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MAJOR ARTICLE

Effects of Hepatitis C Virus (HCV) Eradication on Bone Mineral Density in Human Immunodeficiency Virus/HCV-Coinfected Patients

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Background. Little is known about the effects of eradication of hepatitis C virus (HCV) on bone mineral density (BMD) and biomarkers of bone remodeling in human immunodeficiency virus (HIV)/HCV-coinfected patients.

Methods. We prospectively assessed standardized BMD (sBMD) at the lumbar spine and femoral neck, World Health Organization BMD categories at both sites, and plasma concentrations of soluble receptor activator of NF-κβ ligand (sRANKL), and osteoprotegerin (OPG) at baseline (the date of initiation of anti-HCV therapy) and at 96 weeks.

Results. A total of 238 patients were included. The median age was 49.5 years, 76.5% were males, 48.3% had cirrhosis, 98.3% were on antiretroviral therapy, median $CD4^+$ cell count was 527 cells/μL, and 86.6% had HIV-1 RNA <50 copies/mL. The prevalence of osteoporosis at baseline at the lumbar spine (LS) and femoral neck (FN) was 17.6% and 7.2%, respectively. Anti-HCV therapy comprised pegylated interferon (peg-IFN) and ribavirin (RBV) plus 1 direct-acting antiviral in 53.4%, peg-IFN/RBV in 34.5%, and sofosbuvir/RBV in 12.2%. A total of 145 (60.9%) patients achieved sustained virologic response (SVR). No significant effect of SVR was observed on sBMD for the interaction between time and SVR either in the LS ($P = .801$) or the FN ($P = .911$). Likewise, no significant effect of SVR was observed in plasma levels of sRANKL ($P = .205$), OPG ($P = .249$), or sRANKL/OPG ratio ($P = .123$) for the interaction between time and SVR. No significant correlation was found between fibrosis by transient elastography, and LS and FN sBMD, at baseline and week 96.

Conclusions. SVR was not associated with significant changes in BMD nor biomarkers of bone remodeling in HIV/HCV-coinfected persons.

Keywords. HIV; hepatitis C; osteoporosis; bone; biomarkers.

Osteoporosis and bone fractures are common among patients with liver cirrhosis [1, 2], particularly among those of advanced age, smokers, postmenopausal women, individuals with alcoholism, malnourished individuals, and in liver disease of cholestatic etiology [3].

Nevertheless, viral hepatitis has been associated with reduced bone mineral density (BMD), and several factors have been hypothesized to contribute to it. On the one hand, elevated serum levels of inflammatory cytokines could inhibit bone formation and increase bone resorption. On the other hand, progressive hepatitis-induced liver dysfunction may be associated with reduced hepatic hydroxylation of vitamin D, hypogonadism, and impaired hepatic production of insulin-like growth factor 1 and osteoprotegerin, both of which promote bone formation. The association between noncirrhotic chronic hepatitis C virus (HCV) infection and osteoporosis is, however, controversial [4].

The prevalence of osteoporosis in human immunodeficiency virus (HIV)–infected persons is approximately 15%, which is >3 times greater than reported in HIV-uninfected controls [5]. HCV infection has been associated with an increased risk of osteoporosis and bone fractures in persons with HIV [6]. However, the mechanism is not well understood and may involve the severity of the liver disease, a reduction in BMD, microstructural abnormalities associated with HCV infection, and increased levels of inflammatory markers [7–10]. Nevertheless, unmeasured confounders, including behavioral and nutritional factors, have not been completely ruled out.

Little is known about the effects of sustained virologic response (SVR) on BMD in patients with HCV infection [11–13],

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and to the best of our knowledge, the subject has not been assessed in patients coinfected with HIV/HCV. Our study's main objective was to assess the effects of eradication of HCV on BMD and biomarkers of bone remodeling in HIV/HCV-coinfected individuals. Knowing the effects of therapeutic HCV eradication on BMD may contribute to understanding better the etiopathogenesis of bone loss among HIV/HCV-infected individuals. In addition, this research question is clinically relevant in the current era of direct-acting anti-HCV agents in which SVR can be achieved in most HIV/HCV-infected individuals, even in those with advanced liver disease [14].

METHODS

Design and Patient Selection

Ours was a multicenter prospective study of HIV/HCV-coinfected patients initiating anti-HCV therapy between February 2012 and February 2016 in 14 centers distributed across 5 regions in Spain: Madrid (n = 7), Valencia (n = 3), Catalonia (n = 2), Basque Country (n = 1), and Andalusia (n = 1). The cohort included both naive and anti–HCV therapy–experienced HIV/HCV-coinfected patients with compensated liver disease, who initiated treatment against HCV. Infectious diseases physicians decided to administer anti-HCV therapy and regimen selection at each institution according to contemporary guidelines. The ethics committees of Hospital General Universitario Gregorio Marañón and Hospital Universitario La Paz approved the study. This work was conducted following the Declaration of Helsinki. All patients provided written informed consent for the study. All the centers were monitored (following the last patient's first visit, interim monitoring, and following the last patient's last visit) to verify that all the information in the case report form was consistent with the patient's clinical history.

Investigation

Baseline was the date of anti-HCV treatment initiation. Patients with an undetectable serum HCV RNA level 12 weeks after discontinuation of anti-HCV therapy were classified as having SVR. Clinical and laboratory variables were collected at baseline, every 4 weeks during anti-HCV treatment, and every 12 weeks after discontinuation of therapy, until 96 weeks after the initiation of treatment. All the information was recorded at each institution using a shared database via an online form, which satisfied local requirements of data confidentiality.

Baseline variables included demographics, height and weight, and systolic and diastolic blood pressure. It also included clinical information related to HIV, HCV-related liver disease, other comorbid conditions, prior anti-HCV therapy, whether or not patients were receiving combination antiretroviral therapy (cART) or other medications (including opioid substitution therapy), smoking history, and ongoing alcohol intake >50 g/day. Laboratory tests included complete blood counts, coagulation tests, a comprehensive serum biochemistry profile (including serum lipids, alanine aminotransferase, aspartate aminotransferase, albumin, creatinine, 25-OH vitamin D, thyroxine, thyrotropin, and parathyroid hormone), HIV-1 RNA and HCV RNA quantification, HCV genotype, hepatitis B surface antigen, and determination of $CD4^+$ and $CD8^+$ T lymphocytes.

Assessment of Liver Fibrosis

Liver fibrosis at baseline was assessed using transient elastography (FibroScan; Echosens, Paris, France) performed by trained operators. Results were expressed in kilopascals (kPa) with a range of 2.5–75 kPa. We considered 10 acquisitions with a success rate of at least 60% and an interquartile range (IQR) <30% of the median value as representative measurements of liver stiffness. Fasting was not routinely required before the examination. Cirrhosis was defined as a liver stiffness value ≥12.5 kPa [15] or by liver biopsy.

Assessment of Bone Mineral Density

BMD was assessed using dual-energy X-ray absorptiometry (DXA) at the lumbar spine and femoral neck. In accordance with World Health Organization (WHO) definitions, osteoporosis was defined as a T score of −2.5 standard deviations (SD) or lower and osteopenia as a T score between −1 and −2.5 SD [16]. Normal status was defined as a T score above −1 SD. As different densitometers were used (Hologic [n = 8], Lunar [n = 3], and Norland [n = 2]), standardized BMD (sBMD) was also calculated based on published equations [17, 18].

Biomarkers of Bone Remodeling

Plasma samples were taken at baseline and 96 weeks after the initiation of therapy and stored frozen in the HIV BioBank of Hospital General Universitario Gregorio Marañón (http://hivhgmbiobank.com). Two biomarkers of bone formation and resorption were determined in these samples: soluble receptor activator of NF-κβ ligand (sRANKL) and osteoprotegerin (OPG) [19]. These biomarkers are implicated in the pathogenesis of low bone density in people living with HIV [20, 21], and different studies have found an association between imbalances of these biomarkers and BMD loss in antiretroviral therapy (ART)–treated people living with HIV [22, 23]. Both biomarkers were measured by ProcartaPlex multiplex immunoassay (Bender MedSystems GmbH, Vienna, Austria) using a Luminex 200 analyzer (Luminex Corporation, Austin, Texas) and according to the manufacturer's specifications [24]. After all clinical tests were completed, the sRANKL/OPG ratio was calculated.

Statistical Analysis

For the descriptive study, values were expressed as absolute number and percentage or median and IQR. Differences between groups were analyzed using the χ^2 test, *t* test, or Mann-Whitney test, as appropriate. Normality was analyzed using the Kolmogorov-Smirnov test. Pearson correlation and Spearman

ρ were used to assess the correlations between sBMD and liver stiffness. Univariable and multivariable logistic regression analyses were used to identify variables associated with osteoporosis at the lumbar spine and the femoral neck at baseline. Variables analyzed included age, sex, body mass index (BMI), current smoking, alcohol intake >50 g/day, methadone use, history of injection drug use, Centers for Disease Control and Prevention clinical category, tenofovir disoproxil fumarate use, HIV RNA, $CD4^+$ cell count, nadir $CD4^+$ cell count, $CD4^+/CD8^+$ ratio, HCV genotype, HCV RNA, liver stiffness, 25-OH vitamin D, thyroxine, thyrotropin, and parathyroid hormone. Variables included in the multivariable analysis included age, sex, and those with a *P* value < .1 in univariable analysis. Linear mixed models for longitudinal data were used in analyses to account for repeated measures of sBMD, with SVR and time and their interaction taken as fixed effects, and the patient as a random effect. Least-square means and their 95% confidence intervals (CIs) are reported for these models. In addition, to study changes between baseline and week 96 WHO BMD categories, tests for repeated measures were used (paired *t* test and Wilcoxon test). IBM SPSS Statistics for Windows, version 21.0, was used for all calculations. All statistical tests were performed 2-sided, and a *P* value of < .05 was considered statistically significant.

RESULTS

Overall, 238 patients were included in the study, 145 of whom (60.9%) achieved SVR; the full characteristics of these patients, categorized by whether or not they achieved SVR, are shown in Table 1. In brief, 76.5% were male, the median age was 49.5 years, 27.3% had had prior AIDS-defining conditions, 98.3% were on cART, the median baseline CD4 cell count was 527 cells/μL, 86.6% had an undetectable HIV viral load, 62.6% were infected with HCV genotype 1, the median HCV RNA load was 6.4 $\log_{10}$ IU/mL, and 3.4% were hepatitis B surface antigen positive. Liver cirrhosis was detected in 48.3% of patients, and 11.3% had received prior anti-HCV therapy. Alcohol intake >50 g/d was reported by 2.1% patients, 68.1% were current smokers, 13.0% had arterial hypertension, and 8.4% had diabetes mellitus. Anti-HCV regimens included pegylated interferon (peg-IFN) plus ribavirin (RBV) 34.5%, peg-IFN plus RBV plus a first-generation anti-HCV protease inhibitor 47.5%, peg-IFN plus RBV plus daclatasvir 5.9%, and sofosbuvir plus RBV 12.2%. HCV RNA and the type of anti-HCV therapy were the only baseline variables in which significant differences were found between responders and nonresponders.

The overall prevalence of osteoporosis at the lumbar spine and femoral neck in HIV/HCV-coinfected patients was 17.6% and 7.2%, respectively, without significant differences between responders and nonresponders (Table 1). Logistic regression analyses of variables associated with osteoporosis at the lumbar spine and the femoral neck at baseline are shown in Table 2. Lower BMI was the only variable independently associated with osteoporosis at the lumbar spine (adjusted odds ratio, 0.853 [95% CI, .735–.991]; *P* = .037). BMI was also associated with osteoporosis at the femoral neck in the univariable analysis but not in multivariable analysis.

No significant correlation was found between liver fibrosis, assessed by transient elastography, and lumbar spine and femoral neck sBMD, at baseline and week 96 Figure 1.

Changes in sBMD in the lumbar spine and femoral neck from baseline to 96 weeks in responders and nonresponders are shown in Figure 2. No significant effect of SVR was observed on sBMD for the interaction between time and SVR in the lumbar spine (*P* = .801) or the femoral neck (*P* = .911). The median change from baseline to week 96 in sBMD at the lumbar spine was −0.007 (95% CI, −.033 to .032) g/cm^2 in responders and −0.006 (95% CI, −.028 to .027) g/cm^2 in nonresponders. Likewise, the change in sBMD at the femoral neck was −0.023 (95% CI, −.065 to .012) g/cm^2 in responders and −0.024 (95% CI, −.052 to .019) g/cm^2 in nonresponders. Two sensitivity analyses were carried out, the first without the 8 patients that were coinfected with hepatitis B virus, and the second without the 4 patients not on ART; the results of these sensitivity analyses did not change the results found in the primary analysis (data not shown).

No significant changes in BMD WHO categories from baseline to 96 weeks were found in the lumbar spine and femoral neck either in responders or nonresponders (Figure 3).

Changes in plasma levels of sRANKL, OPG, and sRANKL/OPG ratio from baseline to 96 weeks in responders are shown in Figure 4. No significant effect of SVR was observed in plasma levels of sRANKL (*P* = .205), OPG (*P* = .249), or sRANKL/OPG ratio (*P* = .123) for the interaction between time and SVR.

DISCUSSION

In this prospective study with 238 HIV/HCV-coinfected patients, half of whom had liver cirrhosis and approximately one-third of whom achieved SVR following anti-HCV therapy, we did not observe an association between HCV eradication and significant changes in sBMD, BMD WHO categories, and plasma levels of biomarkers of bone remodeling. To the best of our knowledge, ours is the largest prospective study analyzing the effects of HCV eradication after anti-HCV therapy in patients with chronic HCV infection, and the first analyzing this issue among HIV/HCV-coinfected patients.

We found that among the study population, the prevalence of osteoporosis at the lumbar spine and femoral neck was 17.6% and 7.2%, respectively, differences that can be explained because lumbar spine consists of trabecular bone, which is more metabolically active with a higher rate of turnover and may be affected earlier and more severely in comparison with the cortical bone of the femoral neck [25]. Lower BMI was the only baseline variable associated with osteoporosis. It must be

Table 1. Baseline Characteristics of the Study Population

Characteristic	No SVR (n = 93)	SVR (n = 145)	*P* Value	Total (N = 238)
Male sex	66 (70.9)	116 (80.0)	.109	182 (76.5)
Age, y, median (IQR) (baseline)	49.1 (46.6–52.6)	49.7 (46.3–53.2)	.629	49.5 (46.4–53)
BMI, kg/m^2, median (IQR)	24.1 (22.1–26.1)	24.4 (21.5–27.3)	.460	24.2 (21.8–26.6)
Prior injection drug use	73 (78.5)	107 (73.8)	.410	180 (75.6)
Methadone therapy	13 (14.0)	16 (11.0)	.707	29 (12.2)
CDC disease category C	31 (33.3)	34 (23.4)	.229	65 (27.3)
CD4$^+$, nadir, cells/μL, median (IQR)	160 (69–253)	162 (84–246)	.901	160 (72–250)
cART during anti-HCV treatment[a]	92 (98.9)	142 (97.9)	.708	234 (98.3)
Antiretroviral drugs			.561	
Tenofovir disoproxil fumarate	56 (60.9)	86 (60.6)		142 (60.7)
Emtricitabine	53 (57.6)	83 (58.5)		136 (58.1)
Lamivudine	14 (15.2)	35 (24.6)		49 (20.9)
Abacavir	13 (14.1)	32 (22.5)		45 (19.2)
Raltegravir	35 (38.0)	54 (38.0)		89 (38.0)
Darunavir/ritonavir	21 (22.8)	22 (15.5)		43 (18.4)
Atazanavir/ritonavir	14 (15.2)	24 (16.9)		38 (16.2)
Lopinavir/ritonavir	4 (4.3)	5 (3.5)		9 (3.8)
Fosamprenavir/ritonavir	3 (3.3)	1 (0.7)		4 (1.7)
Etravirine	18 (19.6)	23 (16.2)		41 (17.5)
Efavirenz	15 (16.3)	19 (13.4)		34 (14.5)
Rilpivirine	3 (3.3)	6 (4.2)		9 (3.8)
Nevirapine	2 (2.2)	5 (3.5)		7 (3.0)
Maraviroc	0 (0)	2 (1.4)		2 (0.9)
CD4$^+$, baseline, cells/μL, median (IQR)	550 (372–822)	518 (385–772)	.556	527 (380–803)
Undetectable HIV RNA load at baseline	77 (82.8)	129 (89)	.230	206 (86.6)
Prior anti-HCV therapy	9 (9.7)	18 (12.4)	.516	27 (11.3)
HCV genotype			.334	
1	55 (59.1)	94 (64.9)		149 (62.6)
2	3 (3.2)	2 (1.4)		5 (2.1)
3	19 (20.4)	24 (16.6)		43 (18.1)
4	10 (10.8)	10 (6.9)		20 (8.4)
Other/mixed	6 (6.5)	14 (9.7)		20 (8.4)
Unknown	0 (0)	1 (0.7)		1 (0.4)
HCV RNA, $\log_{10}$ IU/mL, median (IQR)	6.5 (6.1–6.9)	6.3 (5.8–6.6)	.001	6.4 (5.9–6.7)
HBsAg positivity	3 (3.2)	5 (3.4)	.655	8 (3.4)
Serum albumin, g/dL, median (IQR)	4.2 (3.95–4.5)	4.3 (4.1–4.6)	.030	4.3 (4.0–4.6)
Liver cirrhosis (Metavir 4 or TE >12.5)	41 (44.1)	74 (51)	.295	115 (48.3)
Current alcohol intake >50 g/d	1 (1.1)	4 (2.8)	.560	5 (2.1)
Diabetes mellitus[b]	9 (9.7)	11 (7.6)	.570	20 (8.4)
Current smoking	65 (69.9)	97 (66.9)	.910	162 (68.1)
Arterial hypertension[b]	14 (15.1)	17 (11.7)	.456	31 (13)
Anti-HCV therapy			.003	
Peg-IFN + RBV	29 (31.2)	53 (36.6)		82 (34.5)
Peg-IFN + RBV + HCV protease inhibitor	36 (38.7)	77 (53.1)		113 (47.5)
Peg-IFN + RBV + daclatasvir	6 (6.5)	8 (5.5)		14 (5.9)
Sofosbuvir + RBV	22 (23.7)	7 (4.8)		29 (12.2)
WHO diagnostic classification at the lumbar spine[c], No./No. with data (%)			.825	
Osteoporosis	13/82 (15.9)	24/128 (18.8)		37/210 (17.6)
Osteopenia	32/82 (39.0)	42/128 (32.8)		74/210 (35.2)
Normal	37/82 (45.1)	62/128 (48.4)		99/210 (47.1)
WHO diagnostic classification at femoral neck[d], No./No. with data (%)			.260	
Osteoporosis	7/81 (8.6)	8/127 (6.3)		15/208 (7.2)
Osteopenia	44/81 (54.3)	54/127 (42.5)		98/208 (47.1)
Normal	30/81 (37.0)	65/127 (51.2)		95/208 (45.7)

Data are presented as no. (%) unless otherwise indicated.

Abbreviations: BMI, body mass index; cART, combination antiretroviral therapy; CDC, Centers for Disease Control and Prevention; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; Peg-IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virologic response;, TE, transient elastography; WHO, World Health Organization.

[a]Three patients were not receiving antiretroviral therapy (ART) because they were elite controllers of HIV (ie, persons with HIV with spontaneous control of HIV replication at undetectable levels). One patient was not receiving ART because he was a long-term nonprogressor with a CD4$^+$ cell count of 633 cells/μL and an HIV RNA load of 299 copies/mL.

[b]Diabetes and hypertension were defined as diagnoses included in the medical record.

[c]Baseline bone mineral density (BMD) at the lumbar spine was determined in 210 patients.

[d]Baseline BMD at the femoral neck was determined in 208 patients.

Table 2. Logistic Regression Analysis of Variables Associated With Osteoporosis at the Lumbar Spine and the Femoral Neck at Baseline

	Lumbar Spine						Femoral Neck					
	Univariable Analysis			Multivariable Analysis			Univariable Analysis			Multivariable Analysis		
Variable	OR	(95% CI)	*P* Value	aOR	(95% CI)	*P* Value	OR	(95% CI)	*P* Value	aOR	(95% CI)	*P* Value
Age	1.029	(.95–1.114)	.485	1.037	(.933–1.152)	.505	1.063	(.99–1.142)	.091	1.100	(.984–1.231)	.095
Male sex	1.199	(.509–2.826)	.678	1.400	(.559–3.503)	.472	0.608	(.197–1.877)	.387	0.404	(.089–1.825)	.239
BMI	0.853	(.751–.969)	.015	0.853	(.735–.991)	.037	0.833	(.712–.975)	.023	0.830	(.678–1.017)	.072
History of IDU	0.893	(.399–1.998)	.783	…	…		0.910	(.276–3.001)	.877	…	…	
$CD4^+/CD8^+$	1.116	(.913–1.365)	.285	…	…		0.159	(.022–1.164)	.070	0.100	(.010–1.038)	.054
Methadone	2.707	(1.059–6.922)	.038	2.246	(.858–5.877)	.099	NA	NA	NA	…	…	
TDF use	0.684	(.334–1.397)	.297	…	…		0.568	(.197–1.636)	.295	1.444	(.392–5.328)	.581
Current smoking	2.181	(.901–5.282)	.084	1.796	(.647–4.980)	.261	1.815	(.492–6.696)	.371	…	…	
Alcohol intake >50 g/d	0.556	(.257–1.203)	.136	…	…		1.028	(.342–3.085)	.961	…	…	
Clinical category C (CDC)	0.926	(.415–2.069)	.852	…	…		1.255	(.408–3.855)	.692	…	…	
Nadir $CD4^+$ cell count	0.999	(.996–1.002)	.471	…	…		0.998	(.994–1.002)	.275	…	…	
$CD4^+$ cell count	0.999	(.998–1.000)	.197	…	…		0.999	(.996–1.001)	.360	…	…	
HIV RNA BLQ	1.254	(.47–3.343)	.651	…	…		0.982	(.209–4.622)	.982	…	…	
HCV genotype 3	0.853	(.328–2.221)	.744	…	…		1.701	(.509–5.678)	.388	…	…	
Log_{10} HCV RNA	1.572	(.895–2.761)	.115	…	…		0.740	(.386–1.417)	.363	…	…	
Liver stiffness	5.502	(.468–64.65)	.175	…	…		1.000	(.347–2.883)	1.000	…	…	
Vitamin D	0.976	(.946–1.007)	.133	…	…		0.985	(.949–1.023)	.436	…	…	
T4	1.025	(.887–1.183)	.739	…	…		0.926	(.738–1.162)	.508	…	…	
TSH	0.961	(.863–1.07)	.467	…	…		0.753	(.462–1.228)	.256	…	…	
PTH	1.006	(.996–1.015)	.249	…	…		1.005	(.996–1.015)	.273	…		

Abbreviations: aOR, adjusted odds ratio; BLQ, below the limit of quantification; BMI, body mass index; CDC, Centers for Diseases Control and Prevention; CI, confidence interval; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDU, injection drug use; OR, odds ratio; PTH, parathyroid hormone; T4, thyroxine; TDF, tenofovir disoproxil fumarate; TSH, thyrotropin.

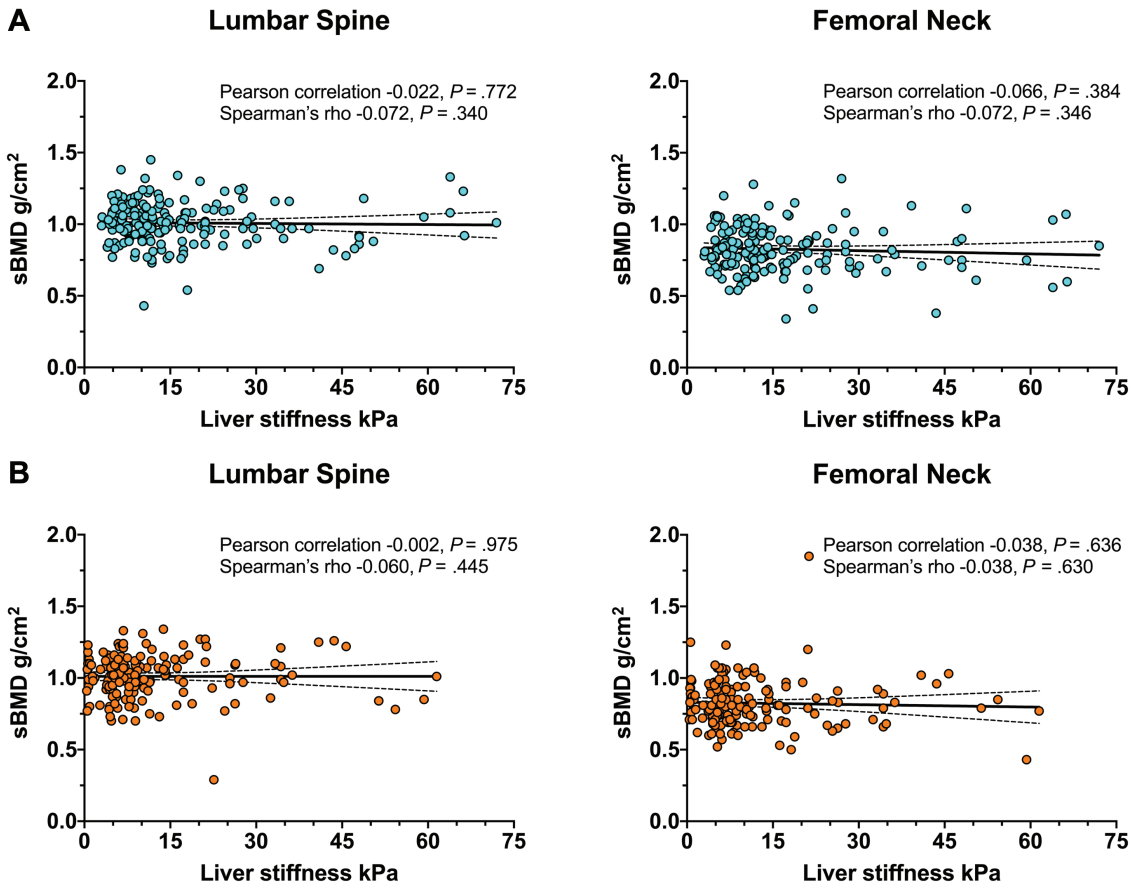
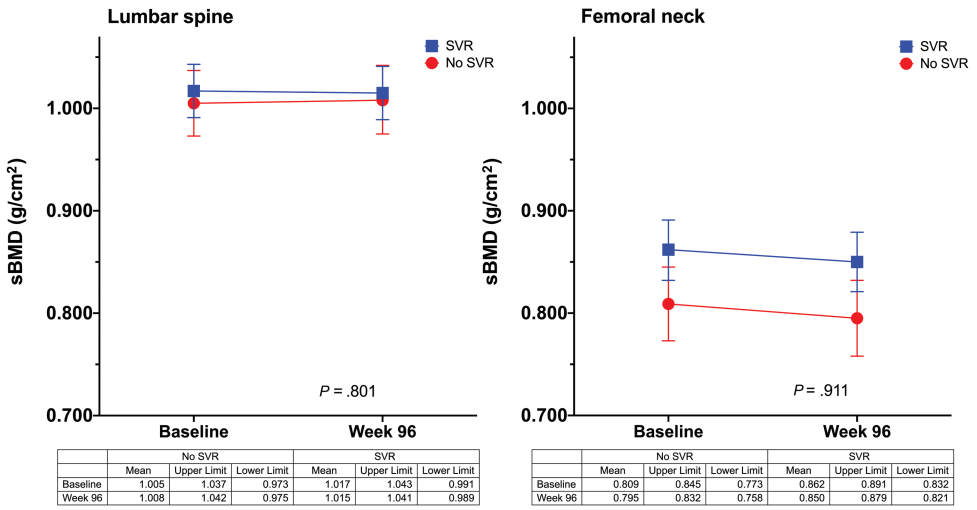


Figure 1. Pearson and Spearman correlations between lumbar spine and femoral neck bone mineral density and liver fibrosis assessed by transient elastography at baseline (*A*) and at 96 weeks (*B*). Abbreviation: sBMD, standardized bone mineral density.



	No SVR			SVR		
	Mean	Upper Limit	Lower Limit	Mean	Upper Limit	Lower Limit
Baseline	1.005	1.037	0.973	1.017	1.043	0.991
Week 96	1.008	1.042	0.975	1.015	1.041	0.989

	No SVR			SVR		
	Mean	Upper Limit	Lower Limit	Mean	Upper Limit	Lower Limit
Baseline	0.809	0.845	0.773	0.862	0.891	0.832
Week 96	0.795	0.832	0.758	0.850	0.879	0.821

Figure 2. Estimated means (95% confidence interval) of bone mineral density in the lumbar spine and femoral neck at baseline and 96 weeks in responders and nonresponders to anti–hepatitis C virus therapy. Abbreviations: sBMD, standardized bone mineral density; SVR, sustained virologic response.

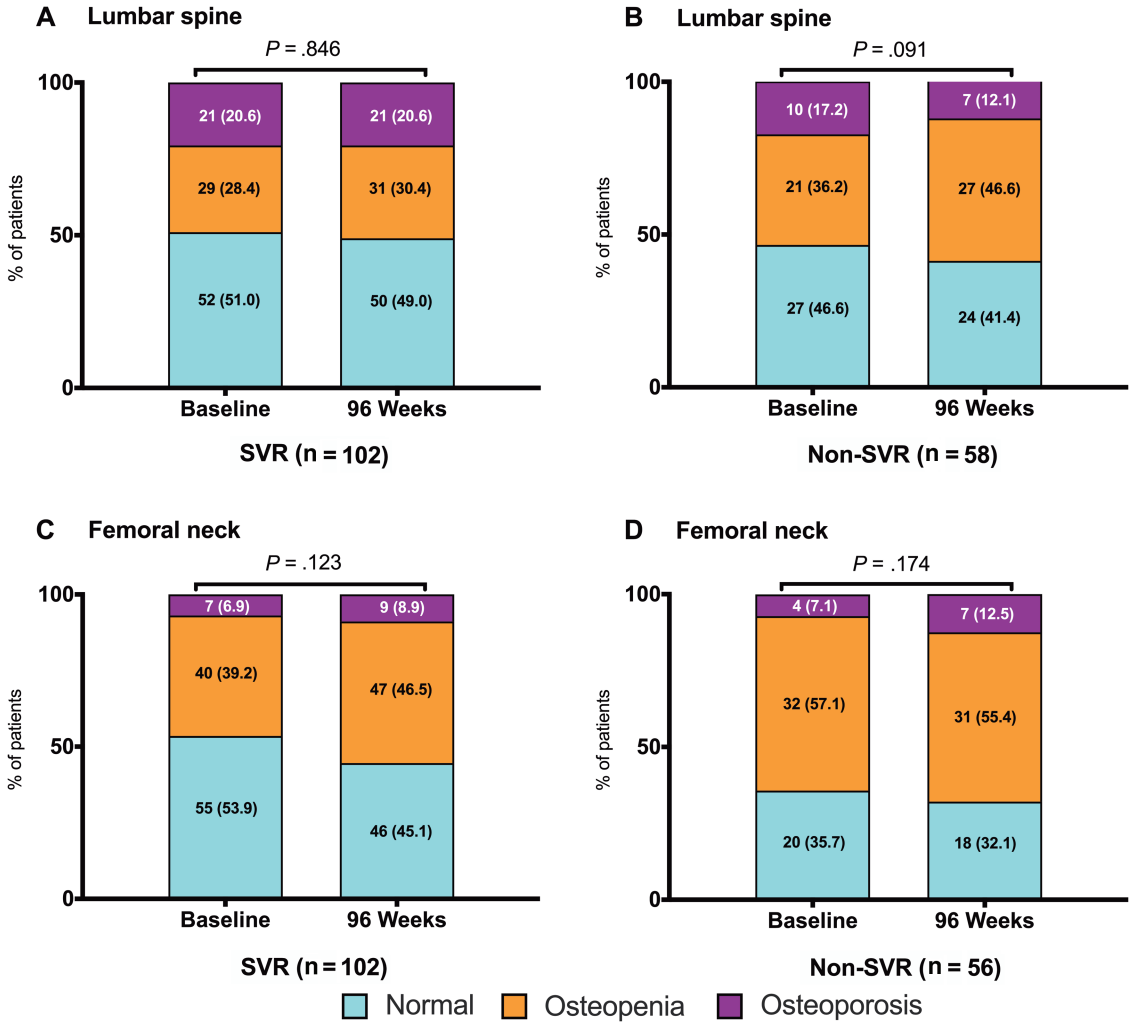
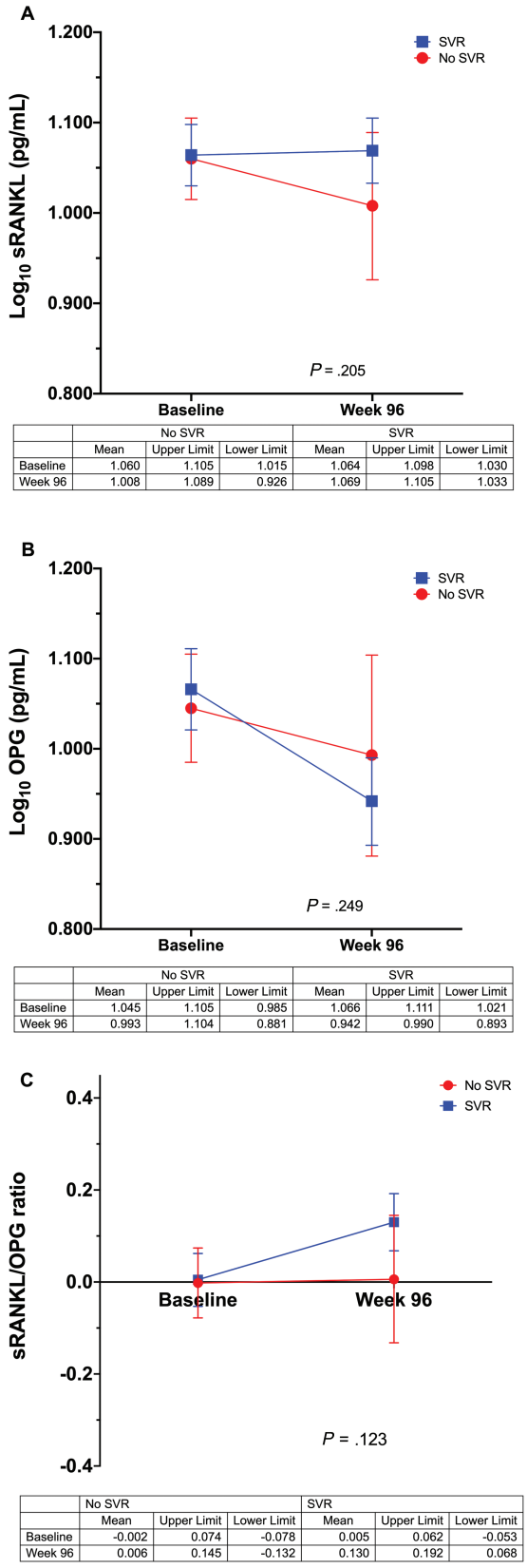


Figure 3. Changes in bone mineral density according to World Health Organization categories from baseline to 96 weeks at the lumbar spine for responders (*A*) and nonresponders (*B*), and at the femoral neck in responders (*C*) and nonresponders (*D*). Abbreviation: SVR, sustained virologic response.



	No SVR			SVR		
	Mean	Upper Limit	Lower Limit	Mean	Upper Limit	Lower Limit
Baseline	1.060	1.105	1.015	1.064	1.098	1.030
Week 96	1.008	1.089	0.926	1.069	1.105	1.033

	No SVR			SVR		
	Mean	Upper Limit	Lower Limit	Mean	Upper Limit	Lower Limit
Baseline	1.045	1.105	0.985	1.066	1.111	1.021
Week 96	0.993	1.104	0.881	0.942	0.990	0.893

	No SVR			SVR		
	Mean	Upper Limit	Lower Limit	Mean	Upper Limit	Lower Limit
Baseline	-0.002	0.074	-0.078	0.005	0.062	-0.053
Week 96	0.006	0.145	-0.132	0.130	0.192	0.068

Figure 4. Estimated means (95% confidence interval) of plasma biomarkers concentrations at baseline and 96 weeks in responders and nonresponders to anti–hepatitis C virus therapy: soluble receptor activator of NF-κβ ligand (sRANKL; *A*), osteoprotegerin (OPG; *B*), and sRANKL/OPG ratio (*C*). Abbreviations: OPG, osteoprotegerin; SVR, sustained virologic response; sRANKL, soluble receptor activator of NF-κβ ligand.

considered, however, that we could not appropriately assess advanced age and female sex, as patients in our cohort were within a narrow age range in their forties and fifties, and more than three-quarters were male. We did not find a correlation between liver fibrosis, assessed by transient elastography, and sBMD at baseline and week 96 after initiation of anti-HCV therapy, consistent with what has been found in previously published studies with a limited number of patients [11–13].

The effects of SVR on BMD have been previously assessed in small studies, including HCV-monoinfected patients. In a prospective study with 36 treatment-naive noncirrhotic patients with chronic hepatitis C with SVR following peg-IFN plus RBV, BMD values at the lumbar spine and the femoral neck at 24 and 48 weeks of antiviral therapy and 48 weeks after the end of treatment were significantly higher in comparison with baseline values [11]. A limitation of this study is the nonavailability of comparable data on the patients who did not achieve SVR. In a prospective study with 46 noncirrhotic children with chronic hepatitis C treated with peg-IFN plus RBV, 23 of whom achieved SVR, follow-up DXA scan results 24 weeks after the end of treatment showed statistically significant improvement in BMD from baseline; however, an effect of SVR on BMD could not be evaluated [12]. In a study with 30 noncirrhotic patients with chronic hepatitis C treated with peg-IFN plus RBV, 19 of whom achieved SVR, BMD increased significantly by the end of 48-week therapy in comparison with baseline but decreased by the end 24-week follow-up period without significant differences between patients with SVR and patients with viral relapse [13]. The authors also found a decrease in osteocalcin and C-terminal propeptide of type I collagen during peg-IFN plus RBV therapy, supporting the findings of the DXA measurements.

Our study is limited because we used different densitometers to assess BMD, something that we tried to correct by calculating sBMD according to published equations. Another limitation is the lack of central reading of DXA scans. It also needs to be mentioned that almost 90% of our patients received peg-IFN and 100% RBV, both of which modulate bone metabolism, with IFN inhibiting excessive osteoclastogenesis resulting in reduced bone resorption [26, 27], and RBV exhibiting oppositional effects [28, 29]. Other limitations include the lack of an HCV-monoinfected group and the lack of an age-, sex-, and race-matched control group. However, the study has the strengths of the prospective design, the large sample size, the assessment of both sBMD and biomarkers of bone remodeling at baseline and 96 weeks after treatment initiation, and the fact that responders and nonresponders were well matched in many relevant characteristics, limiting the possibility of confounding.

In conclusion, in this prospective cohort of HIV/HCV-coinfected patients, half of whom had compensated cirrhosis, no significant correlation was found between liver stiffness and BMD at baseline and week 96 after initiation of anti-HCV

therapy. Besides, in the medium term, eradication of HCV following anti-HCV therapy was not associated with significant changes in sBMD, BMD WHO categories, and plasma levels of biomarkers of bone remodeling. Our findings do not support a causal association between HCV infection and reduced BMD in HIV/HCV-coinfected persons with compensated liver disease. They suggest that lifestyle and other factors may have a more significant impact on BMD than the severity of liver fibrosis in this population.

Notes

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