Bone microarchitecture in patients with autoimmune hepatitis

Constantin Schmidt,1,2 Julian Stürzinkel,1 André Strahl,2 Ralf Oheim,1,3 Christina Weiler-Normann,3,4 Marcial Sebode,4 Florian Barvencik,1,3 Ansgar W. Lohse,3,4 Thorsten Schinke,1 Michael Amling,1,3 Christoph Schramm,3,4 and Tim Rolvien1,2

1 Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
2 Division of Orthopaedics, Department of Trauma and Orthopaedic Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
3 Martin Zeitz Center for Rare Diseases, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
4 1st Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

ABSTRACT

In patients with autoimmune hepatitis (AIH), osteoporosis represents a common extrahepatic complication, which we recently showed by an assessment of areal bone mineral density (aBMD) via dual-energy x-ray absorptiometry (DXA). However, it is well established that bone quality and fracture risk does not solely depend on aBMD, but also on bone microarchitecture. It is currently not known whether AIH patients exhibit a site-specific or compartment-specific deterioration in the skeletal microarchitecture. In order to assess potential geometric, volumetric, and microarchitectural changes, high-resolution peripheral quantitative computed tomography (HR-pQCT) measurements were performed at the distal radius and distal tibia in female patients with AIH (n = 51) and compared to age-matched female healthy controls (n = 32) as well as to female patients with AIH/primary biliary cholangitis (PBC) overlap syndrome (n = 25) and female patients with PBC alone (PBC, n = 36). DXA at the lumbar spine and hip, clinical characteristics, transient elastography (FibroScan) and laboratory analyses were also included in this analysis. AIH patients showed a predominant reduction of cortical thickness (Ct.Th) in the distal radius and tibia compared to healthy controls (p < .0001 and p = .003, respectively). In contrast, trabecular parameters such as bone volume fraction (BV/TV) did not differ significantly at the distal radius (p = .453) or tibia (p = .508). Linear regression models revealed significant negative associations between age and Ct.Th (95% confidence interval [CI], −14 to −5 μm/year, p < .0001), but not between liver stiffness, cumulative prednisolone dose (even after an adjustment for age), or disease duration with bone microarchitecture. The duration of high-dose prednisolone (>7.5 mg) was negatively associated with trabecular thickness (Tb.Th) at the distal radius. No differences in bone microarchitecture parameters between AIH, AIH/PBC, and PBC could be detected. In conclusion, AIH patients showed a severe age-dependent deterioration of the cortical bone microarchitecture, which is most likely the major contribution to the observed increased fracture risk in these patients. © 2021 The Authors. Journal of Bone and Mineral Research published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: AUTOIMMUNE HEPATITIS; AUTOIMMUNE LIVER DISEASE; BONE MICROARCHITECTURE; HR-pQCT; OSTEOPOROSIS

Introduction

Autoimmune hepatitis (AIH) is a chronic autoimmune liver disease with potentially severe flares, but is characterized by a good response to immunosuppressive therapy.(1) Although it may occur in patients of all ages and ethnic groups,(2,3) it most commonly affects middle-aged female patients.(2,5) The etiology of AIH is understood to be a multifactorial event in which genetic and environmental factors play a key role.(6) Its pathogenesis is based on a T-cell–mediated immune response to hepatic autoantigens. Indicators for inflammatory activity are the elevated serum transaminases alanine aminotransferase (ALT) and aspartate aminotransferase (AST), whereas a complete laboratory remission of AIH is defined as a normalization of the transaminase levels together with a normalization of immunoglobulin G (IgG).(7–9) Some of these patients also have features of the cholestatic autoimmune liver diseases primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC). The prevalence of AIH-PBC variant syndromes differs depending on the clinical scoring system and lies between 2.1% and 19%.(10)
Untreated AIH can lead to liver fibrosis, cirrhosis, and its complications.\textsuperscript{14}\n\nA severe extrahepatic complication of autoimmune liver diseases is systemic bone loss (i.e., osteoporosis) accompanied by an increased risk for fragility fractures.\textsuperscript{11–15}\nWe recently performed a dual-energy x-ray absorptiometry (DXA) study in a large cohort of 211 AIH patients in which we detected an osteoporosis prevalence of 19.2% for patients older than 50 years.\textsuperscript{15}\nWe identified independent risk factors for osteoporosis, such as prolonged glucocorticoid use >90 months, body mass index (BMI) < 23 kg/m\textsuperscript{2}, and liver fibrosis as diagnosed by transient elastography values >8 kilopascal (kPa).

Although widely performed and easy to assess, bone mineral density (BMD) evaluation by DXA has potential limitations. For instance, DXA does not allow a differentiation between poor mineralization (i.e., osteomalacia) and the actual loss of bone mass and/or bone microarchitecture. In addition, degenerative changes may cause false-high values in the lumbar spine.\textsuperscript{16}\nImportantly, DXA provides no information about the three-dimensional bone microarchitecture (i.e., trabecular or cortical bone compartments). Bone microarchitecture is of major clinical importance as the various parameters influence bone strength and, importantly, fracture risk independently of areal bone mineral density (aBMD).\textsuperscript{17–19}\nIn fact, nearly half of the postmenopausal women with fragility fractures have values above the World Health Organization (WHO) definition for osteoporosis (i.e., T-score ≤ −2.5), reflecting the low predictive value of DXA measurements.\textsuperscript{20}\nTherefore, it appears essential to obtain and evaluate data of the bone microarchitecture in order to adequately assess the risk for fractures in patients with AIH. High-resolution peripheral quantitative computed tomography (HR-pQCT) is a noninvasive technique that generates three-dimensional data of the bone microarchitecture, the volumetric BMD, including volumetric trabecular BMD (Tb.BMD) and cortical BMD (Ct.BMD), as well as bone geometry. We have recently shown that patients with PBC showed a severe deterioration of both trabecular and cortical bone parameters compared to a reference cohort, although there were no significant differences in aBMD assessed by DXA at the lumbar spine.\textsuperscript{21}\nThis underlines the clinical importance of this measurement in patients with liver diseases.

Hence, the primary goal of this study was to perform HR-pQCT measurements in a cohort of patients with AIH to assess the bone microarchitecture. Furthermore, we compared AIH patients with age-matched, BMI-matched, and sex-matched healthy controls, as well as patients with an AIH/PBC overlap syndrome, and PBC only to identify possible differences between these autoimmune liver diseases. In addition, we evaluated several clinical parameters (e.g., disease duration, liver stiffness, corticosteroid therapy) regarding their prediction quality for microarchitectural deterioration in AIH patients.

**Patients and Methods**

**Study cohort**

We retrospectively analyzed 51 consecutive female patients with AIH who underwent HR-pQCT at our specialized outpatient clinic. AIH was diagnosed in all patients as defined in the European Association for the Study of the Liver (EASL) practice guidelines\textsuperscript{22} by the 1st Department of Medicine, University Medical Center, Hamburg-Eppendorf, Germany based on clinical, biochemical, immunological, and histological criteria. We compared the obtained data with 25 age-matched female AIH/PBC overlap patients, 36 age-matched female PBC patients, and 32 healthy age-matched female controls from a previous study.\textsuperscript{15}\n
The demographic characteristics including age, sex, weight, height, and body mass index (BMI) as well as disease-specific characteristics such as disease duration and severity of liver disease were obtained from clinical examination or medical records and analyzed in an anonymized fashion in all patients. To evaluate potential treatment-induced bone loss, the current dose (within the last 6 months), cumulative dose (over the last 3 years) and the duration of high dose (>7.5 mg) prednisolone treatment before HR-pQCT measurements were extracted from the medical records. Furthermore, budesonide treatment was evaluated. All patients with underlying diseases predisposing for secondary osteoporosis (e.g., hyperparathyroidism, renal disease, cancer treatment-induced bone-loss) were excluded before analysis. All included patients were additionally examined by DXA (Lunar iDXA; GE Healthcare, Madison, WI, USA), with measurements at the lumbar spine and hip. This study was approved by the local ethics committee (WF57/17 and PV4081) and was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

**Laboratory assessment and liver elastography**

Biochemical analyses were performed in our local laboratory and included serum levels of bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (γGT), immunoglobulin G (IgG), immunoglobulin M (IgM), 25-hydroxyvitamin D3 (25-OH-D3), calcium, parathyroid hormone (PTH), bone-specific ALP (bALP), and osteocalcin. The definition of vitamin D insufficiency was a 25-OH-D3 level below 30 μg/L and that of vitamin D deficiency as less than 20 μg/L. Liver stiffness reflecting the stage of liver fibrosis was assessed using transient elastography (FibroScan\textsuperscript{16}; EchoSens, Paris, France) after determining the target area of the right liver lobe using ultrasound as reported.\textsuperscript{23} Procedures with at least 10 valid measurements, an interquartile range (IQR)/median ratio of less than 30%, and a success rate of at least 60% were included and the median value of liver stiffness measurements is recorded in kilopascals (kPa).

**HR-pQCT**

All patients underwent an HR-pQCT measurement (XtremeCT; SCANCO Medical AG, Brüttisellen, Switzerland) at the nondominant distal radius and opposing distal tibia using the default in vivo settings, namely, 60 kVp, 900 μA, 100 ms integration time, and a voxel size of 82 μm as described.\textsuperscript{24}\nAll scans were acquired by the same trained technician in order to achieve optimal standardization. The images were carefully examined for motion artifacts and repeated if motion artifacts were present that were previously categorized as grade 4 or 5.\textsuperscript{25}\nDue to previous fractures, metal implants or persistent motion artifacts, few measurements (n = 2 distal radius scans in the AIH group, n = 1 distal tibia scan in the AIH/PBC overlap group) had to be excluded, resulting in a slightly varying number of HR-pQCT measurements of the radius and tibia for some of the patient cohorts. We used the manufacturer’s standard protocol to generate three-dimensional microarchitectural data of the cortical and trabecular compartment and to analyze bone geometry, vBMD,
and bone microarchitecture. We controlled the quality of the scans by using a manufacturer’s daily calibration phantom.

Bone geometric parameters including total bone area (Tt.Ar, mm²), cortical area (Ct.Ar, mm²) and trabecular area (Tb.Ar, mm²), as well as vBMD measurements including total BMD (Tt.BMD, mg HA/cm³), trabecular BMD (Tb.BMD, mg HA/cm³), and cortical BMD (Ct.BMD, mg HA/cm³) were assessed according to the guidelines of the International Osteoporosis Foundation–American Society for Bone and Mineral Research–European Calfified Tissue Society (IOF-ASBMR-ECTS) working group. Bone microarchitecture values are comparable to those used in bone histology and include bone volume to total volume ratio (BV/TV), trabecular number (Tb.N, 1/mm), trabecular thickness (Tb.Th, mm), and trabecular separation (Tb.Sp, mm), as well as cortical thickness (Ct.Th, mm). The four study groups were additionally compared with normative HR-pQCT values from the literature expressed as the percentage of the age-specific, sex-specific, and site-specific normative data.

Statistical analysis
SPSS 22 software® (version 22.0; IBM Corp, Armonk, NY, USA) and GraphPad Prism® (version 7.0; GraphPad Software, La Jolla, CA, USA) were used for statistical analyses. Quantitative characteristics are presented as mean ± standard deviation (SD) or the number (proportion) of patients with condition. Normal distribution of the data was tested by using the D’Agostino and Pearson normality test. We used ordinary one-way analysis of variance (ANOVA) and Tukey’s multiple comparison test on normally distributed data while Kruskal-Wallis test and Dunn’s multiple comparison test was used on nonparametric-distributed data to identify significant differences within the four groups. To determine potential predictors of microarchitectural changes (BV/TV, Ct.BMD, and Ct.Th) in AIH patients, we performed linear regression models including age, FibroScan, and laboratory values such as ALT and IgG serum levels. Data was also tested for significant outliers and the respective data was excluded if applicable. Values of $p < .05$ were considered as statistically significant.

Results

Demographic, disease-specific, and bone-specific characteristics

Our AIH-study cohort consisted of 51 female patients. These patients were similar in age, height, weight, and BMI compared to patients with AIH/PBC overlap syndrome ($n = 25$), PBC only ($n = 36$), and healthy controls ($n = 32$) (Table 1). At the time point of HR-pQCT measurement, the mean disease duration of the AIH patients was 90.4 months. Although the disease duration was similar in all groups, the AIH cohort presented with significantly higher FibroScan values compared to PBC patients ($p = .027$).

When comparing the laboratory values of all three patient groups, significant higher levels of ALP were detected in patients with PBC compared to AIH patients ($p < .001$). Moreover, AIH patients had significantly lower levels of IgM compared to patients with AIH/PBC and PBC (both $p < .001$). There were no significant differences for laboratory values of calcium and bone metabolism parameters except for significantly higher levels of 25-OH-D₃ in patients with AIH compared to AIH/PBC and PBC patients ($p = .020$, $p = .018$) (Table 1). In total, 11 AIH patients (21.6%) had insufficient vitamin D levels and six patients (11.8%) were vitamin D-deficient with vitamin D levels lower than 20 μg/L.

At the time of assessment, a considerable frequency of AIH patients was treated with glucocorticoids within the last 6 months (prednisolone (43.1%)) and/or with budesonide (11.8%). The mean dose of prednisolone was 13.1 ± 17.9 mg, and the mean dose of budesonide was 5.1 ± 2.3 mg per day. The mean cumulative duration of prednisolone treatment was 1171 ± 1528 days and the mean cumulative dose of prednisolone in the last 3 years was 3503 ± 3053 mg. In contrast, only 10 of 25 (40.0%) of the AIH/PBC and 0 of 36 (0.0%) of the PBC patients received prednisolone within the last 6 months, respectively. Most of the AIH patients were treated with azathioprine (66.7%), whereas 56% of the AIH/PBC patients and only 13.9% of the PBC patients received azathioprine.

The mean aBMD T-score was significantly lower in AIH patients at the lumbar spine ($p = .004$) and the hip ($p = .030$) compared to controls, but similar compared to patients with PBC or AIH/PBC. Among all AIH patients, 14 (27.5%) had a history of fragility fractures, whereas only three (12.0%) of the AIH/PBC patients and six (16.7%) of the PBC patients had previously suffered from a fragility fracture (Table 1).

Bone volumetric, microarchitectural, and geometric alterations in AIH patients

Visualizations of the skeletal microarchitecture showed that AIH patients primarily had a cortical bone loss syndrome at the distal radius and tibia compared to healthy controls (Fig. 1A,E). Specifically, we first compared the HR-pQCT data of AIH patients with healthy controls. Compared with the controls, Tt.BMD was significantly lower in AIH patients both at the distal radius (282.3 mg HA/cm³ vs. 362.0 mg HA/cm³, $p < .0001$) and the distal tibia (264.4 mg HA/cm³ vs. 307.1 mg HA/cm³, $p = .004$) (Fig. 1B,F; Table 2). Tb.BMD did not significantly differ between patients with AIH and healthy controls at either location. Ct.BMD was significantly lower at the distal radius (radius 783.3 mg HA/cm³ vs. 865.0 mg HA/cm³, $p < .0001$) but not at the distal tibia ($p = .101$). Analysis of the bone microarchitecture parameters revealed no significant differences in the trabecular compartment (i.e., BV/TV, Tb.Th, and Tb.N) (Fig. 1C,G; Table 2), but a severe reduction of Ct.Th (radius 0.62 mm vs. 0.91 mm and tibia 0.92 mm vs. 1.15 mm; $p < .0001$ and $p = .003$, respectively) in AIH patients compared to controls (Fig. 1D,H; Table 2). Furthermore, Ct.Ar was significantly lower at both locations (radius 46.5 mm² vs. 61.5 mm² and tibia 95.7 mm² vs. 117.8 mm²; $p < .0001$ and $p = .004$, respectively).

Additionally, we compared HR-pQCT parameters of AIH patients with those of AIH/PBC and PBC patients but detected no significant differences in any of the HR-pQCT parameters. We additionally confirmed the reduction of cortical parameters in patients with AIH versus controls by comparing the measurement results to the previously published normative HR-pQCT data (Supplementary Fig. S1).

Variables associated with microarchitectural deterioration in AIH patients

Linear regression models were performed to investigate the effects of different variables on the microarchitectural changes in patients with AIH. For that purpose, TLBMD, BV/TV and Ct.Th were used as dependent variables, while age, BMI, disease
| Parameter                              | Reference range | AIH (n = 51) | AIH/PBC (n = 25) | PBC (n = 36) | CO (n = 32) | AIH versus AIH/PBC p | AIH versus PBC p | AIH versus CO p |
|---------------------------------------|-----------------|--------------|------------------|--------------|-------------|----------------------|-----------------|----------------|
| Demographic characteristics           |                 |              |                  |              |             |                      |                 |                |
| Age (years)                           | 52.4 ± 16.7     | 57.9 ± 13.1  | 52.1 ± 7.6       | 56.1 ± 8.1  | .288        | .999                | .561            |                |
| Height (cm)                           | 165.6 ± 6.9     | 164.8 ± 7.2  | 165.8 ± 6.3      | 164.0 ± 8.0 | .964        | .999                | .737            |                |
| Weight (kg)                           | 75.4 ± 15.8     | 72.2 ± 12.8  | 70.5 ± 16.0      | 73.8 ± 15.6 | .827        | .455                | .964            |                |
| BMI (kg/m²)                           | 27.6 ± 6.3      | 26.7 ± 4.7   | 25.9 ± 5.6       | 27.5 ± 5.9  | .902        | .506                | .999            |                |
| Disease characteristics               |                 |              |                  |              |             |                      |                 |                |
| Duration of disease (months)          | 90.4 ± 84.5     | 78.6 ± 63.3  | 81.6 ± 70.8      | .799         | .855        |                      |                 |                |
| FibroScan (kPa)                       | 8.6 ± 6.4       | 7.0 ± 2.8    | 5.6 ± 2.1        | .322         | .027        |                      |                 |                |
| Laboratory values                     |                 |              |                  |              |             |                      |                 |                |
| Bilirubin (mg/dl)                     | 0.3–1.2         | 1.0 ± 1.7    | 0.6 ± 0.4        | 0.5 ± 0.2    | .400        | .169                |                 |                |
| AST (U/L)                             | <35.0           | 33.1 ± 44.6  | 27.4 ± 10.5      | 29.8 ± 17.1 | .748        | .881                |                 |                |
| ALT (U/L)                             | <35.0           | 33.6 ± 29.2  | 31.0 ± 22.0      | 33.1 ± 14.2 | .893        | .993                |                 |                |
| ALP (U/L)                             | 46.0–116.0      | 87.5 ± 39.6  | 99.7 ± 49.8      | 135.6 ± 78.2 | .658        | <.001               |                 |                |
| γGT (U/L)                             | <38.0           | 59.6 ± 86.7  | 101.0 ± 295.3    | 69.8 ± 78.2 | .529        | .952                |                 |                |
| IgG (g/L)                             | 65–160          | 13.4 ± 6.1   | 12.7 ± 3.8       | 11.5 ± 3.5  | .828        | .183                |                 |                |
| IgM (g/L)                             | 0.5–3.0         | 1.2 ± 0.5    | 2.2 ± 1.5        | 2.1 ± 1.1   | <.001       | <.001               |                 |                |
| 25-OH-D₃ (μg/L)                       | >30.0           | 37.5 ± 14.2  | 28.5 ± 12.1      | 29.4 ± 13.3 | .020        | .018                |                 |                |
| Calcium (mMol/L)                      | 2.08–2.65       | 2.3 ± 0.1    | 2.3 ± 0.1        | 2.3 ± 0.1   | .801        | .391                |                 |                |
| PTH (ng/L)                            | 17.0–84.0       | 57.8 ± 24.8  | 77.2 ± 29.7      | 81.9 ± 51.0 | .237        | .077                |                 |                |
| bALP (μg/L)                           | 5.5–22.9        | 14.4 ± 8.8   | 17.8 ± 6.4       | 21.9 ± 13.8 | .658        | .057                |                 |                |
| Osteocalcin (μg/L)                    | 12.0–52.1       | 21.1 ± 8.0   | 20.4 ± 3.2       | 18.2 ± 6.1  | .965        | .394                |                 |                |
| Treatment regime                      |                 |              |                  |              |             |                      |                 |                |
| Prednisolone (within last 6 months)   | 22/51 (43.1)    | 10/25 (40.0) | 0/36 (0.0)       | 0/32 (0.0)  | .066        | <.001               |                 |                |
| Budesonide (within last 6 months)     | 6/51 (11.8)     | 2/25 (8.0)   | 0/36 (0.0)       | 0/32 (0.0)  | .066        | <.001               |                 |                |
| Vitamin D substitution (IU per week)  | 15908 ± 6931    | 12042 ± 6403| 10250 ± 7141     | .020         | .018        |                      |                 |                |
| Azathioprine                          | 34/51 (66.7)    | 14/25 (56)   | 5/36 (13.9)      | .965        | .394        |                      |                 |                |
| DXA                                   |                 |              |                  |              |             |                      |                 |                |
| T-score lumbar spine                  | −1.2 ± 1.4      | −1.0 ± 1.6   | −0.8 ± 1.5       | −0.1 ± 1.6  | .961        | .573                | .004            |                |
| Minimum T-score hip                   | −1.1 ± 1.1      | −1.4 ± 0.8   | −1.2 ± 1.1       | −0.5 ± 1.1  | .620        | .999                | .030            |                |
| History of fractures                  | 14/51 (27.5)    | 3/25 (12.0)  | 6/36 (16.7)      | .965        | .394        |                      |                 |                |

Note: Results are presented as mean ± SD, or n/n (%) Bold indicates significant differences (p < .05).

Abbreviations: γGT, gamma glutamyl transferase; 25-OH-D₃, 25-hydroxyvitamin D₃; AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; bALP, bone-specific alkaline phosphatase; BMI, body mass index; CO, healthy controls; DXA, dual-energy x-ray absorptiometry; IU, international unit; IgG, immunoglobulin G; IgM, immunoglobulin M; PTH, parathyroid hormone; SD, standard deviation.
duration, FibroScan values, treatment with glucocorticoids (cumulative prednisolone dose, duration of high-dose treatment) as well as laboratory values such as ALT and IgG served as independent variables. At the distal tibia, the results revealed that age was strongly negatively associated with Tt.BMD and Ct.Th. However, a significant influence of age on BV/TV could not be detected (Fig. 2A). To further investigate the influence of age on Ct.Th in patients with AIH, we compared the age-associated reduction of Ct.Th in patients with AIH with that of the healthy population by expressing the values as the percentage of the age-specific, sex-specific, and site-specific normative HR-pQCT data. We observed that the percentage of Ct.Th in patients with AIH compared to Ct.Th in the reference population decreased with age, supporting the observation that age represents an additional risk factor for cortical bone loss in AIH (Supplementary Fig. S2). We next tested for the effects of FibroScan values, cumulative prednisolone dose, and the serum levels of ALT and IgG on bone microarchitecture, but no associations were detected in any of the parameters (Fig. 2B–E). When evaluating the HR-pQCT parameters of the distal radius in association with the same clinical variables, we observed a similar pattern with significant associations between age and Ct.Th (p < .005), but no associations between any of the other parameters (data not shown).

In the PBC cohort, disease duration and stage were negatively associated with most bone microarchitecture parameters, although no associations with age were detected at the distal tibia (Supplementary Fig. S3). In the AIH/PBC overlap cohort, age and disease stage were negatively associated with Tt.BMD and Ct.Th, although disease duration was not associated with the bone microarchitecture at the distal tibia (Supplementary Fig. S4). Together, the cortical bone loss syndrome in the AIH cohort is primarily influenced by age, whereas in the PBC cohort it is primarily determined by disease stage and disease duration, and in the AIH/PBC overlap cohort by age and disease stage.

In the AIH cohort, other linear regression models (e.g., BMI, disease duration, and 25-OH-D₃ serum levels) did not reach any significant relationships at either location. Furthermore, no significant associations were detected between the duration of prednisolone treatment and any of the bone microarchitecture

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Fig. 1. Bone microarchitecture determined by HR-pQCT in AIH patients compared to AIH/PBC, PBC, and healthy controls. (A) Representative 3D reconstruction of the distal radius in an AIH patient compared to healthy control. (B) Quantification of total BMD of the radius. (C) Quantification of BV/TV of the radius. (D) Quantification of Ct.Th of the radius. (E) Representative 3D reconstruction of the distal tibia in an AIH patient compared to healthy control. (F) Quantification of total BMD of the tibia. (G) Quantification of BV/TV of the tibia. (H) Quantification of Ct.Th of the tibia. Ordinary one-way ANOVA and Tukey’s multiple comparison test was used on normally distributed data while Kruskal-Wallis test and Dunn’s multiple comparison test was used on non-parametric distributed data, *p < .05; **p < .01; ***p < .001; ****p < .0001. Abbreviations: 3D, three-dimensional; AIH, autoimmune hepatitis; ANOVA, analysis of variance; BMD, bone mineral density; BV/TV, bone volume to total volume; Ct.Th, cortical thickness; HR-pQCT, high-resolution quantitative computed tomography; PBC, primary biliary cholangitis.
TABLE 2. Bone microarchitecture assessed by HR-pQCT

| Parameter          | AIH     | AIH/PBC | PBC     | CO      | AIH versus AIH/PBC p | AIH versus PBC p | AIH versus CO p |
|--------------------|---------|---------|---------|---------|----------------------|------------------|-----------------|
| Radius             | n = 49  | n = 25  | n = 36  | n = 32  |                      |                  |                 |
| Tb.BMD (mg HA/cm³) | 282.3 ± 66.4 | 269.7 ± 78.2 | 294.6 ± 56.1 | 362.0 ± 54.3 | >.999                | >.999            | <.0001          |
| Tt.BMD (mg HA/cm³) | 144.1 ± 42.4 | 126.1 ± 37.1 | 151.6 ± 35.4 | 154.2 ± 40.2 | .921                 | >.999            | .631            |
| Cl.BMD (mg HA/cm³) | 783.3 ± 80.0 | 784.2 ± 78.8 | 783.8 ± 67.9 | 865.0 ± 39.9 | >.999                | >.999            | <.0001          |
| BV/TV              | 0.12 ± 0.0 | 0.11 ± 0.0 | 0.13 ± 0.0 | 0.13 ± 0.0 | >.999                | >.999            | .453            |
| Tb.N (1/mm)        | 1.9 ± 0.4 | 1.8 ± 0.5 | 2.1 ± 0.3 | 1.9 ± 0.4 | .990                 | .727             | >.999           |
| Tb.Th (mm)         | 0.06 ± 0.0 | 0.06 ± 0.0 | 0.06 ± 0.0 | 0.07 ± 0.0 | >.999                | >.999            | .174            |
| Tb.Ar (mm²)        | 235.7 ± 80.7 | 234.0 ± 60.9 | 219.0 ± 54.2 | 186.4 ± 51.2 | >.999                | >.999            | .021            |
| Ct.Ar (mm²)        | 95.7 ± 31.3 | 95.5 ± 30.1 | 99.1 ± 23.4 | 117.8 ± 26.7 | >.999                | >.999            | <.0001          |

Note: Results are presented as mean ± SD. Bold indicates significant differences (p < .05).
Abbreviations: AIH, autoimmune hepatitis; AIH/PBC, AIH/PBC overlap syndrome; BV/TV, bone volume/total volume; CO, healthy control; Ct.Ar, cortical area; Ct.BMD, cortical bone mineral density; Ct.Th, cortical thickness; HA, hydroxyapatite; HR-pQCT, high-resolution peripheral quantitative computed tomography; PBC, primary biliary cholangitis; SD, standard deviation; Tb.Ar, trabecular area; Tb.BMD, trabecular bone mineral density; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.BMD, trabecular bone mineral density.

values (Supplementary Fig. S5). Based on the assumption that high doses of corticosteroids may lead to more severe bone loss, we also evaluated the relationship between the duration of high-dose prednisolone treatment (i.e., ≥7.5 mg/day for at least 3 months) and HR-pQCT parameters. The duration of high-dose treatment was not associated with a deterioration of bone microarchitecture parameters at the tibia, although a significant negative association was observed with the Tb.Th at the distal radius (Fig. 3A,B). We furthermore tested for the associations between the cumulative prednisolone dose in the last 3 years and the age- and sex-adjusted values for Tb.Th and Ct.Th. Although a trend towards lower Tb.Th with higher duration of high-dose prednisolone was noted, no significant associations could be detected (Supplementary Fig. S6).

Discussion

In the recent years, the importance of bone microarchitecture analyses for the evaluation of bone quality and fracture risk has been outlined in a variety of disorders potentially affecting skeletal integrity. Although we have previously shown that AIH patients are at high risk of developing osteoporosis, a detailed analysis of bone microarchitecture in patients with AIH had not yet been performed. In the present study, we demonstrated that patients with AIH display pronounced alterations in the cortical bone microarchitecture primarily associated with age but not with disease stage, disease duration, or markers of hepatitis activity. The magnitude of cortical bone loss in AIH was comparable to that observed in patients with PBC and AIH/PBC overlap syndrome.

Cortical thickness is known to be an important contributor to bone strength and thus the risk of fractures independently of aBMD, and the majority of bone loss after an age of 65 years is cortical. As we have recently demonstrated, age is also a critical factor for bone loss measured by DXA in patients with AIH. However, it was unknown whether this age-associated bone loss in patients with AIH is due to cortical, trabecular, or combined loss of structure. Here we show that progressing age in patients with AIH leads to a reduction of cortical thickness, but not of trabecular parameters. In addition, our data suggest that age is also a risk factor for cortical bone loss in AIH that exceeds age-associated bone loss in healthy controls. However, for further verification of this hypothesis, investigations with larger group sizes should be conducted in the future. Interestingly, although a predominantly trabecular bone loss has been described for women with an age of 50 to 64 years, the presented female AIH patients similar of age showed a cortical bone loss and mostly intact trabecular microarchitecture, which might give further insights into the possible pathological changes in bone remodeling in patients with AIH. This can be also of clinical relevance as these patients might benefit most from treatment.
Fig. 2. Associations between clinical/laboratory characteristics and bone microarchitecture at the distal tibia. (A) Associations between age and the microarchitectural parameters Tt.BMD, BV/TV, and Ct.Th. (B) FibroScan and Tt.BMD, BV/TV, and Ct.Th. (C) Associations between cumulative prednisolone dose in the last 3 years and Tt.BMD, BV/TV, and Ct.Th. (D) Serum levels of ALT and Tt.BMD, BV/TV, and Ct.Th. (E) IgG serum levels and Tt.BMD, BV/TV, and Ct. Th. Linear regression models were calculated in all panels. Abbreviations: ALT, alanine aminotransferase; BV/TV, bone volume to total volume; Ct.Th, cortical thickness; IgG, immunoglobulin G; Tt.BMD, total bone mineral density.
with the receptor activator of nuclear factor-κB (NF-κB) ligand (RANKL) antibody denosumab, which has been shown to increase cortical thickness to a large extent. (32) In fact, it was recently shown that short-term and long-term therapy with denosumab significantly increased BMD in patients with both AIH and PBC. (33)

In other autoimmune diseases such as rheumatoid arthritis, Crohn’s disease, and ulcerative colitis, a predominant cortical bone loss has been shown suggesting inflammation as a possible cause for a lower cortical thickness in AIH. (34) This is in line with elevated pro-inflammatory cytokines that were found in patients with AIH. (35-36) In fact, the idea of a reciprocal influence of immune and bone cells led to the interdisciplinary research field of osteoimmunology and RANKL appears to be an important link between the bone and immune system. (37,38) The production of RANKL is mainly mediated by T helper 17 (Th17) cells and interleukin 17 (IL17). (38,39) We recently found that the reduction of cortical bone mass in patients with PSC is associated with Th17 cell frequency. (12) In these patients, the Th17 cell frequency correlated positively with bone resorption indicated by increased levels of urinary deoxypyridinoline crosslinks. Although Th17 cells and IL17 also seem to play a role in the pathogenesis of AIH, (40) it is likely that an association exists with high bone resorption as well as the cortical bone loss observed in our AIH patient collective. However, these associations remain to be studied with further analyses including assessments of Th17 cells and bone resorption parameters. The serum level of the transaminase ALT and IgG serve as an indicator for the inflammatory activity in the liver, also being a part of the diagnostic score by the International Autoimmune Hepatitis Group. (41) We were not able to detect a significant association of ALT and IgG serum levels with cortical parameters, which may have been influenced by the low number of patients with active disease.

Another cause for bone loss in patients with autoimmune hepatitis may be explained by their treatment with glucocorticoids. (42,43) We could recently show that the duration of prednisolone therapy correlated negatively with aBMD assessed by DXA in AIH patients. (15) In our study cohort, treatment duration with prednisolone was not associated with bone microarchitecture parameters (even after adjusting for patient age), suggesting that the treatment with glucocorticoids may not fully explain the bone loss in AIH patients. Nonetheless, because our detailed analyses revealed that the duration of high-dose prednisolone treatment was associated with trabecular thinning at the distal radius, we found evidence of a microarchitectural pattern compatible with glucocorticoid-induced osteoporosis, (44) although the cortical deterioration seemed to be not affected by glucocorticoids in our study cohort. Furthermore, one might speculate if the potentially negative effects of prednisolone were compensated by its reduction of inflammation, therefore counteracting the AIH-associated (cortical) bone resorption. In this regard, it is important to note that the extent of cortical bone loss was similar in AIH and PBC patients, although PBC patients received no glucocorticoids. In contrast to AIH, the cortical bone loss syndrome in PBC patients is primarily influenced by disease stage and duration but not patient age. (21) Besides inflammation and glucocorticoid treatment, liver fibrosis is an independent risk factor for bone loss in patients with AIH. (15) However, the majority of our patients showed fibrosis values within the reference range, indicating a rather early AIH stage in our cohort, suggesting that additional factors might have played the leading role for the development of the observed microarchitectural alterations.

**Fig. 3.** Relationship between the duration of high-dose (≥7.5 mg) prednisolone treatment and bone microarchitecture parameters. (A) Negative association between high-dose prednisolone treatment and Tb.Th but not Tt.BMD, BV/TV, and Ct.Th at the distal radius. (B) Associations between high-dose prednisolone treatment and bone microarchitecture parameters at the distal tibia. Linear regression models were calculated in all panels. Abbreviations: BV/TV, bone volume to total volume; Ct.Th, cortical thickness; Tb.Th, trabecular thickness; Tt.BMD, total bone mineral density.
The significant difference in vitamin D serum levels between AIH patients and PBC patients is most likely explained by the higher weekly vitamin D supplementation in AIH patients. Similarly, the significant difference in ALP levels between AIH and PBC patients can be explained by the fact that higher levels of ALP are known in patients with PBC and therefore serve as a diagnostic criteria. However, lower ALP values in AIH could also be attributed to higher vitamin D levels. In this context, it is also possible that the (missing) associations between prednisolone treatment and some of the bone microarchitecture could have been influenced by vitamin D treatment, because vitamin D supplementation is often initiated in a close temporal context with prednisolone initiation. Together, the differences in laboratory markers between the groups were essentially not associated with differences in the bone microarchitecture between AIH, PBC, and AIH/PBC overlap syndrome.

Although the assessment of bone microarchitecture by HR-pQCT represents one of the most accurate clinical methods to determine fracture risk, it is known that fracture risk does not solely depend on bone microarchitecture. Other relevant factors include bone qualitative changes that are not covered by HR-pQCT such as the mineral or collagen quality, but also factors that are not directly related to bone quality, such as the patients’ individual fall risk. In our patient collective, the fact that fragility fractures had been observed more frequently in AIH than in PBC patients could be attributed to the considerable frequency of AIH patients treated with prednisolone. In glucocorticoid-induced osteoporosis, bone qualitative changes include lower mineral content as well as collagen fibril deformation at the nanoscale, which may not be fully depicted by the microarchitectural deficit observed in HR-pQCT. In the future, studies with larger cohorts are needed to verify the trend of higher fracture prevalence in AIH compared to PBC.

The present study has a few limitations that need to be acknowledged. First, we did not measure biomarkers of bone resorption; therefore, we cannot make definitive statements concerning the bone-resorption related mechanisms of bone loss in AIH. Second, although around 25% of AIH patients are male, we did not include male AIH patients because we aimed to provide a homogenous study cohort with available reference data. Therefore, we cannot make any statement on the possible loss of bone microarchitecture in male patients. Third, we did not measure cortical porosity as an important additional parameter of cortical bone quality. We used the first-generation HR-pQCT scanner, which has a lower nominal isotropic resolution compared to the second generation (82 μm vs. 61 μm). Because recent studies described a high degree of resolution dependence with particularly poor accuracy of cortical porosity parameters with lower voxel size, we decided not to measure or include the cortical porosity. Fourth, we were not able to assess the menopausal status in all of the included patients. However, the differences in the mean age of the AIH and the control group were only minor (and nonsignificant), also the menopausal status likely does not differ in a relevant manner.

Taken together, this is the first assessment of bone microarchitecture in patients with AIH showing a severe deterioration of the cortical compartment that was associated with age. Based on our data, HR-pQCT measurements are useful to identify AIH patients with a high risk for fractures who would benefit from a bone-specific therapy. More clinical and experimental studies are now needed to gain a better understanding of the pathologic changes in bone remodeling in AIH patients as well as their treatment response to bone-specific agents.

Disclosures

All authors state that they have no conflicts of interest.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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