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Title

Chronic viral hepatitis is associated with low bone mineral density in HIV-infected patients, ANRS CO 3 Aquitaine Cohort

Authors, Academic degrees

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Running head

Bone mineral density, HIV and viral hepatitis

2,038 words, 4 tables

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Appendix

The Groupe d'Epidémiologie Clinique du SIDA en Aquitaine (GECSA) coordinating the Aquitaine Cohort is organized as follows:

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Introduction

The prognosis of HIV-infected patients treated with antiretroviral therapy (ART) has dramatically improved over the past 15 years, and HIV infection is now a chronic disease with life-long treatment plans. Concomitantly, many studies have reported long-term complications of HIV infection, ART or both, involving systemic organs, and metabolic bone mineral diseases. High prevalence rates of osteopenia and osteoporosis have thus been observed in HIV-infected people [1] and the prediction of low bone mineral density (BMD) requires careful investigation within this population. Mechanisms involving either HIV itself or ART exposure, particularly the use of protease inhibitors (PIs) have been suggested [1-10]: bone loss has been more frequently observed in HIV-infected patients than negative controls, but the precise mechanisms by which ART affects BMD remain unclear. Antiretroviral drugs have also been exonerated [11].

In the general population, osteoporosis occurs frequently among the elderly and especially among postmenopausal women [12]. Cross-sectional surveys have suggested that osteoporosis was frequent among younger HIV-infected patients of both genders [13]. Risk factors for HIV-associated BMD loss are not well established and few studies have examined the role of chronic viral hepatitis. Nevertheless, osteoporosis is frequent in the course of liver diseases [14]. The prevalence of osteoporosis, based on the World Health Organization (WHO) diagnosis criteria [15], varies from 8% to 56% in surveys among patients with chronic liver disease (CLD) [16, 17], with numerous hypotheses about its determinants [18], but the exact mechanism whereby liver disease affects BMD remains to be elucidated. In addition,

many studies conducted among cirrhotic patients have suggested reduced bone formation and increased bone loss, both related to low levels of insulin-like growth factors (IGF) [14, 19-21].

Many studies reported a relationship between an increased risk of fracture and hepatitis co-infection [22, 23] supporting the hypothesis of an association between chronic viral hepatitis and low BMD in HIV-infected patients, and even a differential gender effect was found [24].

We aimed to compare BMD in HIV-infected patients of both genders, with or without viral hepatitis, and investigated the relationship between BMD and cirrhosis as an indicator of an advanced stage of CLD.

Methods

Study design and population

Prevalence of osteopenia and osteoporosis has been previously studied through a cross sectional study in HIV-infected patients consecutively enrolled in the ANRS CO3 Aquitaine Cohort between December 2004 and May 2005 but most of them were free of hepatitis B or C viruses (HBV or HCV) co-infection [13]. We updated this study sample by further enrolling patients co-infected with HBV or HCV, and who presented between November 2008 and October 2009 at Bordeaux Hospital outpatient clinics. The protocol was approved by the research ethical committee, and all patients provided informed consent to participate in the study.

Epidemiological, clinical, biological and therapeutic variables were recorded through patients' medical records and interview of patients.

BMD measurements

BMD of the lumbar spine, femoral neck, and total body were measured using the same instrument, dual energy X-ray absorptiometry (DXA; Hologic, Bedford, Connecticut, USA). Scans were analyzed by the same technician. Results were compared with normative curves, and expressed as T-score and Z-score. WHO diagnosis classification was used to categorize bone status as normal if T-score >-1, osteopenia if T-score between -1 and -2.5, and osteoporosis if T-score <-2.5. Furthermore, because bone abnormalities affected rather young HIV-infected patients [13], Z-scores were used to compare patients' BMD to the average value for a person of same age and gender. Thus a Z-score <-2.0 was used to characterize HIV-infected patients having less bone mass than expected.

Viral hepatitis and cirrhosis

Positive hepatitis B surface antigen (HBsAg) for at least six months defined chronic HBV infection. Chronic HCV infection was confirmed by positive anti-HCV antibodies and HCV RNA. Cirrhosis was diagnosed based on a hierarchical algorithm used in ANRS co-infection studies [25]: liver biopsy results (F4 in the METAVIR system) or if non available, the presence of indirect clinical signs of cirrhosis (ascites, oesophageal varices with or without bleeding, hepatic encephalopathy). Otherwise, non-invasive methods were used: the FibroScan™ result (with an elasticity value >12.5 KPa) [26] in priority and the result of FibroTest™ (F4 in the METAVIR system) [27] in absence of atazanavir or indinavir-related hyperbilirubinemia.

Statistical analysis

Statistical analysis was carried out using SAS software (SAS Institute Inc., North Carolina, USA). Comparative analysis between groups was based on Kruskall Wallis test for median values and their interquartile ranges [IQR] if continuous data; Chi square test was used to analyze differences in categorical data. Statistical significance was set with a p-value <0.05.

Univariable regression (stepwise forward) was performed to identify variables predictive of osteopenia and osteoporosis. All variables with p-value <0.25 were included in a multivariable polytomial logistic regression model to examine the potential determinants of osteopenia or osteoporosis. Modelling was stratified by gender because BMD standards (scores and curves) differed among women and men.

Results

Patients' characteristics

Four hundred and eighty six HIV-infected patients were consecutively enrolled in the initial survey and 140 viral hepatitis co-infected patients were additionally recruited.

Additional co-infected patients were more likely to be older (43 *versus* 45 years, $p=5.10^{-4}$) and be contaminated by intravenous drug use (IDU) route (42.6% *versus* 58.6%, *p=0.009*) than the mono-infected ones. There were significant differences with the original sample in HIV infection duration (15.0 *versus* 17.9 years, $p=10^{-4}$), and osteoporosis prevalence (27.1% *versus* 41.4%, *p=0.014*). Not surprisingly, co-infected patients newly recruited experienced longer antiretroviral drugs, especially PI, nucleoside reverse transcriptase inhibitor (NRTI) and tenofovir drugs (3.0 *versus* 2.0 years, *p=0.027*; 9.4 *versus* 7.8 years, $p=3.10^{-4}$; and 1.2 *versus* 0.0 year, $p=10^{-4}$

respectively). No significant differences were found in gender, postmenopausal status, AIDS stage, bone mass index (BMI), HIV viro-immunological markers and previous hepatitis C treatment.

Altogether, 626 patients were included in the present study: 357 had HIV alone and 269 had also chronic viral hepatitis (208 with HCV, 45 with HBV, and 16 with both HBV and HCV). Characteristics of patients according to viral hepatitis infection status are shown in Table 1. The overall median age was 44 years (IQR: 39; 49 years) and 26.5% of patients were female, 31.3% being postmenopausal. HIV RNA was undetectable in 70.9% of patients and the median CD4 count was 506/µL (IQR: 346; 696/µL). Low BMI was more common, and the duration of HIV infection and ART exposure were longer in co-infected patients. Cirrhosis was diagnosed in 61 (22.7%) co-infected and in two only (0.6%) of the HIV mono-infected patients, both related to excessive alcohol consumption.

Postmenopausal women were older than premenopausal ones (51.0 *versus* 40.0 years, $p=10^{-4}$), and they presented more frequently HIV RNA < 500 copies/mL (98.1% *versus* 75.7%, $p<10^{-4}$). Other variables were significantly comparable among these two groups.

Bone mineral density, hepatitis and cirrhosis

Osteopenia was diagnosed in 320 patients (51.1%) without any difference according to hepatitis co-infection status and osteoporosis in 187 (29.9%) (Table 1). Osteoporosis was present in 34.6% of co-infected patients and 26.3% of HIV-infected patients without chronic viral hepatitis (*p=0.032*). Osteoporosis appeared to be less frequently observed in co-infected patients without cirrhosis (69/208, 33.2%) than in cirrhotic ones (24/61, 39.3%) (*p=0.054)*.

DXA measurements were compared for each anatomic site according to viral hepatitis and cirrhosis status (Table 2). Median BMD values were significantly different between the HIV mono-infected group and the patients co-infected with viral hepatitis when measured at femoral neck, but not at lumbar spine in both males and females. Using T-score and Z-score values, low BMD was predominant at femoral neck but did not significantly differ by hepatitis co-infection and cirrhotic status.

Determinants of low BMD

Polytomial logistic regression investigated patients' determinants of osteopenia and osteoporosis, and results are reported by gender in Tables 3 and 4. After adjustment, the final model showed that, for both male and female patients, levels of low BMD were significantly associated with older age and BMI <20 kg/m². Osteopenia and osteoporosis were also particularly frequent among men who had reported sex with men (MSM): 52.6% and 38.3%, respectively. The association between chronic viral hepatitis and osteoporosis was only present in women (OR=19.0, *p=0.0474*). Among both genders, the multivariable analysis failed to identify any association between cirrhosis and either osteopenia or osteoporosis. ART and exposure duration to each antiretroviral class were not predictive of low BMD (data not shown).

Discussion

We documented high prevalence rates of osteoporosis, considering the average age of the HIV-infected patients recruited, but these are comparable to prevalence findings previously reported in our cohort [13] and by others [28]. The frequency of

osteoporosis was higher in HIV-infected patients with chronic viral hepatitis than among HIV-mono-infected ones but did not differ according to cirrhotic status.

Older age and low BMI were associated with osteoporosis in both women and men. Low BMI is the only known modifiable risk factor of low BMD documented in this study as shown in a previous meta-analysis [29]; bone mass loss was also higher in MSM patients than in any other patients as previously reported [13].

Most patients were clinically asymptomatic at the time of their DXA measurements and no marker of HIV disease progression was associated with osteopenia or osteoporosis in the multivariable model. Furthermore, neither infection duration nor advanced immunosuppression (CD4 count <200/μL) was associated with osteoporosis. Unlike some other reports [30, 31], ART use was not found to be associated with reduced BMD in our analysis. We investigated specifically the nucleosidic and nucleotidic ART class, including lamivudine and tenofovir, as these specific drugs are known to be effective against HBV; but we could not identify any link.

The hypothesis of an epidemiological association between viral hepatitis and osteoporosis was the central aim of our study and was only confirmed in our HIV-infected female patients. Our results corroborate other findings published by Lo Re III *et al* [24] who hypothesized that this association resulted from gender differences in serum levels of hepatitis-associated cytokines, markers of bone resorption, or other factors that maintain bone balance (such as IGF1 and osteoprotegerin (OPG)). In healthy subjects the increase in the serum receptor activator of nuclear factor kappaB ligand (RANKL)/osteoprotegerin ratio leading to BMD loss was shown related to age, with gender differences [32]. We previously identified in this cohort an association between decreased BMD and cardiovascular risk which also support

common physio-pathological mechanisms involving inflammatory factors [33]. Our study was not designed to evaluate OPG and RANKL levels.

Furthermore, our results do not match with published reports indicating an association between bone mass or mineral losses and liver fibrosis [34]. Cirrhosis in HIV-infected patients with chronic viral hepatitis was not associated with BMD alteration in our series. The grading of cirrhosis severity we used did not detect a critical point of decline in liver disease progression as suggested in non HIV-infected populations [18, 20, 34].

Our study had some limitations. The selection of co-infected patients occurred over two recruitment periods but was systematic in each period. Although we believed selection bias may existed, introduced by differences with the original co-infected patients the cross-sectional study design did not allow a full causal assessment, but only independant association. Moreover, some potential confounders like smoking, alcohol use, methadone use had not been collected at the time of the analysis, and the full multivariable model did not adjust on these variables.

Finally, the absence of markers of bone metabolism (such variables are not routinely measured) did not allow us to conclude about underlying mechanisms of association between chronic viral hepatitis and osteoporosis in women.

In conclusion, our study strongly suggests that chronic viral hepatitis is associated with osteoporosis in HIV-infected women; but concludes that cirrhosis is not a condition significantly associated with osteoporosis in both genders. However the contributive effects of cirrhosis and its consequences were not evaluated. Our findings have direct implications for clinical and therapeutical care of HIV-infected patients. For osteopenic or osteoporotic co-infected patients, preventive programs to

reduce the BMD decline and the risk of fracture may be considered, while considering the specific needs of women, and should take into account the well-known risk factors (low BMI and older age). Attending physicians should encourage prevention and early treatment of bone loss in order to reduce fractures rate and ultimately improve patients' qualify of life.

Further longitudinal studies, are needed to elucidate the relationship between osteoporosis and chronic viral hepatitis, including inflammatory process accelerating bone mass loss.

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Table 1. Patients' characteristics according to HBV or HCV associated co-infection, ANRS CO3 Aquitaine Cohort of HIV-infected patients

	HIV-infected patients without chronic hepatitis (n=357)		HIV-infected patients with chronic hepatitis (n=269)		*p-value*
Epidemiological and clinical variables					
Female gender	94	(26.3)	72	(26.8)	*0.90*
Postmenopausal status*	32	(34.0)	20	(29.0)	*0.49*
Age (years)	43.0	[38.0; 51.0]	44.0	[41.0; 48.0]	*0.22*
Mode of transmission					
Homosexual **	179	(68.1)	51	(25.9)	*$<10^{-4}$*
IDU	12	(3.4)	137	(50.9)	*$<10^{-4}$*
Body mass index† <20 kg/m²	49	(15.4)	57	(24.2)	*0.009*
CDC stage C‡	72	(20.2)	68	(25.3)	*0.13*
HIV infection duration (years)	9.9	[5.0; 14.1]	16.8	[12.3; 19.1]	*$<10^{-4}$*
Cirrhosis	2	(0.6)	61	(22.7)	*$<10^{-4}$*
HIV viro-immunological markers					
Median CD4 cells count/µL	512	[340; 688]	497	[360; 737]	*0.53*
CD4 cells count ≥500/µL	185	(51.8)	134	(49.8)	*0.62*
HIV RNA <50 copies/mL	247	(69.2)	197	(73.2)	*0.27*
HIV treatment duration (months)					
PI	13.5	[0; 48.6]	31	[0; 73.7]	*10^{-4}*
NRTI	63.3	[23.7; 97.4]	99.6	[67.2; 130.5]	*$<10^{-4}$*
NNRTI	5.7	[0; 26.4]	12.6	[0; 38.5]	*0.013*
Tenofovir	0	[0; 11.2]	8.5	[0; 26.9]	*$<10^{-4}$*
Previous hepatitis C treatment					
Interferon	-		147	(54.7)	*$<10^{-4}$*
Ribavirin	-		128	(47.6)	*$<10^{-4}$*
Bone abnormalities					
None	74	(20.8)	45	(16.7)	*0.25*

Osteopenia[$]	189	(52.9)	131	(48.7)	*0.33*
Osteoporosis[$]	94	(26.3)	93	(34.6)	*0.03*
Z-scores					
Lumbar spine	-0.5	[-1.2; 0.2]	-0.6	[-1.5; 0.2]	*0.07*
Femoral neck	-0.9	[-1.6; -0.2]	-1.0	[-1.8; -0.4]	*0.11*
Total body	-0.3	[-1.0; 0.6]	-0.2	[-1.2; 0.6]	*0.78*

Data are no. (%) or median [inter-quartile range] for qualitative or quantitative variables, respectively;
* percentage of females; ** percentage of males; † calculated by dividing weight by squared height;
‡ US Centers for Disease Control classification; $ see definitions in text
HBV: hepatitis B virus: HCV: hepatitis C virus; IDU: intravenous drug users; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non nucleoside reverse transcriptase inhibitor; PI: protease inhibitor

Table 2. Comparison of T-scores and Z-scores according to viral hepatitis infection and cirrhosis status, ANRS CO3 Aquitaine Cohort of HIV-infected patients

	Patients without chronic viral hepatitis **(n=357)**		Patients with chronic viral hepatitis and without cirrhosis **(n=208)**		Patients with chronic viral hepatitis and with cirrhosis **(n=61)**		*Overall p-value*
Lumbar spine							
BMD (g/cm²)	0.98	[0.90; 1.06]	0.98	[0.87; 1.06]	1.00	[0.84; 1.08]	*0.55*
T-score	-0.70	[-1.40; 0.00]	-0.90	[-1.80; 0.10]	-0.80	[-2.20; 0.00]	*0.16*
Z-score	-0.50	[-1.20; 0.20]	-0.70	[-1.50; 0.10]	-0.50	[-1.70; 0.20]	*0.17*
Femoral neck							
BMD (g/cm²)	0.74	[0.67; 0.82]	0.84	[0.75; 0.99]	0.85	[0.74; 0.99]	$<10^{-4}$
T-score	-1.90	[-2.50; -1.00]	-1.90	[-2.65; -1.30]	-2.10	[-2.80; -1.30]	*0.25*
Z-score	-0.90	[-1.60; -0.20]	-1.00	[-1.80; -0.40]	-1.10	[-1.70; -0.50]	*0.27*
Total body							
BMD (g/cm²)	1.12	[1.06; 1.18]	1.12	[1.06; 1.19]	1.12	[1.05; 1.20]	*0.97*
T-score	-0.60	[-1.40; 0.30]	-0.55	[-1.40; 0.20]	-0.65	[-1.80; 0.25]	*0.63*
Z-score	-0.30	[-1.00; 0.60]	-0.20	[-1.00; 0.60]	-0.35	[-1.70; 0.70]	*0.62*

Data are median values and their interquartile ranges

Table 3. Factors associated with reduced BMD in osteopenic and osteoporotic male patients (n=404), univariable and multivariable analysis† (final model), ANRS CO3 Aquitaine Cohort of HIV-infected patients

Univariable analysis	Osteopenia (n=234)		Osteoporosis (n=170)	
	OR [95% CI]	*p-value*	OR [95% CI]	*p-value*
Age (per 10 years more)	1.2 [0.8; 1.6]	*0.3656*	1.6 [1.1; 2.2]	*0.0136*
MSM group	1.8 [1.0; 3.3]	*0.0543*	1.9 [1.0; 3.5]	*0.0506*
Body mass index <20 kg/m²	2.8 [0.6; 12.5]	*0.1682*	8.0 [1.9; 34.7]	*0.0052*
CDC stage C	3.4 [1.3; 9.0]	*0.0121*	3.7 [1.4; 9.8]	*0.0093*
HIV infection duration (years)	1.0 [1.0; 1.1]	*0.1712*	1.1 [1.0; 1.1]	*0.0040*
HIV RNA <50 copies/mL	1.0 [0.5; 1.9]	*0.9127*	1.3 [0.6; 2.5]	*0.4835*
CD4 cells count <500/µL	1.0 [0.6;1.9]	*0.9004*	1.4 [0.7; 2.5]	*0.3189*
Chronic viral hepatitis	1.0 [0.6; 1.9]	*0.9498*	1.4 [0.8; 2.7]	*0.2450*
Cirrhosis	0.7 [0.3; 1.7]	*0.4241*	1.2 [0.5; 3.0]	*0.6821*
Previous Interferon treatment	0.6 [0.3; 1.2]	*0.1538*	0.8 [0.4; 1.5]	*0.4570*
Previous Ribavirin treatment	0.6 [0.3; 1.2]	*0.1599*	0.8 [0.4; 1.5]	*0.4390*
NRTI exposure duration (years)	1.1 [1.0; 1.2]	*0.0653*	1.1 [1.0; 1.2]	*0.0082*
NNRTI exposure duration (years)	1.1 [0.9; 1.2]	*0.4916*	1.1 [0.9; 1.3]	*0.2926*
PI exposure duration (years)	1.2 [1.1; 1.3]	*0.0053*	1.2 [1.1; 1.4]	*0.0012*
Tenofovir exposure duration (years)	1.4 [1.0; 1.9]	*0.0370*	1.6 [1.1; 2.1]	*0.0046*

Multivariable analysis (final model)	Osteopenia (n=234)		Osteoporosis (n=170)	
	OR [95% CI]	*p-value*	OR [95% CI]	*p-value*
Age (per 10 years more)	1.2 [0.9; 1.8]	*0.2582*	2.0 [1.3; 3.0]	*10^{-3}*
MSM group	2.6 [1.2; 5.3]	*0.0120*	3.6 [1.6; 8.2]	*0.0021*
Body mass index <20 kg/m²	3.0 [0.7; 14.0]	*0.1543*	12.1 [2.6; 56.2]	*0.0015*
Chronic viral hepatitis	1.6 [0.6; 4.0]	*0.3256*	1.3 [0.4; 3.5]	*0.6602*
Cirrhosis	0.5 [0.1; 2.2]	*0.5924*	1.7 [0.4; 7.7]	*0.4729*

†: Ref. normal BMD (N=56), polytomial logistic regression
BMD: Bone mineral density; OR: Odds ratio; CI: Confidence interval; MSM: Men who had reported sex with men

Table 4. Factors associated with reduced BMD in osteopenic and osteoporotic female patients (n=103), univariable and multivariable analysis† (final model), ANRS CO3 Aquitaine Cohort of HIV-infected patients

Univariable analysis	Osteopenia (n=86)		Osteoporosis (n=17)	
	OR [95% CI]	*p-value*	OR [95% CI]	*p-value*
Age (per 10 years more)	1.6 [1.1; 2.3]	*0.0248*	3.6 [2.0; 6.4]	*<10^{-4}*
Menopausal status	2.3 [1.1; 4.9]	*0.037*	6.9 [2.1; 22.9]	*0.0014*
Body mass index <20 kg/m²	2.7 [1.2; 6.2]	*0.0191*	8.5 [2.3; 30.7]	*0.0012*
CDC stage C	1.8 [0.7; 4.5]	*0.195*	2.9 [0.8; 10.3]	*0.1071*
HIV infection duration (years)	1.1 [1.0; 1.1]	*0.0296*	1.1 [1.0; 1.2]	*0.0598*
HIV RNA <50 copies/mL	0.9 [0.4; 1.7]	*0.651*	0.4 [0.1; 1.5]	*0.1827*
CD4 cells count <500/μL	1.4 [0.7; 2.7]	*0.3059*	0.9 [0.3; 2.7]	*0.8324*
Chronic viral hepatitis	1.4 [0.7; 2.7]	*0.347*	3.2 [1.0; 9.8]	*0.0423*
Cirrhosis	0.6 [0.1; 2.2]	*0.4109*	0.7 [0.1; 6.7]	*0.7765*
Previous Interferon treatment	1.2 [0.6; 2.7]	*0.587*	1.6 [0.5; 5.4]	*0.4442*
Previous Ribavirin treatment	1.0 [0.5; 2.4]	*0.9127*	1.3 [0.4; 4.7]	*0.6823*
NRTI exposure duration (years)	1.1 [1.0; 1.2]	*0.0975*	1.1 [1.0; 1.3]	*0.184*
NNRTI exposure duration (years)	1.1 [0.9; 1.3]	*0.3201*	1.2 [1.0; 1.5]	*0.0711*
PI exposure duration (years)	1.2 [1.0; 1.3]	*0.0233*	1.2 [1.0; 1.4]	*0.0707*
Tenofovir exposure duration (years)	1.3 [1.0; 1.9]	*0.0879*	1.8 [1.1; 2.8]	*0.014*

Multivariable analysis (final model)	Osteopenia (n=86)		Osteoporosis (n=17)	
	OR [95% CI]	*p-value*	OR [95% CI]	*p-value*
Age (per 10 years more)	1.6 [1.0; 2.5]	*0.0301*	15.2 [3.6; 65.5]	*2.10^{-4}*
Body mass index <20 kg/m²	2.5 [0.9; 6.5]	*0.0699*	18.4 [1.6; 217.9]	*0.0208*
Chronic viral hepatitis	0.8 [0.3; 2.3]	*0.7269*	19.0 [1.0; 349.8]	*0.0474*
Cirrhosis	0.3 [0.0; 4.1]	*0.3669*	0.6 [0.0; 12.2]	*0.7259*

†: Ref. normal BMD (N=63), polytomial logistic regression
BMD: Bone mineral density; OR: Odds ratio; CI: Confidence interval