

High prevalence of reduced bone mineral density in primary HIV-1-infected men

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Objective: To assess the bone mineral density (BMD) in a cohort of men with primary HIV-1 infection (PHI).

Methods: Thirty-three men with PHI had a dual-energy X-ray absorptiometry (DXA) of the lumbar spine, femoral neck and total hip. Osteopenia and osteoporosis were defined according to WHO criteria as T-scores between -1 and -2.5 and -2.5 or less, respectively. The association between clinical and laboratory parameters and BMD was investigated using multivariable linear regression analysis.

Results: Mean age was 38 (SD 9) years and mean body mass index (BMI) 22.7 (SD 3.3) kg/m². Twenty-four men (73%) had a negative or indeterminate Western blot, 32 men (97%) were combination antiretroviral therapy-naïve. Mean plasma HIV-1 RNA was 5.0 (SD 1.2) log₁₀ copies/ml. Mean lumbar spine T (-0.8 , SD 1.3, $P=0.001$) and Z-scores (-0.7 , SD 1.3, $P=0.004$) and femoral neck T-score (-0.5 , SD 0.9, $P=0.003$) were significantly lower compared to the reference population. 15/33 men (45%) had osteopenia and 2/33 (6%) osteoporosis. Markers of bone turnover did not differ between patients with or without osteopenia/osteoporosis. Age was negatively associated with femoral neck (β -coefficient = -0.05 , $P < 0.001$) and total hip T-scores ($\beta = -0.03$, $P = 0.04$). BMI was associated with lumbar spine ($\beta = 0.3$), femoral neck ($\beta = 0.2$) and total hip ($\beta = 0.2$) T-scores ($P < 0.001$) and thyroid-stimulating hormone (TSH) with lumbar spine ($\beta = 0.5$, $P = 0.045$) and femoral neck T-scores ($\beta = 0.4$, $P = 0.005$). Increased plasma viral load was associated with lower total hip T-scores ($\beta = -0.2$, $P = 0.02$).

Conclusions: Reduced BMD was prevalent in PHI men and was associated with increased age, lower BMI and TSH levels, and higher levels of HIV-1 viremia.

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Background

Multiple cross-sectional studies have shown an increased prevalence of reduced bone mineral density (BMD) among HIV-infected individuals [1,2]. A systematic review revealed a 6.4-fold increased odds of reduced BMD in HIV-infected patients and a 3.7-fold increased odds of osteoporosis compared to HIV-uninfected controls [2]. A recent population-based study confirmed a higher prevalence of bone fractures in HIV-infected men and women compared to HIV-uninfected controls [3].

Although the cause and pathogenesis underlying this bone loss are not completely understood, the reduced BMD in HIV-infected individuals is most likely of multifactorial origin [4]. Apart from conventional risk factors, which might be more prevalent among HIV-infected persons, HIV infection itself and combination antiretroviral therapy (cART) might each contribute [5–8]. In order to gain further insight into the contribution of HIV infection *per se*, we assessed BMD in a cohort of men with primary HIV-1 infection (PHI).

Methods

Study population

We evaluated the BMD of 33 men with PHI who presented at the Academic Medical Center (AMC) in Amsterdam between 1 February 2008 and 31 October 2009. All participants were enrolled in the Primo-SHM cohort, a multicentre prospective cohort study in the Netherlands, with an embedded randomized trial, that investigates the natural course of HIV-1 infection, and the effects of early cART in patients with PHI [9]. Main inclusion criteria are age at least 18 years and laboratory evidence of PHI, defined as having a negative or indeterminate Western blot in combination with detectable plasma HIV-1 RNA, or, in case of a positive Western blot, a proven negative HIV-screening test result within the previous 180 days. Exclusion criteria for the present study were medical conditions known to affect bone metabolism (e.g. hypercalcemic), and corticosteroid therapy for at least 3 months. The study was approved by the Medical Ethics Committee of the AMC. All participants provided written informed consent.

Screening procedures at enrolment

A questionnaire was administered evaluating sociodemographic characteristics, risk factors for reduced BMD and occurrence of symptoms compatible with an acute retroviral syndrome (ARS). Blood was collected for HIV-related parameters, hepatitis B/C and syphilis serology and biochemical markers of bone metabolism, including bone formation markers (total alkaline phosphatase, osteocalcin, and procollagen type 1 N-terminal propep-

tide) and bone resorption markers (C-terminal telopeptide of type 1 collagen and C-telopeptide crosslink of type 1 collagen).

Bone mineral density of the lumbar spine, femoral neck and total hip were measured by dual-energy X-ray absorptiometry (DXA) using a Hologic QDR 4500W densitometer, software version 12.4. BMD was expressed in T and Z-scores. T-scores refer to the difference in standard deviations (SDs) between a patient's BMD and that of young healthy adults matched for sex and ethnic group. Z-scores represent the difference in SD compared with an age-matched reference population. Osteopenia and osteoporosis were defined according to the WHO criteria as T-scores measured at lumbar spine, femoral neck and/or total hip between -1 and -2.5 SD and -2.5 SD or less, respectively. The overall fracture risk was assessed by FRAX, a tool that calculates the 10-year probability of a major osteoporotic (spine, humerus, wrist) or hip fracture, based on validated clinical risk factors and the femoral neck T-score [10]. FRAX algorithms have not specifically been validated for relatively young HIV-infected persons. Since FRAX was not available for the Netherlands, we used the German algorithm (www.shef.ac.uk/FRAX).

Data analysis

Mean T and Z-scores of lumbar spine, femoral neck and total hip were calculated and compared to the reference population by one sample T-tests. Data of osteopenic and osteoporotic patients were combined in further analyses. Sociodemographic characteristics and laboratory values were compared between patients with normal and reduced BMD using independent sample T-tests and Wilcoxon rank-sum tests for continuous data and chi-squared tests for categorical data. The association of the various parameters with lumbar spine, femoral neck and total hip T-scores was examined using multivariable linear regression analysis. All variables were evaluated separately and those associated ($P < 0.1$) with reduced BMD were stepwise entered into a linear regression model for all three measured bone sites. All models were adjusted for patient age and BMI. Median FRAX scores were calculated for the total group of PHI men and for patients with a normal or reduced BMD. Data were analysed using SPSS statistical software version 16.0 (SPSS Inc., Chicago, Illinois, USA). *P* values less than 0.05 were considered statistically significant.

Results

Patient characteristics

Most patients (91%) were men who have sex with men (MSM). Seventy-three percent were diagnosed with PHI based on a negative or indeterminate Western blot combined with detectable plasma HIV-1 RNA. One

Table 1. Patient characteristics of 33 PHI men.

	Total (N = 33)	Normal BMD (n = 16)	Reduced BMD ^a (n = 17)	P value ^b
Demographic data and risk factors for BMD				
Age (years), mean (SD)	38 (9)	37 (9)	39 (10)	0.7
Weight (kg), mean (SD)	76.0 (12.1)	78.3 (13.4)	73.8 (10.8)	0.3
BMI (kg/m ²), mean (SD)	22.7 (3.3)	24.7 (3.4)	22.0 (2.7)	0.02
Caucasian race, n (%)	26 (79)	13 (81)	13 (77)	0.7
Current smoking, n (%)	18 (55)	9 (56)	9 (53)	0.8
Alcohol (≥ 3 units/day), n (%)	7 (21)	2 (13)	5 (29)	0.2
Current drug use, n (%)	22 (67)	12 (75)	10 (59)	0.3
Dairy food intake (≥ 3 products /week), n (%)	30 (91)	15 (94)	15 (88)	0.6
Multivitamin use, n (%)	17 (52)	8 (50)	9 (53)	0.9
History of bone fracture, n (%)	12 (36)	6 (38)	6 (35)	0.9
Strenuous physical activity (20 min ≥ 3 x/week), n (%)	19 (58)	9 (56)	10 (59)	0.9
Stage of PHI ^c , n (%)				
I	24 (73)	11 (69)	13 (76)	0.6
II	9 (27)	5 (31)	4 (24)	
Symptoms during PHI				
Acute retroviral syndrome, n (%)	27 (82)	13 (81)	14 (82)	0.9
Weight loss ≥ 5 kg, n (%)	8 (24)	4 (25)	4 (24)	0.9
CRP (mg/l) ^d , median (IQR)	1 (1–1.5)	1 (1–2.2)	1 (1–1.2)	0.1
CD4 cell count (cells/ μ l), mean (SD)	551 (251)	643 (280)	465 (190)	0.08
Plasma HIV-1 RNA (log ₁₀ copies/ml), mean (SD)	5.0 (1.2)	5.1 (1.2)	4.9 (1.3)	0.7
Biochemical markers relevant for bone metabolism				
	Reference ranges			
Calcium ^e , mean (SD)	2.2–2.6 mmol/l	2.19 (0.07)	2.18 (0.08)	0.8
Phosphate, mean (SD)	0.7–1.45 mmol/l	0.94 (0.19)	0.91 (0.16)	0.7
ALP, mean (SD)	40–120 U/l	72 (26)	70 (17)	0.8
25-hydroxyvitamin D, mean (SD)	19–126 nmol/l	66 (35)	81 (36)	0.2
1,25-dihydroxyvitamin D, median (IQR)	40–140 pmol/l	99 (83–123)	97 (87–131)	0.4
PTH, mean (SD)	0.6–6.7 pmol/l	3.8 (1.5)	3.6 (1.8)	0.8
TSH ^f median (IQR)	0.5–5 mE/l	1.5 (1.2–1.9)	1.4 (0.9–1.8)	0.9
Testosterone, mean (SD)	11–35 nmol/l	21 (6)	23 (6)	0.5
SHBG, mean (SD)	12–75 nmol/l	34 (15)	42 (18)	0.2
FAI, median (IQR)	20–90	63 (48–93)	63 (41–73)	0.9
Osteocalcin, median (IQR)	2.4–23.5 μ g/l	4.8 (1.7–9.2)	7.4 (0.6–10.3)	0.4
P1NP, mean (SD)	22–87 μ g/l	40 (18)	43 (12)	0.7
1CTP, mean (SD)	2.1–5.0 μ g/l	3.6 (1.4)	3.1 (1.1)	0.3
CTX, median (IQR)	<584 ng/l	203 (129–317)	332 (118–378)	0.3

ALP, alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; CRP, C-reactive protein; 1CTP, C-terminal telopeptide of type 1 collagen; CTX, C-telopeptide cross-link of type 1 collagen; FAI, free androgen index [FAI = (testosterone/SHBG) \times 100]; P1NP, procollagen type 1 N-terminal propeptide; PHI, primary HIV-1 infection; PTH, parathyroid hormone; SHBG, sex hormone-binding globulin; TSH, thyroid-stimulating hormone.

^aReduced BMD defined as a T-score ≤ -1 in either the lumbar spine and/or the hip.

^bP-value based on independent T-test, Wilcoxon rank sum test or χ^2 test for proportions.

^cI: HIV-1 RNA positive and negative or low HIV-antibodies or indeterminate western blot; II: HIV-1 RNA, HIV-1 specific antibodies and western blot positive and a documented HIV-1 negative test in preceding 180 days.

^dOne patient with missing result.

^eSerum calcium was corrected for serum albumin (g/l) using the equation: corrected calcium = calcium + ((40 – albumin) \times 0.025).

^fTwo patients with missing results.

patient had been on cART since 9 days, the remaining 32 patients were cART-naïve. Five patients (15%) were hospitalized for several days during the ARS, none had been exposed to i.v. drug use or was coinfecting with syphilis or hepatitis B/C. Patient characteristics and biochemical markers relevant for bone metabolism are summarized in Table 1. None of the patients had reduced levels of testosterone or vitamin D metabolites. Ten patients, equally distributed among patients with normal and reduced BMD, had low levels of osteocalcin, possibly indicating a decrease in bone formation. Levels of bone formation and resorption markers were not significantly different between patients with or without reduced BMD.

Prevalence of reduced bone density

The median number of days between the first HIV-1-positive test and the DXA scan was 32 [interquartile range (IQR) 20–45] days. Mean lumbar spine T (–0.8, SD 1.3, $P=0.001$) and Z-scores (–0.7, SD 1.3, $P=0.004$) and mean femoral neck T-score (–0.5, SD 0.9, $P=0.003$) were significantly lower compared to the reference population. The mean total hip T-score (–0.3, SD 0.8), femoral neck (–0.1, SD 0.8) and total hip (–0.1, SD 0.8) Z-scores were also lower than the reference values, but the differences did not reach statistical significance. Of the 33 men, 17 had reduced BMD of whom 15 (45%) had osteopenia and 2 (6%) had osteoporosis. For those

patients with low BMD, bone loss predominated at the lumbar spine (mean T-score -1.8 , SD 0.8).

Factors associated with reduced bone mineral density

Age was negatively associated with femoral neck [β -coefficient = -0.05 , (95% CI -0.07 to -0.02), $P < 0.001$] and total hip T-scores [$\beta = -0.03$ (-0.05 to -0.001), $P = 0.04$]. BMI was positively associated with lumbar spine [$\beta = 0.3$ (0.2 – 0.4), $P < 0.001$], femoral neck [$\beta = 0.2$ (0.1 – 0.2), $P < 0.001$] and total hip [$\beta = 0.2$ (0.1 – 0.2), $P < 0.001$] T-scores, and thyroid-stimulating hormone (TSH) levels with lumbar spine [$\beta = 0.5$ (0.01 – 0.9), $P = 0.045$] and femoral neck T-scores [$\beta = 0.4$ (0.1 – 0.6), $P = 0.005$]. Increased levels of plasma HIV-1 RNA were associated with a lower total hip T-score [$\beta = -0.2$ (-0.4 to -0.04), $P = 0.02$]. In our study population of PHI men, differences in BMI accounted for a substantial part of the observed differences in BMD: 43, 45 and 56% for the lumbar spine, femoral neck and total hip T-scores, respectively, as patients with reduced BMD were on average 2.7 kg/m^2 lighter than patients with normal BMD. The three remaining covariates, although significantly associated with BMD, did not explain much of the observed differences in BMD.

Fracture risk

Ten-year probabilities of major osteoporotic fractures and hip fractures as predicted by the German FRAX algorithm are shown in Table 2. Three men (9%) had a 10-year risk of a major osteoporotic fracture above 7.5%, the threshold at which in the UK antiresorptive treatment is considered to be cost effective at the age of 50 years [11,12].

Discussion

Reduced BMD is increasingly being recognized among HIV-infected populations. It remains unknown whether this is caused by HIV infection itself, cART, traditional risk factors or a combination of all three [13]. The present study provides the first data on the frequency of osteopenia and osteoporosis in a cohort of untreated PHI men. Half of them had reduced BMD, of whom 45% had osteopenia and 6% osteoporosis. These numbers are much higher than would be expected in a relatively young male population like ours, but are in agreement

with frequency rates of 40–83% reported in patients with chronic HIV-1 infection [2].

In our cohort, reduced BMD was especially seen in the lumbar spine, which mostly consists of trabecular bone. Osteoporosis in men usually involves the cortical bone (e.g. femoral neck), which is less sensitive to acute changes in health and medication [14]. Studies describing the site of bone loss in HIV-infected patients are limited and have been inconclusive [15,16]. No differences were seen in biochemical markers of bone metabolism between patients with or without osteopenia/osteoporosis. None of our patients had low vitamin D metabolites, a finding that is frequently reported among chronic HIV-1-infected patients [17].

Not surprisingly, reduced BMD was independently associated with age and BMI at the measured bone sites, confirming findings obtained from the general population and studies evaluating bone disorders in HIV-1-infected populations [18,19]. MSM have a lower body weight than heterosexual men [20] and might therefore be more at risk to develop a reduced BMD. Among our study population, the linear regression models showed that differences in BMI between patients with or without osteopenia/osteoporosis accounted for only 50% of the reduced BMD. TSH was positively associated with lumbar spine and femoral neck T-scores, as confirmed by the literature [21]. The total hip T-score was associated with the degree of HIV-1 replication. Previous studies have shown an association between bone loss and high plasma viral load levels [22]. As PHI is associated with high levels of HIV-1 viremia [23], this may suggest a direct role of HIV infection on osteoblast and osteoclast activity [24]. Reduced serum osteocalcin levels have been described in patients with acute viral hepatitis [25], suggesting that acute viral infections as such may affect bone turnover.

The study has several limitations. It is a cross-sectional analysis, which can not establish causal relationships between HIV infection and reduced BMD. Second, the majority of our patients have been identified as having PHI based on symptoms. Since ARS is known to be an independent prognostic factor of AIDS progression [26], the results may not be generalizable to those who are asymptomatic during PHI. Finally, it is remarkable that low BMD develops so quickly during PHI. Rapid bone loss can be seen during immobilization [27,28]. On the

Table 2. Ten-year fracture risk assessment using the FRAX algorithm for Germany computed with the femoral neck T-score.

	Total ($N = 33$)	Normal BMD ($n = 16$)	Reduced BMD ^a ($n = 17$)
Major osteoporotic fracture, median (IQR)	2.9 (2.7–5.7)	2.8 (2.4–4.6)	4.0 (2.9–6.8)
Hip fracture, median (IQR)	0.3 (0.1–0.9)	0.2 (0.1–0.3)	0.6 (0.3–2.0)

^aReduced BMD defined as a T-score ≤ -1 in either the lumbar spine and/or the hip.

contrary, a study from San Francisco among 209 healthy HIV-seronegative MSM reported a high proportion of bone thinning, which was associated with popper and amphetamine use [29]. This raises the question whether it is actually the recent HIV-1 infection causing rapid bone loss shortly after transmission, or whether these bone disorders predate HIV infection and are caused by other risk factors. Of note, the reduced BMD present in our PHI men was not associated with biochemical evidence of increased bone turnover or systemic inflammation as measured by CRP, which might have been expected in the context of the generalized inflammation associated with PHI. In patients with inflammatory bowel disease for example, bone turnover was found to be elevated in relation to the underlying disease activity and inversely related with BMD, suggesting that bone metabolism was affected by the underlying disease [30,31].

In conclusion, this study shows a high rate of osteopenia and osteoporosis early in the course of HIV infection, before the possible influence of cART. Reduced BMD was associated with older age, lower BMI and TSH levels, and a higher degree of HIV-1 replication. Longitudinal studies are needed to evaluate changes over time. Studies involving HIV-seronegative controls will be a key in understanding whether these findings relate to the presence of HIV or other risk factors affecting bone health among MSM.

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Authors' contributions: M.L.G. and J.M.P. drafted the manuscript. M.L.G., F.W.N.M.W and J.M.P. conducted the statistical analysis. M.L.G., R.S. and J.M.P. established the cohort. S.M.E.V., R.S., P.L. and P.R. provided valuable input into protocol development, interpretation of data and critically revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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