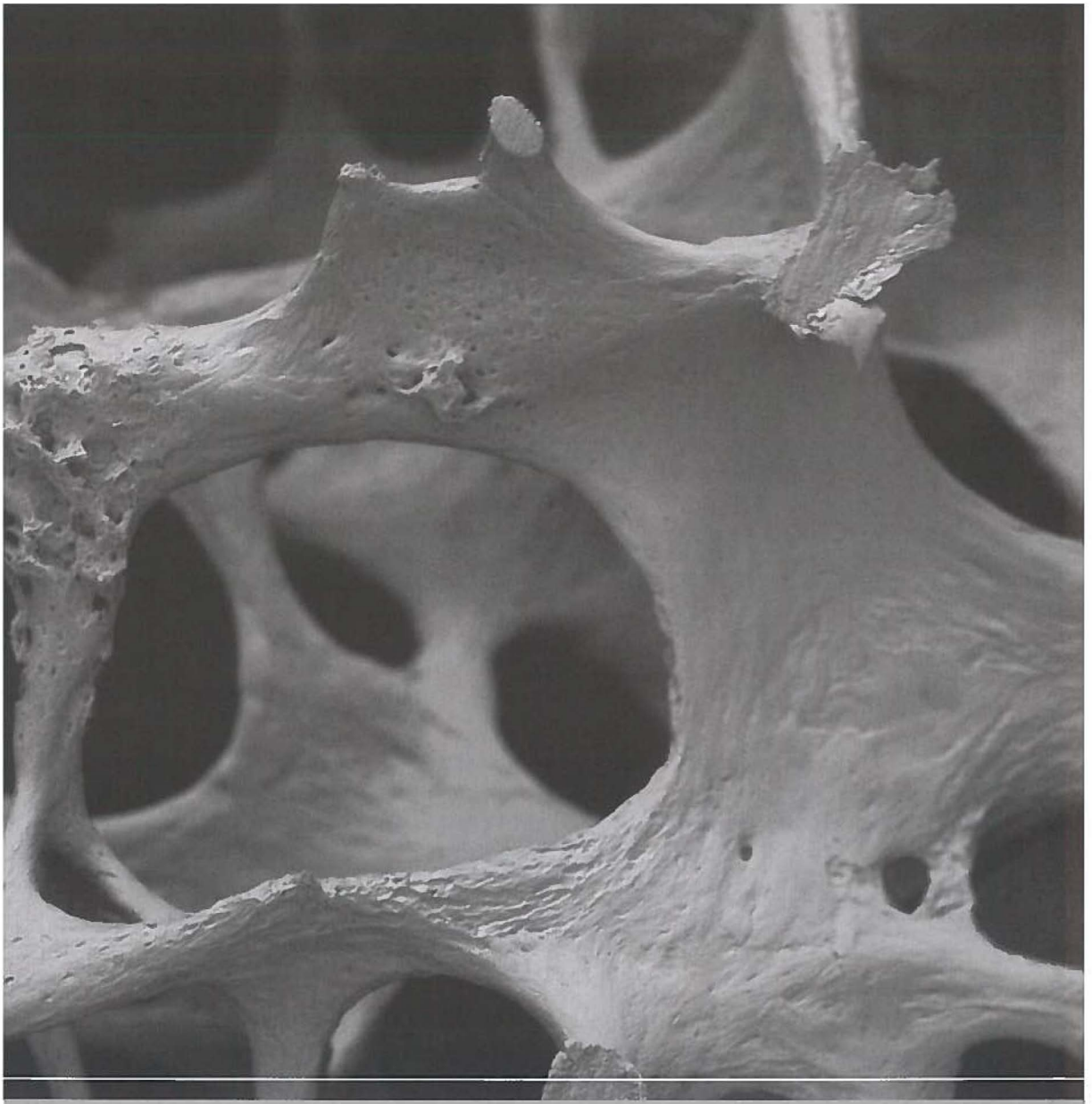

Bone disease after liver transplantation and its management

Submitted

Introduction

Patients with advanced chronic liver disease, especially cholestatic disease, have a high incidence of osteopenia and osteoporosis, resulting into atraumatic fractures. Pretransplant bone disease is thought to be multifactorial, including effects of cirrhosis,[1,2] hypogonadism,[1,3-5] decreased calcium and vitamin D metabolism and secondary hyperparathyroidism,[6,7] poor nutritional and physical status,[8-10] immobility,[11,12] cholestasis,[13,14] alcohol[3,15] and glucocorticoid intake.[9,16]

Orthotopic liver transplantation (OLT) offers the opportunity to reverse many of these abnormalities with normalization of liver function. However, the procedure, as with other solid organ transplants, is accompanied by an exacerbation of bone loss with an increase in fracture rates. In addition, some patients will suffer from avascular necrosis (AVN), a further cause of significant morbidity.

This review will detail the bone mineral density (BMD) changes both early and late after OLT, the histomorphometric changes accompanying bone loss and gain and provide details of our present knowledge of etiologic mechanisms for these changes. The clinical consequences of fracturing and AVN will then be described, together with known predictive factors for these complications. Finally, from the basis of our present knowledge, consideration will be given to management of these patients.

Posttransplant changes of BMD

Following OLT, BMD at the lumbar spine (BMD-LS) is lost in a biphasic pattern of rapid bone loss early after OLT (0 - 4 months) followed by slower bone gain. This early posttransplant bone loss occurs in patients who already have abnormal bone remodeling due to the failing liver.

Bone mass (BMD) at time of OLT

At the time of OLT, 20 - 40% patients with chronic liver disease have osteoporosis,[1,3,17,18] and 15 - 30% have pretransplant fractures.[1,2,19] The highest prevalence of fractures is seen in cholestatic cirrhotic females, but fracturing also occurs in male patients and in patients with cirrhosis of other etiologies. Trabecular (or cancellous) bone (i.e. lumbar spine) has a faster rate of bone remodeling than cortical bone (i.e. femur); in an abnormal metabolic environment such as chronic cholestasis or cirrhosis, bone loss may therefore be more evident at sites of trabecular bone. But after several years of liver disease, both cancellous and cortical bone sites may develop bone loss. Several studies[8,20-24] have assessed both BMD-LS and femoral BMD (BMD-F) in end-stage liver disease patients, suggesting that BMD-F at time of OLT is almost as low as BMD-LS, with T-scores around -2.0.[8,21,22]

Pretransplant osteoporosis may be decreasing in recent years, perhaps due to greater physician awareness and better management of risk factors in liver disease patients. This was recently confirmed in cholestatic patients, where a mean BMD-LS T-score of -2.5 was seen in 93 cholestatic patients transplanted before 1990, compared to -1.7 in 115 cholestatic patients transplanted after 1996.[25] Whether this improvement of

pretransplant osteoporosis will be maintained in the MELD era with shortage of donors is unknown; a longer waiting time, increasing age and pretransplant debility may off-set these beneficial temporal changes.

Early posttransplant BMD loss

An accelerated loss of BMD-LS occurs in most patients during the first 4-6 months posttransplant.[23,26-30] Studies of (mainly cholestatic) patients transplanted between 1985 - 1989 showed a BMD-LS loss at a rate of 20% - 30%/yr.[26-28] Later studies in mainly non-cholestatic patients, transplanted between 1992 - 1995, found BMD-LS losses of 11 - 24%/yr during the first months,[23,29] whereas patients transplanted between 1991-2000 had a posttransplant bone loss rate of 11%/yr[30]. It may seem from these studies that early posttransplant bone loss is decreasing with time, but comparison of the studies is difficult due to differences in populations, gender ratios and methods. However, a recent study[25] of 360 cholestatic patients transplanted between 1985 - 2001, indicated that despite temporal increases in baseline BMD-LS and reduction in posttransplant glucocorticoid doses, no significant change in posttransplant bone loss occurred with time. Posttransplant changes of BMD-F are different from those seen with BMD-LS, showing a longer period of bone loss for at least the first 12 posttransplant months.[20-24]

Later posttransplant BMD gain

After this first phase of bone loss, bone metabolism improves with a consequent gain in BMD-LS.[20,23,27] A study[28] of 20 female PBC patients transplanted between 1985 - 1989 showed that bone gain from 3 - 12 months was 5.9%/yr, after which the rate of bone gain decreased to 1.2%/yr. Less bone gain (2% in months 3 - 12 after OLT) was reported by Floreani et al[29] in mainly non-cholestatic patients transplanted before 1996. Monegal et al[23] showed no increase in BMD-LS in 45 mainly non-cholestatic patients during the first year, although BMD-LS started to increase thereafter. These findings may suggest that posttransplant BMD gain is more in cholestatic patients than non-cholestatic patients. A possible reason for this may be the low BMD values in cholestatic patients at time of OLT, since some studies suggest that patients with low BMD experience more bone gain after OLT. In contrast, a study[25] in cholestatic patients showed that patients with increased pretransplant BMD had increased BMD gain from 4-24 months after OLT. However, these patients with improved posttransplant bone gain were transplanted more recently (>1996) with concomitant less posttransplant hospitalization days, less glucocorticoid doses, less rejection and better kidney function after OLT. Posttransplant BMD-LS gain may therefore have improved in recent years.
The timing and extent of bone gain in the lumbar spine is not seen in the femur; after a longer period of bone loss,[20-24] BMD-F remains stable or decreases further.[20-22,28] A few studies report some long-term recovery of posttransplant BMD-F,[23,24] but values do not reach baseline BMD-F values.

Long-term follow-up of BMD

Only a few studies[25,31] have evaluated long-term BMD-LS changes following OLT, but present evidence suggests that the improvement in BMD is lasting. Feller et al[31] followed 28 patients (14 cholestatic) for up to 7 years posttransplant, and showed that BMD-LS increased for up to 46 months, after which a slight decrease started. After

adjusting for age and sex (Z-scores) a continuing increase of BMD-LS was seen. Similar findings were seen in 74 cholestatic patients who were followed for more than 8 years posttransplant; Z-scores continued to slowly increase whereas T-scores remained stable despite an aging and postmenopausal population.[25] Long-term follow-up of BMD-F has not been studied.

Etiology of bone loss and bone gain after OLT

While DEXA measurements identify changes in BMD, histomorphometric assessment of bone biopsies provides insight into the actual bone remodeling abnormalities. A few studies[3,15,32-37] have studied pretransplant bone metabolism, mainly in female PBC patients[32-37] with scarce data in male patients.[3,15] The most consistent finding is osteoporosis with normal mineralization, and decreased bone formation rates with decreased numbers of osteoblasts[15,34-37] in both female and male patients.[3,15] Bone resorption rates seem also increased, especially in female patients (both pre- and postmenopausal), with increased number of osteoclasts.[3,33,35,38]

Posttransplant bone metabolism has been even less studied, but some histomorphometric findings suggest that early posttransplant bone loss is mainly related to a further reduction in bone formation rates early after OLT.[39] This early reduction in bone formation was also shown by assessment of serum bone formation markers (osteocalcin, bone alkaline phosphatase, PICP) during the first months posttransplant.[21,40] These biochemical studies also report a concomitant increase of serum bone resorption makers (urinary hydroxyproline and deoxypyridinoline) but whether this is of clinical significance is not evident from histomorphometric assessment.[39,41-43]

The further deterioration of bone metabolism after OLT lasts for only a short time, since by 4 months, improved bone metabolism is seen with increased bone formation and increased activation frequency of new bone remodeling units towards the normal ranges.[39,41-43] Biochemical studies have confirmed increases in bone formation as shown by increases in biochemical bone formation markers (osteocalcin, bone alkaline phosphatase) at 4 months posttransplant.[14,21,39] Bone biopsies at later posttransplant time points have not been studied, but osteocalcin (biochemical formation marker) continues to increase after OLT towards values above normal.[9,14,21] Increased osteocalcin values has been reported up to 85 months posttransplant[14] probably implying a continuous state of increased bone formation. In addition, bone biochemical resorption markers (urinary hydroxyproline and deoxypyridinoline) decrease towards normal during the second half-year posttransplant[40,44] probably depicting a recovery of bone resorption towards normal.

The actual etiologic factors responsible for the above changes remain elusive. After OLT, pretransplant factors influencing bone metabolism, such as cholestasis or abnormalities of calcium metabolism, may resolve due to recovery of liver function. However, several other pretransplant factors may still be in effect after OLT and continue to influence bone metabolism, such as poor nutritional status, physical debility,

low BMI, and hypogonadal status. The following etiologic entities are some of the postoperative factors with the potential to influence bone metabolism and therefore BMD after OLT.

Immunosuppression

Glucocorticoids are well known for their deleterious effects on bone metabolism by mainly decreasing bone formation, but also by increasing bone resorption. These negative effects of glucocorticoids on bone remodeling are caused by several glucocorticoid-induced changes, including secondary hyperparathyroidism, inhibition of osteoblast function, increased apoptosis of osteoblasts, increased RANKL expression with decreased apoptosis of osteoclasts, and inhibition of gonadal hormone production.[45,46] Glucocorticoid-induced apoptosis of osteocytes results in changes in mechanosensory function of bone, which probably contributes to the development of osteonecrosis.[47-49]

After OLT, both histomorphometric and (bone) biochemical studies have confirmed changes in bone metabolism applicable to those seen with glucocorticoid use, with a further reduction of bone formation early after OLT.[9,21,40] Studies identified correlations between glucocorticoid doses and posttransplant bone metabolism, BMD losses and fractures.[9,29,24,50,51] In addition, tapering posttransplant glucocorticoids contributed to more posttransplant bone gain, further stressing the important role of glucocorticoids in posttransplant bone disease.[25]

Calcineurin inhibitors (tacrolimus and cyclosporine) have been shown to increase bone turnover in rats.[52-54] Animal studies comparing the effects of tacrolimus (TAC) and cyclosporine (CyA) have been contradictory, with some studies indicating similar effects on bone metabolism and bone loss,[52,55] while others suggested that tacrolimus is more potent in stimulating bone osteogenesis and bone formation.[56-58] Although some clinical studies in transplanted patients have suggested less posttransplant BMD loss and fractures with TAC treatment, these findings were not adjusted for differences in glucocorticoid doses. After adjusting for glucocorticoid doses, one histomorphometric study indicated improved bone formation and trabecular bone architecture in 4-month bone biopsies which was not seen in CyA pts.[9] These bone remodeling improvements were not reflected by increased BMD values, but patient numbers were small. In addition, the effects of calcineurin inhibitors on BMD are currently difficult to estimate due to the overwhelming effects of glucocorticoids and due to the lack of a control group of patients not treated by these agents.

Hyperparathyroidism and changes in calcium and vitamin D metabolism

Levels of parathyroid hormone (PTH) increase early after OLT[59] continuing during the first posttransplant years[14,23,29] with most changes occurring within the normal ranges. However, Feller et al[31] showed that with long-term follow-up after OLT, 9 of 28 patients had PTH levels above normal at 7 years posttransplant. The clinical significance of this is however unknown. Posttransplant changes of PTH may be caused by deficiencies of vitamin D, glucocorticoid-induced decreases of serum calcium, or deteriorating kidney function.[23] In general, hyperparathyroidism causes more losses of cortical bone than that of trabecular bone, which may explain the reported minimal recovery and even

continuing losses of BMD-F after OLT. This was further supported by direct correlations between PTH and BMD-F in both liver[59] and cardiac transplant patients.[60]
In addition to changes in PTH, 25(OH)D increases after OLT in most studies from low to normal values[23,26,31,61,62] which correlated positively with BMD-F and BMD-LS.[62] The metabolically active compound, 1,25 di(OH)D, as well as ionized calcium, seems to increase after OLT, but these changes are less well studied.

Table 1. Etiologic factors influencing posttransplant bone metabolism

Etiologic factors
Pretransplant factors
Pre-existing bone disease
Genetic profile
Posttransplant factors
Immunosuppression
Hyperparathyroidism, calcium and vitamin D disturbances
Hypogonadism
Imbalances of the RANKL – osteoprotegerin system
Immobility
Recurrence of disease, retransplantation

Hypogonadism

Hypogonadism with low free testosterone is present in the majority of male patients with end-stage liver disease, irrespective of their alcohol intake.[31,61,23,45] Low free testosterone levels in combination with low to normal gonadatrophin hormone levels, indicate a hypothalamic disturbance,[4,5] as also seen in female patients.[9] Free testosterone levels increase in male patients after OLT but recovery of male gonadal function is incomplete[9,23,31] with low free testosterone levels for years posttransplant.[23,31] Male patients with less posttransplant recovery of free testosterone showed a trend towards decreased posttransplant BMD values,[31] and increased bone resorption.[9] In female patients, estradiol and FSH levels increases after OLT[9,23,31] and this correlated with increased bone formation.[9] It therefore seems that although gonadal function may recover after OLT, the ongoing decreased levels of gonadal hormones in males and postmenopausal females may still contribute to bone loss after OLT.

Genetic polymorphisms

In the general population, the individual genetic profile determines to a large degree the level of peak bone mass and the development of osteoporosis. Although liver transplant patients have clear risk factors for the development of osteoporosis, the genetic profiles of some patients may make them more susceptible to lose BMD. Studies have attempted to assess the association between allelic variants of the vitamin D receptor and hepatic osteoporosis and this has yielded conflicting results. A correlation between pretransplant bone loss and vitamin D receptor (VDR) polymorphisms was found in 72

female PBC patients[63], but this was not confirmed by a second study in 61 female PBC patients.[64] In this latter study, collagen type 1 polymorphism was associated with lower BMD-LS at baseline, but the reduction of BMD-LS during their follow-up period of 2.5 years correlated with the severity of cholestasis. In 55 males with liver disease, the VDR genotype did not correlate with pretransplant bone loss, but did influence the rate of bone loss after liver transplantation.[65] Larger studies have not yet confirmed these findings.

Imbalance of the RANKL-osteoprotegerin system

RANKL (receptor activator of nuclear factor-kappaB ligand) and OPG (osteoprotegerin) are both cytokines, and are responsible for interaction between osteoblasts and osteoclasts. RANKL is expressed on osteoblasts, T cells and stromal cells and can bind to RANK on osteoclasts, which leads to osteoclastogenesis. OPG is produced by osteoblasts and acts as a soluble decoy receptor for RANKL as is blocks its effects. Imbalances in RANKL-OPG functions have been related to osteoporosis. The most consistent finding in chronic liver disease patients is increased OPG,[66-71] with increased values in cirrhotic versus non-cirrhotic patients.[66,69] The increases of OPG correlated with serum bone formation markers (bone alkaline phosphatase, osteocalcin, C terminal type 1 collagen C) in some studies[70,71] and may be a mechanism to compensate for the negative bone balance and increased bone resorption. However, it is also possible that inflammatory and immunological factors play a role in stimulating OPG. A recent study[67] identified that shortly after OLT (< 14 d) both OPG and RANKL increase. No correlations were found with glucocorticoids and calcineurin inhibitors, which are known to decrease OPG and increase RANKL. Only one study[69] identified a correlation between OPG/RANKL ratio and osteoporosis in cirrhotic patients. Although the role of RANKL-osteoprotegerin imbalances in transplant-related osteoporosis is currently not well established, RANKL-OPG studies may provide new insights in osteoporosis and new therapeutic aims.

Other posttransplant factors

During the first months posttransplant, hospitalization and immobility probably further contribute to the early phase of profound bone loss. The contribution of posttransplant immobility to bone loss was shown in a few clinical studies indicating negative correlations between hospitalization days and BMD-LS[21,26] and BMD-F[21], although most studies did not find such a correlation. Other posttransplant clinical events that may lead to further disturbances in bone metabolism are non-anastomotic biliary strictures, recurrence of the pre-existing disease and retransplantation.[8,25,51]

Clinical consequences of posttransplant bone disease

Posttransplant fractures

Although the period of posttransplant bone loss is relatively short, it occurs in a population of which the majority is osteopenic or osteoporotic and this causes an increased incidence of posttransplant fractures. Most fractures occur during the first 2 years after OLT, with reported posttransplant incidences of 25-40%.[4,18,26-28,72,73] A few studies have assessed posttransplant fractures in a protocolised fashion, with the remaining studies assessing fractures by symptoms. Leidig et al[18] studied vertebral fractures in a protocolised fashion in 130 liver transplant recipients (17 cholestatic pts); 33% of patients had vertebral fractures during the first 2 posttransplant years. Similar posttransplant vertebral fracture rates were shown in 360 cholestatic patients who underwent protocolised screening of spine, chest and pelvic fractures. Twenty-three percent of these cholestatic patients had posttransplant rib fractures, 8% pelvic fractures and 10% other fractures.[4] Overall, after OLT most fractures occur at sites of trabecular bone (vertebral spine and ribs).

Posttransplant BMD losses alone cannot explain the increased fracture rates and no correlations are seen between posttransplant BMD-LS losses and fractures. It is possible that changes in microarchitecture which are not fully assessed by DEXA are in effect. Overall, studies have indicated that independent predictors for posttransplant fractures are pre- and posttransplant BMD, pretransplant fractures, and posttransplant glucocorticoid doses.[18,51] With increasing BMD at baseline and thus also after OLT, less posttransplant fractures were seen in cholestatic patients transplanted in recent years. Nonetheless, still 25% of patients sustain fractures during the first 2 posttransplant years.

Posttransplant avascular necrosis

Avascular necrosis is regarded as a different etiologic entity from osteopenic fracturing, and has been related to insults to the vascular supply of bone, hyperlipidemia and glucocorticoid administration.[84-89] Glucocorticoid-induced AVN has been related to several mechanisms, including increased fat emboli in microvasculature of bone, increased size of intraosseous adipocytes, and increased apoptosis of osteocytes affecting the mechanosensory function of bone.[47,48,74,75] Glucocorticoid administration may also contribute to posttransplant AVN, but risk factors and incidences of posttransplant AVN have not been well established. The few available data have indicated that incidences of posttransplant AVN range between 3-10%.[27,76,77] A recent study[51] in 360 cholestatic liver transplant patients showed a posttransplant AVN incidence of 9%, and confirmed glucocorticoid use as an important risk factor. In addition, AVN correlated with pre- and posttransplant lipid metabolism and pre- and posttransplant BMD and fractures. This latter mechanism may relate to changes in micro-architecture predisposing to AVN.

Diagnosing posttransplant bone disease.

Diagnosing and preventing pretransplant bone disease is one of the main targets to minimize posttransplant bone loss and fractures. In most patients, at time of diagnosis of the liver disease, BMD is not different from that of the normal population. However,

with chronic cholestasis, postmenopausal status, cirrhosis, corticosteroid therapy (> 3 mo) and a previous fragility fracture, the chance of having osteoporosis increases. BMD should be measured in patients with these risk factors, normally done by DEXA.[78,79] In addition, male patients with low free testosterone should also undergo DEXA screening due to a higher prevalence of osteoporosis.

The American Gastroenterological Association (AGA) guidelines[78] are currently recommending that patients with risk factors and a normal BMD should undergo repeat DEXA screening every 2-3 years, whereas shorter screening intervals should be used with corticosteroid therapy. There are no clear guidelines about radiologic assessment pretransplant, but patients with bone pain should undergo radiologic assessment to determine if an osteoporotic fracture is the cause of pain. In addition, patients activated for OLT are recommended to undergo radiologic assessment of lumbar spine, chest and pelvis to determine their baseline status. Following OLT there are no established guidelines but, in general, annual DEXA screening and radiologic assessments are reasonable in the early years after OLT with later screening depending on risk factors.

Management of posttransplant bone disease

Identification and treatment of ongoing risk factors for bone loss

Several metabolic disturbances improve after orthotopic liver transplantation (OLT) due to normalization of liver function. These metabolic improvements may not occur immediately after OLT or only partially. Poor nutritional status, muscle wasting and low BMI have all been correlated to low BMD and fractures both before and after OLT Optimizing nutritional status and adequate weight-bearing exercises are therefore required in all transplant recipients to increase muscle strength and BMD. Calcium supplements (1.5 g/day) and adequate vitamin D (according to serum levels) are recommended in all liver transplant recipients in order to optimize bone remodeling.[78,79] This is especially important in patients with pretransplant osteopenia, as these patients have the potential after OLT to gain bone mass for which adequate calcium and vitamin D is essential.

Hypogonadism is found in the majority of female and male patients with end-stage liver disease and its recovery after OLT may occur slowly or only partially. Hormone replacement therapy (HRT) has been shown to increases BMD before and after OLT in postmenopausal women,[9,80] but its use is becoming less popular due to recent concerns over increased risk of venous thromboembolism and gynecologic cancers.[81] Raloxifene, a SERM (selective estrogen receptor modulator) may become of more importance in the future to treat postmenopausal osteoporosis before and after OLT, but its use in this setting is currently not well established. Neither the efficacy nor safety of testosterone therapy in male hypogonadal patients has been established after OLT. A small study of monthly intramuscular testosterone injections in 5 hypogonadal hemochromatosis patients showed increases in BMD,[82] but there is at least some theoretical concern over the risk of hepatocellular cancer.

Tapering posttransplant immunosuppression

Most liver transplant recipients receive high doses of glucocorticoids during OLT (up to 1000 mg i.v.) and during the early posttransplant days to avoid early rejection. Glucocorticoid doses are then tapered gradually and in most patients eventually withdrawn. Histomorphometric studies have indicated that the major insult to bone metabolism occurs immediately after OLT, suggesting an important role for early high-dose glucocorticoids. A few studies have successfully tapered glucocorticoids to zero in the very early posttransplant period (14 days of less), but unfortunately did not assess effects on preventing BMD loss and fractures.[83-85] For the prevention of posttransplant bone loss the early reduction of posttransplant glucocorticoids will be of utmost importance. The effects of calcineurin inhibitors, immobility and other effects related to the liver transplantation may be less, but are currently not well established due to the overwhelming effects of posttransplant glucocorticoids.

Therapeutic measures for posttransplant bone disease

Unfortunately, therapeutic studies after OLT have been small, with different disease populations, and different treatment strategies. Currently only a few studies in OLT recipients have investigated the effects of anti-resorptive agents (i.e. bisphosphonates and calcitonin) on preventing and treating posttransplant bone disease

Calcitonin therapy

Hay et al[86] studied the effect of subcutaneous salmon calcitonin (100 IU/day, starting on day 7th for 6 months after OLT) after randomization of patients early after OLT; calcitonin-treated patients (n = 29) had similar bone loss and fracture rates when compared to the 34 control patients and no effect was seen on posttransplant bone metabolism, including bone resorption.[39] Valero et al[87] treated posttransplant patients who had low baseline BMD Z-scores (< -2) with salmon calcitonin (40 mg/d intramuscular) or etidronate (400 mg/d for 15 d every 3 mo); BMD was measured at the beginning and at the end of the 12 - months treatment and calcitonin-treated patients had 6.4% increase in BMD, compared to 8.2% in bisphosphonate- treated patients. The effect of treatment on early preventing posttransplant bone loss and fractures was not assessed.

Oral bisphosphonate therapy

Pretransplant cyclical etidronate (400 mg/d for 14 d, repeated every 14 wk) starting at time of activation for OLT did not prevent bone loss at the lumbar spine and femur after OLT.[44] Thirty-four postmenopausal PBC patients were randomized to alendronate therapy (70 mg once a week) early after OLT showing that at 1 year posttransplant BMD-LS and BMD-F was significantly higher than in control patients.[88] Due to their high rate of postmenopausal patients (> 95%) this effect may reflect the known beneficial effects of bisphosphonates in treating postmenopausal osteoporosis. Atamaz et al[89] randomized 98 pts (70% men) to alendronate (70 mg weekly) or placebo. After 2 years of treatment bone gain in treated patients was greater than that seen in controls at both BMD-LS (8.9% vs 1.4%) and BMD-F (6.2% vs 0.3%); the difference in fracture rate was not significant.

Intravenous bisphosphonate therapy
Pamidronate (i.v.) at the time of activation for OLT did not affect BMD loss or posttransplant fractures in 71 patients,[9091] but the overall low rate of posttransplant bone loss and fractures and small patient numbers in this study makes assessment difficult. A beneficial effect of repeated pamidronate administration (60 mg infusion) before and every 3 months after OLT on preventing posttransplant fractures was suggested by Reeves et al.[92] The 13 treated patients had no signs of fractures (by symptoms not radiologic studies), which was a significant improvement from historic controls. BMD was not measured in this study.

Repeated pamidronate infusion (30 mg every 3 mo) for 2 years in 21 patients (which started at a mean of 2 years posttransplant) increased both BMD-LS (10.4%) and BMD-F (7%) (control patients respectively 1.8% and -1.1%).[93] Pennisi et al[94] studied the effects of repeated pamidronate infusions (30 mg every 3 mo) for 1 year after OLT in 43 patients (osteopenic – osteoporotic) and compared this to 42 controls (with normal BMD). BMD-LS increased from baseline to 12 months in the treated population, whereas no changes were seen in the control population. BMD-F decreased in both groups. Crawford et al[40] showed that cyclical zoledronic acid (4 mg intravenously within 7 days of OLT and repeated at months 1, 3, 6, 9) prevented early losses of posttransplant BMD-LS and BMD-F compared to untreated patients but the beneficial effect of treatment on preventing fractures was not assessed. Interestingly, cyclical zoledronic acid (4 mg intravenously within 7 days of OLT and repeated at months 1, 3, 6 and 9) showed a preventive effect on posttransplant bone loss at both the femur and lumbar spine.[40] The beneficial effects of bisphosphonate treatment after other solid organ transplantations were also reported with reduction of bone loss[95-97] and fractures.[98]

Other potential therapies
Teriparatide (human recombinant PTH) is an anabolic agent which has been shown to be effective in postmenopausal osteoporosis to increase BMD and reduce fracture risk.[99] Its use has been limited in other immunosuppressed patient populations due to concerns of a possible increased risk of sarcoma. However, this has not been confirmed in a recent large study[100], and more data is necessary to confirm or reject this concern. Denosumab, a humanized monoclonal antibody that functions like OPG, has been shown to decrease bone resorption and increase BMD-LS in postmenopausal osteoporosis[101], trials are underway to assess prevention of postmenopausal fractures.

Conclusions

Osteopenia and osteoporosis are commonly present in patients with end-stage liver disease at the time of OLT, due to decreased bone formation with/without increased resorption. Several abnormalities associated with chronic liver disease probably contribute to bone metabolism abnormalities and bone loss, including hypogonadism, nutritional and physical deficits, mineral and vitamin abnormalities, and effects of cholestasis, alcohol and glucocorticoid intake. Following OLT, bone metabolism is further compromised, resulting in profound loss of BMD-LS during the first 4 posttransplant months. Histomorphometric analysis suggests that this early posttransplant bone loss is mainly related to a further decrease in bone formation, which is concordant with the known effects of glucocorticoids on bone. Other factors possibly contributing to early posttransplant bone loss including calcineurin inhibitors and immobility are probably overwhelmed by the profound effects of glucocorticoids.
Although BMD-LS values are still decreased at 4 months posttransplant, bone metabolism has improved with normalization of bone formation towards a more coupled bone remodeling balance. This leads to a fast rate of bone gain from 4 - 24 months. Possible factors contributing to bone gain are tapering of glucocorticoids, recovery of gonadal function, and optimal calcium and vitamin D metabolism. The improvements of bone metabolism and BMD gain after OLT are a durable change in both cholestatic and non-cholestatic patients with improved BMD up to a decade after OLT. The biphasic pattern of bone loss and bone gain as seen at the lumbar spine is not seen at the femur; BMD-F values decreases during the first posttransplant year, after which BMD-F remains decreased in most patients and hardly recovers. Posttransplant bone loss leads to a high incidence of posttransplant vertebral and non-vertebral fractures especially during the first 2 years, with significant morbidity.

Although in recent years, improvements in pre- and posttransplant bone disease have been shown in cholestatic patients, it is uncertain whether this will remain in the future with current deceased donor organ allocation. New treatment strategies are therefore needed to prevent posttransplant fractures. In addition, alternative immunosuppression to replace high-doses glucocorticoids during and early after OLT would be a major step to prevent posttransplant BMD losses and subsequent fractures. Currently, a few studies have reported some beneficial effects of bisphosphonate treatment on posttransplant BMD, but studies are small, and effects on fracture prevention are not established. However results are promising and it will be of interest to see the effects of the new potent bisphosphonates with or without anabolic agents, including human recombinant PTH (teriparatide).

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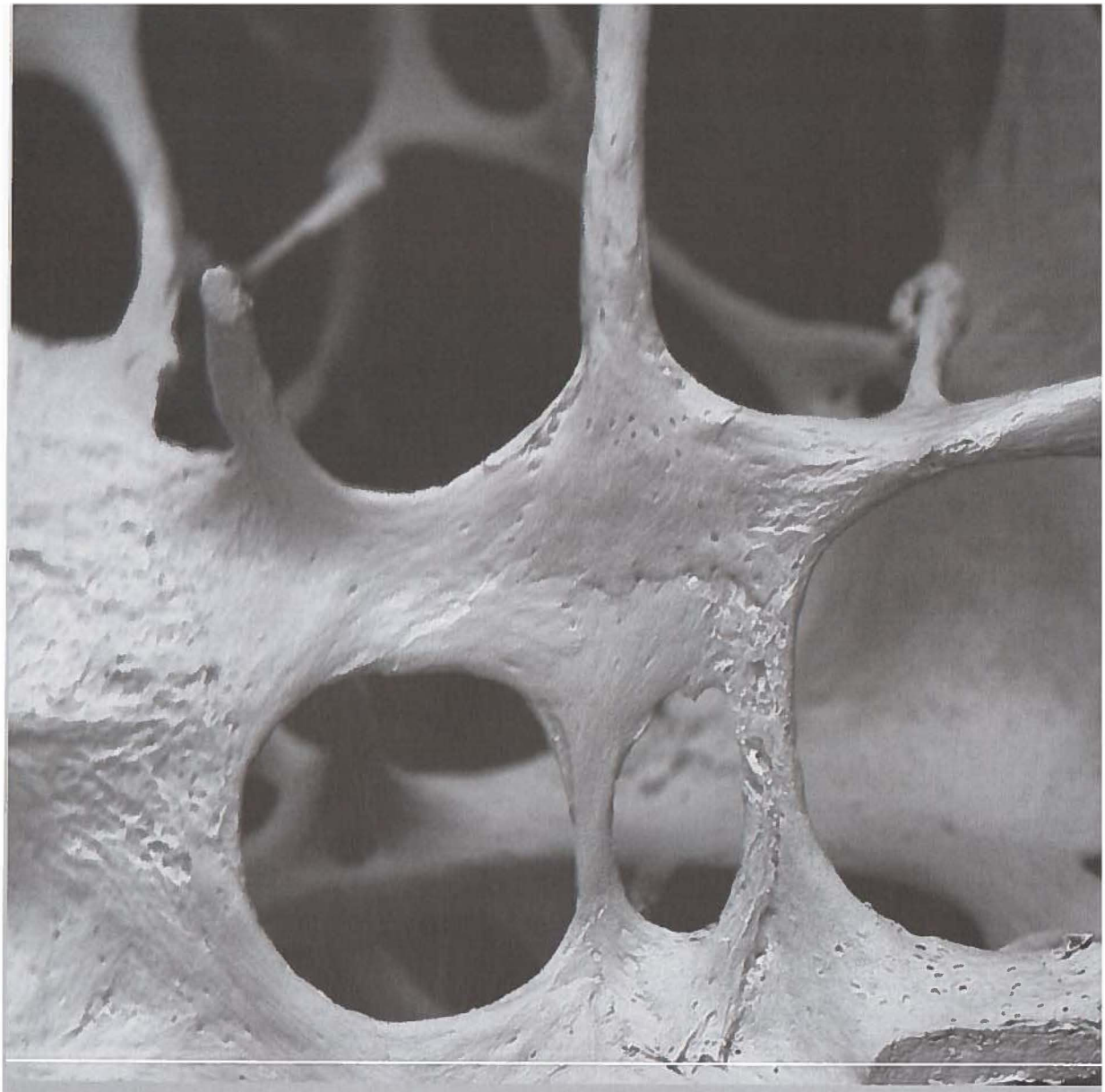
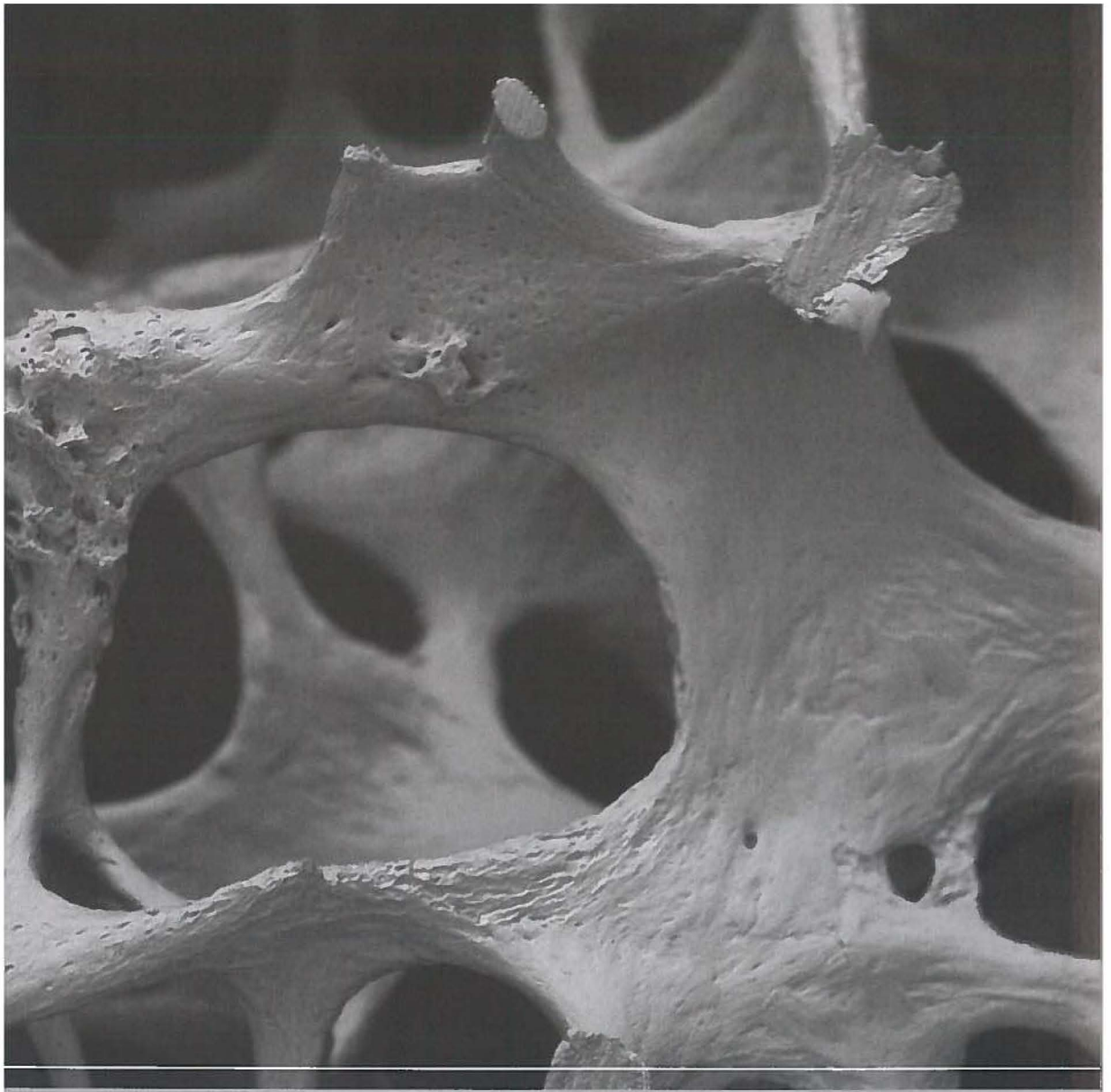
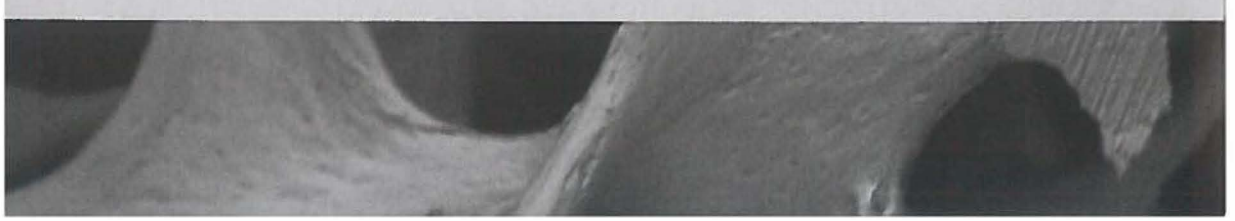


Chapter 9



Summary and discussion



Summary of the Thesis

Osteoporosis and atraumatic fracturing are well known complications of chronic liver disease, especially chronic cholestatic liver disease, but little is known of the underlying etiologic mechanisms. The aim of this thesis was to establish the natural history of metabolic bone disease both before and after orthotopic liver transplantation (OLT) and to elucidate etiologic mechanisms and risk factors. The study population consisted of end-stage primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC) who were selected to undergo liver transplantation (OLT).

Part 1 (chapters 2 - 4) is a prospective study of pre- and posttransplant bone biopsies from 50 osteopenic patients with advanced PBC and PSC. Histomorphometric analysis of bone biopsies allows assessment of bone volume, resorption and formation and is considered the "gold standard" to study bone metabolism. Patients had iliac crest bone biopsies taken at time of OLT to study parameters of bone metabolism in advanced chronic cholestatic liver disease. At 4 months postoperatively, the study population underwent a second bone biopsy to study changes of bone metabolism after OLT. In addition, multiple clinical and laboratory parameters were prospectively studied in order to assess risk factors for abnormalities in bone metabolism before and after OLT.

Part 2 (chapters 5 and 6) is a retrospective patient chart study of all 360 consecutive cholestatic patients (PBC and PSC) transplanted at the Mayo Clinic from 1985 – 2001. In these patients clinical, biochemical and radiologic data were prospectively collected from time of activation for OLT to 8 years posttransplant. The aim of the study was to identify changes after OLT and to investigate etiologic mechanisms of pre- and posttransplant osteoporosis, fractures and avascular necrosis.

Part 3 (chapters 7 and 8) provides an overview of the literature of "hepatic" bone disease and its management, before (chapter 7) and after (chapter 8) OLT.

Bone metabolism in chronic cholestatic liver disease (Chapter 2)

Fifty potential liver transplant recipients with advanced PBC (n = 22) or PSC (n = 28) were prospectively selected for iliac crest bone biopsy at the time of OLT. The mean BMD-LS T-score in this study population was -1.9 and 32% of the patients had osteoporosis. Bone biopsies were taken after tetracycline labeling, which allowed assessment of dynamic parameters of bone formation and mineralization (i.e. bone formation rates, adjusted apposition rates, mineralization lag time and mineralization rates). Other histomorphometric parameters included static parameters of bone volume, bone resorption (osteoclasts and eroded surface areas), bone formation (osteoblast numbers, newly formed bone called osteoid), and parameters of trabecular architecture (indirect parameters of bone resorption). Histomorphometric parameters in the cholestatic study population were expressed as raw values and as Z-scores (standard deviations from normal female and male histomorphometric reference values).

Bone histomorphometric parameters confirmed reduced bone volume parameters in end-stage cholestatic liver disease and this is consistent with the low BMD-LS measured by dual energy X-ray absorptiometry (DEXA). Both dynamic and static parameters of bone formation were decreased, and this was seen in both male and female patients. In female patients, both direct and indirect markers of bone resorption were increased when compared to normal and this combination of effects contributed to a profound negative balance of bone remodeling. There were no differences between patients with PBC or PSC suggesting a similar etiologic basis for bone loss through a mechanism associated with cholestasis and/or cirrhosis.

The majority of male patients had low free testosterone levels before liver transplantation reflecting a hypogonadal status. However, no clear evidence of hypogonadism as a cause of bone loss in either male or female patients was extrapolated from our data, since bone resorption and formation parameters did not correlate with gonadal hormones. In addition, premenopausal women had at least as severe histomorphometric abnormalities as postmenopausal women. None of the postmenopausal women were on hormone replacement therapy. The only correlation between histomorphometric and laboratory data was a positive correlation between vitamin D and osteoblast number, probably reflecting the role of vitamin D in osteoblastic proliferation. Pretransplant assessment of bone metabolism by serum (osteocalcin, bone alkaline phosphatase) and urinary biochemical markers (hydroxyproline) was not seen to be useful to predict bone resorption and formation.

Changes of bone metabolism after liver transplantation (Chapter 3)

Thirty-three (22 PBC, 11 PSC; 21 females, 12 males) of the above 50 patients with end-stage cholestatic liver disease had a second bone biopsy at 4 months posttransplant which allowed assessment of posttransplant bone remodeling abnormalities and predictive factors.

During the first 4 months posttransplant, BMD-LS and histomorphometric bone volume parameters decreased significantly. Despite the nadir in BMD-LS and bone volume parameters at 4 months posttransplant, histomorphometric analysis of iliac crest bone biopsies at that time suggested improvement in bone remodeling. Static and dynamic parameters of bone formation had significantly increased from decreased pretransplant values to posttransplant values within the normal range, with the exception of mean wall thickness. Mean wall thickness reflects bone formation during the months preceding the bone biopsy, which is in contrast to the remaining bone formation parameters which depict bone formation closer to or at the time of the bone biopsy procedure. The posttransplant reduction of mean wall thickness probably depicts an additional insult to bone formation which happened early after OLT (first 1 - 2 months). By 4 months however, bone formation had increased to normal. The additional reduction in bone formation in the first 1 - 2 months after OLT may be the key factor for early posttransplant bone loss.
A further increase in bone resorption in this same early posttransplant period was not evident from our results by both direct and indirect markers of bone resorption

(parameters of trabecular architecture). A clinically important increase in posttransplant osteoclastic activity may therefore not have occurred, although the ongoing state of increased bone resorption from pre- to posttransplant undoubtedly contributed to a negative bone remodeling balance. The normalization of bone formation parameters and activation frequency of new bone remodeling units by 4 months posttransplant correlated with BMD-LS gain thereafter (4 - 12 months). The improvements of bone metabolism by 4 months posttransplant were seen in all transplanted cholestatic patients, with no effects of gender, disease or menopausal status.

Hierarchical cluster analysis, a statistical method used to assess functional similarity among variables, suggested that prior to OLT bone resorption occurred independently of bone formation. This "uncoupling" of bone resorption and formation may explain the unusual finding of increased bone resorption and decreased bone formation in cholestatic patients. Interestingly, hierarchical cluster analysis of posttransplant histomorphometric parameters indicated improvements toward a more integrated and "coupled" balance, showing additional evidence for improvements in posttransplant bone metabolism.

Clinical correlations with posttransplant bone metabolism (Chapter 4)

In the third study involving histomorphometric analyses of bone biopsies, we sought to identify etiologic mechanisms for the observed changes in bone metabolism after OLT. Early posttransplant bone loss correlated with decreased levels of pretransplant serum vitamin D and urinary calcium. In addition, pretransplant cholestasis correlated with increased posttransplant loss of bone volume, whereas posttransplant cholestasis correlated with less formation of newly formed bone (osteoid). The hypogonadal status of two-thirds of our male cholestatic patients before OLT recovered fully or partially after OLT in some but not all males. Posttransplant low free testosterone values correlated with more bone resorption. In female patients, follicle-stimulating hormone increased after OLT in both pre- and postmenopausal women, and this correlated with increased posttransplant osteoblasts and bone formation.

Univariate and multivariate analyses confirmed the important etiologic role of glucocorticoids in causing posttransplant bone volume loss and reduced bone formation. Glucocorticoids may also contribute to the correlation of reduced posttransplant calcium with decreased bone formation and increased bone resorption. Patients treated with cyclosporine or tacrolimus had similar losses of BMD-LS after OLT. However, tacrolimus patients had improved trabecular bone architecture, increased apposition rate of bone and increased mean wall thickness at 4 months posttransplant when compared to cyclosporine patients. These findings suggest that tacrolimus-treated patients may have an earlier recovery of bone metabolism after the initial phase of bone loss. These study findings require further investigation.
Serum and urinary bone biochemical markers were studied to assess their usefulness in assessing the changes in posttransplant bone metabolism. Osteocalcin increased after OLT to above normal values at 1 year posttransplant, and correlated with all posttransplant histomorphometric bone formation parameters. It therefore seems that

osteocalcin may be useful to assess changes in posttransplant bone formation, whereas bone alkaline phosphatase and urinary hydroxyproline were ineffective in predicting posttransplant changes of bone remodeling.

Bone mineral density before and after OLT: long-term follow-up and predictive factors (Chapter 5)

A direct consequence of bone remodeling abnormalities is osteoporosis, which can be identified by measuring BMD-LS with dual energy X-ray absorptiometry (DEXA). We studied changes in BMD-LS in 360 consecutive patients with PBC (n = 156) and PSC (n = 204) who underwent OLT at the Mayo Clinic between 1985 and 2002. All patients had protocolised follow-up of clinical, laboratory and BMD-LS (by DEXA) at time of activation for OLT, 4 and 12 months after OLT, and yearly thereafter. This allowed us to study pretransplant prevalence of osteoporosis with long-term posttransplant changes of BMD-LS. In addition, predictive factors were sought for pretransplant bone disease, posttransplant bone loss and bone gain.

Assessment of pretransplant BMD-LS by DEXA in 360 end-stage cholestatic patients activated for OLT revealed that only 23% had normal BMD; 39% of patients had osteopenia (BMD T-score -1.0 to -2.5) and 38% had osteoporosis (T-score < -2.5). Osteoporosis was significantly more frequent in PBC than PSC (44% vs 33%) and in female than male patients (40% vs 33%). However, after adjusting for age, similar Z-scores were seen in these populations, suggesting a similar effect of cholestatic disease/cirrhosis. Risk factors for pretransplant decreased BMD values were older age, PBC, female gender, postmenopausal status, low OLT number, muscle wasting, decreased BMI, longer duration of disease, decreased albumin and increased alkaline phosphatase levels. Multivariate analysis suggested independent risk factors for decreased BMD before OLT; decreased BMI, low OLT number, female gender, an older age, increased alkaline phosphatase and decreased albumin.

During the first 4 months posttransplant, a high rate of bone loss is seen (BMD-LS loss of 15.9 ± 18.9 %/yr), resulting in osteoporosis in 51% of transplanted patients at 4 months posttransplant. This bone loss occurred in most patients (82%) and was significantly more in PSC than PBC patients. Other risk factors for posttransplant bone loss were younger age, increased pretransplant BMD-LS, absence of inflammatory bowel disease, shorter duration of liver disease, and smoking. It therefore seems that those patients with less pretransplant morbidity lose more BMD-LS after OLT. Multivariate analysis suggested independent risk factors for posttransplant BMD loss; PSC disease and a shorter duration of disease. The only posttransplant factors negatively associated with bone loss were bilirubin levels at 4 months and non-anastomotic strictures (trend). No correlations were seen with posttransplant immunosuppressive doses, number of hospitalization days and rejection episodes.

After the early loss of BMD-LS (0 - 4 months posttransplant), BMD-LS starts to increase at an annual rate of 7% (4 – 24 months posttransplant). Factors favoring posttransplant BMD gain were lower pre- and posttransplant BMD-LS, premenopausal status, high

OLT number, absence of posttransplant cholestasis or kidney failure, absence of non-anastomotic biliary strictures, and increased posttransplant levels of vitamin D and parathyroid hormone. In addition, tapering posttransplant glucocorticoid doses in more recently transplanted patients and hormone replacement therapy in postmenopausal patients increased this rate of bone gain. Long-term follow-up of BMD-LS indicated that the improvement in BMD status is lasting; after the rapid increase of BMD-LS during the first 2 posttransplant years, BMD-LS T-scores remained stable throughout the 8 years of the study, while BMD-LS Z-scores continued to increase. It appears that the cholestatic patients in this study did not experience a decrease in BMD-LS at the usual rate of 1 - 2% per year of the general population.

Fractures and avascular necrosis before and after OLT; long-term follow-up and predictive factors (Chapter 6)

The 360 cholestatic patients with long-term BMD-LS follow-up after OLT also underwent protocolised radiologic screening for fractures (of pelvis, spine, and chest) at multiple pre- and posttransplant time points (4, 12 months, yearly) and when clinically indicated. At time of OLT, 20% of the patients had radiologic evidence of fractures and 1.4% avascular necrosis. Of all pretransplant clinical and laboratory parameters, only BMD correlated with pretransplant fractures.

After OLT the fracture rate increased sharply during the first posttransplant year (cumulative incidence of fractures: 30% in first year). After this first posttransplant year the rate of fracturing leveled off, resulting in a cumulative incidence of 50% of patients with fractures at 8 years posttransplant. Non-vertebral fractures (rib, pelvis, femur and other) and vertebral fractures occurred at similar rates. Overall, most fractures occurred at sites of trabecular bone with the spine and ribs accounting for more than 90% of total fractures.

Pretransplant risk factors for posttransplant fractures were pretransplant older age, PBC disease, female gender and postmenopausal status, poor nutritional status, muscle wasting, low body mass index (BMI), low BMD-LS, fractures and increased alkaline phosphatase levels. Posttransplant fractures correlated with posttransplant glucocorticoid doses. Multivariate analysis suggested independent risk factors for posttransplant fractures; PBC disease, pretransplant fractures, low BMD, low OLT number, and posttransplant glucocorticoid doses. Cyclosporine-treated patients sustained more posttransplant fractures when compared to tacrolimus patients. This is probably a glucocorticoid effect with higher doses of glucocorticoids in cyclosporine-treated patients. Although female postmenopausal patients on hormone replacement therapy (HRT) had more posttransplant BMD-LS gain when compared to those without HRT, no difference in posttransplant fracture rates was seen. However, HRT was started later after OLT (> 2 yrs posttransplant), at a time after which posttransplant bone loss has had its major effect on fracturing.

Avascular necrosis after OLT was seen in 9% of transplanted patients, mainly at the femoral neck. Posttransplant avascular necrosis was not influenced by gender or type

of liver disease, but correlated with pre- and posttransplant BMD and fractures, low BMI, changes in lipid metabolism (increase of triglycerides from pre- to posttransplant, posttransplant cholesterol) and posttransplant non-anastomotic biliary strictures, kidney failure, rejection episodes and glucocorticoid doses. Multivariate analysis suggested independent risk factors for posttransplant AVN; pretransplant fractures, low BMI, low OLT number, low triglycerides, posttransplant non-anastomotic strictures, and fractures before and after OLT. Eighty-five percent of patients with posttransplant avascular necrosis also sustained fractures after OLT. We report a novel association of avascular necrosis with cholestatic parameters, which may have also contributed to the increased incidence of avascular necrosis (1.4%) in patients with advanced cholestatic liver disease (avascular necrosis is seen in less than 0.01% in the USA). The etiologic mechanism is not obvious from our study, but may be related to the known negative effect of hyperbilirubinemia on osteoblastogenesis.

Temporal changes of pre- and posttransplant BMD and fractures (Chapters 5 and 6)

Over the last two decades changes have occurred in the management and follow-up of advanced liver disease and in immunosuppressive regimens. Transplant recipients have become older with more advanced disease and with a longer waiting time. Whether these effects have influenced BMD and fractures before and after OLT have not been studied.

The 360 study patients were transplanted during a 16-year period from March 1985 till January 2001. During this study period, mean spinal T-scores improved from –2.5 before 1990 to –1.7 after 1996; this was mainly caused by improvements in pretransplant T-scores of PSC patients. PBC patients had stable pretransplant T-scores over time, despite an older and more menopausal population with longer duration of disease before OLT. The improvements in pretransplant bone status with time occurred with concomitant clinical improvements, including increased body mass index (BMI), less muscle wasting, less poor nutritional status, less glucocorticoid use, increased vitamin D levels, and less cholestasis with time. The improvements in pretransplant BMD with time led to less pretransplant fractures (23% when transplanted before 1989, 13% when transplanted after 1996), and less posttransplant fractures (at 2 years; 36% when transplanted before 1989, 24% when transplanted after 1996). The temporal reduction in pretransplant fractures mainly occurred in PSC patients, whereas the posttransplant reduction in fractures was seen in both PBC and PSC patients.

Posttransplant bone loss did not change with time in both PBC and PSC despite tapering of glucocorticoids. It is likely that the high glucocorticoid doses still used during and early after OLT causes early posttransplant bone loss. Tapering of glucocorticoid doses after this early phase correlated with increased posttransplant bone gain (4 – 24 months posttransplant) in the more recent transplanted patients. Other beneficial factors after OLT possibly contributing to more posttransplant bone gain were less rejection episodes, less hospitalization days, and less non-anastomotic biliary strictures. Moreover, the more recent transplanted patients had lower posttransplant

creatinine and phosphorus levels, and higher PTH levels. Although posttransplant fractures decreased with time, still 25% of patients with chronic cholestatic liver disease develop de novo fractures after OLT.

General discussion and future perspectives

By bone histomorphometry and radiologic studies we have assessed bone metabolism, BMD, fractures and avascular necrosis both before and after OLT. The study population consisted of patients with advanced chronic cholestatic liver disease activated for OLT. This population was chosen due to its high incidence of pre- and posttransplant osteoporosis and related fractures. Nonetheless, other patients with chronic liver disease also have osteopenic bone disease and experience bone loss after OLT; it is likely therefore that the study results will have a wider application than solely the cholestatic population.

All transplanted cholestatic study patients had protocolised evaluation of BMD (by DEXA) and fractures (by radiographs of the chest, pelvis and spine) at fixed time points before and after OLT. To date, this represents the largest study population of both end-stage PBC and PSC patients. The study confirmed the high incidence of osteoporosis and fractures in PBC before and after OLT, and has expanded present knowledge in several ways. After adjusting for age and sex, BMD values were similarly low in PSC and male patients, as those seen in PBC and female patients. In addition, both PBC and PSC patients had signs of uncoupled bone metabolism with decreased bone formation and increased bone resorption for which risk factors were identified. Furthermore, this study strongly suggests that the deleterious events causing posttransplant bone loss are in effect very early after OLT (first 1-2 months), mainly resulting from an additional insult to bone formation. The study identified early high dose glucocorticoids as a major risk factor for posttransplant bone metabolism changes, less posttransplant bone gain, increased fractures and avascular necrosis. In addition, other risk factors for avascular necrosis were identified, including the novel association with cholestasis. Long-term follow-up of BMD indicated that bone gain after the first phase of bone loss is a durable change, with stable T-scores up to 8 years posttransplant. The study correlations stressed the negative effects of poor nutritional status, muscle wasting and cholestasis on bone metabolism, and as these health issues have improved with time, so has "hepatic" osteoporosis and fractures even with the same severity of liver disease. In addition, with improved pretransplant bone status, posttransplant fractures decreased with time, but no significant improvement in early posttransplant bone loss was seen.

Since we studied radiologic assessments retrospectively, it is possible that some posttransplant fracturing and avascular necrosis was missed and therefore underestimated. Nonetheless, the large consecutive number of transplanted study patients and protocolised follow-up of patients, contributes to the validity of the study findings. The histomorphometric analysis of bone biopsy specimens was performed in a smaller subset of the total cholestatic study population and was prospective in design. This allowed us to reduce the biases of intraobserver variation (mean of 4 readings was used for all measured histomorphometric parameters) and interobserver variation (technicians were regularly assessed for accuracy). For consistency, the same technicians and laboratory techniques were used to assess the study patients and histomorphometric controls. In addition, unlike many other studies, the histomorphometric study population was selected on the basis of homogeneous

disease (patients with end-stage cholestatic liver disease) with no other confounding illnesses or medications known to influence bone metabolism. However, not all flaws could be prevented. Evaluation of dynamic bone formation and mineralization rates required double labeling by tetracycline at fixed time points before the bone biopsy date. Despite a special tetracycline labelling schedule, accurate double tetracycline labelling could not be achieved in all patients due to uncertainty of OLT date, and thus the bone biopsy date.

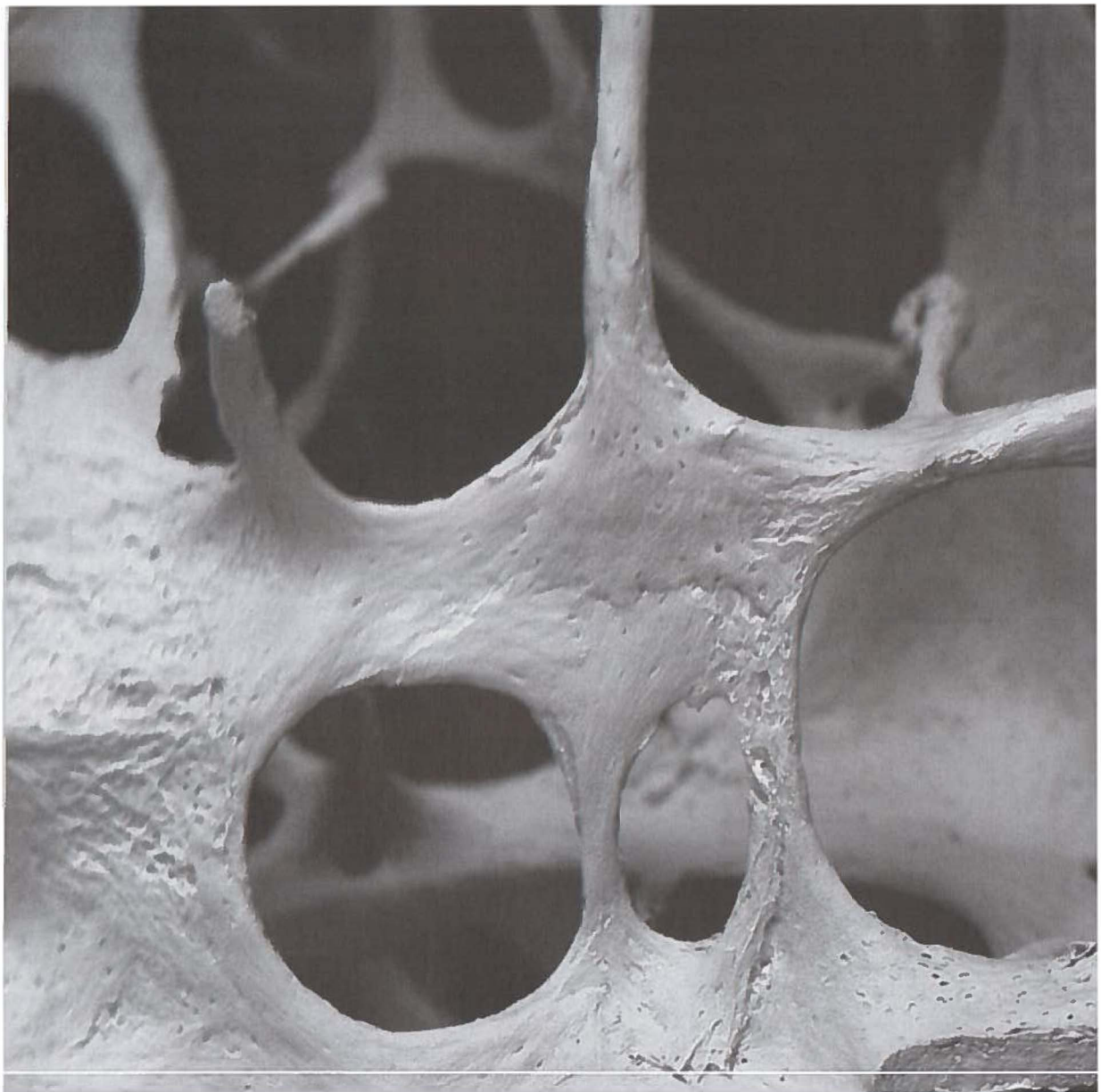
Histomorphometric studies are complex and invasive and this limits their application to small numbers of patients. Unfortunately there is currently no other accurate technique to assess bone metabolism. Bone biochemical parameters have not been shown to be useful in patients with liver disease, and are of limited use after OLT, as shown in our study. Future studies should be aimed at developing more accurate non-invasive ways to study bone metabolism. Currently, BMD assessment by DEXA is used extensively to diagnose osteoporosis and fracture risk. However, fracture risk is not only predicted by BMD, but also by bone architecture and bone remodeling, which is not reflected by DEXA. Newer techniques, such as micro-CT and MRI, may assess changes of trabecular architecture and may shed new light on bone metabolism. However, such methods are expensive and this may limit their application to large numbers of patients.

Hepatic osteopenia and osteoporosis are improved by liver transplantation and this uniquely "reversible" form of osteoporosis may serve as a model by which to test other hypotheses about osteoporosis in general. There is an array of hormones, growth factors and cytokines (e.g. osteoprotegerin, IGF-1) which are proving to effect bone metabolism. Measurement of these parameters may provide new etiologic information. In addition, extension of our study with the inclusion of other liver diseases and measurements of femoral BMD, may lead to further insights into bone remodeling disturbances, identification of additional risk factors, and perhaps disease-specific risk models.

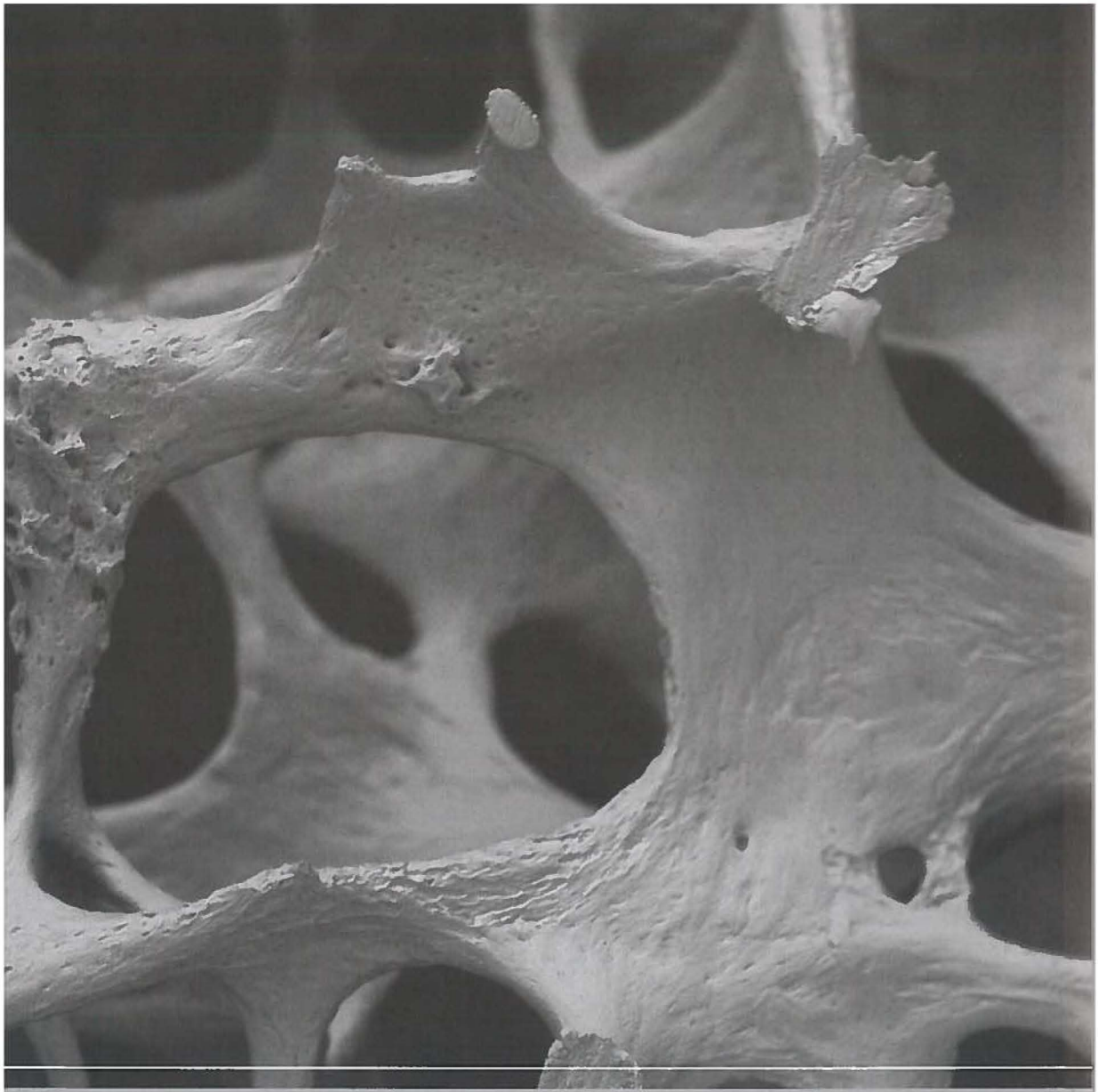
Although the temporal improvement in pre- and posttransplant bone disease is encouraging, it is uncertain if this will continue in the future. A further reduction in pre- and posttransplant bone disease may be effected by reducing the time that the failing liver influences bone metabolism. However, this is not likely to happen in the present climate of organ allocation. Specific treatment regimens may establish further improvements in osteoporosis and fracture rates. A few studies have shown some promising results with the anti-resorption agents, bisphosphonates, to prevent pre- and posttransplant bone loss. However, future studies are necessary to establish whether or not bisphosphonates also result in reduced fracture rates. In addition, the optimal dosing, timing of therapy and its safety are not well established. More effective therapy to prevent or treat bone loss may depend on the anabolic treatment of reduced bone formation. The most potent anabolic agent currently on the market is human recombinant-PTH (teriparatide), which has shown beneficial effects in postmenopausal osteoporosis. Its use in patients with end-stage liver disease is unknown as well as its use in patients on immunosuppression. However, the biggest step in preventing posttransplant bone loss will be to minimize or eliminate posttransplant glucocorticoids.

Currently, there are no medications available to replace glucocorticoids during and early after OLT.

In summary, new light has been shed on abnormalities of bone metabolism and their natural history before and after OLT in cholestatic cirrhosis. New risk factors are established and previously reported risk factors are confirmed for pre- and posttransplant bone loss, fractures and avascular necrosis. Long-term improvement in BMD after OLT is a durable change, although fractures still continue. While the temporal improvement in osteoporosis, fractures and avascular necrosis in cholestatic patients is encouraging, it is unclear whether this trend can be maintained in the future, with the current organ allocation system and shortage of donors. In addition, early posttransplant bone loss did not change over time, despite tapering glucocorticoid doses. Preventive and therapeutic regimens are sorely needed, as are new diagnostic tools to evaluate bone metabolism by non-invasive means. Moreover, the avoidance of posttransplant glucocorticoids with the development of new immunosuppressive regimens will be a major step in the prevention of posttransplant bone disease. Although major breakthroughs in the future may depend on better understanding of osteoporosis in general, it is hoped that with time, pre-and posttransplant osteoporosis will be both preventable and treatable.



Chapter 10



Samenvatting en discussie

Samenvatting van het proefschrift

Osteoporose ("botontkalking") en botfracturen zijn bekende complicaties van chronische leverziektes. De hoogste prevalentie van "hepatische" osteoporose wordt gezien bij patiënten met galwegobstructie (cholestase). Over de etiologische mechanismen van "hepatische" osteoporose is niet veel bekend. Het doel van deze studie is het onderzoeken van de prevalentie, etiologie en risicofactoren van botziekten voor en na orthotopische levertransplantatie (OLT) bij cholestatische patiënten met eindstadium primaire biliaire cirrose (PBC) en primaire scleroserende cholangitis (PSC).

In deel 1 (hoofdstukken 2 - 4) worden de resultaten beschreven van een prospectieve studie naar afwijkingen van botmetabolisme voor en na levertransplantatie. Deze afwijkingen werden geanalyseerd aan de hand van histomorfometrische analyse van botbiopsiëen, wat wordt gezien als de "gouden standaard" om botmetabolisme te onderzoeken. Vijftig patiënten ondergingen een botbiopsie tijdens levertransplantatie om botmetabolisme van patiënten met eindstadium cholestatische levercirrose te onderzoeken. Vier maanden na de levertransplantatie werd een tweede botbiopsie genomen om veranderingen van botmetabolisme na levertransplantatie te onderzoeken. Naast een prospectieve analyse van afwijkingen en veranderingen in het botmetabolisme, werden vele klinische en laboratoriumparameters prospectief onderzocht om risicofactoren te analyseren voor de stoornissen in het botmetabolisme in cholestatische levercirrose patiënten.

Deel 2 (hoofdstukken 5 en 6) is een retrospectieve studie naar botafwijkingen van 360 cholestatische levercirrose patiënten. Deze 360 patiënten zijn getransplanteerd in de Mayo Clinic tussen 1985 en 2001 en ondergingen een geprotocolleerde follow-up voor en na levertransplantatie. Dit protocol omvatte o.a. het meten van botmineraaldichtheid van de lumbale wervelkolom (BMD-LS) en radiologische evaluaties van botfracturen op vastgestelde tijdstippen voor en na levertransplantatie. Deze gegevens werden verzameld vanaf plaatsing op de levertransplantatielijst tot 8 jaar na levertransplantatie. Naast het onderzoeken van de prevalentie en incidentie van "hepatische" osteoporose, botfracturen en avasculaire necrose, werden etiologische mechanismen en risicofactoren geanalyseerd.

In deel 3 (hoofdstukken 7 en 8) worden literatuurstudies beschreven over botziektes voor levertransplantatie (hoofdstuk 7) en over botziektes na levertransplantatie (hoofdstuk 8).

Botmetabolisme in cholestatische patiënten met eindstadium levercirrose (hoofdstuk 2)

Vijftig patiënten met eindstadium PBC (n = 22) of PSC (n = 28) werden prospectief onderzocht naar stoornissen in botmetabolisme. DEXA-metingen (dual energy X-ray absorptiometry) van botmineraaldichtheid (BMD) toonden aan dat de studiepopulatie een gemiddelde BMD T-score had van -1.9 voor levertransplantatie (32% van de

patiënten leed aan osteoporose). Alvorens de botbiopsie werd verkregen (tijdens levertransplantatie), ondergingen de patiënten tetracycline labelling. Aan de hand van deze tetracycline markeringen konden dynamische parameters van botformatie en botmineralisatie geanalyseerd worden (snelheid van botaanmaak, tijd tussen botaanmaak en mineralisatie, snelheid van mineralisatie). Daarnaast werden statische parameters van botmetabolisme gemeten: variabelen van botvolumes (o.a. volumes van trabeculair en corticaal bot), botformatie (hoeveelheid osteoblasten, aanmaak van osteoid), botresorptie (hoeveelheid osteoclasten en diepte botresorptie-holte), en van trabeculaire architectuur (dienen tevens als indirecte parameters van botresorptie). De meetwaarden van deze variabelen in de onderzoekspopulatie werden vergeleken met de normaalwaarden van een gezonde referentiegroep (histomorfometrische Z-scores). De waarde van de Z-score geeft aan hoeveel standaard deviaties de onderzoekspopulatie afwijkt van de histomorfometrische normaalwaarden voor mannen en vrouwen.

De histomorfometrische analyse van de botbiopsiëen bevestigde verminderde botvolumes in patiënten met eindstadium cholestatische levercirrose. Deze bevindingen stemden overeen met de lage botmineraaldichtheid gemeten met DEXA. Zowel mannelijke als vrouwelijke patiënten hadden verlaagde statische en dynamische parameters van botformatie. Daarnaast hadden vrouwelijke patiënten verhoogde (directe en indirecte) parameters van botresorptie, wat verder bijdroeg aan een negatieve botbalans. Er waren geen verschillen tussen PBC of PSC patiënten wat betreft de histomorfometrische parameters. Deze bevinding duidt op een gezamenlijke etiologische basis van "hepatische" osteoporose, waarschijnlijk geassocieerd met de negatieve effecten van levercirrose en cholestase op botmetabolisme. De meerderheid van mannelijke patiënten had een laag vrij-testosteron gehalte voor levertransplantatie, passende bij een hypogonadale status. Of hypogonadisme ook significant bijdroeg aan de afwijkingen in botmetabolisme is in onze studie niet duidelijk geworden. Er waren geen significante correlaties tussen afwijkingen van botmetabolisme en gonadale hormonen, en tevens waren er geen histomorfometrische verschillen tussen pre- en postmenopauzale vrouwen. Geen van deze postmenopauzale vrouwen gebruikte hormonale supplementen. De enige correlatie tussen histomorfometrische en laboratoriumparameters was een positieve correlatie tussen vitamine D en osteoblasten. Mogelijk dat deze correlatie de stimulerende rol van vitamine D weergeeft bij de proliferatie van osteoblasten. De studieresultaten laten tevens zien dat serum parameters van botformatie (osteocalcine en bot-alkalische fosfatase) en botresorptie (urine hydroxyproline) niet correleerden met de gevonden histomorfometrische afwijkingen van botmetabolisme.

Veranderingen in botmetabolisme na levertransplantatie (hoofdstuk 3)

Van de eerder genoemde vijftig patiënten ondergingen 33 cholestatische patiënten (22 PBC, 11 PSC; 21 vrouwen, 12 mannen) 4 maanden na levertransplantatie een tweede botbiopsie. Door deze opeenvolgende botbiopsiëen was het mogelijk het effect van levertransplantatie op botmetabolisme te onderzoeken. Tijdens de eerste 4 maanden na levertransplantatie daalden de histomorfometrische gemeten botvolumes evenals de

botmineraaldichtheid. Ondanks lage waarden van botmineraaldichtheid en histomorfometrische botvolumes 4 maanden na levertransplantatie, liet analyse van de overige histomorfometrische parameters een verbetering van het botmetabolisme zien. Dit bleek onder andere uit een toename van alle statische en dynamische parameters van botformatie tot normale waarden, met uitzondering van de gemiddelde wanddikte. De gemiddelde wanddikte is een maat van de hoeveelheid botformatie voorafgaande aan de botbiopsie. Daarentegen geven de overige parameters van botformatie de botaanmaak weer ten tijde van de botbiopsie. De daling van de gemiddelde wanddikte suggereert dat botformatie verder afneemt spoedig na levertransplantatie (1-2 maanden posttransplantatie), waarna botformatie herstelt tot normale waarden (4 maanden posttransplantatie). De verdere reductie in botformatie spoedig na levertransplantatie is waarschijnlijk een belangrijke oorzaak van het verlies van botmineraaldichtheid na levertransplantatie. Of er tegelijkertijd (spoedig na levertransplantatie) een verdere stijging van botresorptie heeft plaatsgevonden komt in onze resultaten niet naar voren. Wel wordt gezien dat botresorptie onveranderd hoog blijft na levertransplantatie, wat zonder twijfel verder bijdraagt aan de negatieve botbalans.

Het normaliseren van botformatie en de toename van botmetabolisme 4 maanden na levertransplantatie correleerden met de daaropvolgende toename in botdichtheid (4-12 maanden post-levertransplantatie). Deze histomorfometrische verbeteringen werden gezien in alle getransplanteerde cholestatische patiënten, zonder effecten van geslacht, type levercirrose (PBC of PSC) of menopauzale status. Door middel van hiërarchische clusteranalyse is gekeken naar de functionele gelijkenis tussen histomorfometrische variabelen. Hieruit bleek dat botresorptie afzonderlijk van botformatie functioneert. Deze "ontkoppeling" van botresorptie en botformatie verklaart waarschijnlijk de ongebruikelijke bevinding van verhoogde botresorptie en verlaagde botformatie in cholestatische levercirrose patiënten. Interessant is dat hiërarchische clusteranalyse na levertransplantatie een functionele verbetering laat zien. Deze analyse versterkt de evidentie voor verbetering van botmetabolisme na levertransplantatie.

Klinische correlaties met botmetabolisme na levertransplantatie (hoofdstuk 4)

Het doel van deze studie is het onderzoeken van etiologische verklaringen voor de gevonden veranderingen in botmetabolisme na levertransplantatie. Vrij testosteron was verlaagd in ⅔ van de mannelijke studiepatiënten voor levertransplantatie. Deze waarden verbeterden langzaam na levertransplantatie, maar niet in alle getransplanteerde mannelijke patiënten. Verlaagde waarden van vrij testosteron na levertransplantatie correleerde met meer botresorptie. FSH (follikel-stimulerend hormoon) steeg na levertransplantatie in zowel pre- en postmenopauzale vrouwen. Deze stijging correleerde met meer osteoblasten en meer botformatie. Cholestase en deficiënties van calcium en vitamine D voor levertransplantatie correleerden met corticaal botverlies na levertransplantatie, terwijl cholestase na levertransplantatie correleerde met minder nieuw aanmaak van bot (= osteoid).

De statistische analyses bevestigen de belangrijke etiologische rol van glucocorticoïden bij het reduceren van BMD en botformatie na levertransplantatie. Glucocorticoïden dragen waarschijnlijk ook bij aan de correlatie tussen verlaagd calcium met verlaagde botformatie en verhoogde botresorptie na levertransplantatie. Patiënten die behandeld zijn met ciclosporine of tacrolimus toonden gelijke dalingen in botmineraaldichtheid na levertransplantatie. Echter, 4 maanden na levertransplantatie hadden patiënten behandeld met tacrolimus meer verbetering van trabeculaire botarchitectuur en botformatie ten opzichte van patiënten behandeld met ciclosporine. Deze bevindingen bleven significant ook na het bijstellen voor verschillen in prednisongebruik. De studieresultaten suggereren dat patiënten die behandeld zij met tacrolimus een snellere verbetering hebben van botmetabolisme ten opzichte van patiënten die behandeld zijn met ciclosporine. Deze resultaten behoeven nader onderzoek.

Botmarkers in serum en urine werden onderzocht op hun precisie in het weergeven van botformatie en botresorptie. Serum osteocalcine is een parameter van botformatie en steeg na levertransplantatie. De stijging van osteocalcine correleerde met alle histomorfometrische verbeteringen in botformatie na levertransplantatie. Deze bevindingen suggereren een rol voor osteocalcine in het weergeven van veranderingen in botformatie na levertransplantatie. De overige biochemische parameters (bot alkalisch fosfatase en urine hydroxyproline) waren echter ineffectief in het weergeven van botmetabolisme na levertransplantatie.

Botmineraaldichtheid voor en na levertransplantatie: natuurlijk beloop en etiologische factoren (hoofdstuk 5)

Osteoporose is het gevolg van een insufficiënt botmetabolisme, en de aanwezigheid van osteoporose kan worden vastgesteld aan de hand van DEXA-metingen van botmineraaldichtheid. Wij bestudeerden BMD-LS in 360 patiënten met PBC (n = 154) en PSC (n = 204) die opeenvolgend een levertransplantatie ondergingen in de Mayo Clinic tussen 1985 en 2002. Alle patiënten hadden een geprotocolleerde follow-up van klinische en biochemische variabelen en botmineraaldichtheid op vastgestelde tijdstippen (bij activatie voor levertransplantatie, 4 en 12 maanden na levertransplantatie en jaarlijks daarna). Door deze uniforme follow-up was het mogelijk osteoporose te bestuderen voor en na levertransplantatie in een grote populatie cholestatische patiënten. Daarnaast werden risicofactoren bestudeerd voor "hepatische" osteoporose en voor de veranderingen in botmineraaldichtheid na levertransplantatie.

DEXA-metingen in de 360 studiepatiënten toonden aan dat 23% van de patiënten een normale botmineraaldichtheid had, echter 39% van de patiënten leed aan osteopenie (BMD T-scores tussen -1.0 en -2.5) en 38% aan osteoporose (T-score < -2.5). De prevalentie van osteoporose was significant meer in PBC dan in PSC patiënten (44% vs 33%), wat waarschijnlijk het gevolg was van de verhoogde prevalentie van osteoporose in vrouwelijke ten opzichte van mannelijke patiënten (40% vs 33%). Na het bijstellen van de botmineraaldichtheid voor verschil in leeftijd (Z-scores) werd een gelijke Z-score gezien in alle patiëntenpopulaties, wat een zelfde effect suggereert van

cholestatische levercirrose op BMD. Univariate analyse toonde aan dat lage BMD waarden werden gezien bij oudere patiënten, PBC, vrouwelijk geslacht, laag OLT nummer, postmenopauzale status, spierverlies, verlaagde BMI (body mass index), langere ziekteduur voor OLT, verlaagde albumine en verhoogde alkalische fosfatase. Aan de hand van multivariate analyse werden onafhankelijke risicofactoren voor verlaagde BMD waarden (voor levertransplantatie) bepaald; laag BMI, vrouwelijk geslacht, oudere leeftijd, laag OLT nummer, verhoogde waarden van alkalisch fosfatase en albumine.

Tijdens de eerste 4 maanden na levertransplantatie verloren de patiënten veel botmineraaldichtheid. Dit verlies van BMD resulteerde in een stijging van de hoeveelheid patiënten met osteoporose van 38% (voor levertransplantatie) naar 51% (4 maanden na levertransplantatie). Het verlies van BMD werd gezien in de meerderheid van de patiënten (82%) en was significant hoger in PSC dan in PBC patiënten. Andere univariate risicofactoren voor verlies van BMD na levertransplantatie waren jongere leeftijd, verhoogde BMD-metingen voor levertransplantatie, afwezigheid van IBD (inflammatory bowel disease), kortere ziekteduur, en roken. Deze statistische univariate analyse suggereert dat meer botverlies optreedt na levertransplantatie in patiënten met minder pretransplantatie morbiditeit. Aan de hand van multivariate analyse werden onafhankelijke risicofactoren voor verlies van BMD (na levertransplantatie) bepaald; PSC en een kortere ziekteduur voor OLT. Na levertransplantatie correleerde bilirubine met het verlies van BMD. Er waren geen correlaties tussen verlies van BMD en doseringen van immunosuppressiva, opnameduur of rejectie-episodes.

De periode van verlies van BMD (0 - 4 maanden posttransplantatie) werd opgevolgd door een periode van toename van BMD (jaarlijkse toename 7% t.o.v. baseline BMD). De univariate analyse toonde aan dat de toename in botmineraaldichtheid significant hoger was in patiënten met lage (pre- en posttransplantatie) BMD waarden, hoge OLT nummer, minder cholestase, hogere vitamine D en PTH waarden, en in premenopauzale vrouwen t.o.v. postmenopauzale vrouwen. Daarnaast correleerden de gereduceerde glucocorticoïd-doseringen, afwezigheid van nonanastomose stricturen en hormonale therapie (in postmenopauzale vrouwen) met een hogere toename van BMD-LS. Langdurige follow-up van BMD waarden na levertransplantatie toonde aan dat de positieve veranderingen in BMD een blijvende verbetering is; na de eerste periode van snelle toename in BMD-LS (4 - 24 mnd posttransplantatie) blijven de BMD T-scores gering stijgen totdat de waarden stabiliseren (24 - 96 mnd posttransplantatie). De BMD Z-scores stegen gering gedurende deze hele studieperiode. Dit is in tegenstelling tot de bekende BMD dalingen van 1 - 2% per jaar die normaal optreden in de ouder wordende (gezonde) populatie.

Botfracturen en avasculaire necrose voor en na levertransplantatie: natuurlijk beloop en voorspellende factoren (hoofdstuk 6)

De 360 cholestatische patiënten met langdurige follow-up na levertransplantatie ondergingen tevens geprotocolleerde radiologische follow-up ten aanzien van de opsporing van botfracturen (van bekken, lumbale wervelkolom en thorax). Deze

radiologische screening werd verricht bij plaatsing op de levertransplantatielijst, 4 en 12 maanden na levertransplantatie, jaarlijks daarna en wanneer het klinisch relevant was. Op het moment van levertransplantatie had 20% van de patiënten radiologisch vastgestelde botfracturen en 1.4% avasculaire necrose (AVN). Van alle gemeten parameters voor levertransplantatie correleerde alleen BMD negatief met de aanwezigheid van botfracturen.
Na levertransplantatie werd er gedurende het eerste jaar een sterke stijging gezien in de incidentie van botfracturen (30% van getransplanteerde patiënten had 1 of meer nieuwe botfracturen in het eerste jaar na transplantatie). Na deze sterke stijging stabiliseerde deze incidentie op een lager niveau, wat uiteindelijk resulteerde in een cumulatieve incidentie van 50% (percentage patiënten met fracturen) gedurende de eerste 8 jaar na levertransplantatie. Vertebrale- en niet-vertebrale fracturen (ribben-, bekken-, femurfracturen) hadden na levertransplantatie een gelijke incidentie. Meer dan 90% van de totale fracturen waren botfracturen van de wervels en ribben, oftewel botfracturen van trabeculair bot. Univariate risicofactoren voor post-transplantatie botfracturen waren oudere leeftijd, PBC, het vrouwelijke geslacht en postmenopauzale status, slechte voedingstoestand, verlies van spiermassa, lage BMI, lage OLT nummer, lage BMD, botfracturen en verhoogde waarden van alkalisch fosfatase. Aan de hand van multivariate analyse werden onafhankelijke risicofactoren voor botfracturen (na levertransplantatie) bepaald; PBC, botfracturen ontstaan voor levertransplantatie, verminderde botmineraaldichtheid (voor en na levertransplantatie) en glucocorticoïd-doseringen na levertransplantatie.

Er werden meer botfracturen gezien in patiënten behandeld met ciclosporine in vergelijking met patiënten behandeld met tacrolimus. Dit verschil in incidentie van botfracturen wordt mogelijk veroorzaakt door de hogere doseringen glucocorticoïden in de ciclosporine-regimes. Hormonale therapie (HRT) in postmenopauzale vrouwen droeg bij aan BMD stijging na levertransplantatie, maar dit leidde niet tot een significante vermindering van botfracturen. Echter, HRT werd gestart enkele jaren na levertransplantatie (> 2 jaar posttransplantatie), reeds nadat de meeste botfracturen waren gediagnosticeerd.

Avasculaire necrose na levertransplantatie werd gezien in 9% van de getransplanteerde patiënten, vooral ter hoogte van de proximale femur. De incidentie van post-transplantatie AVN werd niet beïnvloed door geslacht of type levercirrose (PBC of PSC), maar correleerde met botfracturen en een laag BMD, laag BMI, veranderingen in lipidenmetabolisme (stijging van triglyceriden en cholesterolwaarden na OLT), aanwezigheid van non-anastomose galwegstricturen, rejectie-episodes en glucocorticoïddoseringen. Aan de hand van multivariate analyse werden onafhankelijke risicofactoren voor post-transplantatie AVN bepaald; lage BMI-waarden, lage triglyceriden, laag OLT getal, non-anastomose galwegstricturen (na OLT) en botfracturen voor en na levertransplantatie. Botfracturen werden gezien in 85% van de patiënten met AVN na levertransplantatie. Tevens bleek uit de analyse een nieuwe associatie tussen cholestatische parameters en AVN. Deze associatie draagt mogelijk tevens bij aan de verhoogde incidentie van AVN voor levertransplantatie (1.4%) in onze cholestatische studiepatiënten (AVN wordt gezien in minder dan 0.01% van de algemene Amerikaanse bevolking). Het etiologische mechanisme tussen cholestase en

AVN is niet duidelijk in onze studie, maar is mogelijk gerelateerd aan het bekende inhiberende effect van hyperbilirubinemie op de osteoblastogenese.

Veranderingen in botziekten voor en na levertransplantatie gedurende een periode van 16 jaar (hoofdstukken 5 en 6)

Gedurende de laatste 2 decennia hebben vele veranderingen plaatsgevonden in behandeling en follow-up van patiënten met eindstadium levercirrose. In de loop van de tijd zijn de transplantatiepatiënten ouder geworden en patiënten hebben een langere wachttijd voor levertransplantatie. Tevens is de behandeling na levertransplantatie veranderd met o.a. minder gebruik van immunosuppressiva. Of deze veranderingen invloed hebben gehad op botmineraaldichtheid en het ontstaan van botfracturen voor en na levertransplantatie is niet bekend.

De 360 studiepatiënten werden getransplanteerd gedurende een periode van 16 jaar (van maart 1985 tot januari 2001). Tijdens deze studieperiode zijn de gemiddelde BMD-LS T-scores verbeterd van -2.5 (in patiënten getransplanteerd voor 1990) tot -1.7 (in patiënten getransplanteerd na 1996). Deze veranderingen weerspiegelen vooral de verbeteringen in BMD van PSC patiënten. De PBC patiënten behielden stabiele T-scores gedurende de studieperiode, ondanks dat ze in de loop van de tijd ouder werden, meer postmenopauzaal en een langere ziekteduur hadden voor levertransplantatie. De verbeteringen van botmineraaldichtheid gedurende de studieperiode zijn waarschijnlijk het gevolg van de verschillende klinische verbeteringen die zich tegelijkertijd hebben afgespeeld; o.a. stijging in BMI, minder verlies van spiermassa, betere voedingstoestand, hogere vitamine D waarden en minder cholestase. De verbeteringen van pretransplantatie BMD leidde tot een vermindering van pre-transplantatie botfracturen (23% in patiënten getransplanteerd voor 1989, 13% in patiënten getransplanteerd na 1996) en minder post-transplantatie botfracturen (2 jaar na levertransplantatie; 23% van de patiënten getransplanteerd voor 1989 hadden botfracturen in vergelijking met 13% in patiënten getransplanteerd na 1996). De vermindering van botfracturen voor levertransplantatie werd met name gezien in PSC patiënten, terwijl de verbetering van botfracturen na levertransplantatie zich afspeelde in zowel PBC als PSC patiënten.

Ondanks het verminderen van de glucocorticoïd doseringen na levertransplantatie is het verlies van BMD niet significant veranderd gedurende de studieperiode. Dit is waarschijnlijk het gevolg van de nog hoge doseringen glucocorticoïden die gegeven worden tijdens de levertransplantatie en vroeg daarna. Wel bleek dat de toename van BMD (na de periode van BMD verlies) significant groter was geworden gedurende de studieperiode. Het is waarschijnlijk dat de reductie van glucocorticoïd doseringen gedurende de studieperiode heeft bijgedragen aan deze toename in BMD, gezien de correlatie tussen lagere doseringen glucocorticoïden en hogere BMD waarden. Andere post-transplantatie factoren die mogelijk bijdroegen aan meer toename van BMD waren minder rejectie-episodes, minder lange ziekenhuisopnames, minder cholestase, en minder non-anastomose galwegstricturen. Tevens hadden de recent getransplanteerde patiënten lagere waarden van creatinine en fosfaat en hogere waarden van PTH

(parathyroïd hormoon) na levertransplantatie. Ofschoon onze studie een vermindering van botfracturen laat zien gedurende de 16 transplantatie-jaren, hebben nog steeds 25% van de recent (na 1996) getransplanteerde cholestatische patiënten nieuwe fracturen na levertransplantatie.

Discussie en toekomstperspectieven

Aan de hand van histomorfometrische en radiologische studies hebben we botmetabolisme, BMD, botfracturen en avasculaire necrose onderzocht voor en na levertransplantatie. De studiepopulatie bestond uit patiënten met eindstadium cholestatische levercirrose. Deze populatie is gekozen vanwege de hoge incidentie van pre- en posttransplantatie osteoporose en geassocieerde botfracturen. Patiënten met andere vormen van levercirrose lijden echter ook aan osteoporose; het is aannemelijk dat de resultaten van deze studie een bredere toepasbaarheid hebben dan alleen de cholestatische patiënten.

Alle studiepatiënten ondergingen een geprotocolleerde follow-up van BMD (aan de hand van DEXA-metingen) en botfracturen (aan de hand van radiologische foto's van thorax, bekken en lumbale wervelkolom) op vastgestelde tijdstippen voor en na levertransplantatie. Tot op heden is deze studiepopulatie de grootste studie met niet alleen eindstadium PBC patiënten maar tevens met de inclusie van PSC patiënten. De studie bevestigt de hoge incidentie van osteoporose en botfracturen in PBC patiënten voor levertransplantatie, en draagt daarnaast op verschillende manieren bij aan de huidige kennis van "hepatische" osteoporose. Na het bijstellen voor verschil in leeftijd en geslacht tussen PBC en PSC patiënten (Z-scores), bleken er geen verschillen te bestaan van BMD waarden tussen PBC en PSC, en mannen en vrouwen. Daarnaast hadden zowel PBC als PSC patiënten tekenen van "ongekoppelde" botmetabolisme met verlaagde botformatie en verhoogde botresorptie, waarvoor risicofactoren werden geanalyseerd. Tevens toonde de studie aan dat direct na levertransplantatie (de eerste 1-2 maanden post-transplantatie) de belangrijkste negatieve effecten op botmetabolisme zich afspelen. Analyse toonde aan dat dit mogelijk leidt tot een verdere verlaging van botformatie. Ook bleek uit de studieresultaten dat de hoge doseringen glucocorticoïden direct na levertransplantatie een belangrijke risicofactor zijn voor stoornissen in botmetabolisme, verlies van botmineraaldichtheid, minder botaanmaak, meer botfracturen en AVN. De reductie van de latere glucocorticoid-doseringen heeft bijgedragen aan een toegenomen stijging van BMD na OLT. Daarnaast werden risicofactoren voor AVN geïdentificeerd, met onder andere een nieuwe associatie tussen cholestase en AVN. Langdurige follow-up van BMD na levertransplantatie toonde aan dat de toename in BMD na de eerste fase van botverlies een blijvend herstel is, met stabiele BMD-LS T-scores gedurende 8 jaren na levertransplantatie. De studiebevindingen onderstrepen verder de negatieve effecten van een slechte voedingstoestand, verlies van spiermassa, en de negatieve effecten van cholestase op botmetabolisme. Met het verbeteren van deze factoren in de loop van de tijd werd een verbetering gezien van "hepatische" osteoporose en botfracturen terwijl de ernst van de leverziekte hetzelfde bleef. Daarnaast toonde de studie aan dat de verbetering van pretransplantatie botstatus leidde tot minder posttransplantatie botfracturen, ondanks dat het verlies van BMD na levertransplantatie onveranderd bleef gedurende de studieperiode.

De radiologische studies naar osteoporose en botfracturen waren retrospectief, en het is daarom mogelijk dat sommige botfracturen en AVN gemist en daardoor onderschat zijn. Daarentegen dragen de grootte van de studiepopulatie en het includeren van alle

opeenvolgende getransplanteerde patiënten, met allen dezelfde geprotocolleerde follow-up, bij aan de validiteit van de studiebevindingen. De histomorfometrische analyse van botbiopsieën werd uitgevoerd in een subgroep van de onderzoekspopulatie en was prospectief in opzet. Hierdoor was het mogelijk de effecten van studie-biases voor aanvang van de studie te minimaliseren. Om intra-observer variatie te verkleinen werd een gemiddelde van 4 histomorfometrische metingen genomen als uiteindelijke waarde. Daarnaast werden de histomorfometrische labtechnici frequent gecontroleerd op hun nauwkeurigheid om inter-observer variatie te verkleinen. De botbiopsieën van de studiepatiënten en de controle patiënten werden geanalyseerd door dezelfde labtechnici met gebruik van dezelfde laboratoriumtechnieken. In tegenstelling tot eerdere studies werd de studiepopulatie gekozen op basis van homogeniciteit (alleen patiënten met eindstadium cholestatische leverziekte, zonder andere aandoeningen of medicatie die botstofwisseling beïnvloeden). Desalniettemin was het niet mogelijk om alle biases te voorkomen. Markering van botformatie door tetracycline is nodig om dynamische waarden van botformatie en mineralisatie te kunnen meten. Dit vereist nauwkeurige markering van tetracycline op vastgestelde tijdstippen waarna de botbiopsie verricht kon worden. Een speciaal tetracycline markeringsschema werd opgesteld, echter bij niet alle patiënten was adequate markering mogelijk mede door de onzekerheid over de uiteindelijke levertransplantatiedatum en daardoor de botbiopsiedatum.

Het verrichten van histomorfometrische studies is complex en invasief van karakter waardoor het gebruik ervan in studies beperkt is. Momenteel is er echter geen andere nauwkeurige techniek om botmetabolisme te onderzoeken. Biochemische parameters van botmetabolisme bleken in andere studies (alsmede onze studie) niet nauwkeurig voor het evalueren van botmetabolisme afwijkingen voor levertransplantatie en zijn van beperkte waarde na levertransplantatie. Toekomstige studies zijn nodig om niet-invasieve methoden te onderzoeken waarmee botmetabolisme bestudeerd kan worden. Momenteel worden DEXA-metingen veelvuldig gebruikt om osteoporose en daarmee het risico op botfracturen vast te stellen. Echter, het krijgen van botfracturen wordt niet alleen voorspeld door BMD waarden maar is mede afhankelijk van botarchitectuur en botmetabolisme. Deze factoren worden niet door DEXA geanalyseerd. Nieuwere methoden, zoals micro-CT en micro-MRI, geven meer inzicht in veranderingen van trabeculaire architectuur en geven daardoor mogelijk meer inzicht in botmetabolisme. Echter, deze methoden zijn nog niet goed onderzocht en het gebruik is duur, waardoor momenteel het gebruik van micro-CT en micro-MRI in studies beperkt is.

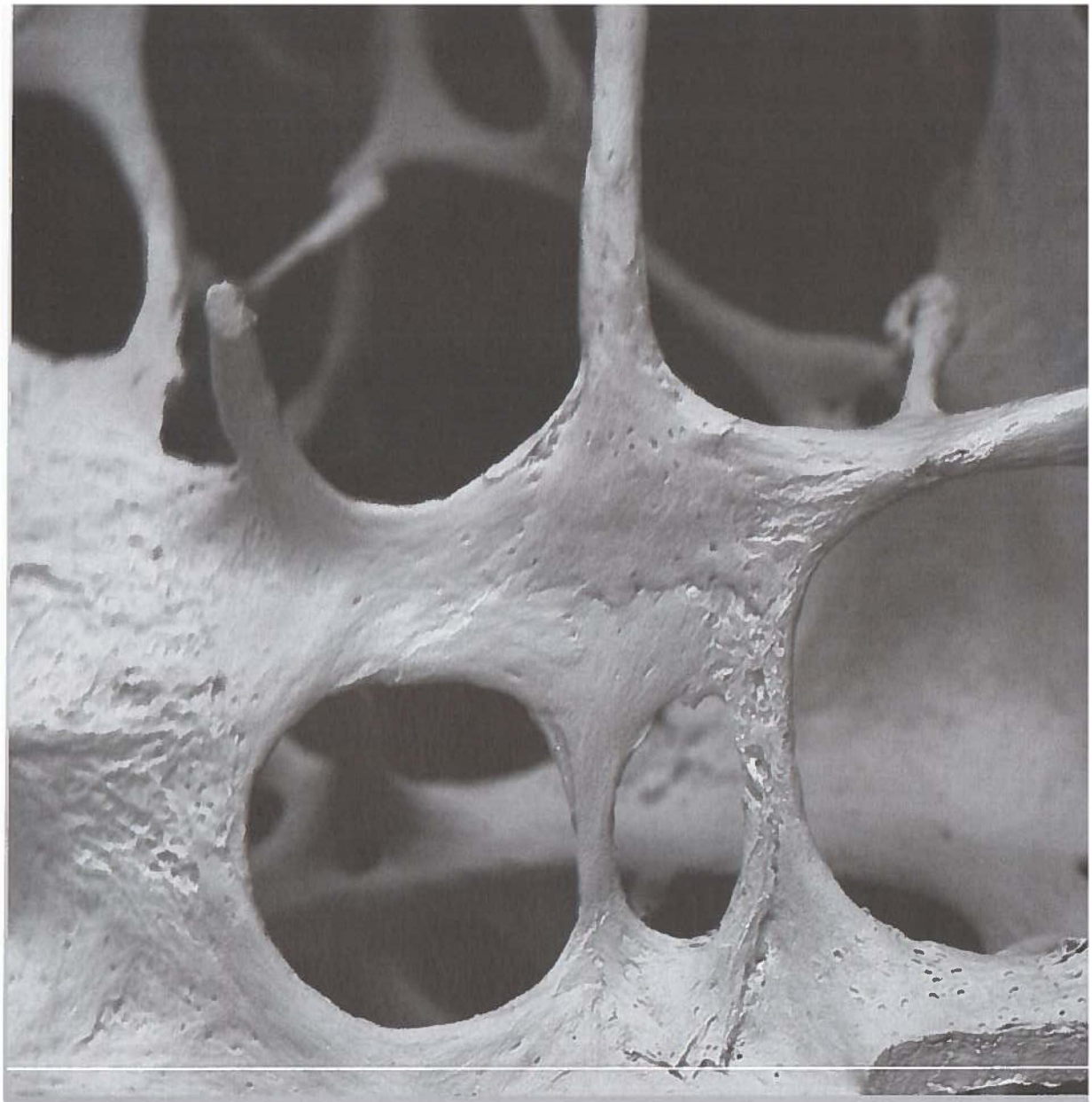
Onze studie onderstreept de herstellende effecten van levertransplantatie op "hepatische" osteopenie en osteoporose. Deze unieke "reversibele" vorm van osteoporose kan mogelijk dienen als studiemodel voor andere hypotheses over osteoporose in het algemeen. Tevens is er een scala aan hormonen, groeifactoren en cytokines (bijv. osteoprotegerin en insuline-like growth factor 1) die botmetabolisme beïnvloeden en waarvan de effecten op BMD in leverpatiënten niet goed onderzocht zijn. Mogelijk dat het verder onderzoeken van deze factoren in "hepatische" osteoporose kan leiden tot nieuwe etiologische mechanismes. Daarnaast zal de uitbreiding van onze studie met niet-cholestatische levercirrose patiënten en het meten

van femur-BMD mogelijk leiden tot verdere inzichten in botmetabolisme stoornissen, het identificeren van nieuwe risicofactoren, en ziekte-specifieke risicomodellen.

De verbetering in pre- en posttransplantatie botstatus gedurende de transplantatieperiode (1985 - 2001) is bemoedigend, echter het is onduidelijk of deze verbetering gehandhaafd blijft in de toekomst. Een verdere verbetering van de botstatus kan mogelijk bewerkstelligd worden door het reduceren van de tijd dat de falende lever zijn effecten uitoefent op het botmetabolisme. Echter, dit is momenteel niet te realiseren door het huidige allocatiesysteem van organen en het tekort aan orgaandonoren. Een verdere verbetering van "hepatische" osteoporose en botfracturen kan mogelijk bewerkstelligd worden door betere preventieve en therapeutische modaliteiten. Enkele studies hebben veelbelovende resultaten laten zien van antiresorptie-medicatie (bisfosfonaten) in het voorkomen van BMD-verlies voor en na levertransplantatie. Echter, toekomstige studies zijn nodig om aan te tonen dat deze therapie tevens leidt tot een reductie van botfracturen. Daarnaast is er geen duidelijkheid over de optimale dosis, timing van therapie, en veiligheid van gebruik van bisfosfonaten bij levercirrose patiënten. Het behandelen van verminderde botaanmaak met anabolische therapie kan mogelijk bijdragen aan de preventie dan wel behandeling van "hepatische" osteoporose. Teriparatide (rhPTH (1–34), het actieve fragment van endogeen humaan parathyroïd hormoon) heeft in studieverband gunstige effecten laten zien bij de behandeling van postmenopauzale osteoporose. Over de veiligheid en de werkzaamheid van teriparatide in patiënten met eindstadium levercirrose als mede over het gebruik na levertransplantatie is tot op heden niet veel bekend. Desalniettemin blijft de belangrijkste stap in het verder reduceren van BMD-verlies na levertransplantatie het verminderen en elimineren van glucocorticoïden. Echter, momenteel zijn er geen medicijnen op de markt die de effecten en daarmee het gebruik van glucocorticoïden kunnen vervangen.

Samenvattend, is er nieuw licht gaan schijnen op de botmetabolisme abnormaliteiten en het natuurlijk beloop ervan voor en na levertransplantatie in cholestatische levercirrose. Nieuwe risicofactoren voor hepatische osteoporose, botfracturen en AVN zijn geanalyseerd, terwijl de rol van andere risicofactoren bevestigd zijn. Tevens werd aangetoond dat de verbetering van BMD na levertransplantatie een aanhoudende verbetering is, terwijl botfracturen continueren te ontstaan. Gedurende de 16 transplantatie-jaren bleek dat osteoporose, fracturen en AVN in cholestatische patiënten verbeteren, maar het is onduidelijk of deze verbetering gehandhaafd kan worden door het aanhoudende tekort van orgaandonoren en het huidige allocatiesysteem van organen. Daarnaast is er ondanks vermindering van post-transplantatie glucocorticoïden geen significante vermindering gekomen in het verlies van botmineraaldichtheid vroeg na levertransplantatie. In de toekomst zijn betere modaliteiten nodig voor de preventie en behandeling van "hepatische" osteoporose en botfracturen voor en na levertransplantatie. Hiervoor is het tevens van belang om betere diagnostische mogelijkheden te ontwikkelen om botmetabolisme te onderzoeken. Met de komst van nieuwe immunosuppressiva in de toekomst wordt het misschien mogelijk het gebruik van glucocorticoïden te elimineren, wat een belangrijke stap zal zijn in het minimaliseren van verlies van botmineraaldichtheid na transplantatie.

Ofschoon de verdere verbetering van "hepatische" osteoporose afhangt van vele facetten, wordt het hopelijk in de toekomst mogelijk "hepatische" osteoporose voor en na levertransplantatie te voorkomen en te behandelen.



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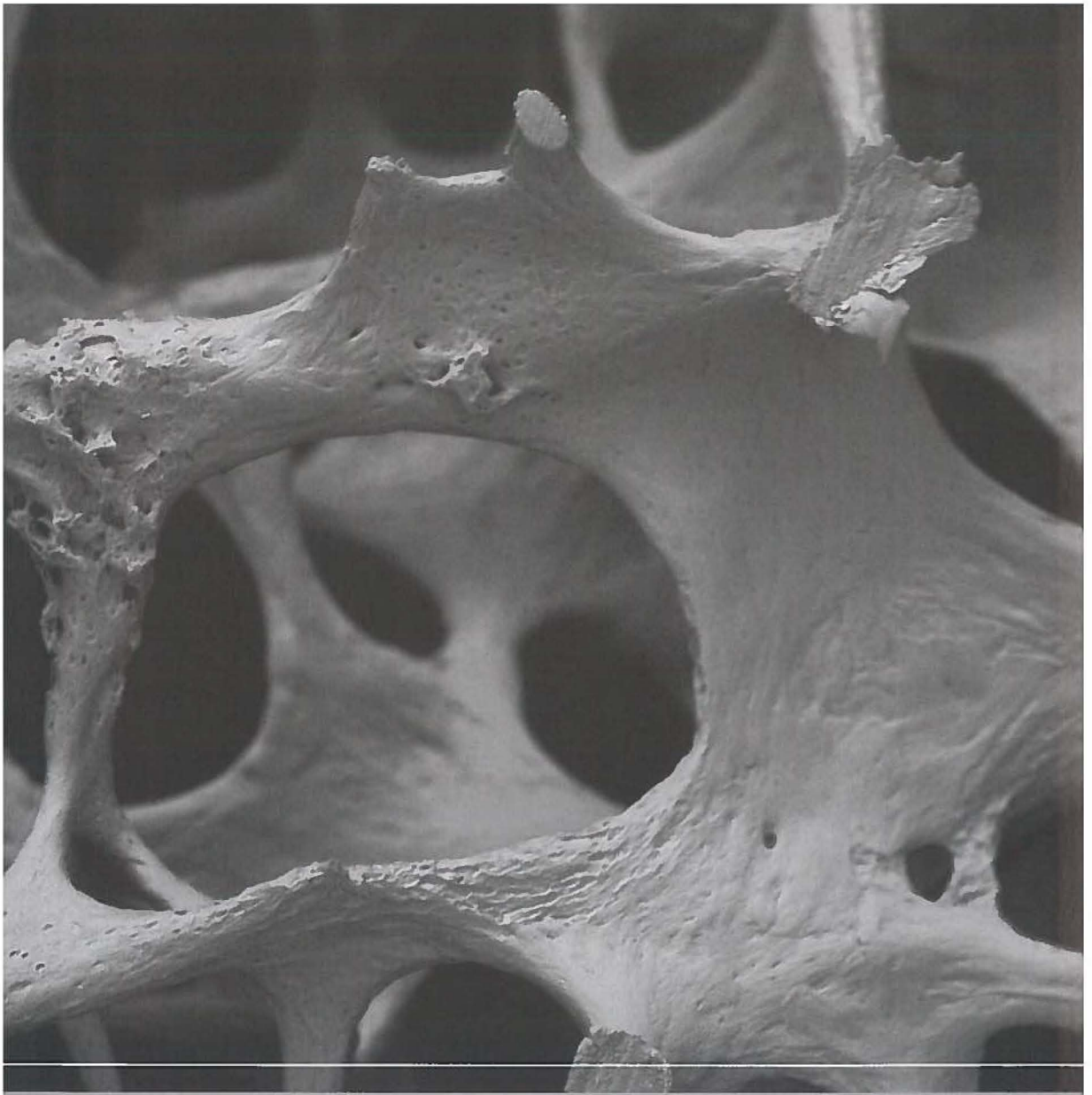

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About the author

Maureen Guichelaar was born on February 11, 1977, in Oldenzaal, the Netherlands. Here she attended elementary and high school, and became interested in sports, arts, music and outdoor activities. In 1995 she moved to Groningen to start her medical study at the University of Groningen. As a student she was involved in several student organizations and committees. In 1997 she joined the Gips International School of Hepatology and Tropical Medicine (GISH-T) under the supervision of Professor Chris H. Gips. Through GISH-T she went in 1998 to Ga-Rankuwa hospital, Pretoria, South-Africa to work on a research project (Nutritional status in Gauteng Province and assessment of severity of liver disease: a pilot study) under the supervision of Professor Tian van der Merwe and dr. Elwin Buchel. In 1999 she went through GISH-T to the Mayo Clinic in Rochester Minnesota, USA, to work on a research project (Bone disease before and after liver transplantation) under the supervision of Professor J. Eileen Hay. After her return to the Netherlands she started her clinical electives at Medisch Spectrum Twente, Enschede, with her final elective at the surgical ICU at the University Medical Center Utrecht. On January 31, 2002, she graduated with honors (cum laude) from the study of medicine.
After her graduation she obtained a 1-year position as a research fellow at the University Medical Center Groningen (under the supervision of Professor Maarten J.H. Slooff) with the aim to continue the research studies at the Mayo Clinic for a PhD-thesis. In this context she worked as a research fellow in the Liver Transplantation program of the Mayo Clinic from May 2002 until November 2003, under the supervision of Professor J. Eileen Hay. After her return to the Netherlands she worked for 6 months on implementing a prospective, multicentre, longitudinal study (Carotid IMT and IMT-PROgression as Predictors of Vascular Events in a High Risk European Population, IMPROVE-study) at the Isala Clinics, Zwolle, under the supervision of Professor Henk Bilo and dr. Andries J. Smit. In July 2004 she started her residency in Internal Medicine and Gastroenterology at Medisch Spectrum Twente, Enschede, and continued to work on the studies for the PhD-thesis. In July 2008 she will start her last 2 years of training in Gastroenterolgy and Hepatology at the University Medical Center Groningen.