The Differential Effects of Human Immunodeficiency Virus and Hepatitis C Virus on Bone Microarchitecture and Fracture Risk

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Background. Human immunodeficiency virus (HIV)/hepatitis C virus (HCV)–coinfected individuals have a significantly greater osteoporotic fracture risk than HIV-monoinfected persons, despite the fact that HIV/HCV coinfection has not been associated with lower bone mineral density (BMD) than HIV or HCV alone. To evaluate if changes in bone microarchitecture, measured by trabecular bone score (TBS), could explain these differences, we performed a prospective, cross-sectional cohort study of virologically suppressed HIV-infected subjects, untreated HCV-infected subjects, HIV/HCV-coinfected subjects, and uninfected controls.

Methods. We enrolled 532 male subjects: 57 HIV/HCV coinfected, 174 HIV infected, 123 HCV infected, and 178 controls. We conducted analysis of covariance comparing BMD and TBS between groups, controlling for age, race, body mass index, and smoking. We used linear regression to evaluate predictors of BMD and TBS and evaluated the effects of severity of HCV infection and tenofovir disoproxil fumarate use.

Results. Despite both infections being associated with decreased BMD, only HCV, but not HIV, was associated with lower TBS score. Also, HIV/HCV-coinfected subjects had lower TBS scores than HIV-monoinfected, HCV-monoinfected, and uninfected subjects. Neither the use of TDF or HCV viremia nor the severity of HCV liver disease was associated with lower TBS.

Conclusions. HCV infection is associated with microarchitectural changes at the lumbar spine as assessed by the low TBS score, suggesting that microstructural abnormalities underlie some of the higher fracture risk in HCV infection. TBS might improve fracture risk prediction in HCV infection.

Keywords. HIV; HCV; bone mineral density; trabecular bone score; fracture risk.

Bone mineral density (BMD) is lower among human immunodeficiency virus (HIV)–infected patients than in age-matched uninfected subjects, and patients on antiretroviral therapy have even lower BMD than antiretroviral-naive patients [1, 2]. This lower BMD translates into a higher incidence of osteoporotic fractures (OFs) among HIV-infected patients than age-matched uninfected subjects [3–5]. About 15%–30% of HIV-infected patients are coinfected with hepatitis C (HCV), which further increases OF risk [6–8]. HIV/HCV-coinfected patients have a 3-fold higher fracture incidence compared with uninfected individuals [8, 9], and up to twice the fracture risk of HIV-monoinfected subjects [6, 8, 10]. Despite being consistently associated with higher OF risk in several reports [5, 8, 10–12], HCV coinfection has only been associated with further reductions in BMD among HIV-infected patients in some [13, 14], but not all studies [15, 16]. These findings raise the possibility that the higher fracture risk observed in patients with chronic HCV infection might not be due to low BMD alone, but could involve other mechanism(s). While cirrhosis and advanced liver disease are known risk factors for osteoporosis [17], the deleterious impact of HCV on BMD [18] and fracture risk [10] appears to occur prior to the development of severe liver fibrosis or cirrhosis [10, 19], but the exact mechanism(s) remain to be elucidated.

Possible contributors to the increased fracture risk among HCV-infected subjects likely include an overrepresentation of “traditional” risk factors for osteoporosis in these populations (including tobacco use, alcohol consumption, and corticosteroid use), or “nontraditional” behavioral factors (such as substance abuse and intoxication leading to traumatic fractures) [8, 20, 21]. However, we hypothesized that HCV may be associated with architectural changes beyond what can be assessed by planar BMD that lead to increased bone fragility.

Trabecular bone score (TBS) is a novel measurement of bone microarchitecture from dual-energy x-ray absorptiometry (DXA)

Received 30 June 2017; editorial decision 5 November 2017; accepted 13 November 2017; published online November 14, 2017.

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Clinical Infectious Diseases® 2018;66(9):1442–7

Published by Oxford University Press for the Infectious Diseases Society of America 2017. This work is written by (a) US Government employee(s) and is in the public domain in the US. DOI: 10.1093/cid/cix1011

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METHODS

Subjects
We prospectively enrolled HIV/HCV-coinfected patients (HIV/ HCV), as well as HIV-monoinfected (HIV), HCV-monoinfected (HCV), and uninfected Veterans (controls) receiving care at the Veterans Affairs North Texas Health Care System (VANTHCS) in Dallas, Texas. All patients seen at the VANTHCS HIV clinic (a clinic of about 1000 patients, roughly one-third of which are HCV coinfected) were offered enrollment in the study if they met inclusion criteria. Consecutive HCV-monoinfected and uninfected patients seen at the Liver and General Medicine clinics, respectively, were also offered enrollment into the study if they met enrollment criteria. Patients were included in the cohort if they were aged ≥40 years, had no known metabolic bone disease, multiple myeloma, cancer, endocrinopathies, celiac or inflammatory bowel disease, or chronic kidney disease (defined as estimated glomerular filtration rate <45 mL/minute/1.73 m²).

For the HIV and control groups, HCV antibody testing and HCV RNA level were performed at enrollment to confirm HCV-negative status. Similarly, enzyme-linked immunosorbent assay testing was used to verify HIV-negative status in HCV-monoinfected and control subjects. The Internal Review Board of VANTHCS approved the study, and all subjects provided informed consent.

Treatment History
HIV-infected and HIV/HCV-coinfected patients were enrolled only if they had been virologically suppressed on combination antiretroviral therapy for at least 6 months. There was no CD4 cell count threshold for inclusion into the cohort. Based on antiretroviral therapy exposure, these patients were classified as (1) current tenofovir disoproxil fumarate (TDF) users, (2) past TDF users, or (3) never TDF users. Minimum duration of exposure for consideration in the first 2 categories was 1 month. HCV-infected and HIV/HCV-coinfected patients were enrolled only if they were HCV treatment naive.

Bone Mineral Density and Trabecular Bone Score
All patients underwent BMD testing by DXA at the lumbar spine (LS; L1–L4), femoral neck (FN), and total hip (TH) on a QDR4500 bone densitometer (Hologic, Bedford, Massachusetts). Osteoporosis and osteopenia were defined by World Health Organization criteria for postmenopausal women: T scores of −2.5 or less represent osteoporosis; T scores between −1.0 and −2.49 represent osteopenia [24].

TBS was evaluated in the same regions of measurement as those used for LS BMD using TBS Insight version 2.0 (Med-Imaps, Pessac, France; http://www.medimapsgroup.com/product/technology/). A vertebral exclusion process was performed using the following criteria: (1) if there was >1.0 T-score difference between the vertebra in question and adjacent vertebrae, or (2) if the vertebra was abnormal or nonassessable [25].

Laboratory Analyses
We calculated the AST-to-platelet ratio index (APRI) [aspartate aminotransferase (AST) in (U/L)/upper normal x 100 / platelet count (10⁹/L)] [26]. APRI is a validated surrogate marker of liver fibrosis and predictive of HCV-related liver mortality in monoinfected and HIV/HCV-coinfected individuals [27].

CD4 counts were measured by flow cytometry (FACSCalibur; Becton Dickinson, San Jose, California). HIV-1 RNA was quantified by polymerase chain reaction (PCR) using the AMPLICOR HIV-1 MONITOR Ultrasensitive Test (version 1.5) with a linear range of 50–100,000 copies/mL (Roche Diagnostics). HCV RNA was also quantified by AMPLICOR PCR.

Statistical Analysis
We compared group characteristics with the Kruskal-Wallis test for continuous variables and χ² test for categorical variables. We conducted analysis of covariance comparing BMD and TBS between groups, controlling for known risk factors for low BMD: age, race, body mass index (BMI), and smoking.

We evaluated the predictors of BMD and TBS in the cohort with linear regression, further controlling for, as warranted, CD4 count, HIV viral load, HCV viral load, antiretroviral drugs (TDF use and duration), HCV viremia and genotype, systemic glucocorticoid therapy (for ≥3 months at doses equivalent to 5 mg prednisone daily, or more), and conditions associated with secondary osteoporosis.

We used SAS version 9.4 software (SAS Institute, Cary, North Carolina) for statistical analyses.
heavy alcohol use (≥3 alcoholic drinks daily) and the prevalence of tobacco use varied significantly between the groups (68% in uninfected controls, 31% in HIV). The mean CD4 count in the HIV and HIV/HCV patients was 550 cells/µL. Among these groups, 78% were current or past users of TDF, and the median duration of TDF use at the time of BMD assessment was 4.3 years.

**Effects of Human Immunodeficiency Virus and Hepatitis C Virus on Bone Mineral Density and Trabecular Bone Score**

We built a multivariable regression base model to predict BMD and TBS using traditional risk factors for osteoporosis: age, race, BMI, and smoking status. HIV and HCV infection status and HIV and/or HCV viremia were also added to the model. We confirmed previous observations that HIV and HCV infections were each independently associated with lower TH BMD (adjusted β coefficient = −.04, P < .001 for HIV and β = −.04, P = .001 for HCV), and lower FN BMD (β = −.05, P < .001 and β = −.03, P = .006, respectively). However, neither infection was associated with a significantly lower LS BMD (β = −.03, P = .10 and β = −.02, P = .21) (Table 2; Figure 1).

HIV/HCV coinfection was associated with lower BMD TH and LS than HIV (covariate adjusted P = .02 and P = .03) and HCV (P = .02 and P = .02). There was a nonsignificant association of coinfection with lower BMD at FN than HIV or HCV alone (P = .21 and P = .06; Figure 1).

Despite both infections being associated with lower total hip and femoral neck BMD, only HCV infection was associated with significantly lower TBS score (β = −.03, P = .005). HIV infection was not (β = −.02, P = .10; Table 2). Also, HIV/HCV-coinfected subjects had lower TBS scores than HIV-monoinfected (P < .01), HCV-monoinfected (P = .04), and uninfected subjects (P < .01) (Figure 1). We also conducted a sensitivity analysis excluding HIV/HCV and HCV patients with detectable viremia (HCV viral load [VL] ≥43 copies/mL). Again, viremic HCV infection was associated with significantly lower TBS score (β = −.03, P = .010), but HIV infection was not (β = −.02, P = .09).

Similarly, excluding patients with heavy alcohol use or corticosteroid exposure did not alter the findings. HCV remained associated with decreased TBS (P = .02), whereas HIV was not (P = .13).

**Other Predictors of Lower Trabecular Bone Score**

We built additional models to evaluate among HIV-infected patients if current or past TDF use was associated with decreased BMD (Table 2). TDF use was associated with lower BMD at TH (β = −.05, P = .03), FN (β = −.05, P = .02), and LS (β = −.06, P = .03). However, TDF use was not associated with decreased TBS (β = −.02, P = .24; Table 2). Adding TDF to the HIV base model of BMD increased the R² significantly.

We also built regression models to evaluate if the severity of liver disease and the presence of HCV viremia (VL ≥43 vs <43 copies/mL) was associated with lower BMD or TBS. Among HIV-infected patients, high APRI score was associated with lower FN BMD (β = −.03, P = .02) and a trend toward lower TH BMD (β = −.02, P = .06) (Table 2). It was not associated with lower LS BMD (β = −.02, P = .12) or lower TBS score (β = −.01, P = .24). Detectable HCV viremia (≥43 copies/mL) was associated with higher TH BMD (β = −.08, P = .04) and lower FN BMD (β = −.09, P = .01), but no difference in LS BMD (β = .03, P = .47) or TBS score (β = −.02, P = .44) (Table 2).

**DISCUSSION**

In this analysis, we confirmed our previous observations that HIV and HCV independently reduce BMD. We further show that only HCV (but not HIV) infection is associated with lower TBS, suggesting that microstructural abnormalities may underlie...
Table 2. Predictors of Bone Mineral Density and Trabecular Bone Score From Multiple Linear Regression Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Hip BMD</th>
<th>Femoral Neck BMD</th>
<th>LS Spine BMD</th>
<th>TBS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>PValue (95% CI)</td>
<td>β</td>
<td>PValue (95% CI)</td>
</tr>
<tr>
<td>All patients Base model</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>.000 (-0.002 to 0.002)</td>
<td>.93</td>
<td>-0.002 (-0.004 to 0.003)</td>
<td>.02</td>
</tr>
<tr>
<td>African American race</td>
<td>.007 (0.05–0.09)</td>
<td>&lt;.0001</td>
<td>.09 (0.07–0.12)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>.02 (0.10–0.15)</td>
<td>&lt;.0001</td>
<td>.01 (0.08–0.01)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.02 (-0.04 to -0.005)</td>
<td>.13</td>
<td>-0.02 (-0.04 to -0.009)</td>
<td>.21</td>
</tr>
<tr>
<td>$R^2$ base model</td>
<td>0.26</td>
<td>0.27</td>
<td>0.10</td>
<td>0.21</td>
</tr>
<tr>
<td>HIV</td>
<td>-0.04 (-0.07 to -0.02)</td>
<td>.0004</td>
<td>-0.05 (-0.08 to -0.03)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HCV</td>
<td>-0.04 (-0.07 to -0.02)</td>
<td>.0001</td>
<td>-0.03 (-0.06 to -0.01)</td>
<td>.006</td>
</tr>
<tr>
<td>$R^2$ base model + HCV + TDF</td>
<td>0.29</td>
<td>0.29</td>
<td>0.11</td>
<td>0.23</td>
</tr>
</tbody>
</table>

n = 501 n = 501 n = 502 n = 482

HIV patients only $R^2$ base model 0.32 0.33 0.11 0.29
TDF use -0.05 (-0.09 to -0.006) 0.03 -0.05 (-0.09 to -0.007) 0.02 -0.06 (-11 to -0.007) 0.03 -0.02 (-0.06 to .01) 0.24

n = 216 n = 215 n = 218 n = 212

HCV patients only $R^2$ base model 0.25 0.23 0.17 0.25
APRI score (log) -0.02 (-0.05 to -0.001) 0.66 -0.03 (-0.06 to -0.006) 0.02 -0.02 (-0.06 to -0.006) 0.12 -0.01 (-0.04 to -0.009) 0.24
HCV viremia (VL ≥43 vs <43) 0.08 (0.004–0.15) 0.04 0.09 (0.07–0.16) 0.01 0.03 (-0.05 to -0.12) 0.47 -0.02 (-0.08 to -0.04) 0.44

$n = 135 n = 135 n = 135 n = 134$

Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio index; BMD, bone mineral density; BMI, body mass index; CI, confidence interval; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LS, lumbar spine; TBS, trabecular bone score; TDF, tenofovir disoproxil fumarate; VL, viral load.

Some of the higher fracture risk in HCV-monoinfected individuals and might also help explain the significantly increased fracture risk associated with HIV/HCV coinfection [6, 8].

Interestingly, although the use of TDF (median duration of >4 years) was associated with lower BMD, as has been shown in multiple studies [28–31], it was not associated with lower TBS score, suggesting that the increased risk of fractures observed with the use of TDF is not mediated by microarchitectural changes of the bone.

Although our study demonstrated that HCV infection is associated with low TBS, neither the severity of liver disease (as assessed by the APRI score) nor HCV viremia was predictive of decreased TBS. These data suggest that successful HCV treatment (clearing of HCV viremia with or without improvement of fibrosis score) might not necessarily result in reversal of HCV-related bone fragility, although this will require prospective validation in HCV treatment studies. Consistent with this observation, a recent analysis of the Swiss cohort suggests that, unlike most other chronic complications, the OF risk in HIV/HCV coinfected subjects was not lower among those who either spontaneously cleared their infection or achieved sustained virologic response following anti-HCV with direct-acting antivirals [32]. Our study also complements our previous observation in suggesting that the mechanism of increased fracture risk is different in HIV- and HCV-infected individuals. We had previously showed that, unlike HIV, there is no significant increase in bone resorption in men with HCV [15]. Now we are showing that, unlike HIV, HCV is associated with deleterious microarchitectural changes. These findings might imply that different therapeutic interventions to improve bone health may be more adequate depending on the underlying infection. For example, the commonly used antiresorptive agents might not necessarily be adequate in management of HCV-related increased OF risk.

The major strengths of our study include the large and diverse number of patients (HIV infected, HCV infected, HIV/HCV coinfected, and uninfected) comprehensively evaluated about number of patients (HIV infected, HCV infected, HIV/HCV coinfected, and uninfected) comprehensively evaluated about different potential mechanisms of bone disease. A major limitation is the exclusive male composition of the study population, limiting the generalizability of our findings to women. Also, the cross-sectional nature of the study precludes the evaluation of longitudinal changes in BMD and TBS in the study population and whether BMD or TBS is predictive of actual OF events. The design nonetheless allows us to suggest significant differences in the effects of HIV and HCV on bone health and support further research into ways to mitigate fracture risk in these populations. Finally, it remains possible that unmeasured confounders,
including behavioral and nutritional factors, could account for at least part of the association of HCV infection with bone microarchitectural changes.

In summary, we show that HCV infection is associated with microarchitectural changes at the lumbar spine as assessed by the low TBS score, suggesting that microstructural abnormalities underlie some of the higher fracture risk in HCV infection but not in HIV.

Notes
Acknowledgments. This work was done as part of the corresponding author's functions at the Veterans Affairs North Texas Health Care System (US government employee).

Financial support. This study was funded by the Department of Veterans Affairs (MERIT grant number 101 CX000418-01A1).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

Figure 1. Effects of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections on bone mineral density (BMD) and trabecular bone score (TBS). P values represent comparisons of mean values in HIV/HCV vs HIV or HCV/HCV vs uninfected.