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ORIGINAL ARTICLE

Bone mineral density and trabecular bone score in treatment-naïve patients with non-cirrhotic hepatitis C virus infection

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Abstract

Summary We studied 112 treatment-naïve chronic HCV patients without cirrhosis, and we found that, especially HCV+ postmenopausal women, they had lower TBS and BMD values than healthy controls. This suggests that HCV infection is an independent risk factor for osteoporosis, and therefore, screening for osteoporosis in postmenopausal HCV+ women should be considered.

Purpose To know whether patients in earlier stages of chronic HCV infection are at increased risk of developing low bone mass and bone microarchitectural changes and whether there is an association between bone metabolism and the severity of the liver disease.

Methods We studied 112 treatment-naïve chronic HCV outpatients and 233 healthy age- and sex-matched controls. Bone mineral density (BMD) and trabecular bone score (TBS) were assessed by DXA. Serum 25(OH)D, PTH, P1NP, and CTX were determined by electrochemiluminescence.

Results TBS values were significantly lower in HCV patients than in controls, both considering the population as a whole (1.337 ± 0.119 vs. 1.377 ± 0.122; $p < 0.005$) and after stratifying by sex (1.347 ± 0.12 vs. 1.381 ± 0.13 in men and 1.314 ± 0.10 vs. 1.369 ± 0.11 in women). The difference remained significant ($p < 0.0001$ in all cases) after adjusting for confounders. BMD was also lower in HCV patients (lumbar spine, 0.935 ± 0.151 vs. 0.991 ± 0.143 g/cm^2, p 0.001; femoral neck, 0.764 ± 0.123 vs. 0.818 ± 0.123 g/cm^2, p 0.0001; total hip, 0.926 ± 0.148 vs. 0.963 ± 0.132 g/cm^2, p 0.02), although, after adjustment, differences kept a clear trend towards statistical significance in women at the lumbar spine and femoral neck. However, in men and at the total hip in women, differences were no longer significant. We find no relationship between these parameters and the severity of the disease. No significant difference was observed in PTH and 25OHD status after adjustment. Finally, serum P1NP, but not CTX, was higher in HCV patients.

Conclusions Our findings suggest that HCV infection is an independent risk factor for osteoporosis, especially among postmenopausal women. Therefore, the appropriateness of screening for osteoporosis in postmenopausal HCV-positive women should be considered.

Keywords TBS · BMD · HCV infection · 25(OH)D · PTH · Osteoporosis

Introduction

Hepatitis C virus (HCV) is recognized as one of the main causes of chronic liver disease (CLD) [1]. Over 70 million individuals worldwide are chronically infected [2, 3]; many of whom are unaware of their infection, contributing to its perpetuation [4].

Despite a new era of direct-acting antiviral (DAA) therapies, which have made elimination strategies theoretically feasible, HCV still represents a leading cause of cirrhosis, hepatocellular carcinoma (HCC), liver transplantation, and liver-

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related death worldwide [5, 6]. Furthermore, chronic HCV infection is associated with an important number of extrahepatic manifestations, mainly autoimmune and lymphoproliferative disorders [7].

Bone manifestations are another well-known extrahepatic complications of CLD. The term "hepatic osteodystrophy" refers to bone disorders associated with CLD, and includes osteoporosis; osteopenia; and, less frequently, osteomalacia [8, 9]. Its highest prevalence is found in cholestatic and alcoholic liver disease, where it reaches up to 50% [9, 10].

However, few studies have evaluated the possible disorders of bone mineral metabolism that may occur in patients with CLD who have not developed cirrhosis [11–14]. This number is still substantially lower when only patients with chronic HCV infection without other causes of liver diseases are considered. Thus, we have only been able to find information about this subject in a small number of studies [15–18]. They frequently show discordant results, though most of them tend to find an association between HCV infection and bone disease [15, 16, 18, 19]. Moreover, some methodological issues related to suboptimal osteoporosis diagnosis (i.e., lack of bone densitometry [19]), the treatment some patients were on [11], and the absence of a healthy control group [15] have limited the value of their results.

Trabecular bone score (TBS) is an imaging technique developed to indirectly evaluate the state of the trabecular microarchitecture based on the information provided by the standard DXA [20]. It consists of a texture parameter that assesses pixel gray-level variations in the projected lumbar spine DXA image. Thus, the TBS may be considered an overall index of bone quality and low values have been associated with a worse bone structure and a higher risk of fracture [20, 21]

Accordingly, our study aimed to determine whether patients in earlier stages of chronic HCV infection are at increased risk of losing bone mass and microarchitectural damage, as well as whether there is an association between bone metabolism and the severity of the underlying liver disease.

Patients and methods

Study design and participants

From April 2016 to October 2017, treatment-naïve chronic HCV outpatients who were about to start DAA therapy were recruited from the Gastroenterology Department at the University Hospital Marqués de Valdecilla in Northern Spain. Chronic HCV infection was defined as positivity of anti-HCV antibodies and serum HCV RNA for more than 6 months. HCV genotype, years of known infection, and viral load before treatment were collected in all the patients. Liver elastography was also performed using FibroScan® 502 50-Hz touch system device (Echosens, North America Inc.). Fibrosis was considered significant when patients had a FibroScan result ≥ 2 ($F \geq 2$).

Exclusion criteria were age under 18 or over 75 years; liver cirrhosis (defined as follows: (a) Child-Pugh score > 6; (b) FibroScan® result of F4 with altered platelet count; (c) prothrombin time or bilirubin; and (d) history of end-stage liver disease complications [e.g., varices, ascites, or hepatic encephalopathy]); human immunodeficiency virus (HIV) co-infection; hepatitis B virus co-infection; autoimmune liver disease; genetic liver disease (e.g., Wilson disease, hemochromatosis); or any other causes of hepatic disease. Other exclusion criteria were alcohol consumption > 30 g/day, active intravenous drug use, chronic kidney disease (defined as estimated glomerular filtration rate < 45 ml/min/1.73 m^2), active bone metabolic diseases, or concomitant use of drugs known to affect bone metabolism.

Healthy age- and sex-matched subjects (ratio close to 1:2) randomly selected from the Camargo Cohort Study were included as a control group. Participants of this cohort, of which more details have been previously published [22, 23], are a representation of the general population. Moreover, we used data from 30 healthy young volunteer fellows from our hospital, to complete the youngest part of the control group, as the Camargo Cohort Study does not include individuals under 40 years of age.

At the baseline visit, subjects were interviewed by the investigators and all participants provided data regarding the risk factors for osteoporosis and fractures using a structured questionnaire which included age; race; weight; height; body mass index (BMI); personal history of fractures in adulthood (> 40 years); family history of osteoporotic fractures among first-degree relatives; tobacco use; consumption of dairy products; alcohol use (g/day); history of diseases such as cardiovascular disease, diabetes mellitus, dyslipidemia, or renal dysfunction; and present or past consumption of medications. Height and weight were measured with subjects wearing light indoor clothing and without shoes. BMI was defined as weight (kg) divided by squared height (m^2). Tobacco smoking was categorized as current smoker, former smoker (patients who had quit smoking more than 1 year earlier), or never smoker. Menopause was defined as the complete cessation of menstruation for 12 months or more.

The study was approved by the local Ethics Committee (Comité Ético de Investigación Clínica de Cantabria-IDIVAL, internal code: 2016.003), and all the subjects gave written informed consent.

Biochemical tests

Blood was drawn in the morning after overnight fasting. Routine tests included complete blood cell count, glucose, creatinine, aspartate transaminase (AST), alanine

transaminase (ALT), total alkaline phosphatase, total serum calcium, phosphate, and albumin. They were measured using standard methods (ADVIA 2400 Chemistry System autoanalyzer; Siemens, Germany). Serum concentrations of 25-hydroxyvitamin D (25OHD), intact parathyroid hormone (PTH), aminoterminal propeptide of type I collagen (PINP), and C-terminal telopeptide (CTX) of type I collagen were determined by a fully automated Roche electrochemiluminescence system (Elecsys 2010, Roche Diagnostics, GmbH, Mannheim, Germany). The detection limit of serum 25OHD was 4 ng/ml, its intraassay coefficient of variation (CV) was 5%, and its interassay CV was 7.5%. Regarding intact PTH, the detection limit was 6 pg/ml, with a normal range of 15–65 pg/ml. Intraassay and interassay CVs were 3.4% and 5.9%, respectively. The PINP limit of detection was 5 ng/ml (reference range between 15 and 78 ng/ml in men, and between 19 and 100 ng/ml in postmenopausal women), and its intraassay and interassay CVs were 3.9% and 4.1%, respectively. Intraassay and interassay CVs for CTX were 4.2% and 4.7%, and the detection limit was 0.01 ng/ml. Its reference range were 0.069–0.760 ng/ml in men and 0.112–1.018 ng/ml in postmenopausal women [22, 23]

DXA measurements

All subjects underwent BMD testing by DXA at the lumbar spine (LS), femoral neck (FN), and total hip (TH) (Hologic QDR 4500, Bedford, MA, USA). In vivo precision was 0.4–1.5% at the different measurement sites. Results were expressed as grams per square centimeter. Results were also expressed as *T*-score (defined as the number of standard deviations (SDs) below the mean value for young people) and *Z*-score (defined as the number of SDs below the mean for people of the same age). Osteoporosis was defined as a *T*-score ≤ -2.5 and osteopenia as a *T*-score ≤ -1 and > -2.5 following the WHO classification [24]. Quality control was performed according to the usual standards [22].

Trabecular bone score

Spine TBS measurements were performed with the TBS software installed in our DXA equipment (TBS iNsight® v2.1, Med-Imaps, Pessac, France). The TBS was calculated based on the data acquired by the DXA scan, assessing the same vertebrae at which the LS-BMD had been measured [25]. As a rule, the measurement of BMD in LS was performed in L1–L4, except for those cases in which the vertebral morphology advised its exclusion.

Statistical analysis

Baseline characteristics of the population were recorded as a whole and for men and women separately. All continuous variables were tested for normality. Variables non-normally distributed underwent a logarithmic transformation before statistical analyses. Results were expressed as mean ± SD, median (interquartile range), or percentages, as appropriate. Student's *t* test or Mann-Whitney *U* test was used to determine the differences between groups for continuous variables and χ^2 test for categorical variables.

Multivariable general linear analyses were performed to compare the site-specific BMD, bone serum markers, and TBS of HCV patients and controls adjusting for BMD- and TBS-related covariates, including age, BMI, tobacco use, and diabetes.

Univariate and multivariate regression models were built to analyze the association between HCV infection with site-specific BMD and TBS results. All p values < 5% were considered significant.

Results

One-hundred and seventy-one treatment-naïve chronic HCV outpatients were recruited. Figure 1 shows the flow chart of the study patients. Finally, a total of 112 patients completed the study: seventy-seven men between 19 and 74 years old and 35 postmenopausal women. The demographic and clinical characteristics of the population according to gender are presented in Tables 1 and 2. The mean age of HCV patients and controls was similar, but BMI was lower in HCV patients. There were significantly more smokers among HCV+ population, while alcohol consumption was higher in control males but not in females, although as stated, patients with alcohol consumption greater than 30 g/day were excluded. The prevalence of dyslipidemia and diabetes mellitus were similar in patients and controls, although hypertension prevalence was higher among controls.

Among HCV patients, the most frequent genotype was 1a and 1b (64%) with an average viral load (VL) of 6.07×0^6 IU/ml, 7.04×10^6 IU/ml in men, and 3.96×10^6 IU/ml in women. VL > 10^6 UI/l was detected in 67% of patients. Almost half of the patients (56% of men and 40% of women) had significant liver fibrosis (F2–F4), while the remaining had fibrosis of low degree (F0 and F1). The known duration of HCV infection was about 15 years in both, men (15 ± 12 years) and women (16 ± 14 years).

Serum 25OHD levels were higher in patients than in controls although these differences disappeared when adjusted for confounders. Conversely, PTH levels were significantly lower in both men and women HCV patients, although these differences also disappeared after adjusting. P1NP was higher in

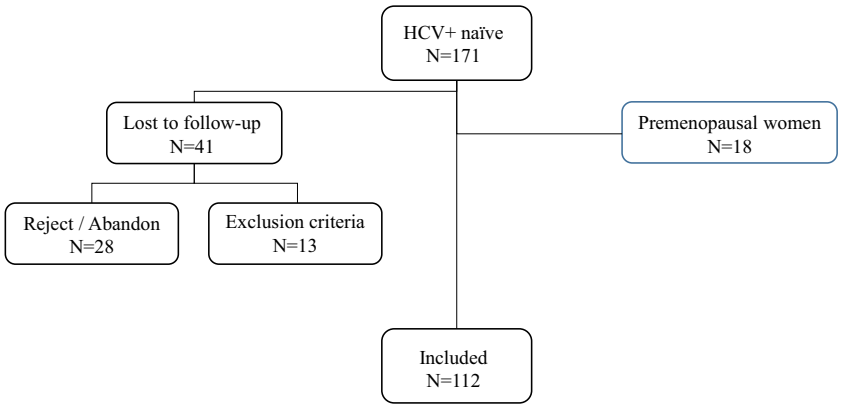

Fig. 1 (supplement) Flow chart of the study patients

patients, both men and women, and these differences persisted ($p = 0.02$ in men and $p = 0.04$ in women) after adjusting for confounders. There was no difference in CTX between HCV patients and controls (Table 1).

Table 2 summarizes the LS-, FN-, and TH-BMD values (g/cm^2 and *T*- and *Z*-scores) in patients and controls, both in the whole population and in men and women separately. BMD was significantly lower in HCV patients. Differences were greater in women than in men. In women, BMD at the lumbar spine was lower in patients than in controls by 9.0% ($p = 0.001$), while in men, the difference only amounted to 4.3% ($p = 0.03$). The corresponding values at the femoral neck were 12.0% and 4.5% ($p < 0.05$), and at the total hip 8.7% and 1.9% ($p = 0.001$ in women, and not significant in men). At the femoral neck, the difference in women almost reaches a standard deviation (*T*-score, − 1.47 vs. − 0.62). After adjustment for age, BMI, tobacco use, and diabetes status, the significant difference was lost in men. In women, the significant difference was lost at the total hip, although it remained significant at the lumbar spine and femoral neck ($p < 0.05$). Nevertheless, since the number of years since menopause was different in HCV+ women than in the controls, we have performed an additional adjustment adding this factor. After that, the significance was borderline at the lumbar spine ($p = 0.05$) and showed a trend towards it ($p = 0.06$) at the femoral neck (Table 3).

Overall, 18.8% of HCV patients and 12.9% controls had osteoporosis (the difference not being significant), whereas osteopenia was observed in 52.7% of the patients and 27.5% of the controls ($p = 0.001$). When stratifying by sex, 15.6% of HCV+ men and 25.7% of HCV+ postmenopausal women had osteoporosis, whereas these figures were 12.7% ($p = 0.3$) and 13.2% ($p = 0.09$) among control subjects. About half (49.1%) of VHC+ males and 63% of postmenopausal women showed *T*-scores compatible with osteopenia (Figs. 2 and 3). These percentages were higher than those seen in healthy, age-matched controls (22.3% in men; $p < 0.0001$, and 38.2% in women; $p = 0.015$). Altogether, 64% and 75% of men and postmenopausal women HCV+ reached *T*-scores consistent with osteoporosis or osteopenia, compared with 35% ($p = 0.0001$) and 51.2% ($p = 0.015$) in men and women in the control group.

No relationship was found between BMD values or the percentage of osteopenia and osteoporosis with the presence of significant fibrosis ($F \geq 2$), high VL ($> 10 \times ^6$ IU/ml), or HCV genotype. Moreover, the risk for osteopenia or osteoporosis was not related to the estimated duration of HCV infection.

TBS values were significantly lower in HCV patients than in controls (Table 2), both considering the population as a whole and after stratifying by sex. The difference remained significant ($p < 0.0001$ in all cases) after adjusting for confounders (Table 3). Again, we do not find any association between TBS values and liver fibrosis, VL, HCV genotype, and the duration of the viral infection.

Among HCV patients, 49 men (63%) and 15 women (44%) had a TBS value > 1.310, which is considered to be normal; 12 men (16%) and 10 women (29%) had values between 1.230 and 1.310, consistent with partially degraded microarchitecture; and 12 men (16%) and 7 women (22%) had a TBS value < 1.230, which defines degraded microarchitecture [20, 21]. In controls, these figures were 117 men (74%) and 56 women (74%); 17 men (10%) and 14 women (18%); and 23 men (14%) and 6 women (8%; $p = 0.041$), respectively.

The association between TBS and serum PTH levels was negative in HCV patients ($r = - 0.227$; $p < 0.03$), although it was canceled after adjusting for age and BMI.

BMD at the hip was also negatively related with PTH in HCV patients (FN: $r = - 0.137$ [$p < 0.0001$]; TH: $r = - 0.101$ [$p < 0.0001$]), but not at the lumbar spine. After adjustment for age and BMI, the association was canceled at the femoral neck, but not at the total hip. A positive relationship was also found between 25(OH)D and BMD at the femoral neck ($r = 0.054$ [$p = 0.04$]) and the total hip ($r = 0.066$ [$p = 0.01$]). Again, after adjustment for age and BMI, the association was canceled at the femoral neck, but not at the total hip.

Table 1 Baseline characteristics of HCV patients and controls

	Total			Men			Postmenopausal women		
	HCV+ (*N*, 112)	Control (*N*, 233)	*p*	HCV+ (*N*, 77)	Control (*N*, 157)	*p*	HCV+ (*N*, 35)	Control (*N*, 76)	*p*
Age (year)	53.0 ± 9.1	52.8 ± 8.8	0.84	50.9 ± 8.8	51.6 ± 8.9	0.57	57.7 ± 8.0	55.4 ± 8.0	0.16
BMI (kg/m^2)	25.6 ± 4.5	28.6 ± 4.5	0.0001	26.0 ± 4.3	28.6 ± 4.0	0.0001	24.8 ± 4.9	28.5 ± 5.3	0.001
Current smoking[a]	60.7	21.0	0.0001	63.6	23.9	0.0001	54.3	15.8	0.0001
Current alcohol[a,b]	29.5	39.5	0.07	28.6	54.5	0.0001	31.4	13.2	0.02
HCV RNA (IU ml/10^6)	6.07 ± 2.02	-	-	7.04 ± 2.5	-	-	3.96 ± 5,2	-	-
Significant fibrosis $F \geq 2$[a]	48.6	-	-	56.6	-	-	40.0	-	-
Menopause (years)	47.1 ± 6.7	48.2 ± 4.4	0.43	-	-	-	47.1 ± 6.7	48.2 ± 4.4	0.43
Years since menopause	-	-	-	-	-	-	10.8 ± 8.8	7.3 ± 8.3	0.04
Hypertension[a]	24.1	35.2	0.04	20.8	38.9	0.006	31.4	27.6	0.68
Dyslipidemia[a]	18.8	25.1	0.56	19.5	20.4	0.87	17.1	23.7	0.44
Diabetes mellitus[a]	11.6	8.6	0.37	14.3	10.8	0.44	5.7	3.9	0.65
Glucose (mg/dl)	95.8 ± 29.5	96.3 ± 29.9	0.88	98.7 ± 34.1	99.6 ± 34.4	0.86	89.3 ± 13.7	90.5 ± 18.3	0.73
Creatinine (mg/dl)	0.8 ± 0.4	1.0 ± 0.2	0.0001	0.9 ± 0.4	1.0 ± 0.2	0.001	0.7 ± 0.1	0.9 ± 0.1	0.0001
AST (U/l)	48.9 ± 28.9	-	-	47.4 ± 28.5	-	-	52.3 ± 29.6	-	-
ALT (U/l)	65.1 ± 49.5	-	-	68.1 ± 55.0	-	-	58.5 ± 34.1	-	-
Alkaline phosphatase (U/l)	78.9 ± 26.4	63.6 ± 19.6	0.0001	74.9 ± 22.6	63.3 ± 19.5	0.001	87.7 ± 31.8	772.3 ± 18.6	0.01
Total calcium (mg/dl)	9.2 ± 0.4	9.6 ± 0.4	0.0001	9.2 ± 0.4	9.6 ± 0.3	0.001	9.2 ± 0.3	9.7 ± 0.4	0.0001
Albumin (g/dl)	4.3 ± 03	4.5 ± 0.3	0.0001	4.3 ± 0.2	4.6 ± 0.3	0.001	4.4 ± 0.2	4.5 ± 0.3	0.05
cCa (mg/dl)	8.9 ± 0.4	9.1 ± 0.3	0.0001	8.9 ± 0.5	9.1 ± 0.3	0.001	8.9 ± 0.4	9.3 ± 0.3	0.0001
25OHD (ng/ml)	28.4 ± 14.8	24.8 ± 10.7	0.03	28.4 ± 15.8	25.0 ± 9.7	0.13	28.2 ± 12.5	24.6 ± 12.4	0.11
PTH (pg/ml)	39.9 ± 32.1	47.9 ± 20.3	0.007	40.1 ± 37.2	48.5 ± 18.2	0.0001	39.3 ± 16.9	46.9 ± 23.6	0.03
PINP (ng/ml)	55.8 ± 25.4	43.1 ± 20.5	0.0001	53.4 ± 27.4	38.9 ± 18.7	0.0001	60.7 ± 20.1	50.4 ± 21.7	0.01
CTX (ng/ml)	0.342 ± 0.191	0.303 ± 0.176	0.07	0.311 ± 0.187	0.278 ± 0.160	0.12	0.410 ± 0.186	0.348 ± 0.195	0.10

In VHC patients, there were some significant relationships between BMD and both P1NP and CTX (P1NP and lumbar spine BMD, − 0.075 [p = 0.45]; P1NP and femoral neck BMD, − 0.233 [p = 0.02]; P1NP and total hip BMD, − 0.192 [p = 0.05]; CTX and lumbar spine BMD, − 0.228 [p = 0.02]; CTX and femoral neck BMD, − 0.328 [p = 0.001]; and CTX and total hip BMD, − 0.327 [p = 0.001]). However, there was no association between TBS and either P1NP (r = − 0.100 [p = 0.33]) or CTX (r = − 0.030 [p = 0.56]). After adjusting for age and BMI, only the associations between femoral neck BMD and both PINP and CTX (p = 0.04 and p = 0.03, respectively), and between total hip BMD and serum CTX (p = 0.03) remained significant in patients with HCV infection. No correlation between serum bone turnover markers and VL was found.

Discussion

We have shown that TBS values are decreased in HCV patients, even after adjusting for possible confounding factors such as age, BMI, tobacco use, and diabetes status. BMD is also lower than normal in these patients, although the difference after adjusting only remains significant at the lumbar spine and femoral neck in women. However, we find no relationship between these parameters and the severity of the disease.

Hepatic osteodystrophy is a well-recognized extrahepatic complication of cirrhosis that leads to the development of low BMD in the setting of CLD [8, 9]. The prevalence of osteoporosis in CLD varies widely, ranging from 15 to 55% [8–10]. This variability depends, on the one hand, on the population studied and, on the other hand, on the type and severity of the underlying liver disease. The highest prevalence occurs in advanced cirrhosis and disorders of cholestatic nature. According to Nakchbandi [26], the estimated prevalence of liver-related osteoporosis is between 20 and 420/100000 of the general population and fractures between 60 and 880/100000. Given the high prevalence of osteoporosis and the increased risk of fracture in CLD, screening for metabolic bone diseases has been recommended in patients diagnosed with cirrhosis [27].

Table 2 BMD and TBS findings by sex in HCV patients and controls

	Total			Men			Postmenopausal women		
	HCV+ (112) m ± SD	Control (233) m ± SD	*p*	HCV+ (77) m ± SD	Control (157) m ± SD	*p*	HCV+ (35) m ± SD	Control (76) m ± SD	*p*
Lumbar BMD	0.935 ± 0.151	0.991 ± 0.143	0.001	0.971 ± 0.148	1.015 ± 0.146	0.03	0.854 ± 0.128	0.941 ± 0.124	0.001
T-score	− 1.29 ± 1.33	− 0.98 ± 1.27	0.04	− 1.07 ± 1.34	− 0.85 ± 1.32	0.23	− 1.71 ± 1.19	− 1.24 ± 1.22	0.05
Z-score	− 0.61 ± 1.37	− 0.30 ± 1.32	0.04	− 0.66 ± 1.41	− 0.41 ± 1.34	0.21	− 0.48 ± 1.24	− 0.07 ± 1.24	0.11
FN-BMD	0.764 ± 0.123	0.818 ± 0.123	0.0001	0.800 ± 0.120	0.836 ± 0.118	0.03	0.687 ± 0.09	0.781 ± 0.12	0.0001
T-score	− 1.10 ± 0.91	− 0.65 ± 0.96	0.0001	− 0.93 ± 10.90	− 0.66 ± 0.89	0.03	− 1.47 ± 0.82	− 0.62 ± 1.10	0.0001
Z-score	− 0.22 ± 0.87	0.23 ± 0.96	0.0001	− 0.17 ± 0.92	− 0.12 ± 0.88	0.02	− 0.32 ± 0.74	0.45 ± 1.10	0.0001
TH-BMD	0.926 ± 0.148	0.963 ± 0.132	0.02	0.975 ± 0.136	0.995 ± 0.126	0.27	0.819 ± 0.113	0.897 ± 0.117	0.001
T-score	− 0.56 ± 0.95	− 0.28 ± 0.89	0.007	− 0.36 ± 0.91	− 0.23 ± 0.85	0.30	− 1.00 ± 0.86	− 0.37 ± 0.96	0.001
Z-score	− 0.03 ± 0.93	0.21 ± 0.91	0.02	0.01 ± 0.95	0.12 ± 0.84	0.36	− 0.12 ± 0.89	0.40 ± 0.99	0.009
TBS L1–L4	1.337 ± 0.119	1.377 ± 0.122	0.005	1.347 ± 0.12	1.381 ± 0.13	0.04	1.314 ± 0.10	1.369 ± 0.11	0.01

The main pathogenic mechanism involved in the development of osteoporosis in liver disease is a deficient bone formation. It has been proposed that this decrease in bone formation is due to the harmful effects of substances related to liver metabolism, such as bilirubin and bile acids, or to substances with a toxic effect on both the liver and the bone (osteoblasts), such as alcohol or iron [28, 29]. On the other hand, changes in molecular systems involved in the regulation of bone metabolism, such as the RANKL/RANK/OPG system [30], have also been reported. Similarly, decreases in IGF1 and vitamin D have been considered to play a role [8]. Besides, CLD patients may present with risk factors that are known as contributing to the development of osteoporosis (smoking, malnutrition, physical inactivity, or hypogonadism [8, 9]).

Bone metabolism in viral hepatitis has been much less studied than in alcoholic or cholestatic liver disease, especially in patients without cirrhosis. Whether chronic HCV infection in the absence of cirrhosis or viral treatment represents a risk factor for the development of the bone disease is a matter of debate, and negative [17] or positive [15, 16, 18, 19, 31] results have been published. Although studies are difficult to compare because of differences in their design, most of them have concluded that HCV infection is associated with a decrease in bone mass. Our results support this conclusion.

We have further shown that HCV patients had low TBS values compared with controls, even though neither the severity of liver disease (assessed by elastography) nor VL or HCV genotype was related to this decrease of TBS values. As stated before, TBS is a parameter that can be obtained from bone densitometry images provided by some types of devices. It is related to the bone microarchitecture and gives additional information to conventional densitometry on bone texture [20, 21, 32]. TBS is lower in postmenopausal women with a history of osteoporotic fractures than in those without, regardless

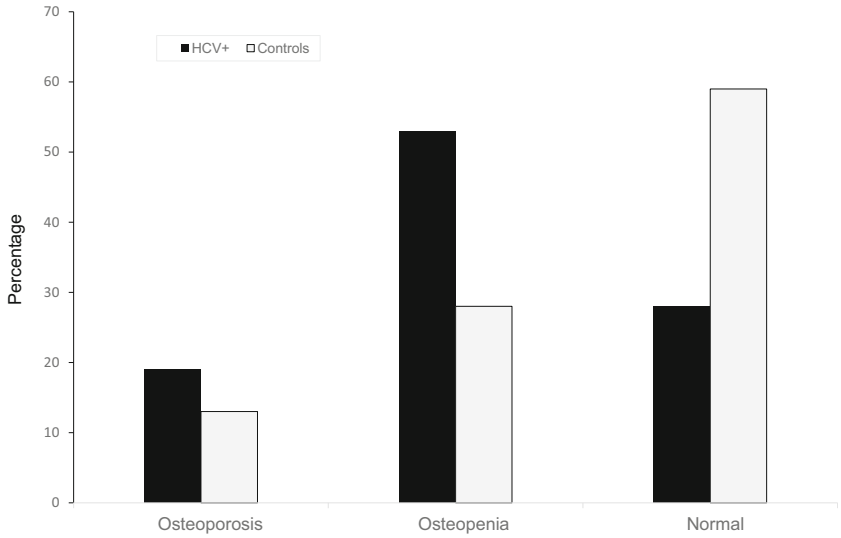

Fig. 2 Comparison of the prevalence of osteoporosis, osteopenia, or normal bone mineral density, according to *T*-score values, between HCV patients and healthy controls

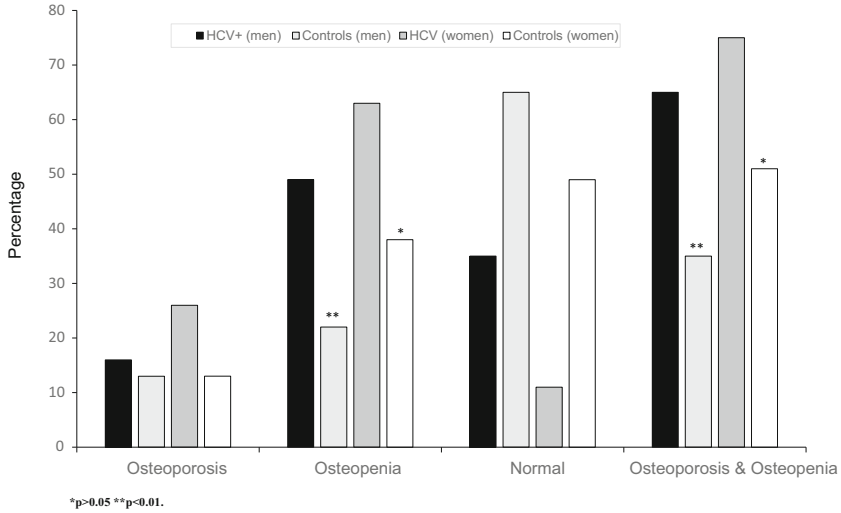

Fig. 3 Comparison of the prevalence of osteoporosis, osteopenia, and normal or reduced bone mineral density (osteoporosis and osteopenia) in HCV patients and controls according to gender

of the lumbar BMD, and various prospective studies have reported that TBS is an independent risk factor for fractures [20, 21, 32]. Therefore, the low TBS found in our HCV patients suggests that microstructural abnormalities may be implicated in the higher fracture risk described in HCV infection [33].

Our results agree with those recently reported by Bedimo et al. [34] who found that HCV infection in men is associated with a low TBS score at the lumbar spine. The authors assessed BMD and TBS in male subjects with HIV/HCV co-infection, HIV infection, HCV infection, and controls. They showed that both HIV and HCV infections were associated with decreased BMD, but only HCV, and not HIV, was related to a lower TBS after adjustment for possible confounders.

Besides, our data also confirm that non-cirrhotic HCV patients did not present any clinically relevant abnormality concerning serum calciotropic hormones [10, 18]. No significant difference was observed in 25OHD status between HCV patients and healthy controls, after adjusting for confounders, suggesting that liver injury was not severe in our patients, at least in terms of vitamin D hydroxylation. The difference in PTH levels in univariate analysis was also canceled after adjustment for BMI and tobacco use.

Noteworthy, despite high normal vitamin D status and low or normal PTH levels, serum P1NP, but not CTX, was higher in HCV patients. In this regard, Lai et al. [15] have previously observed high serum P1NP levels in non-cirrhotic HCV patients with low BMD. Besides, Lin et al. [16] reported high serum levels of bone alkaline phosphatase in HCV+ osteoporotic patients. These findings would be in contrast with the mechanism considered to be responsible for bone loss in cirrhotic patients which would be mainly associated with low bone turnover, with a decrease in bone formation [8, 9]. On the other hand, in our study, the slight increase in serum CTX levels found in HCV patients did not reach statistical significance. This does not exclude the possibility of an increase in bone turnover, but it makes it unlikely since remodeling is a coupled process where resorption precedes bone formation. A mechanism for bone loss different from that involved in cirrhosis would be under the idea of HCV as an independent risk factor for osteoporosis. This idea seems possible if we take into account that the duration of the infection and the presence of significant fibrosis were not related to an increased prevalence of bone disorders in our patients. In HCV liver disease, an inflammatory response dominates the pathophysiologic response to the infection with an association with increased cytokine release [35]. Thus, it could be speculated that systemic inflammation associated with HCV infection could explain the changes observed in bone metabolism [35, 36]. However, Lai et al. [15] assessed this association by comparing serum high sensitivity CRP, TNF-α, and IL-6 in 28 HCV+ non-cirrhotic patients with osteoporosis or osteopenia and 32 HCV+ subjects with normal BMD, and they did not find significant differences.

Our study has several limitations. Firstly, as an observational study, it has the limitations inherent to these types of studies, mainly the impossibility of establishing a causal relationship. Secondly, since the moment when the patient becomes infected may be difficult to establish, the associations we have described may not be precise, as we cannot say with certainty how much time has elapsed since the beginning of the infection. Finally, the cross-sectional nature of the study precludes the evaluation of longitudinal change in BMD and TBS in the participants. Among the strengths, we want to emphasize that the participants were well-characterized, and all subjects were carefully studied from the mineral and bone metabolism point of view after excluding any disease or treatment known to affect bone metabolism. Moreover, all the samples were obtained at the same time of the day and in a fasting state and all BMD and TBS measurements were

performed with the same device. Thus, factors to minimize biological variability have been controlled.

In summary, we found that non-cirrhotic HCV patients had lower BMD and TBS values than healthy controls. After adjustment for confounding factors, these differences persisted except for BMD in men. Although the underlying pathogenic mechanism is not clear, it seems that microarchitectural changes and bone loss are not likely to result from a decrease in bone formation. Therefore, our findings suggest that HCV infection is an independent risk factor for osteoporosis, especially among postmenopausal women, and therefore, the appropriateness of screening for osteoporosis in postmenopausal HCV+ women should be considered.

BMI, body mass index; *HCV RNA*, serum HCV RNA; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *cCa*, corrected calcium; *PTH*, intact parathyroid hormone; *25OHD*, 25-hydroxyvitamin D; *PINP*, aminoterminal propeptide of type I collagen; *CTX*, C-terminal telopeptide of type I collagen

[a]Data are expressed as percentages

[b]Alcohol consumption was lower than 30 g/day in patients and controls

HCV, hepatitis C virus; *BMD*, bone mineral density; *FN*, femoral neck; *TH*, total hip; *TBS*, trabecular bone score

Table 3 Multivariable general linear models showing adjusted BMD at the lumbar spine (LS-BMD), femoral neck (FN-BMD) and total hip (TH-BMD), and TBS values, in HCV patients and controls

	Mena	Postmenopausal womena		Postmenopausal womenb					
	HCV+ (77) m (SE)	Controls (157) m (SE)	*p*	HCV+ (35) m (SE)	Controls (76) m (SE)	*p*	HCV+ (35) m (SE)	Controls (76) m (SE)	*p*
LS-BMD	0.983 (0.02)	1.013 (0.01)	0.26	0.874 (0.02)	0.933 (0.01)	0.046	0.876 (0.02)	0.932 (0.01)	0.05
FN-BMD	0.825 (0.01)	0.818 (0.01)	0.71	0.718 (0.02)	0.766 (0.01)	0.048	0.721 (0.02)	0.765 (0.01)	0.06
TH-BMD	1.000 (0.02)	0.980 (0.01)	0.35	0.850 (0.02)	0.883 (0.01)	0.17	0.863 (0.02)	0.877 (0.01)	0.56
TBS L1–L4	1.304 (0.02)	1.388 (0.01)	< 0.0001	1.290 (0.02)	1.379 (0.01)	< 0.0001	1.289 (0.02)	1.379 (0.01)	< 0.0001

m, least square mean; *SE*, standard error

[a]Model 1: adjusted by age, BMI, tobacco use, and diabetes status

[b]Model 2: adjusted by age, BMI, tobacco use, diabetes status, and duration of menopause

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Compliance with ethical standards

The study was approved by the local Ethics Committee (Comité Ético de Investigación Clínica de Cantabria-IDIVAL, internal code: 2016.003), and all the subjects gave written informed consent.

Conflicts of interest None.

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