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Controlled HIV Viral Replication, Not Liver Disease Severity Associated with Low Bone Mineral Density in HIV/HCV Co-Infection

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Abstract

Objective—To evaluate the prevalence and risk factors for low bone mineral density (BMD) in persons co-infected with HIV and Hepatitis C.

Methods—HIV/HCV co-infected study participants (n=179) were recruited into a prospective cohort and underwent dual-energy X-ray absorptiometry (DXA) within 1 year of a liver biopsy. Fibrosis staging was evaluated according to the METAVIR system. Osteoporosis was defined as a T-score ≤ −2.5. Z-scores at the total hip, femoral neck, and lumbar spine were used as the primary outcome variables to assess the association between degree of liver disease, HIV-related variables, and BMD.

Results—The population was 65% male, 85% Black with mean age 50.3 years. The prevalence of osteoporosis at either at the total hip, femoral neck, or lumbar spine was 28%, with 5% having osteoporosis of the total hip, 6% at the femoral neck, 25% at the spine. The mean Z-scores (standard deviation) were −0.42 (1.01) at the total hip, −0.16 (1.05) at the femoral neck, and −0.82 (1.55) at the lumbar spine. In multivariable models, controlled HIV replication (HIV RNA < 400 copies/mL vs ≥400 copies/mL) was associated with lower Z-scores (mean ± standard error) at the total hip (−0.44±0.17, p=0.01), femoral neck (−0.59±0.18, p=0.001), and the spine (−0.98±0.27, p=0.0005). There was no association between degree of liver fibrosis and Z-score.

Conclusion—Osteoporosis was very common in this population of predominately African-American HIV/HCV co-infected patients, particularly at the spine. Lower BMD was associated with controlled HIV replication, but not liver disease severity.

Keywords

hepatitis C; bone mineral density; hepatic fibrosis; HIV

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Introduction

Osteoporosis is 3-4 times more common in HIV-infected persons compared to HIV-uninfected controls [1] and accounts for an increased risk of fracture among HIV-infected men and women [2]. The etiology of osteoporosis in HIV-infected patients is multifactorial. While the effect of chronic HIV infection and the high prevalence of traditional osteoporosis risk factors, such as low body weight, hypogonadism, and smoking, contribute to osteoporosis among HIV-infected patients, certain antiretroviral therapies and controlled HIV replication have been associated with lower bone mineral density in multiple studies [3-11].

Chronic viral hepatitis may also contribute to the risk of osteoporosis in HIV-infected patients. In the general population, lower bone mineral density has been associated with chronic liver disease, including chronic viral hepatitis [12]. The etiology of this association remains unclear, but may include alterations in vitamin D metabolism, gonadal status, or chronic inflammation. In a study of patients infected with either hepatitis B or C, but not HIV, the severity of osteoporosis was related to the severity of liver disease by concomitant histology, even among those without evidence of cirrhosis [13]. A direct role for chronic HCV infection in the pathogenesis of osteoporosis is further supported by the observation that fracture risk decreased in HCV mono-infected patients who received successful antiviral treatment [14]. Among HIV-infected patients, the impact of chronic viral hepatitis on bone mineral density has not been clearly established. However, HCV co-infection has been identified as an independent risk factor for subsequent fragility fracture in multiple cohorts of HIV-infected persons [15,16].

The goal of this study was to determine the prevalence and risk factors associated with low bone mineral density in a cohort of HIV/HCV co-infected subjects. We hypothesized that histologic evidence of hepatic fibrosis and inflammation would be associated with lower BMD.

Methods

Study Population

Study participants were recruited from the Johns Hopkins University HIV Clinic or the Viral Hepatitis clinical practice into a prospective cohort whose primary aim is to characterize liver disease progression among HIV/HCV co-infected persons. The criteria for study participation were broad, and included HCV/HIV co infection, ability to provide written informed consent, and current or past treatment at the Infectious Diseases outpatient clinic or viral hepatitis clinic. Recruitment occurred by self referral using flyers posted in those clinics, and/or provider referral. Between January 2007 and February 2009, 179 individuals enrolled in the cohort underwent BMD measurements of the hip and spine by dual-energy x-ray absorptiometry (DXA). For those without known cirrhosis (n=143), the DXA was performed within 1 year of the liver biopsy. The median time between the liver biopsy and the DXA was −13 days, (interquartile range [IQR]: 67, 0 days).

For all subjects, information on prescribed medications and laboratory parameters was obtained from clinical and laboratory databases. Data on patient demographics, social practices, clinical and laboratory parameters, and prescribed antiretroviral and other medications were abstracted from charts by trained personnel and transferred electronically from the laboratory database at enrollment and subsequent 6-12 month intervals, as described previously [17]. The designation of injection drug use and alcohol abuse was based on physician diagnosis and self-reports via orally administered questionnaires. Highly active antiretroviral therapy (HAART) was defined as use of a PI, NNRTI, fusion inhibitor

or integrase inhibitor. The study was approved by the Johns Hopkins Institutional Review Board and written informed consent was obtained for all participants.

Laboratory Evaluations

Patients had standard laboratory assessments performed by licensed clinical laboratories, including a complete blood cell count, serum chemistry panels, alanine transaminase (ALT) levels, CD4 cell count, and plasma HIV-RNA level (reverse-transcriptase polymerase chain reaction) measured within 6 months of DXA. HCV genotype testing was performed using reverse-transcriptase polymerase chain reaction.

Liver Histology

A transcutaneous liver biopsy was performed using an 18-gauge needle. Liver tissue was then fixed in 10% formalin, and paraffin-embedded sections were stained with hematoxylin-eosin and trichrome stains. Slides were evaluated by a single pathologist (M.T.). For fibrosis stage, biopsies were evaluated according to the METAVIR system (0 [no fibrosis] to 4 [cirrhosis]) [18]. Fibrosis was defined as METAVIR grades 2, 3, or 4. Necroinflammatory activity was scored based on the 0-18 scale of the modified histologic activity index (MHAI) and scores were divided into quintiles for analysis [19].

Body Composition and Bone Mineral Density

Body mass index was defined as weight (kilograms) divided by height (meters) squared. A wall-mounted stadiometer was used to measure height. Each participant was weighed while wearing minimal clothing. BMD at the total hip, femoral neck, and lumbar spine were measured using a Hologic 4500A machine with QDA4500A software version 9.03 (Hologic Inc, Waltham, MA). T-scores and Z-scores were calculated from the site-specific BMD measures using normative data from the manufacturer matched for gender and race [20,21]. The T-score is the number of standard deviations a participant's BMD falls from the mean BMD at a given site for a gender- and race-matched population at peak bone mass (~ age 30 years). The Z-score is the number of standard deviations a participant's BMD falls from the mean BMD of a gender- , age-, and race-matched population. For descriptive analyses, osteoporosis was defined as a T-score ≤ -2.5. Osteopenia was defined as $-2.5 <$ T-score ≤ -1. Low BMD was defined as Z-score ≤ -2. Given the relatively young age of our population, Z-scores were used as the primary outcome measurement in accordance with National Osteoporosis Foundation guidelines [22].

Statistical Analysis

The prevalence of osteoporosis, osteopenia, and low BMD was compared between men and women using chi-squared tests. Site-specific BMD and Z-scores were compared between men and women using t-tests. Z-scores of total hip, femoral neck, and spine were compared between grades of fibrosis (METAVIR grades 0-4), and quintiles of MHAI using one-way ANOVA tests. Univariable and multivariable linear regression was used to estimate the associations between Z-scores at these sites and various demographic, laboratory, and body composition measurements. For multivariable models, variables that were associated with each site-specific DXA Z-score in univariate analysis with a *P* value less than 0.10 were considered. Age, sex, race, and body mass index (BMI) were included in all multivariable models regardless of statistical significance. HIV RNA was analyzed as whether or not the concentration of HIV RNA was < 400 copies/mL within 6 months of the DXA (median duration −7.5 days [IQR: −40.5, 16.5 days]). Two-sided *P* values < 0.05 were considered statistically significant. With the known prevalence of fibrosis in our cohort of 40%, we estimated that we would be able to detect a difference of Z-score of 0.4 or greater at any site

between those with and with fibrosis with 80% power and an alpha of 0.05. All analyses were performed using SAS, version 9.1 (Cary, North Carolina, USA).

Results

Description of Study Population

The demographic and clinical characteristics of the study population are presented in Table 1. The median age was 50.3 years, 65% were male, 85% were black; 44% had a history of clinician-diagnosed alcohol abuse; 71 % had a history of smoking and 63% were smokers at the time of the DXA. The median BMI was 25.2 kg/m^2, 88% had a CD4 count > 200 cells/mm^3, and 77% had an HIV-RNA level < 400 copies/mL. Of the 39 subjects with uncontrolled HIV replication, 54% were receiving HAART within 6 months of the DXA, 23% had previously received HAART but not within a year of the DXA, and 23% had never received HAART. Cumulative exposure to HAART in the study population was 5.7 years [IQR: 2.5, 9.1], cumulative exposure to PI was 3.6 years [IQR: 0, 8.4] and cumulative tenofovir exposure was 0.9 years [IQR: 0, 3.8]. 98% were infected with HCV genotype 1. Seventy-seven percent (137 of 179) reported history of IV drug use. We were unable to assess the duration of HCV infection estimated from first reported injection drug use, as only 40 subjects answered the question regarding age at first use (12 of whom reported they had never injected). Twenty-seven percent had history of HCV therapy. The majority had received a combination interferon/ribavirin therapy whereas 5 of 49 (10.2%) had received ribavirin monotherapy. The median duration of HCV therapy prior to DXA was 24 weeks [IQR 12-48]; and the median number of years since first HCV treatment was 3.79 [1.95, 6.56]. Fibrosis grade 2, 3 or 4, confirmed by biopsy was present in 41%.

Bone Mineral Density Measures

Bone mineral density measures and prevalence of osteoporosis, osteopenia, and low BMD, stratified by sex are presented in Table 2. Overall, 30.2% (35/116) of men and 25.4% (16/63) of women had osteoporosis (i.e. T-score ≤ −2.5 at any of the 3 sites). The prevalence of osteoporosis was similar between men and women at each of the 3 sites. For both men and women, osteoporosis was most common in the lumbar spine (26.7% of men and 22.2% of women). Mean Z-scores were similar between men and women at for the femoral neck and spine. Mean Z-scores of the total hip were significantly lower for women. The prevalence of low BMD (Z-score ≤ −2 at any of the 3 sites) was similar between men and women (Men: 30.2% vs Women: 19%).

Bone Measures & Histologic Staging of Hepatic Fibrosis and Inflammation

The mean Z-score at each of the 3 skeletal sites is presented by the histologic stage of fibrosis or necroinflammatory activity in Table 3. Z-scores at all sites were similar across categories of fibrosis and inflammation.

Determinants of Hip, Femoral-neck, and Spine Z-Scores

Table 4 shows the relationship between demographic and clinical covariates and site-specific Z-scores. Lower BMI and controlled HIV replication (HIV RNA < 400 copies/mL) were associated with lower Z-scores at all three sites in multivariable models. At the total hip, smoking (ever vs. never) was marginally associated with lower Z-scores in the univariate model (−0.32 ±0.17, p=0.07), but this trend was no longer observed after multivariable adjustment. At the femoral neck, smoking history, nadir CD4 cell count, and the lowest ALT tertile (vs. highest, p=0.09) were marginally associated with lower Z-scores in univariate models, but not after multivariable adjustment. At the lumbar spine, longer cumulative HAART (p=0.002), current EFV use (p=0.08), and ever AZT use (p=0.08) were

associated with higher Z-score in univariable models. There was no association between HCV therapy or injection drug use with BMD at any site. After adjustment for age, sex, BMI, HIV RNA, and cumulative HAART exposure, current EFV use (0.79±0.28; p=0.005), but not AZT use (0.24±0.32; p=0.44), was also associated with higher spine Z-score. Alcohol abuse, hepatic fibrosis, cumulative or current exposure to protease inhibitors (PI), d4T, or tenofovir (TDF) were not associated with Z-scores at the any of the 3 sites (data not shown).

Discussion

In this study of a predominately African-American, HIV/HCV co-infected population in Baltimore, we found a very high prevalence of osteoporosis and low BMD, particularly at the lumbar spine. Contrary to our initial hypothesis, there was no relation between the severity of liver disease, by stage of fibrosis or necroinflammatory activity and the degree of bone loss at the spine or the hip. However, controlled HIV infection (< 400 copies/mL) was consistently associated with lower Z-scores, but no association was seen with any specific antiretroviral drug.

The 28% prevalence of osteoporosis (T-score ≤ −2.5 at the lumbar spine, total hip, or femoral neck) observed in our study was considerably higher than the 10-15% prevalence of osteoporosis observed in many cross-sectional studies of HIV-infected persons [23]. Similarly, the 26% prevalence of low BMD (Z-score ≤ −2 at any of the 3 sites) was higher than the 16% prevalence of low BMD in a large cohort of HIV/HCV co-infected patients from Modena Italy [24] . The explanation for the very high prevalence of low BMD in our cohort deserves further investigation. Although our cohort was similar to the Modena Cohort with respect to HIV/HCV co-infection, smoking, alcohol use, and illicit drug use, the extent to which the two cohorts differed by nutritional, behavioral, and other health-related factors that affect BMD is unclear.

One major difference between the two cohorts was the racial composition. Unlike the Modena Cohort, which was nearly exclusively Caucasian, 85% of our cohort was of African descent. In population-based studies in the US, BMD in African-Americans is 2-18% higher in the hip and spine compared to Caucasians [25-30], which likely accounts for the lower fracture rates among African-Americans, compared to other races [31]. The BMD of the men in our study was approximately 4-8% lower than a community-based cohort in Boston of a similar racial composition [25], and total hip and femoral neck BMD in women was approximately 3-8% lower in our cohort compared to NHANES III data [32]. Interestingly, the prevalence of osteoporosis in this cohort was similar to a separate cohort of participants in inner-city Baltimore, who shared many of the same demographic characteristics and risk factors [33]. Whether this unexpectedly low BMD in both cohorts translates into a higher than expected fracture rate is an important area for further inquiry.

Over 88% of the cases of osteoporosis in our cohort were attributable to osteoporosis at the spine. The lumbar spine is more metabolically active as it consists mainly of trabecular bone and may be affected earlier in disease than cortical bone. Findings of lower lumbar spine BMD have been similarly reported in several studies of cirrhotic patients [34-37], and have been associated with low IGF-1 and low 25OH Vitamin D levels. While not measured in the current study, these factors will be an important target for future investigations in this cohort.

Although 26% had histologic evidence of cirrhosis in our sample, we found no association between liver disease severity and BMD at any site. Several studies in patients with liver disease have found a relation between bone loss and the degree of liver disease [35,37], but this finding has been inconsistent [34,36]. These studies have primarily relied on clinical

classification of disease severity, comparing patients in Child-Pugh Class B or C to Class A [34,35,37]. In contrast to these studies, we used liver histology to categorize liver disease severity and none of our patients had decompensated liver disease. It is possible that the mechanisms inducing bone loss in patients with liver disease are triggered as a response to liver disease decompensation (eg hypogonadism) or as a result failure of synthetic activity (egs, decreased production of IGF-1 or 25OH Vitamin D). Our findings stand in contrast to a small German study of HBV or HCV monoinfected patients which found an association with liver fibrosis stage and BMD [13]. The explanation for the differences between the studies is not clear. In addition to HIV infection and antiretroviral therapy, the cohorts also differed by other important characteristics including race, socioeconomic factors, and likely HCV genotype.

Despite the well-established link between systemic inflammation and bone loss in the general population [38] and in patients with rheumatoid arthritis or inflammatory bowel disease [39,40], our data do not support the hypothesis that liver inflammation associated with HIV/HCV co-infection is related to loss of bone mineral density. Our analysis was limited by the lack of markers of systemic inflammation or cellular immune activation which would be useful to further evaluate the relationship between inflammation, liver disease, and bone mineral density.

One of the most consistent correlates with lower BMD in our study was controlled HIV replication, likely indicative of effective antiretroviral therapy. Although untreated HIV may be associated with increased osteoclast activity and decreased osteoblast activity, initiation of antiretroviral therapy is associated with a rapid increase in bone turnover [41,42] and a 2-6% decrease in BMD over 48-96 weeks [3-5,9-11]. Conversely, interruption of antiretroviral therapy in the SMART study was associated with an attenuation of bone loss compared with continuous viral suppression [6]. The mechanisms underlying this seemingly counter-intuitive association between controlled HIV viral replication and lower BMD deserve further investigation. It is possible that uncontrolled HIV and the resulting systemic inflammation impair osteoblast function, thereby slowing down bone turnover and protecting against BMD loss. Studies investigating bone turnover with antiretroviral interruption and re-initiation may be particularly useful in understanding the potential mechanisms.

Although we observed a consistent relationship between lower BMD and controlled viral replication, we did not observe a similar relationship between BMD and HAART use, per se. This was likely due to the fact that persons receiving HAART may not be compliant with therapy or may have drug-resistant virus. Alternatively, because HIV RNA was assessed within 6 months of the DXA and the medication database is updated every 6-12 months, the laboratory value may provide a more accurate assessment of effective HAART.

In studies investigating the effect of ART initiation on BMD, pre-treatment CD4 cell count has been an important predictor of subsequent bone loss with ART initiation [3,10] and consistent with this finding, we found that nadir CD4 cell count tended to be associated with lower BMD at the femoral neck. Earlier ART initiation may be useful to attenuate the bone loss observed with ART initiation, but this hypothesis has not yet been tested.

Certain antiretroviral medications may contribute to bone loss among HIV-infected patients. Although tenofovir has been consistently associated with a larger decrease in bone density with ART initiation [5,7,8], we did not find any association between TDF use and lower BMD. Tenofovir use, however, was not widespread in our cohort, such that power may have been limited to detect an effect. Similarly, although PIs have also been implicated in the pathogenesis of bone loss among HIV-infected patients [4,7], we did not find an association

with PIs and lower Z-score. We did however find that those receiving efavirenz had a higher spine Z-score compared to those not receiving efavirenz, although this effect was not observed at either the total hip or the femoral neck. This finding is consistent with ACTG 5224s, which showed that efavirenz was associated with a smaller decrease in BMD at the spine with ART initiation compared to atazanavir/ritonavir [7]. However, at the total hip, BMD decreased similarly in both groups. The explanation for this differential effect of efavirenz on the spine and hip is unclear.

It is unknown if low BMD will translate to increased fracture risk in HIV/HCV men and women. A French group that studied an HIV-infected cohort treated with combination ART found that the 2-year incidence rate of bone fractures 3.6-fold higher in those with HCV co-infection than HIV mono-infected subjects [15]. In that cohort, only fractures requiring hospitalization were reported, which could have underestimated fracture risk. Recently, data from HOPS cohort showed that HCV-co-infection was associated with a 60% increased risk of fragility fracture [16]. Our finding of a lack of association between the severity of liver disease and lower BMD may suggest that the increased risk of fracture in HIV/HCV co-infected patients is not mediated by low BMD, but rather compromised bone quality, which is not measured by conventional DXA. Alternatively, HCV-infection may be a marker of other conditions (eg, nutritional factors, opiate exposure) which mediate the relationship between HCV and low BMD, but have not been captured in our study.

There are a few additional limitations to our study. First, our study was cross-sectional and our findings do not exclude the possibility that those with more advanced liver fibrosis may experience accelerated bone loss, or that progression of liver disease may be associated with decreasing BMD. Further longitudinal studies are required to address these issues. Second, our study did not include an otherwise similar HIV-monoinfected or HCV-monoinfected control groups to explore the independent contributions of these chronic infections on bone health. Next, HIV RNA was not always measured at the time that a DXA scan was performed, raising the possibility of misclassification; however, we specified that the HIV RNA assessment occur within 6 months of the DXA scan and the median difference between these two assessments was very short (approximately 1 week). Finally, although hepatic inflammation via liver biopsy was not found to be a determinant of low bone density, this assessment may have limitations when used as a measure of hepatic inflammation in that: 1) it was assessed at a single time point, 2) it is potentially subject to sampling error, and 3) it may not be reflective of systemic inflammation.

In conclusion, we found high prevalence of osteoporosis, particularly at the spine, in this middle-aged population of predominately African-American subjects with HIV/HCV co-infection, which was not related to the severity of liver fibrosis. In the general population, African-Americans are less likely to be screened for osteoporosis compared to Caucasians, perhaps because BMD is higher and fracture risk is comparatively lower [43,44]. Our data would suggest that fracture risk may be higher than expected in African-American HIV/HCV co-infected persons and that more aggressive screening of persons with these risk factors who are 50 years or older may be warranted. In addition, further evaluation of the mechanisms underlying the high prevalence of osteoporosis and its clinical significance is warranted.

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Table 1

Demographics (n=179)

	N (%)	N
Median age in years (IQR)	50.3 (46.4, 53.4)	179
Sex - male	116 (65)	179
Race – black	152 (85)	178
Median BMI (IQR)	25.2 (22.3, 29.3)	175
History of alcohol abuse	79 (44)	179
History of smoking	121 (71)	171
Smoking at DXA	107 (63)	171
History of IV Drug Use	137 (76.5%)	179
HIV RNA < 400 copies/mL at DXA	129 (77)	168
CD4 > 200 at DXA	146 (88)	166
Median CD4 at DXA (IQR):	470 (294, 647)	166
Median nadir CD4(IQR)	148 (43, 265)	178
History of HAART	159 (89)	179
HAART at DXA	114 (64)	179
Median cumulative years on HAART (IQR)	5.7 (2.5, 9.1)	179
Fibrosis (grade 2,3, or 4)	74 (41)	179
History of HCV treatment	49 (27)	179

Table 2

Bone Mineral Density (BMD) Measures

Overall (i.e. total hip, femoral neck, or lumbar spine)	**Male (n=116)**	**Female (n=63)**	**p-value**
Osteopenia N (%)	80 (69.0)	36 (57.1)	0.11
Osteoporosis N (%)	35 (30.2)	16 (25.4)	0.50
Low BMD N (%)	35 (30.2)	12 (19.1)	0.11
Site-Specific Data			
Total hip (n= 175): BMD mean (g/cm^2), (SD)	1.00 (0.17)	0.88 (0.16)	<0.0001
Z-score: Mean (SD)	−0.29 (0.98)	−0.65 (1.03)	0.02
Osteopenia N (%)	48 (42.5)	22 (35.5)	0.37
Osteoporosis N (%)	5 (4.4)	3 (4.8)	F0.90
Low BMD N (%)	4 (3.5)	4 (6.5)	0.38
Femoral neck (n= 175): BMD mean (g/cm^2), (SD)	0.89 (0.15)	0.82 (16)	0.005
Z-score: Mean (SD)	−0.05 (0.99)	−0.37 (1.14)	0.06
Osteopenia N (%)	57 (50.4)	18 (29.0)	0.006
Osteoporosis N (%)	6 (5.3)	5 (8.1)	0.47
Low BMD N (%)	2 (1.8)	5 (8.1)	0.04
Total Spine (n= 179): BMD mean (g/cm^2), (SD)	1.03 (0.17)	0.98 (0.18)	0.07
Z-score: Mean (SD)	−0.93 (1.59)	−0.62 (1.45)	0.20
Osteopenia N (%)	44 (37.9)	24 (38.1)	0.98
Osteoporosis N (%)	31 (26.7)	14 (22.2)	0.51
Low BMD N (%)	34 (29.3)	8 (12.7)	0.01

−Osteopenia defined as −2.5 < T-score ≤ −1; Osteoporosis defined as having T-score ≤ −2.5; low BMD defined as having Z-score ≤ −2

Table 3

Spine and Hip Z-scores (Mean/STD) by Fibrosis and Inflammation Category (n=179)

	Fibrosis					**Inflammation***				
	None (n=38)	**Grade 1 (n=68)**	**Grade 2 (n=19)**	**Grade 3 (n=12)**	**Grade 4 (n=60)**	**Quintile 1 (n=31)**	**Quintile 2 (n=31)**	**Quintile 3 (n=33)**	**Quintile 4 (n=27)**	**Quintile 5 (n=25)**
Spine Z-score	−1.10 (1.33)	−0.63 (1.60)	−0.91 (1.18)	−0.77 (2.16)	−0.86 (1.61)	−0.97 (1.48)	−0.75 (1.54)	−0.67 (1.64)	−0.99 (1.61)	−0.72 (1.41)
Femoral Neck Z-score	−0.25 (0.95)	−0.05 (1.00)	−0.006 (0.99)	−0.15 (1.28)	−0.33 (1.18)	−0.13 (0.95)	−0.02 (1.13)	−0.23 (0.87)	−0.20 (1.16)	0.06 (0.88)
Total Hip Z-score	−0.42 (0.85)	−0.34 (0.96)	−0.32 (1.05)	−0.36 (1.34)	−0.59 (1.14)	−0.34 (0.89)	−0.32 (1.02)	−0.41 (1.06)	−0.42 (1.03)	−0.27 (0.82)

Those with known cirrhosis on previous biopsy were considered to be Metavir grade 4

*Quintile 1= MHAI 1 and 2

Quintile 2=MHAI 3

Quintile 3=MHAI 4

Quintile 4=MHAI 5

Quintile 5=MHAI 6,7,8

Table 4

Correlates of bone mineral density Z-scores at the total hip, femoral neck, and lumbar spine. Coefficients represent the difference in mean Z-score in relation to the reference group.

	Total Hip				Femoral Neck				Lumbar Spine			
	Univariate PR (SE)	**p-value**	**Multivariate PR (SE)**	**p-value**	**Univariate PR (SE)**	**p-value**	**Multivariate PR (SE)**	**p-value**	**Univariate PR (SE)**	**p-value**	**Multivariate PR (SE)**	**p-value**
Age (per 5 years)	0.03 (0.06)	0.64	0.04 (0.06)	0.51	0.01 (0.06)	0.86	0.05 (0.06)	0.44	0.0006 (0.09)	0.99	0.10 (0.09)	0.26
Sex (F vs M (ref))	−0.36 (0.16)	**0.02**	−0.46 (0.16)	**0.004**	−0.31 (0.16)	**0.06**	−0.32 (0.16)	0.05	0.31 (0.24)	0.20	0.40 (0.24)	0.10
Race (Black vs White (ref))	−0.10 (0.22)	0.65			−0.17 (0.22)	0.44			0.51 (0.33)	0.13		
BMI	0.07 (0.01)	**<0.001**	0.08 (0.01)	**<0.001**	0.07 (0.01)	**<0.001**	0.07 (0.01)	**<0.001**	0.06 (0.02)	**0.003**	0.05 (0.02)	**0.02**
Nadir CD4 cell count	−0.00039 (0.00047)	0.42			−0.0009 (0.00049)	**0.06**	−0.0007 (0.00047)	0.12	0.000091 (0.0007)	0.90		
HIVRNA viral load (<400 vs ≥400)	−0.33 (0.18)	**0.07**	−0.44 (0.17)	**0.01**	−0.43 (0.19)	**0.02**	−0.59 (0.18)	**0.001**	−0.59 (0.28)	**0.03**	−0.98 (0.27)	**0.0005**
Cumulative HAART exposure (continuous)	0.03 (0.02)	0.21			0.03 (0.02)	0.13			0.09 (0.03)	**0.002**	0.10 (0.03)	**0.0009**
Any AZT use (yes vs no)	0.13 (0.18)	0.47			0.22 (0.19)	0.24			0.50 (0.27)	**0.07**		
Current EFV use (yes vs no)	0.05 (0.19)	0.81			−0.09 (0.20)	0.66			0.51 (0.28)	**0.08**	0.79 (0.27)	**0.005**
Ever smoker	−0.32 (0.17)	**0.07**	−0.21 (0.16)	0.19	−0.33 (0.18)	**0.07**	−0.19 (0.17)	0.26	−0.25 (0.27)	0.36		
ALT (tertiles)												
Tertile 1	1				1		1		1			
Tertile 2	0.18 (0.19)	0.35			0.006 (0.20)	0.98	1		0.21 (0.29)	0.46		
Tertile 3	0.22 (0.19)	0.25			0.33 (0.20)	**0.09**	0.20 (0.16)	0.22	0.24 (0.29)	0.40		
History of IVDU	−0.19 (0.19)	0.32			−0.21 (0.20)	0.31			−0.47 (0.29)	0.11		
History of HCV treatment	0.03 (0.17)	0.85			0.05 (0.18)	0.80			−0.13 (0.26)	0.62		

Bold for univariate: p<0.1; bold for multivariate: p<0.05