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ORIGINAL ARTICLE

Prevalence of causes of secondary osteoporosis and contribution to lower bone mineral density in HIV-infected patients

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Abstract
Summary Eighty-one percent of human immunodeficiency virus (HIV)-infected patients had one or more of seven evaluated causes of secondary osteoporosis, and this rate increases with age. The type and number of causes were associated with a lower bone mineral density (BMD), and with an increased rate of osteopenia/osteoporosis, regardless of age and body mass index.
Introduction The objective of this study was to determine whether factors of secondary osteoporosis were associated with lower BMD in HIV.
Methods This was a cross-sectional study of 285 HIV-infected patients (25 % females) evaluating the impact of seven different factors of reduced BMD: hyperthyroidism, diabetes, chronic viral hepatitis, chronic kidney disease (CKD), hypovitaminosis D, secondary hyperparathyroidism, and hypogonadism. Dual-energy X-ray absorptiometry scan of the femoral neck was obtained at the clinical visit.
Results Mean age was 45.7 years; osteopenia and osteoporosis were diagnosed in 38 and 6 %, respectively. Overall, 230 patients (81 %) had secondary factors; 107 (38 %) had only 1 cause, 94 (33 %) had 2, and 28 (10 %) had 3 or more, predominantly vitamin D deficiency in 61 %, hepatitis C virus coinfection in 45 %, and secondary hyperparathyroidism in 27 %. The number of secondary factors was closely related to a lower BMD, which is statistically significant for patients having ≥2 causes (0.77 vs 0.73 g/cm^2, $p=0.02$). The rate of osteopenia ranged from 36 % without any cause to 57 % with three or more, osteoporosis from 0 to 19 %, and Z-score <−2 SD from 0 to 27 %, respectively. In a multivariate linear regression, adjusting by age, body mass index, and HIV-related factors, the number of secondary factors was independently associated with a lower BMD (β coefficient −0.134; $p=0.02$), mainly due to patients with hepatitis C virus (HCV) coinfection, secondary hyperparathyroidism, and CKD.
Conclusions A high prevalence of secondary causes of osteoporosis is observed in HIV-infected patients, and its type and cumulative number determine a lower BMD, after adjusting by age and body mass index.

Keywords Bone mineral density · Chronic kidney disease · Dual-energy X-ray absorptiometry · HIV · Secondary hyperparathyroidism · Secondary osteoporosis · Viral hepatitis · Vitamin D

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Introduction

Recent advances in antiretroviral therapy (ART) have led to a substantial decline in AIDS-related opportunistic infections, but comorbidities affecting the liver, kidney, bone, cardiovascular, and central nervous system have become increasingly prevalent [1]. Specifically, low bone mineral density (BMD) has been reported in many studies involving human immunodeficiency virus (HIV)-infected individuals [2–5]. In one meta-analysis, the prevalence of osteoporosis was three times higher among HIV-infected patients than among HIV-negative subjects [6]. This lower BMD may contribute to the increased incidence of fractures in this population [7, 8].

Furthermore, current evidence supports the inclusion of HIV infection among other risk factors [4], and several studies have also shown that BMD decreases by 2–6 % after initiation of various ART regimens [9, 10]. In any case, the causes of low BMD in HIV appear to be multifactorial, including the interaction between HIV infection, traditionally established osteoporosis risk factors, such as low body mass index

(BMI), high rates of tobacco, and alcohol use, and ART-related factors [4, 11].

However, the importance of causes of secondary osteoporosis in HIV patients has not been fully elucidated. Secondary osteoporosis is a condition of reduced bone mass that is caused or exacerbated by a variety of specific and well-defined disorders, such as hyperthyroidism, or hypogonadism, but more than 80 different causes have been involved [12]. HIV-infected patients have a higher prevalence of several of these causes, such as chronic viral hepatitis, kidney disease, or low vitamin D levels, suggesting that they could play an important role on BMD among this population. Moreover, these causes could be related, and patients could present several of them concomitantly. Thus, the aim of our study was to determine the prevalence and the impact on BMD of seven known causes of secondary osteoporosis not previously evaluated together, chronic viral hepatitis, hypogonadism, diabetes mellitus, hyperthyroidism, chronic kidney disease, vitamin D deficiency, and secondary hyperparathyroidism, in relation to smoking, alcohol consumption, other traditional causes of osteoporosis, and HIV-related factors.

Methods

This was a cross-sectional study of consecutive adult HIV-infected patients with regular follow-up in a specialized HIV/AIDS unit in a tertiary university hospital. Between October 2010 and December 2011, HIV-infected individuals were included if they met the criteria of demonstrated HIV infection and were at least 30 years old. Exclusion criteria were pregnancy, recent opportunistic infection, and current treatment with hormonal agents including testosterone, megestrol, or thyroid replacement therapy. All patients gave written informed consent before participating, and the study was approved by our institutional review board.

Variables

In the clinical visit, age, gender, clinical and exploratory data (including weight and height), and the history of HIV infection and antiretroviral therapy were collected. These data included risk practice for HIV acquisition, time of HIV infection (from the first positive serology), the lowest (nadir) and current CD4+ count, current and previous therapy HIV RNA level, number of previous antiretroviral therapies, and the composition and duration of current highly active antiretroviral therapy (HAART). In addition, patients were asked about the presence of traditionally considered risk factors for reduced BMD: smoking, alcohol consumption, personal or family history of no traumatic fractures or osteoporosis, corticosteroid intake, or diagnosis of autoimmune disease, especially rheumatoid arthritis.

Blood samples were collected for the determination of HIV-related parameters (CD4+ lymphocyte count and HIV load). Glomerular filtration rate (GFR) was estimated by using the chronic kidney disease (CKD)-epidemiology collaboration equation. Also, patients were screened for thyroid function (thyroid-stimulating hormone, TSH), hepatitis C virus (HCV) coinfection (if not available at history), diabetes mellitus, gonadal function (total testosterone), vitamin D status (serum determination of 25-dihydroxyvitamin D) by radioimmunoassay (DiaSorin, Stillwater, USA), and parathyroid function (serum parathyroid hormone, PTH) by radioimmunoassay.

The BMD of the femoral neck was measured by dual X-ray absorptiometry (DXA) using the same Hologic densitometer (Hologic 4500, Bedford, USA) at the clinical visit after daily calibration with "gold standard" reference spine and hip phantoms with anatomically correct contours to read BMD within 1 %.

Definitions

BMI was calculated as weight in kilograms divided by height squared in meters. Smoking was defined as current, active consumption. Alcohol intake was considered if there was daily consumption of three units or more (one unit=10 g). Corticosteroid intake was considered if it suppose 5 mg daily of prednisone or similar dose of other corticosteroids after conversion, during 3 months or more. HCV coinfection was considered in presence of a positive serology against HCV and a positive RNA-HCV by polymerase chain reaction or previous positive history and untreated. Diabetes mellitus was considered if the patient had a previous diagnosis or if he was receiving glucose-lowering therapy. Hypogonadism was defined as testosterone deficiency in men, measured by a total testosterone level of less than 300 ng/dL [13]. Hypogonadism was not evaluated in women. Vitamin D deficiency was defined as a value below 20 ng/ml. Secondary hyperparathyroidism was defined as an elevated plasma PTH above 65 pg/ml, and the possibility of primary hyperparathyroidism was ruled out in presence of elevated serum calcium (>11 mg/dL). CKD was established if estimated GFR (eGFR) values were lower than 60 ml/min/1.73 m^2 in two determinations prior to the visit (CKD stage 3 or higher) according to the guidelines for CKD of the National Kidney Foundation [14]. Hyperthyroidism (including subclinical) was defined by TSH levels below 0.50 mU/l, independently of symptoms.

According to BMD at the femoral neck obtained at the DXA scan, the T-scores were calculated as a standard deviation score compared with BMD at peak bone mass (age 30) and categorized according to the World Health Organization criteria as osteoporosis if <−2.5 SD and osteopenia when the T-score <−1.0 SD [15]. Also, Z-score, which compares an individual's BMD to the mean of an age-matched and gender-matched reference population, was also calculated; Z-

scores>−2.0 SD were considered within the normal range for age [16].

The primary study outcome was the value of BMD of the femoral neck, overall, and according to different age strata (<40, 41–50, 51–60, and >60 years), in the absence/presence of secondary causes of osteoporosis. As secondary outcome, the rate of osteopenia, osteoporosis, and Z-score of less than −2 SD was also calculated. We used the femoral neck as the skeletal region of interest, since it has been validated for use with the fracture risk assessment tool equation [17], and the T-score derived from measurement of BMD at the femoral neck by DXA is also the WHO international reference standard for the diagnosis of osteoporosis [18].

Statistical analysis

Means, medians, and range were calculated by each variable, in total and for each decade of life since 30 years. Comparisons were performed by Student's *t* test or Mann–Whitney test for continuous variables and chi-square test for categorical variables. Bivariate correlations were obtained between BMD and the different continuous factors. A linear regression model was created utilizing BMD value as the dependent variable, considered as continuous (grams per square centimeter). The overall fit of the model was assessed and standardized beta coefficients for secondary factors of osteoporosis (isolated and according to the number) were reviewed for statistical significance and contribution to reduced BMD, together with statistically significant variables in the univariate analysis. Age, gender, and BMI were considered to be required covariates. A p value less than 0.05 denoted the presence of statistical significance.

Results

Overall, 285 patients were included in the analysis; 70 (25 %) were women. As shown in Table 1, mean age was 45.7 (30–80) years, median time since HIV diagnosis was 178.5 months (range, 1.6–319.7), median nadir CD4 cell count was 197 cells/mm^3 (range, 3–795), and median current CD4 cell count was 345 cells/mm^3 (4–1,575). A nadir CD4+ cell count <200 cells/mm^3 was documented in 142 cases (53 %). HIV RNA levels were below 50 copies/ml in 215 patients (75 %). Most patients were on ART (98 %), in 125 cases (44 %) based in protease inhibitor therapy, and 215 (75 %) were currently receiving tenofovir in their antiretroviral regimen. Cumulative time on HAART was 551.5 patient-years.

Risk factors for reduced BMD

Traditionally known factors for osteoporosis were uncommon, with only one (0.4 %) and three (1 %) patients having a previous fracture or family history of fractures, respectively. Mean BMI (kilograms per square meter) was 23.8 (14.3–36.6), with 41 patients (14 %) having less than 20 kg/m^2 and 19 (7 %) with more than 30 kg/m^2. Other causes of secondary osteoporosis included in traditional evaluation were rare: no patient had diagnosis of rheumatoid arthritis and none was on corticosteroid therapy, whereas smoking or alcohol consumption equal or higher than three units/day were observed in 146 patients (51 %) and 25 cases (9 %), respectively.

Overall, of the seven contributors to secondary osteoporosis evaluated in this study, 230 patients (81 %) had at least 1 cause, 55 patients (19 %) had none, 107 (38 %) had 1 cause, 94 (33 %) had 2 causes, and 28 (10 %) had 3 or more known factors (Fig. 1). The percent of those with at least one cause increased to 86 % when smoking and alcohol were included. We found vitamin D deficiency in 61 %, HCV coinfection in 128 (45 %), and secondary hyperparathyroidism in 27 %. Hypogonadism was observed in 8 % of males and development of a CKD stage 3 or greater was observed in 7 % before the clinical visit, whereas hyperthyroidism developed in 4 % and diabetes mellitus in 3 %. No patient had serum calcium levels above 10.5 mg/dL, and there were no obvious symptoms or suspicion of primary hyperparathyroidism.

Of note, secondary causes were significantly correlated. HCV coinfection was related to a higher rate of CKD (12 vs 4 %) and lower levels of serum vitamin D (17 vs 20.3 ng/ml; $p=0.05$); CKD was associated with a higher prevalence of hyperparathyroidism (50 vs 19 %; $p<0.01$), whereas hypovitaminosis D was related to hyperparathyroidism (33 vs 19 %; $p=0.01$), and it was more frequent with hyperthyroidism (vitamin D levels, 19.3 vs 11.1 ng/ml; $p<0.01$).

Table 1 also shows the baseline characteristics, traditionally considered risk factors for osteoporosis, and prevalence of secondary causes, stratified by age. As it can be observed, a significantly higher prevalence of CKD, hypogonadism, secondary hyperparathyroidism, and diabetes was progressively observed in older patients. According to age categories, CKD ranged from 0 to 31 %, hypogonadism from 4 to 21 %, diabetes from 0 to 13 %, and hyperparathyroidism from 19 to 38 %. Therefore, the presence of secondary factors increases progressively from 63 to 94 % according to age (Fig. 1).

As expected, women had an overall lower BMD than men (0.71 vs 0.79 g/cm^2, $p<0.01$), with important, although not statistically significant, differences in each strata of age (Table 2). Also, women had a significant lower BMI than men, longer duration since HIV diagnosis, and time on therapy. However, and despite the absence of diabetes and lack of data on hypogonadism, a similar rate of secondary factors was observed, attributable to a higher prevalence of hypovitaminosis D and secondary hyperparathyroidism.

In the overall population, osteopenia and osteoporosis were observed in 38 and 6 %, respectively, ranging from 26 % and 0 cases in younger than 40 years to 55 and 19 % in patients in the

Table 1 Baseline characteristics of the 285 patients included and according to the different decades of life

Variables	Overall (n=285)	<40 years (n=62, 22 %)	41–50 years (n=133, 47 %)	51–60 years (n=74, 26 %)	>60 years (n=16, 6 %)
Mean age (years)	45.7 (30–80)	32.4 (30–35.2)	45.4 (41–48)	53.1 (51–59)	65.7 (61–80)
Gender, male	215 (75)	52 (84)	98 (74)	51 (69)	14 (88)
Risk factors					
IDU	128 (45)	4 (7)	78 (59)	46 (63)	0
MSM	100 (35)	43 (69)	31 (23)	16 (22)	10 (63)
Traditionally considered factors for osteoporosis					
Mean BMI (kg/m^2)	23.8 (14.3–36.6)	23.4 (17.4–33.1)	23.9 (14.3–36.5)	23.4 (15–32.6)	26.4 (21.7–30.3)
Current smoking	146 (51)	25 (40)	76 (57)	41 (55)	4 (25)
Alcohol intake ≥3 units/day	25 (9)	4 (7)	12 (9)	9 (12)	0
Family history of fractures	3 (1 %)	1 (2)	1 (1)	1 (1)	0
Previous fractures	1 (0.4)	0	0	0	1 (6)
Glucocorticoid tx	0	–	–	–	–
HIV-related data					
Nadir CD4+ (cells/mm^3)	197 (3–795)	310 (9–795)	197 (3–741)	146 (4–450)	216 (3–672)
Time since HIV diagnosis (months)	178.5 (1.6–319.7)	46.2 (1.6–292.6)	208.4 (3.5–331.8)	215.3 (47.5–320)	159.8 (11–248)
Current CD4+ (cells/mm^3)	345 (4–1,575)	387 (9–1,575)	341 (13–1,539)	323 (4–1,400)	313 (34–744)
On HAART	280 (98)	59 (95)	132 (99)	73 (99)	16 (100)
Current use of PI	125 (44)	14 (23)	64 (48)	40 (54)	7 (44)
Current use of tenofovir	215 (75)	56 (90)	104 (78)	48 (65)	7 (44)
Time on current HAART (months)	29.2 (0–124.7)	26.2 (0–65.3)	22.4 (0–84.6)	34.4 (0–125)	33.4 (0–95.4)
Secondary causes					
eGFR (ml/min/1.73 m^2)	95.8 (40.5–127)	110.1 (82.6–125)	97.4 (56–123)	80.7 (40.5–107)	72.4 (54–105.1)
CKD	20 (7)	0	3 (2)	12 (16)	5 (31)
HCV coinfection	128 (45)	3 (5)	82 (62)	42 (57)	1 (6)
Hyperthyroidism	10 (4)	5 (8)	4 (3)	1 (1)	0
Mean vitamin D (ng/ml)	19.3 (5–60)	19.1 (5–46.3)	19.2 (7–23.5)	20.1 (5–60)	16.9 (7.8–32)
Deficiency (<20)	175 (61)	36 (58)	82 (62)	45 (61)	12 (75)
Mean PTH (pg/ml)	56.3 (8–189)	50.9 (16.8–90.9)	56.5 (8–140.2)	59.9 (25–189)	62.7 (32–144)
Hyperparathyroidism	76 (27)	12 (19)	35 (26)	23 (31)	6 (38)
Hypogonadism[a]	18 (8)	2 (4)	5 (5)	8 (16)	3 (21)
Diabetes mellitus	8 (3)	0	4 (3)	2 (3)	2 (13)
One or more secondary causes	230 (81 %)	39 (63)	113 (85)	63 (85)	15 (94)

Data are no. (percent) of patients or median (range), unless otherwise specified

IDU intravenous drug user, *MSM* men having sex with men, *BMI* body mass index, calculated as weight in kilograms divided by the square of height in meters, *HIV* human immunodeficiency virus, *HAART* highly active antiretroviral therapy, *PI* protease inhibitor, *eGFR* estimated glomerular filtration rate, *CKD* chronic kidney disease, *HCV* hepatitis C virus, *PTH* parathyroid hormone

[a] Calculated only in men

51–60 strata, respectively. Of note, a Z-score <−2 SD was observed in 7 %, especially in patients between 51 and 60 years (3, 3, 22, and 0 % for the different age strata; p<0.01).

The number of secondary factors was linearly associated with a lower BMD and a high rate of Z-score <−2 SD, osteopenia and osteoporosis (Fig. 2), that was statistically significant for patients having two or more causes (0.77 vs 0.73 g/cm^2, p=0.02) in comparison with no causes. The rate of osteopenia ranged from 36 % if there were no causes to 57 % if there were three or more causes, whereas osteoporosis ranged from 0 to 19 %, respectively. A Z-score lower than −2 SD was not observed in patients without secondary causes, in comparison with 27 % in the presence of three or more causes.

Figure 3 depicted the mean BMD according to the number of secondary factors in each age strata. As shown, statistically significant differences were also observed with two or more secondary factors in all age strata, with the exception of patients older than 60 years, probably due to the small number of patients in this category. These differences were observed independently of BMI, with significantly reduced BMD for

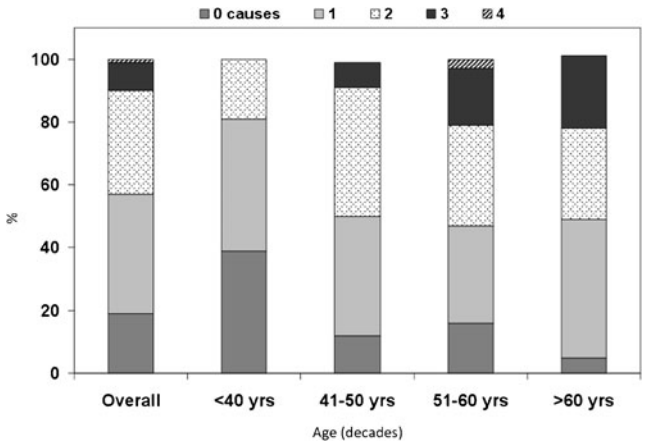

Fig. 1 Prevalence and number of secondary causes of osteoporosis in HIV-infected patients, overall, and according to age categories

patients having at least two secondary factors in patients with BMI below 20 kg/m^2 (0.75 vs 0.57 g/cm^2; $p<0.01$) or above 20 kg/m^2 (0.8 vs 0.74 g/cm^2; $p<0.01$).

Bivariate correlations demonstrate the multifactorial relationships of BMD. There was a significant correlation of BMD with age ($r=-0.38$; $p<0.01$), BMI ($r=0.29$; $p<0.01$), HIV-related factors such CD4+ count nadir ($r=-0.254$; $p<0.01$), or time of HIV infection ($r=-0.36$; $p<0.01$), and a lower BMD was observed in patients with HCV coinfection (0.72 vs 0.8 g/cm^2; $p<0.01$), CKD (0.64 vs 0.75 g/cm^2; $p<0.01$), hyperthyroidism (0.7 vs 0.75 g/cm^2; $p=0.01$), secondary hyperparathyroidism (0.72 vs 0.79 g/m^2; $p<0.01$), use of protease inhibitor (PI) (0.72 vs 0.81 g/cm^2; $p<0.01$), and current use of tenofovir (0.72 vs 0.79 g/cm^2; $p<0.01$), whereas there was a trend to association with smoking (0.75 vs 0.79 g/cm^2; $p=0.09$). This significant association was not found for patients with diabetes (0.73 vs 0.77 g/cm^2; $p=0.45$), or with hypogonadism, as defined (0.76 vs 0.78 g/cm^2; $p=0.6$). Of note, a lower BMD was observed in patients receiving tenofovir plus boosted PI concomitantly, in comparison with tenofovir plus non-nucleoside reverse transcriptase inhibitors (0.73 vs 0.82 g/cm^2; $p<0.01$). The number of secondary factors, as variable, was also correlated ($r=-0.27$; $p<0.01$). In the linear regression analysis (Table 3), the number of secondary factors was independently associated with lower BMD at the femoral neck, adjusting by BMI, age, or significant HIV-related factors. The model was repeated including the type of secondary factor, and the significant role of hyperparathyroidism, HCV coinfection, and chronic kidney disease on BMD remains statistically significant. Of note, age, sex, and BMI were significantly associated with lower BMD in both models, as well as time of HIV infection or nadir CD4+ count below 200 cells/mm^3. However, the use of tenofovir was not further associated

Table 2 Differences between men and women in baseline variables, secondary causes of osteoporosis, and BMD

	Men (215)	Women (70)	*p* value
Mean age	45.4 (30–80)	46.8 (31–68)	0.28
Mean BMI (kg/m^2)	24.4 (17.4–36.5)	21.9 (14.3–32.8)	<0.01
BMI <20 kg/m^2; *n* (%)	21 (10)	20 (29)	<0.01
Nadir CD4+ count (cells/mm^3)	199 (3–741)	187 (6–795)	0.6
CD4+ <200 cells/mm^3	115 (53)	37 (53)	0.9
Time since HIV diagnosis (months)	168 (3–330)	190.7 (6–315)	0.07
Time on current HAART (months)	28.3 (2–135.4)	36.9 (5.9–91.4)	0.9
PI	90 (42)	34 (49)	0.4
Use of tenofovir	160 (74)	55 (79)	0.48
Smoking	117 (54)	29 (41)	0.07
Alcohol ≥3 units/day	23 (11)	2 (3)	0.05
Secondary causes of osteoporosis	174 (81)	56 (80)	0.9
Diabetes mellitus	8 (4)	0	0.1
HCV coinfection	98 (46)	30 (43)	0.7
Hyperthyroidism	7 (3)	3 (4)	0.8
Vitamin D deficiency	126 (59)	49 (70)	0.1
Secondary hyperparathyroidism	54 (25)	22 (31)	0.3
Chronic kidney disease	15 (7)	5 (7)	0.8
Hypogonadism	18 (8)	ND	
Mean BMD (g/cm^2)	0.79 (0.48–1.4)	0.71 (0.53–1.01)	<0.01
<40 years	0.86 (0.61–1.4)	0.81 (0.71–1.01)	0.2
41–50 years	0.78 (0.48–1.19)	0.73 (0.57–0.94)	0.08
51–60 years	0.72 (0.52–1.03)	0.66 (0.53–0.9)	0.05
>60 years	0.66 (0.64–1.07)	0.62 (0.6–0.66)	0.2

Data are no. (percent) of patients or median (range), unless otherwise specified

BMI body mass index, *HAART* highly active antiretroviral therapy, *HCV* hepatitis C virus

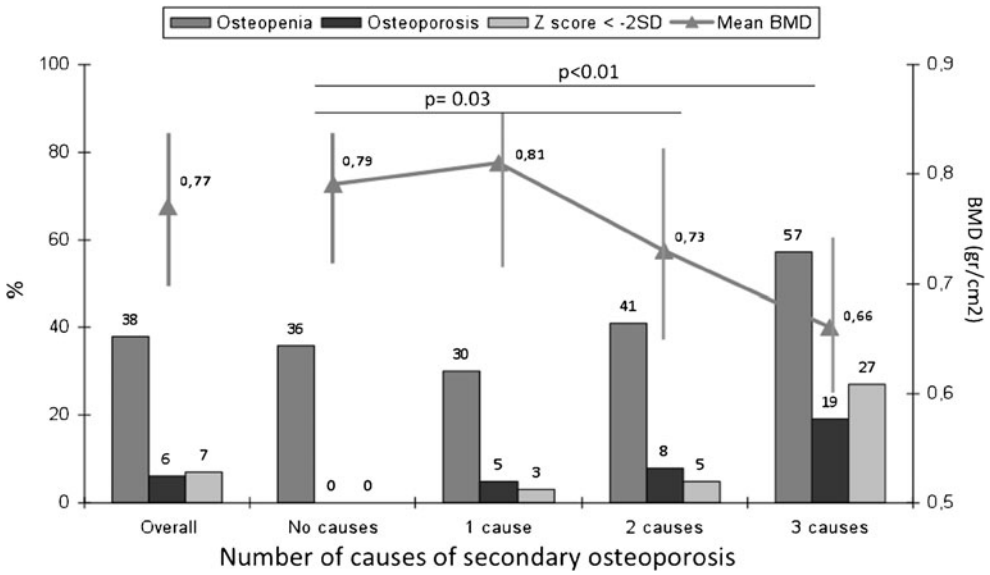

Fig. 2 Rate of osteopenia, osteoporosis, and Z-score <−2 SD and mean bone mineral density (*BMD*), according to the number of secondary factors

when including individual secondary factors, such as CKD or secondary hyperparathyroidism.

Discussion

To our knowledge, this study is the first to examine extensively whether secondary causes of osteoporosis were associated with reduced BMD in HIV-infected patients. A total of 81 % of patients had at least one of the seven causes evaluated, and the cumulative number of factors was related to a lower BMD. Moreover, our study highlights the importance of age when considering the prevalence of secondary factors, taking into account that the prevalence of renal and metabolic diseases increases along the years. Although factors other than low BMD are important in identifying patients at elevated risk for osteoporotic fracture, a low BMD is a predictor of fragility fractures [19], and therefore, our data suggest that the presence of these secondary factors could increase the risk for hip fractures.

In studies including patients with osteoporosis and/or a recent fracture, causes of secondary osteoporosis were found in 7–80 %, depending on the number or type of factors included [20, 21]. Thus, Johnson et al. [22] identified contributors to osteoporosis in 11 % of 180 patients with bone densities lower than expected for age. Tannenbaum et al. [23] identified a prevalence of 32 % of previously unrecognized contributors to osteoporosis in otherwise healthy postmenopausal women. Bours et al. described that 42.5 % of men after age 50 presenting with a clinical fracture had one or more known or new secondary factors [24]. Deutschmann et al. [21] reported a total of previously known and new risk factors for osteoporosis in 63 % of women and 67 % of men, but the largest groups of disorders were lactose malabsorption, hypercalciuria, and renal tubular acidosis type 1.

Thus, although the prevalence of secondary contributors to lower BMD could be comparable in some studies with the results found in the present work, the disorders identified differ. The most common secondary causes in men include hypogonadism, alcoholism, and glucocorticosteroid exposure, accounting for 40–60 % of cases [25], whereas premenopausal estrogen deficiency and glucocorticoid exposure are the most common secondary causes in women [21, 24]. In HIV-infected individuals, low BMD has been linked most frequently to low body weight [26], but it has also been linked to testosterone or estrogen deficiency, glucocorticosteroids, malabsorption, tobacco use, alcohol and opiate abuse, nadir CD4+ cell count, duration of HIV infection, lipodystrophy, insulin resistance, and hyperlactatemia [27]. The traditionally

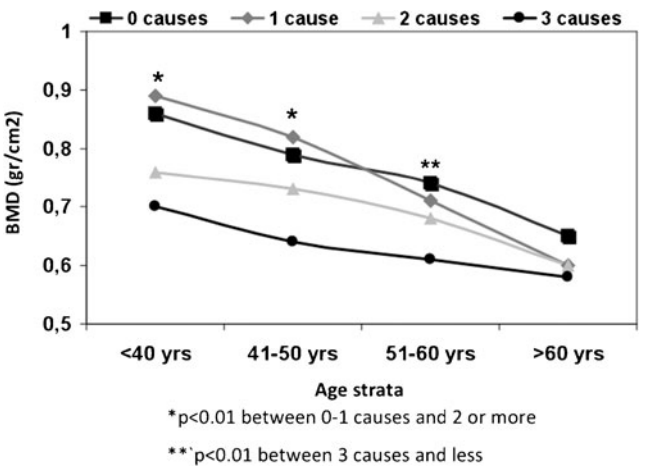

Fig. 3 Mean BMD values according to the number of secondary factors and age strata

Table 3 Relationship of causes of secondary osteoporosis with BMD

Variables	Standardized β coefficient	p value
Model 1		
Age (years)	−0.29	<0.01
Gender, female	−0.17	<0.01
BMI (kg/m^2)	0.32	<0.01
Time since HIV diagnosis (months)	−0.17	<0.01
Current use of tenofovir	−0.11	0.03
CD4+ count <200 cells/mm^3	−0.24	<0.01
Number of secondary factors	−0.134	0.02
Model 2		
Age (years)	−0.27	<0.01
Gender, female	−0.18	<0.01
BMI (kg/m^2)	0.24	<0.01
CD4+ <200 cells/mm^3	−0.36	<0.01
Time since HIV diagnosis (months)	−0.23	<0.01
Current use of tenofovir	−0.09	0.1
Chronic viral hepatitis	−0.16	0.01
Chronic kidney disease	−0.19	<0.01
Secondary hyperparathyroidism	−0.17	0.02

In addition to the variables shown, both models include smoking, time on antiretroviral therapy (months), and use of PI. Model 2 evaluates the different causes of secondary osteoporosis individually, previously included in model 1 as the variable number of secondary factors

BMI body mass index

considered factors for decreased BMD were rare in our population, with the exception of a low BMI in women. Indeed, our study included other secondary factors for osteoporosis, such as smoking, alcohol consumption, rheumatologic or autoimmune disease, or corticosteroid use, but they were rare except from smoking. Even the most powerful predictors of fractures in the general population, a history of fragility fracture, or maternal history of fractures were almost exceptional [28].

To date, few studies have addressed the role of some of these factors on BMD in this population. For example, although not associated with BMD in cross-sectional studies, low vitamin D levels have been associated with lower total hip BMD at baseline or greater reductions in femoral neck BMD during follow-up in longitudinal studies [29], whereas higher PTH levels have been associated with greater reductions in BMD [30]. Viral hepatitis has been associated with reduced BMD among HIV-uninfected individuals [30, 31], with a prevalence of reduced BMD ranging from 10 to 56 % [32], whereas in HIV/HCV, coinfected patients, Lo Re described a 16 % of prevalence [33]. Although the importance of secondary factors included in this work seems to rely on HCV coinfection, chronic kidney disease, and secondary hyperparathyroidism, as shown in the multivariate analysis, our study emphasizes the importance of these factors considered together, an important issue since there was a close relationship between chronic viral hepatitis, CKD, hypovitaminosis D, and secondary hyperparathyroidism.

Of note, the use of PI or tenofovir, as HIV-related factors, with the exception of time of HIV infection and a CD4+ count nadir <200 cells/ml, was not related in the multivariate analysis when introducing secondary factors. Tenofovir disoproxil fumarate (TDF) has been found to be associated with a greater decline in BMD than other antiretroviral drugs, and even prophylactic use of TDF has also been shown to cause a small but significant decline in BMD in HIV-uninfected subjects. Also, earlier studies had suggested that exposure to PIs decreased BMD [10, 11, 34, 35]. Our data, including age, BMI, and multiple factors, confirm the multifactorial nature of reduced BMD in HIV-infected patients and suggest that other risk factors could be more important in predicting fracture risk among HIV-infected patients than antiretroviral exposure, at least in this cohort of patients.

Despite the multiple causes of secondary osteoporosis that have been identified, we have not included other secondary risk factors, such as malabsorption, and other conditions that are rarely prevalent and its usefulness in evaluating BMD is negligible. Thus, although Tannenbaum et al. [22] reported that 6.4 % of women with osteoporosis had low 24-h urine calcium suggesting calcium malabsorption, most of these factors act through low vitamin D or secondary hyperparathyroidism, factors that are already included in our study. In addition, other secondary factors included in our study, such as diabetes or hypogonadism, could be controversial. Several studies support that BMD in type 2 diabetes is similar to or higher than in nondiabetic subjects [36, 37], in probable relationship with higher BMI in diabetic women, although diabetes is associated with a higher fracture risk. Our study includes only eight men with diabetes. They had a lower BMD as previously found in diabetic males [38], and, in any case, the rate of secondary factors and their relationship with BMD remained unchanged after excluding diabetic patients. Also, we defined hypogonadism on the basis of a total testosterone level of <300 ng/dL, as used in previous studies using this value of 300 ng/dL as the lower limit of the normal range for testosterone levels in healthy young men [39, 40]. However, free (bioavailable) testosterone levels are a more accurate measure, since changes in sex hormone-binding globulin levels may result in relative increases in total testosterone that do not reflect bioavailable or free testosterone [41].

Interestingly, we found an important role for CKD. It was observed in 7 % of our patients, and half of them had concurrent secondary hyperparathyroidism. The onset and severity of bone disease are related to the level of GFR, and fracture risk is increased in patients with moderate to severe CKD. In hip fracture patients, 12 and 4 % of patients had CKD stages 3 and 4, respectively [42]. Therefore, GFR decline while on HAART could contribute to a lower BMD.

Of clinical interest, women had lower BMD than men in all the strata of age. Undoubtedly, this fact was related to a lower BMI, with nearly one third of women having less than 20 kg/m^2, but we cannot rule out the role of other factors such as menopause. We did not determine sexual hormones in women, and 25 out of 70 were older than 50 years, supporting a role for estrogen deficiency. However, and despite the absence of diabetes and no data on gonadal function, the rate of secondary factors was similar (80 %), due to a similar percentage of HCV coinfection and CKD, and a slightly higher prevalence of hyperparathyroidism and hypovitaminosis D. Moreover, women were less prone to alcohol or smoking abuse, and probably this fact could explain the lack of significant differences according to gender in different strata of age, taking into account the importance of BMI and menopause on BMD.

Our study had several limitations. First, as cross-sectional studies evaluate exposure and disease status at the same point in time, this study design is limited in its ability to determine causality. Second, a number of potential confounding variables were evaluated and controlled, but multivariable analyses may not entirely eliminate residual confounding from additional unmeasured factors, e.g., we did not have information on the duration of each secondary factor exposure. Third, patients were only from Spain, a population with high prevalence of HCV coinfection or different sun exposure that could influence the rate of hypovitaminosis D and subsequent hyperparathyroidism, potentially limiting the generalizability of our results. Finally, given our small sample size in patients above 60 years, we were unable to fully explore potential common mechanisms of lower BMD between age, BMI, and secondary factors that could explain the observed lack of association in decreasing BMD, taking into account that older patients had the highest number of causes in addition to age.

In conclusion, we found that the presence of secondary causes was highly prevalent in HIV-infected patients, and these causes independently were associated with low BMD. Better understanding of the causes of bone loss would permit targeted interventions to prevent bone loss and mitigate fracture risk. Despite the widespread reports on increased prevalence of reduced BMD in HIV patients, patients with a sum of secondary factors may represent a subgroup with increased risk of osteopenia and osteoporosis and may benefit from individual screening. Thus, these results make consideration of secondary factors when evaluating BMD in HIV-infected patients mandatory, and future studies should evaluate fracture rates according to the type and number of these causes in this population.

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Conflicts of interest None.

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