Osteoporosis International (2019) 30:1501–1510
https://doi.org/10.1007/s00198-019-04942-7

ORIGINAL ARTICLE

CD4 T cell count is inversely associated with lumbar spine bone mass in HIV-infected men under the age of 50 years

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Received: 28 May 2018 / Accepted: 14 March 2019 / Published online: 26 March 2019

Abstract

Summary HIV-infected men under the age of 50 years had a lower bone mass compared to that of HIV-uninfected men. Lower CD4 T cell counts, independent of whether antiretroviral therapy (ART) was used, were associated with lower BMD. HIV-infected patients with low CD4 T cell counts may need follow-up and intervention regarding bone health, including younger patients.

Introduction HIV-infected patients have a low bone mineral density (BMD) owing to multifactorial interaction between common osteoporosis risk factors and HIV-related factors, including chronic inflammation and ART. Although HIV infection and ART might affect bone metabolism, little data is available for patients aged under 50 years. We aimed to investigate the association of HIV infection-induced low CD4 T cell counts and ART with BMD in men aged under 50 years.

Methods We performed an age- and body mass index–matched case–control study. BMD values of HIV-infected and HIV-uninfected men (< 50 years) were compared, and HIV-infected men were stratified by CD4 T cell counts and ART use.

Results After adjusting confounders, HIV-infected men with CD4 T cell counts ≥ 500 cells/μL ($n = 28$) and < 500 cells/μL ($n = 139$) had lower BMD at the femoral neck (FN, $p < 0.001$) and total hip (TH, $p < 0.001$) than HIV-uninfected men ($n = 167$). HIV-infected men with CD4 T cell counts < 500/μL had lower BMD at the lumbar spine (LS, $p = 0.034$) than those with counts of ≥ 500 cells/μL, but not at FN and TH. The CD4 T cell count ($\gamma = 0.169$, $p = 0.031$) was positively correlated with BMD at LS. There was no significant difference in the BMD ($p = 0.499$–> 0.999) between the ART-naïve ($n = 75$) and ART-user group ($n = 92$).

Conclusions Despite their relatively younger age, HIV-infected men had a lower BMD than HIV-uninfected men. Lower CD4 T cell counts, irrespective of ART, might result in lower bone mass.

Keywords Bone mineral density · CD4 T cell counts · HIV infection

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00198-019-04942-7) contains supplementary material, which is available to authorized users.

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Introduction

The prevalence of a lower bone mass in human immunodeficiency virus (HIV)-infected patients is higher than that in HIV-uninfected people [1–4]. Traditional risk factors for osteoporosis in the general population include factors such as old age, female sex, current smoking, low body mass index (BMI), and malnutrition as well as specific risk factors for HIV disease such as chronic inflammation due to viral infection [5]. Recently, the survival rate of HIV-infected patients has been improving [6] due to advancements in medications for HIV treatment. Therefore, many clinicians focus not only on the control of HIV but also on the management of bone health of HIV-infected patients.

Human and animal studies suggested that HIV infection itself and antiretroviral therapy (ART) for the treatment of HIV contribute to bone loss. The suggested mechanisms of HIV-induced bone loss were increased levels of pro-inflammatory cytokines associated with HIV infection [7], immune reconstitution [8], or specific HIV proteins [9–12]. A study on HIV-1 transgenic rats showed increased bone resorption, driven by an increase in osteoclast precursor numbers, and amplified by an elevated receptor activator of nuclear factor-κB ligand (RANKL)/osteoprotegerin (OPG) ratio [13]. The CD40/CD40L co-stimulation between B cells and T cells upregulates OPG production [14, 15], so HIV-transgenic rats also showed that changes in OPG production in HIV infection may be a result of a deficiency in T cell co-stimulation [13]. Besides HIV infection itself, ART also induced bone loss. For example, the nucleoside reverse transcriptase inhibitor (NRTI) of ART has been reported to enhance osteoclast formation [16]. Indeed, ART has been found to increase bone resorption markers in human studies [17]. Immune activation associated with CD4 T cell reconstitution by ART induces increased production of the pro-inflammatory cytokines such as RANKL and tumor necrosis factor alpha (TNFα) by immune cells, driving enhanced bone resorption and loss in BMD in animal models [8] and in humans [18]. Indeed, BMD at the lumbar spine (LS) and hip decreased by 2 to 6% within the first 2 years of ART initiation [19] and then stabilized or improved thereafter [20].

Some studies have demonstrated that lower CD4 T cell counts, an indicator for pharmacologic treatment [21], may be associated with lower bone mass and increased fracture risk [22, 23]. However, these studies were limited in that they did not distinguish by sex and included all age groups, with more than 50% of the study population aged more than 50 years. We hypothesized that if HIV infection-induced low CD4 T cell counts affects bone mass, then HIV-infected patients under the age of 50 years may also have a lower bone mass compared to that of HIV-uninfected patients. In Korea, most HIV-infected patients are men under the age of 50 years [24]. We are therefore concerned that young HIV-infected patients are not recommended for measurement of bone mineral density (BMD) in recent guidelines for bone health in HIV-infected patients [25]. To date, there is no report on the effect of low CD4 T cell counts on bone health in HIV-infected patients under the age of 50 years. Therefore, we aimed to compare the BMD of HIV-uninfected men with that of HIV-infected men under the age of 50 years and to investigate the association of BMD with CD4 T cell counts and the use of ART in HIV-infected men in this age group.

Methods

Study participants

From January 2010 to December 2015, we recruited 229 consecutive patients newly diagnosed with HIV infection. The diagnosis was based on the positivity of an HIV antigen/antibody combination test (Elecsys HIV Combi PT, Roche Diagnostics, Germany) and Western blot. Patients with co-morbid conditions (e.g., rheumatoid arthritis, neoplastic diseases, endocrine disorders, chronic renal disease, and liver disease) or who were taking medication (oral glucocorticoids, antiepileptic drugs, cyclosporine, and cytotoxic drugs) that could affect BMD were excluded from the study ($n = 31$). Men over the age of 50 years were also excluded ($n = 31$). Thus, 167 HIV-infected men under the age of 50 years were eligible for inclusion in this study and were defined as the case group. Information obtained from medical records review and telephone interviews included age, BMI, alcohol intake (≥ 3 U/day), smoking habit (current smoker), regular exercise (≥ 30 min/day), co-morbid conditions, current and previous antiretroviral regimens, and cumulative duration of antiretroviral therapy. The control group was randomly selected from patients who undertook a health screening test at the Health Promotion Center at Soonchunhyang University, Seoul Hospital, and 167 HIV-uninfected men were matched (1:1) to the cases according to both age (within 2.0 years) and BMI (within 1.0 kg/m^2). Since CD4 T cell counts of < 500 cells/μL were suggested as an indication for pharmacologic treatment [26], we divided the HIV-infected men into two groups: those with CD4 T cell counts < 500 cells/μL ($n = 139$) and those with CD4 T cell counts ≥ 500 cells/μL ($n = 28$; Supplemental Fig. 1).

BMD measurement and laboratory measurements

BMD (g/cm^2) at the LS (L1–4), femoral neck (FN), and total hip (TH) was measured using dual-energy X-ray absorptiometry (DXA, GE Lunar, Madison, WI, USA). Because our subjects were men under 50 years of age, we used Z-scores according to the recommendations of the International Society for Clinical Densitometry [27]. Z-score was used after adjustment for sex and race (Korean), which were provided by the equipment manufacturer. The precision values of the machine, from the perspective of the coefficient of variation (CV), were 0.8%, 1.7%, and 0.6% for the lumbar spine, total hip, and femur neck, respectively.

Plasma RNA titers for HIV were measured using real-time polymerase chain reaction (PCR) using the COBAS AmpliPrep/COBAS TaqMan HIV-1 test (Roche Diagnostics International Ltd., Switzerland). CD4 T cell counts were performed using flow cytometry at the Department of Microbiology (Navios Flow Cytometer, Beckman Coulter Life Sciences, IN, USA).

Statistical analysis

All statistical analysis was performed using SPSS version 22.0 for Windows (IBM Corp, Armonk, NY). The Kolmogorov–Smirnov normality test was performed for all continuous variables. The data are shown as the mean ± standard deviation (SD) for normally distributed continuous variables and median (inter-quartile range, IQR) for non-normally distributed continuous variables. Categorical data are expressed as numbers and percentages. Baseline characteristics were compared using a Student's *T* test or the Mann–Whitney *U* test for continuous variables or the χ^2 test for categorical variables.

BMD and Z-score among the HIV-uninfected men, HIV-infected men with CD4 T cell counts ≥ 500 cells/μL, and those with CD4 T cell counts < 500 cells/μL were compared using one-way analysis of variance (ANOVA). Multivariable-adjusted least-square mean levels (95% confidence intervals (CIs)) of BMD and Z-score according to the status of HIV infection and CD4 T cell counts of 500 cells/μL were estimated and compared using analysis of covariance after adjusting for ART use and confounders, which included age, BMI, alcohol use, current smoking status, and regular exercise.

The distributions of CD4 T cell counts and their associations with LS-BMD, FN-BMD, and TH-BMD were investigated using Pearson correlation and scatter plots. Associations of the CD4 T cell count with BMD were investigated through multiple linear regression analyses, after adjustment for confounders. In these analyses, CD4 T cell counts were log-transformed because of their skewed distributions. We performed a power analysis using R version 3.5.1 for Windows ("pwr" package) to investigate whether the different outcomes of BMD according to the site in the HIV-infected men were due to sample size effects.

The distributions of BMD and Z-score at LS, FN, and TH were investigated using box plots with Tukey IQR according to (1) the status of HIV infection and CD4 T cell counts < or ≥ 500 cells/μL, (2) in HIV-infected men according to the use of antiretroviral treatment (ART), and (3) in HIV-infected men according to the use of ART and CD4 T cell counts < or ≥ 500 cells/μL. Multivariable-adjusted least-square mean levels (95% CIs) of BMD and Z-score according to ART were estimated and compared after adjustment for confounders, including age, BMI, alcohol use, current smoking status, and regular exercise. Furthermore, we divided the HIV-infected patients into four subgroups: (1) CD4 T cells ≥ 500 cells/μL and ART-naïve subgroup, (2) CD4 T cells < 500 cells/μL and ART-naïve subgroup, (3) CD4 T cells ≥ 500 cells/μL and ART-user subgroup, and (4) CD4 T cells < 500 cells/μL and ART-user subgroup. BMD and Z-score based on the CD4 T cell counts and the use of ART were compared using one-way analysis of variance (ANOVA). Multivariable-adjusted least-square mean levels (95% CIs) of BMD and Z-score according to the status of the CD4 T cell counts and the use of ART were estimated and compared using analysis of covariance after adjusting for confounders, age, BMI, alcohol use, current smoking status, and regular exercise. A nominal *p* value of 0.05 was regarded as statistically significant.

Results

The baseline characteristics of the 167 HIV-uninfected and HIV-infected men each are listed in Table 1. There was no significant difference in age, height, weight, BMI, and current smoking status between HIV-infected men and HIV-uninfected men. HIV-uninfected men had a higher alcohol intake and a lower amount of regular exercise compared to that of HIV-infected men. Median [IQR] of monthly doses of ART was 84.0 tablets [63.0; 84.0] and that of treatment duration was 26.0 months [9.0; 44.8]. Mean CD4 T cell counts were 373.9 ± 912.2 cells/μL.

Before adjustment, HIV-infected men with CD4 T cell counts ≥ 500 cells/μL ($n = 28$) had a lower BMD than the HIV-uninfected men ($n = 167$) at FN (0.980 ± 0.135 g/cm^2 vs. 1.072 ± 0.130 g/cm^2, $p = 0.001$) and TH (1.003 ± 0.139 g/cm^2 vs. 1.148 ± 0.124 g/cm^2, $p < 0.001$), but not at LS (1.173 ± 0.122 g/cm^2 vs. 1.188 ± 0.131 g/cm^2, $p = 0.563$) (Fig. 1) (Table 2). HIV-infected men with CD4 T cell counts < 500 cells/μL ($n = 139$) also had a lower BMD than HIV-uninfected men at LS (1.115 ± 0.130 g/cm^2 vs. 1.188 ± 0.131 g/cm^2, $p < 0.001$), FN (0.956 ± 0.127 g/cm^2 vs. 1.072 ± 0.130 g/cm^2, $p < 0.001$), and TH (0.989 ± 0.121 g/cm^2 vs. 1.148 ± 0.124 g/cm^2, $p < 0.001$) (Fig. 1a). There was a significant difference between HIV-infected men with the CD4 T cell counts ≥ 500 cells/μL and those with CD4 T cell counts < 500 cells/μL only in the BMD at LS ($p = 0.032$), but not at the FN ($p = 0.373$) and TH ($p = 0.589$) (Fig. 1a). Similarly, HIV-infected men with CD4 T cell counts ≥ 500 cells/μL had a lower Z-score than HIV-uninfected men at LS (− 0.5 ± 1.0 vs.

Table 1 Characteristics of participants with and without HIV infection

	HIV-uninfected men ($n = 167$)	HIV-infected men ($n = 167$)	p
Age (years), median [IQR]	31.0 [26.0; 38.0]	33.0 [27.0; 39.0]	0.475
Height (cm), median [IQR]	174.0 [170.7; 177.8]	174.0 [170.0; 178.0]	0.876
Weight (kg), median [IQR]	66.9 [61.4; 74.4]	67.0 [60.4; 74.0]	0.923
BMI (kg/m^2), median [IQR]	22.3 [20.4; 24.1]	22.0 [20.2; 24.2]	0.818
Alcohol ≥ 3 U/day, *n* (%)	*38 (22.8%)*	*8 (5.3%)*	*< 0.001*
Current smoking, *n* (%)	97 (58.1%)	107 (64.8%)	0.249
Regular exercise ≥ 30 min/day, *n* (%)	*26 (15.6%)*	*68 (40.6%)*	*< 0.001*
HIV duration (months), median [IQR]	NA	10.0 [0.0; 33.5]	
Antiretroviral therapy use, *n* (%)	NA	92 (55.1%)	
Antiretroviral therapy category			
NRTI, *n* (%)	NA	91 (54.5%)	
NNRTI, *n* (%)	NA	37 (22.2%)	
Protease inhibitor, *n* (%)	NA	45 (26.9%)	
Integrase inhibitor, *n* (%)	NA	32 (19.2%)	
ART duration among user (months), median (IQR)	NA	26.0 [9.0; 44.8]	
CD4 T cell counts (cells/μL), median [IQR]	NA	264.0 [129.0; 415.0]	
RNA level (copies/mL), median [IQR]	NA	45.2 [16.7; 156.7] × 10^3	

Values are presented as median (inter-quartile range) for non-normally distributed continuous variables. Categorical data are expressed as numbers and percentages. Baseline characteristics were compared using the Mann–Whitney *U* test for continuous variables or the χ^2 test for categorical variables. Italicized numbers indicate statistically significant values

IQR interquartile range, *BMI* body mass index, *NA* not applicable, *NRTI* nucleoside reverse transcriptase inhibitor, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *ART* antiretroviral therapy

1.7 ± 1.1, $p < 0.001$), FN (− 0.1 ± 0.1 vs. 1.6 ± 1.0, $p < 0.001$), and TH (− 0.2 ± 0.1 vs. 1.2 ± 1.0, $p < 0.001$) (Fig. 1b). HIV-infected men with CD4 T cell counts < 500 cells/μL also had a lower Z-score than HIV-uninfected men at LS (− 1.0 ± 1.0 vs. 1.7 ± 1.1, $p < 0.001$), FN (− 0.4 ± 0.9 vs. 1.6 ± 1.0, $p < 0.001$), and TH (− 0.4 ± 0.8 vs. 1.2 ± 1.0, $p < 0.001$) (Fig. 1b). There was a significant difference between HIV-infected men with CD4 T cell counts ≥ 500 cells/μL and those with CD4 T cell counts < 500 cells/μL only in the Z-score at LS ($p = 0.035$), but not at the FN ($p = 0.216$) and TH ($p = 0.373$) (Fig. 1b).

After adjustment for ART use and confounding factors, including age, BMI, alcohol use, current smoking status, and

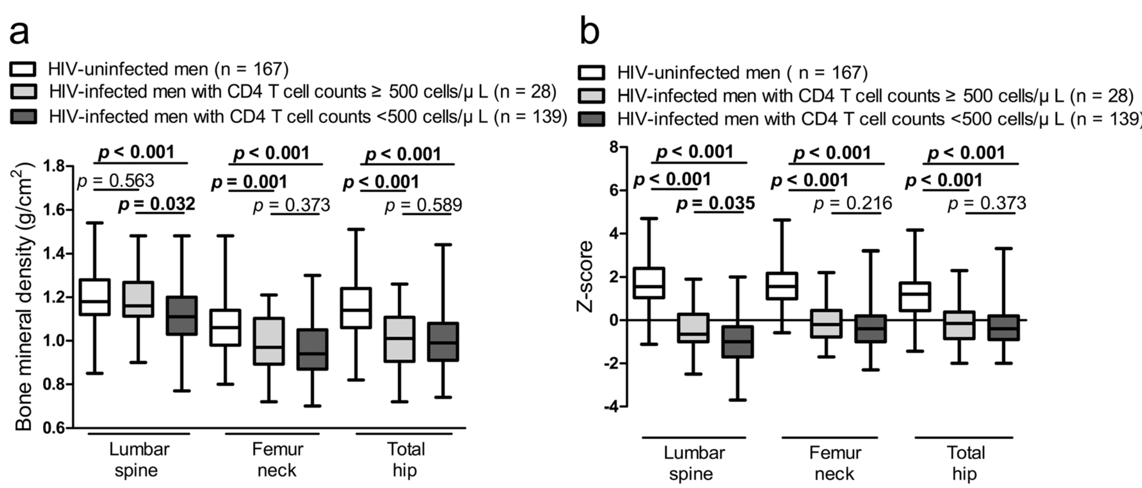

Fig. 1 Box plot with Tukey interquartile range for bone mineral density (BMD) and Z-score according to the status of HIV infection and CD4 T cell counts < or ≥ 500 cells/μL. BMD and Z-score among the HIV-uninfected men, HIV-infected men with CD4 T cell counts ≥ 500 cells/μL, and those with CD4 T cell counts < 500/μL were compared using a one-way analysis of variance (ANOVA)

Table 2 Characteristics of HIV-infected patients according to CD4 T cell counts < or ≥ 500 cells/μL

	CD4 < 500 cells/μL ($n = 139$)	CD4 ≥ 500 cells/μL ($n = 28$)	p
Age (years), median [IQR]	*34.0 [27.0; 40.0]*	*28.0 [25.0; 37.0]*	*0.031*
Height (cm), median [IQR]	174.0 [170.0; 178.0]	175.0 [168.5; 177.0]	0.492
Weight (kg), median [IQR]	68.0 [62.0; 74.0]	65.0 [56.4; 76.5]	0.258
BMI (kg/m^2), median [IQR]	22.1 [20.8; 24.2]	21.4 [19.6; 24.3]	0.285
Alcohol ≥ 3 U/day, *n* (%)	7 (5.6%)	1 (3.7%)	0.684
Current smoking, *n* (%)	87 (63.5%)	16 (57.1%)	0.528
Regular exercise ≥ 30 min/day, *n* (%)	42 (32.0%)	19 (53.8%)	0.148
HIV duration (months), median [IQR]	11.5 [0.0; 37.0]	2.0 [0.0; 23.0]	0.229
Antiretroviral therapy use, *n* (%)	*82 (59.0%)*	*10 (35.7%)*	*0.024*
Antiretroviral therapy category			
NRTI, *n* (%)	*81 (58.3%)*	*10 (35.7%)*	*0.029*
NNRTI, *n* (%)	*36 (25.9%)*	*1 (3.6%)*	*0.006*
Protease inhibitor, *n* (%)	41 (29.5%)	4 (14.3%)	0.109
Integrase inhibitor, *n* (%)	26 (18.7%)	6 (21.4%)	0.738
ART duration among user (months), median (IQR)	*26.5 [10.8; 46.3]*	*11.5 [3.5; 32.25]*	*0.009*
CD4 T cell counts (cells/μL), median [IQR]	*213.0 [106.0; 335.0]*	*709.0 [571.8; 791.5]*	*< 0.001*
RNA level (copies/mL), median [IQR]	*60.8 [19.7; 210.0] ·10^3*	*16.1 [6.3; 45.2] ·10^3*	*0.001*

Values are presented as median [IQR] for non-normally distributed continuous variables. Categorical data are expressed as numbers and percentages. Baseline characteristics were compared using the Mann–Whitney *U* test for continuous variables or the χ^2 test for categorical variables. Italicized numbers indicate statistically significant values

IQR interquartile range, *BMI* body mass index, *NA* not applicable, *NRTI* nucleoside reverse transcriptase inhibitor, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *ART* antiretroviral therapy

regular exercise, HIV-infected men with CD4 T cell counts ≥ 500 cells/μL had a lower BMD (mean, 95% CI) at FN (0.972, 0.925–1.019) and TH (1.001, 0.955–1.047) than HIV-uninfected men at FN (1.071, 1.164–1.208, $p < 0.001$) and TH (1.149, 1.128–1.171, $p < 0.001$), but not at LS (1.173, 1.127–1.220 vs. 1.186, 1.164–1.208, $p = 0.630$). HIV-infected men with CD4 T cell counts < 500 cells/μL had a lower BMD at LS (1.117, 1.034–1.141), FN (0.958, 0.934–0.983), and TH (0.988, 0.964–1.012) than HIV-uninfected men at LS ($p < 0.001$), FN ($p < 0.001$), and TH ($p < 0.001$). HIV-infected men with CD4 T cell counts < 500 cells/μL had a lower BMD than those with CD4 T cell counts ≥ 500 cells/μL at only LS ($p = 0.034$), but not at FN ($p = 0.609$) and TH ($p = 0.616$). Similarly, after adjusting for confounding factors, HIV-infected men with CD4 T cell counts ≥ 500 cells/μL had a lower Z-score (mean, 95% CI) at LS (− 0.5, − 0.9 to − 0.1), FN (− 0.1, − 0.5–0.2), and TH (− 0.2, − 0.5–0.2) than HIV-uninfected men at LS (1.6, 1.5–1.8, $p < 0.001$), FN (1.6, 1.4–1.8, $p < 0.001$), and TH (1.2, 1.0–1.4, $p < 0.001$). HIV-infected men with CD4 T cell counts < 500 cells/μL also had a lower Z-score at LS (− 0.9, − 1.1 to − 0.7), FN (− 0.4, − 0.6 to − 0.2), and TH (− 0.4, − 0.6 to − 0.2) than HIV-uninfected men at LS ($p < 0.001$), FN ($p < 0.001$), and TH ($p < 0.001$). HIV-infected men with CD4 T cell counts < 500 cells/μL had a lower Z-score than those with CD4 T cell counts ≥ 500 cells/μL at only LS ($p = 0.034$), but not at FN ($p = 0.187$) and TH ($p = 0.283$).

There was a positive correlation between CD4 T cell counts and LS-BMD ($\gamma = 0.168$, $p = 0.031$). However, there was no significant correlation of CD4 T cell counts with FN-BMD ($\gamma = 0.190$, $p = 0.810$) or with TH-BMD ($\gamma = -0.010$, $p = 0.904$) (Fig. 2). Multiple linear regression analysis was carried out to investigate the independent association of CD4 T cell counts with BMD. After adjustment for all potential confounders including age, BMI, alcohol use, current smoking status, regular exercise, and ART use, CD4 T cell counts showed a positive association with BMD at LS (unstandardized regression coefficient, $\beta = 0.020$; standard error, SE = 0.010, standardized regression coefficient, $\beta = 0.150$, $p = 0.047$), but not at FN ($\beta = 0.001$; SE = 0.010, $\beta = -0.002$, $p = 0.975$) and TH ($\beta = -0.004$, SE = 0.010, $\beta = -0.031$, $p = 0.691$).

We compared BMD and Z-score in HIV-infected men according to the use of ART (Fig. 3). There was no significant difference in the BMD at LS ($p = 0.261$), FN ($p = 0.784$), and TH ($p = 0.989$) between the ART-naïve group ($n = 75$) and the ART-user group ($n = 92$) (Fig. 3a). There was also no significant difference in Z-score at LS ($p = 0.250$), FN ($p = 0.614$), and TH ($p = 0.811$) between the two groups (Fig. 3b). After adjustment for confounding factors, there was no significant

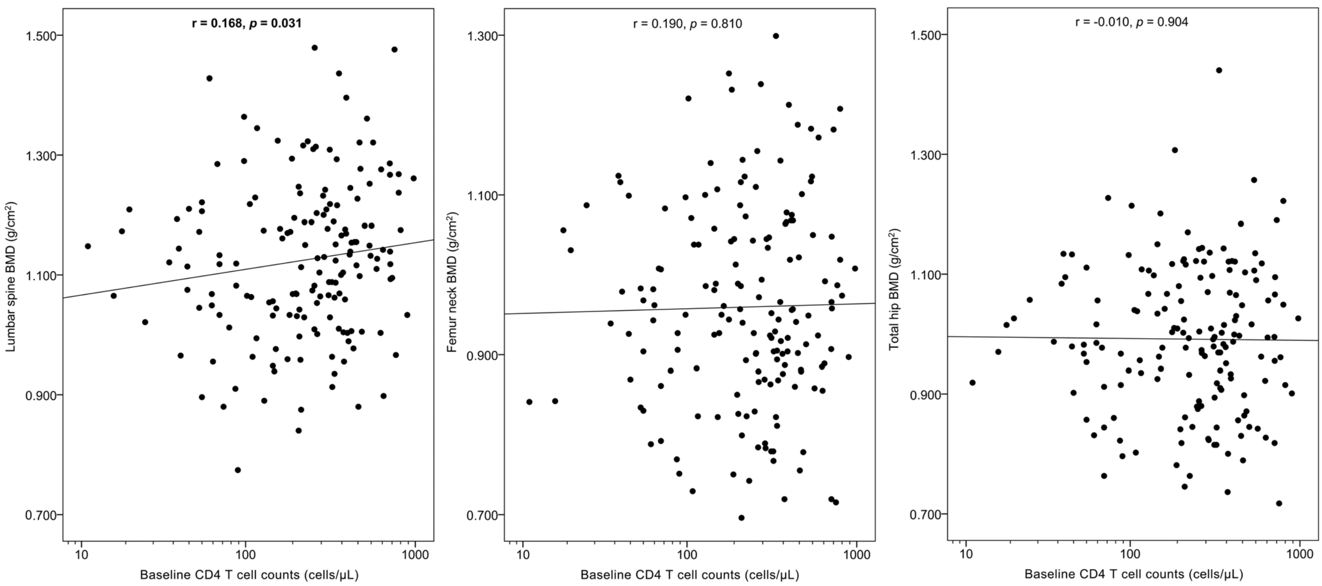

Fig. 2 Correlation of CD4 T cell counts with bone mineral density (BMD) at the lumbar spine (LS), femur neck (FN), and total hip (TH). The distribution of CD4 T cell counts and their associations with LS-BMD, FN-BMD, and TH-BMD were investigated using Pearson correlation and scatter plots

BMD difference between the ART-naïve and the ART-user groups in the BMD (mean, 95% CI) at LS (1.132, 1.103–1.161 vs. 1.119, 1.092–1.145, $p = 0.499$), FN (0.962, 0.933–0.991 vs. 0.958, 0.931–0.984, $p = 0.960$), and TH (0.992, 0.964–1.021 vs. 0.991, 0.965–1.016, $p > 0.999$). There was also no significant difference between HIV-infected men within the ART-naïve and the ART-user groups in the Z-score (mean, 95% CI) at LS (− 0.8, − 1.1 to − 0.6 vs. − 0.9, − 1.2 to − 0.7, $p = 0.522$), FN (− 0.3, − 0.5 to − 0.1 vs. − 0.4, − 0.5 to − 0.1, $p = 0.698$), and TH (− 0.3, − 0.5 to − 0.1 vs. − 0.4, − 0.5 to − 0.1, $p = 0.987$).

We compared the BMD and Z-score in HIV-infected men between the four subgroups: CD4 T cells ≥ 500 cells/μL and ART-naïve subgroup ($n = 18$), CD4 T cells < 500 cells/μL and ART-naïve subgroup ($n = 57$), CD4 T cells ≥ 500 cells/μL and ART-user subgroup ($n = 10$), and CD4 T cells < 500 cells/μL and ART-user subgroup ($n = 82$) according to the use of ART and CD4 T cell counts < or ≥ 500 cells/μL (Fig. 4). Before adjustment, there was no significant difference between the ART-naïve subgroup and the ART-user subgroup within the same CD4 T cell count group in BMD at LS (CD4 T cells ≥ 500 cells/μL and ART-naïve subgroup vs. CD4 T cells ≥ 500 cells/μL and ART-user subgroup, $p = 0.842$; CD4 T cells < 500 cells/μL and ART-naïve subgroup vs. CD4 T cells < 500 cells/μL and ART-user subgroup, $p = 0.355$), FN (within the CD4 T cells ≥ 500 cells/μL groups, $p = 0.122$; within the CD4 T cells < 500 cells/μL groups, $p = 0.584$), and TH (within the CD4 T cell counts ≥ 500 cells/μL groups, $p = 0.158$; within the CD4 T cell counts < 500 cells/μL groups: $p = 0.478$) (Fig. 4a). There was no significant difference within ART-naïve groups according to CD4 T cell counts (CD4 T cell counts ≥ 500 cells/μL and ART-naïve subgroup vs. CD4 T

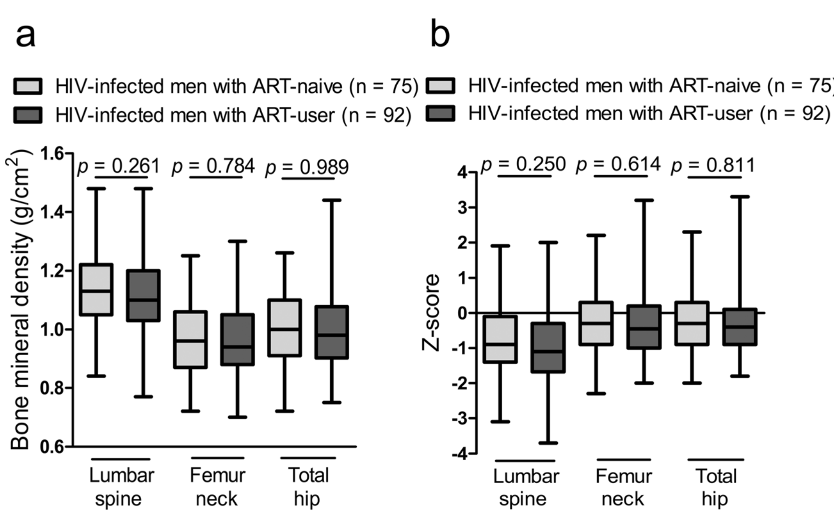

Fig. 3 Box plot with Tukey interquartile range for bone mineral density (BMD) and Z-score in HIV-infected men according to the use of antiretroviral therapy (ART). BMD and Z-score among the HIV-infected men with ART-naïve and those with ART-user were compared using a one-way analysis of variance (ANOVA)

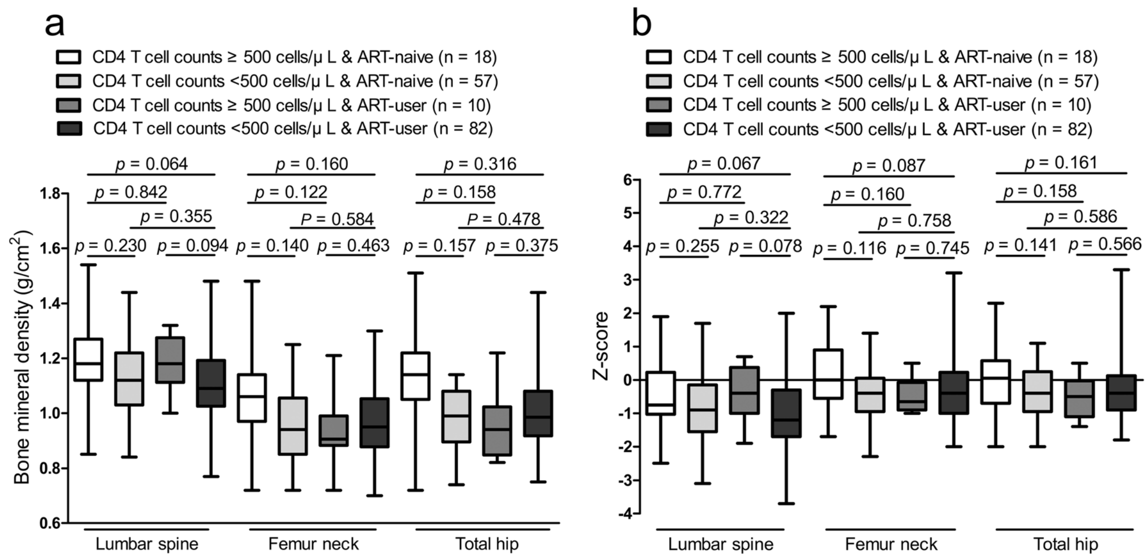

Fig. 4 Box plot with Tukey interquartile range for bone mineral density (BMD) and Z-score in HIV-infected men according to the use of antiretroviral therapy (ART) and CD4 T cell counts < or ≥ 500 cells/μL. HIV-infected patients were divided into four subgroups: CD4 T cells ≥ 500 cells/μL and ART-naïve subgroup, CD4 T cells < 500 cells/μL and ART-naïve subgroup, CD4 T cells ≥ 500 cells/μL and ART-user subgroup, and CD4 T cells < 500 cells/μL and ART-user subgroup. Subgroups were compared using one-way analysis of variance (ANOVA)

cell counts < 500 cells/μL and ART-naïve subgroup) in BMD at LS ($p = 0.230$), FN ($p = 0.140$), and TH ($p = 0.157$). The CD4 T cell counts < 500 cells/μL and ART-user group showed a marginally lower BMD at LS ($p = 0.094$), but not at FN ($p = 0.463$) and TH ($p = 0.375$) than the CD4 T cell counts ≥ 500 cells/μL and ART-user group (Fig. 4a). This pattern was the same with the Z-score (Fig. 4b).

After adjustment for the confounding factors, age BMI, alcohol use, current smoking status, and regular exercise, there was no significant difference between the ART-naïve subgroup and the ART-user subgroup within the same CD4 T cell count group in BMD (mean, 95% CI) at LS (1.161, 1.102–1.220 for CD4 T cell counts ≥ 500 cells/μL and ART-naïve subgroup vs. 1.195, 1.116–1.274 for CD4 T cell counts ≥ 500 cells/μL and ART-user subgroup, $p = 0.489$; 1.123, 1.090–1.156 for CD4 T cell counts < 500 cells/μL and ART-naïve subgroup vs. 1.109, 1.082–1.137 for CD4 T cell counts < 500 cells/μL and ART-user subgroup, $p = 0.532$). There was no significant difference between CD4 T cell counts ≥ 500 cells/μL and ART-naïve subgroup and CD4 T cell counts ≥ 500 cells/μL and ART-user subgroup in BMD (mean, 95% CI) at FN (1.002, 0.942–1.061 vs. 0.921, 0.841–1.001, $p = 0.112$) and TH (1.028, 0.969–1.086 vs. 0.951, 0.873–1.030, $p = 0.125$). There was no significant difference between CD4 T cell counts < 500 cells/μL and ART-naïve subgroup and CD4 T cell counts < 500 cells/μL and ART-user subgroup in BMD at FN (0.950, 0.916–0.983 vs. 0.962, 0.934–0.990, $p = 0.574$) and TH (0.982, 0.949–1.014 vs. 0.995, 0.968–1.022, $p = 0.529$). In addition, there was no significant difference within ART-naïve groups according to CD4 T cell counts (CD4 T cell counts ≥ 500 cells/μL and ART-naïve subgroup vs. CD4 T cell counts < 500 cells/μL and ART-naïve subgroup) in BMD (mean, 85% CI) at LS (1.161, 1.102–1.220 vs. 1.123, 1.090–1.156, $p = 0.267$), FN (1.002, 0.942–1.061 vs. 0.950, 0.916–0.983, $p = 0.136$), and TH (1.028, 0.959–1.086 vs. 0.982, 0.949–1.014, $p = 0.175$). In contrast, CD4 T cell counts < 500 cells/μL and ART-user group had significantly lower BMD at LS (1.109, 1.082–1.137) than CD4 T cell counts ≥ 500 cells/μL and ART-user group (1.195, 1.116–1.274, $p = 0.043$). There was no significant difference between the CD4 T cell counts < 500 cells/μL and ART-user subgroup and CD4 T cell counts ≥ 500 cells/μL and ART-user subgroup in BMD at FN (0.962, 0.934–0.990 vs. 0.921, 0.841–1.001, $p = 0.338$) and TH (0.995, 0.968–1.022 vs. 0.951, 0.873–1.030, $p = 0.298$).

Discussion

This study was conducted to estimate the effect of HIV infection-induced lower CD4 T cell counts and the use of ART in men under the age of 50 years on bone mass. HIV-infected men in this age group had a lower BMD compared to that of HIV-uninfected men independent of CD4 T cell counts. HIV-infected men with CD4 T cell counts < 500 cells/μL had a lower BMD and Z-score at LS than those with CD4 T cell counts ≥ 500 cells/μL before and after adjusting for confounders. The CD4 T cell count exhibited a positive association with LS BMD. Furthermore, there was no significant difference between the ART-naïve group and the ART-user group in BMD and Z-score at all sites before and after adjusting for confounders. Finally, there was no significant difference between the ART-naïve and the ART-user within the same CD4 T cell count group in BMD at all sites.

Collectively, CD4 T cell counts, independent of whether ART was used, were inversely associated with LS BMD, even in the men under the age of 50 years.

In the present study, HIV-infected men had lower BMD compared to that of HIV-uninfected men, even in those under the age of 50 years. To the best of our knowledge, this study is the first that reports the effect of HIV infection-induced low CD4 T cell counts on BMD in men in this age group. Therefore, a lower CD4 T cell count may facilitate the deleterious effect of HIV infection on bone metabolism. Several studies reported that the decline of CD4 T cell counts were associated with reduced bone mass and increased risk for fracture [22, 23, 28, 29]. However, none of the above studies included only patients under the age of 50 years, and recent guidelines for bone health in HIV-infected patients indicate that there is no need to examine BMD in HIV-infected patients under the age of 50 years [25]. Furthermore, most HIV-infected patients in Korea are men under the age of 50 years [24]. Accordingly, we suggest that HIV-infected patients with progression may need an examination regarding bone health, even in patients under the age of 50 years.

Differences between HIV-infected men with CD4 T cell counts < 500 cells/μL and those with CD4 T cell counts ≥ 500 cells/μL were observed only in BMD at LS. The positive associations of CD4 T cell counts with BMD were observed only in LS, but not in the proximal femur. The result of the power analysis showed that LS-BMD (99.9%), FN-BMD (98.9%), and TH-BMD (94.3%) had sufficient power. Therefore, the different BMD outcomes for LS and proximal hip in the HIV-infected men might not be due to sample size effects. Although we cannot explain the exact reason for this finding in our study, the LS, which is composed predominantly of trabecular bone, is metabolically more active than the proximal femur [30]. Other studies have consistently shown an increased risk of vertebral fracture [31–34] or lower trabecular bone score [35] in HIV-infected patients.

HIV infection and the use of ART have been associated with a higher incidence of bone loss [36]. According to a recently published meta-analysis report, the risk of bone loss due to HIV infection is approximately odds ratio (OR) = 2.4 to 2.6, and bone loss between ART-users and ART-naïve patients has been reported as OR = 2.8 to 3.4 [37]. We tried to investigate the effect of HIV and ART on bone separately. First, there was no significant difference between the ART-naïve and the ART-user groups in BMD at all the sites before and after adjustment for confounders. Second, there was no significant difference between the ART-naïve and the ART-user groups within the same CD4 T cell counts group in BMD at all sites. Despite the lack of a significant difference between the ART-naïve and the ART-user groups within the CD4 T cell counts ≥ 500 cells/μL group in BMD at all the sites, CD4 T cells < 500 cells/μL and ART-user group had a significantly lower BMD at LS than CD4 T cells ≥ 500 cells/μL and ART-user group. Although some differences should be considered for interpretation owing to the lack of comparative data regarding CD4 T cell nadir before ART with CD4 T cell counts after ART in the present study, these results were similar with those of previous studies showing that lower CD4 T cells nadir [28] and magnitude of CD4 T cells expansion following ART administration may significantly contribute to the bone loss through immune regeneration [8, 18]. Third, there was a significant correlation between CD4 T cell counts with LS-BMD. Collectively, these findings suggested that the effects of CD4 T cell counts reflecting HIV-related effects on bone might be stronger than those of ART in relatively young HIV-infected men aged under 50 years.

The major strength of the present study was that we enrolled a relatively large number of participants. In addition, our study population was homogeneous, comprising of men under the age of 50 years. Furthermore, by using subgroup analysis, we examined the relationship between HIV infection and BMD and found that it is associated with CD4 T cell counts < 500 cells/μL. Despite these strengths, there are several potential limitations to consider when analyzing our results. First, because this was a matched case–control study, we could not determine whether there was a causal relationship at low CD4 T cell counts and lower bone mass in HIV-infected men. Second, we did not measure testosterone level. However, we reviewed the medical records and identified sexual dysfunction due to hypogonadism. Third, we did not measure levels of parathyroid hormone, inorganic phosphorus, and bone turnover markers. ART might affect bone metabolism. In particular, ART such as tenofovir bind to the membrane of proximal renal tubular cells and inhibit phosphorus reabsorption, inducing hyperphosphaturia. This results in a decrease in blood phosphorus and secondary bone loss [20, 38]. BMD was measured during the first 2 years of ART initiation in about 50% of the patients, so further studies with associated laboratory testing are required to investigate this and a long-term follow-up period will be needed. Fourth, in the subgroup analysis for BMD and Z-score in HIV-infected men according to tART use and CD4 T cell counts < or ≥ 500 cells/μL, low power and high SDs, a result of the small number of patients in some groups, may have contributed to the lack of statistical significance for some comparisons. Therefore, studies using large number of patients will be needed. Finally, we did not measure the level of 25-hydroxyvitamin D (25(OH)D) in all HIV-infected men; vitamin D has a significant effect on bone [39], with observational studies noting a high prevalence of low 25(OH)D levels in HIV-infected populations [40]. We measured 25(OH)D levels in only one-third of the HIV-infected patients (data were not shown), and there was no significant difference in the two groups divided by CD4 T cell counts 500 cells/μL ($p = 0.862$).

In conclusion, HIV-infected men under the age of 50 years had a lower bone mass compared to that of HIV-uninfected men; moreover, lower CD4 T cell counts were associated with lower BMD irrespective of the use of the ART. These results suggest that HIV-infected patients with progression might need to undergo an examination and receive intervention for bone health, even in patients under the age of 50 years.

Acknowledgements We express our sincere gratitude to Suyeon Park, M.S. (Department of Biostatistics, Soonchunhyang University Seoul Hospital, Seoul, Republic of Korea) for her valuable assistance with the statistical analysis.

Funding information This study was supported by grants from the Asan Institute for Life Sciences, Seoul, Republic of Korea (Project Nos. 2016-568 and 2017-568).

Compliance with ethical standards

This study was approved by the Institutional Review Board (IRB) of Soonchunhyang University, Seoul Hospital (Seoul, Republic of Korea) (IRB no. 2017-03-014). The requirement for written informed consent from patients was waived due to the retrospective nature of the study and its impracticability.

Conflicts of interest None.

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