Low bone mineral density and associated risk factors in HIV-infected patients

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Abstract

Background Aging of persons with human immunodeficiency virus (HIV) resulted in high rates of osteopenia and osteoporosis. Multiple cohort studies have reported an increased prevalence of bone demineralization among HIV-infected individuals. The aim of this study was to evaluate bone mineral density (BMD) and risk factors for osteopenia/osteoporosis among HIV-positive patients attending the National Institute for Infectious Diseases "Prof. Dr. Matei Balș", Bucharest, Romania.

Methods We performed a cross-sectional study that enrolled 60 patients with HIV. The association between BMD and lifestyle habits (smoking), body mass index (BMI), nadir cluster of differentiation 4 (CD4) cell count, current CD4 cell count, HIV viral load and history of combination antiretroviral therapy (cART) were investigated. The BMD was measured at the lumbar spine, hips and total body using dual-energy X-ray absorptiometry (DEXA).

Results In the present study, DEXA evaluation showed an overall prevalence of osteoporosis of 16.66% (ten patients) and a prevalence of osteopenia of 48.33% (29 patients). In men, low BMI and cigarette smoking showed significant association with the diagnosis of lumbar spine demineralization ($p=0.034$ and $p=0.041$, respectively). Duration of exposure to cART classes in relation to BMD was also evaluated. The use of non-nucleoside reverse-transcriptase inhibitors (NNRTIs) was associated with low lumbar spine BMD in all patients ($p=0.015$). Reduced BMD was significantly associated with protease inhibitors (PIs)-containing treatment ($p=0.043$) in women.

Conclusion At lumbar spine DEXA, male gender was statistically associated with reduced BMD. At the left hip Ward's area, decreased BMD T scores were significantly associated with aging. The reduced BMD was higher in patients receiving PI- or NNRTI-containing regimens.

Keywords HIV, bone mineral density, osteopenia, osteoporosis, antiretroviral therapy

Background

Increased accessibility and widespread combination antiretroviral therapy (cART) resulted in significant improvement in life expectancy for most HIV-infected patients. But long term use of combination antiretroviral

therapy has also been associated with several metabolic complications including lipodystrophy (fat redistribution), increased insulin resistance, diabetes and dyslipidemia.¹ Multiple studies have proven that reduced bone mineral density is a common metabolic complication in HIV-infected

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persons.

Osteoporosis is defined as a systemic skeletal disorder in which low bone mass and deterioration of bone architecture occur. The reduced bone strength predisposes patients to an increased risk of fragility fractures and is likely to become an important cause of morbidity and mortality as the HIV population ages.²

Recent studies have shown a significantly higher prevalence of low bone mineral density (BMD) in HIV-infected patients. HIV-associated osteopenia/osteoporosis found in patients undergoing antiretroviral therapy is significantly higher compared to non HIV-infected individuals and increased bone resorption is associated with low bone mass.³ A meta-analysis performed by Brown and Qaqish in order to examine bone loss, demonstrated that the odds for osteoporosis were 3.7 times greater in HIV-infected individuals and a prevalence of 15% was found.⁴

Underlying mechanisms leading to these complications are complex and still incompletely elucidated. In addition to traditional determinants of bone demineralization, which seem to be more frequent in this category of patients (low body mass, inadequate physical activity, smoking, alcohol use, opiate use, steroid exposure, hypogonadism, insufficient intake of vitamin D and calcium), persistent activation of proinflammatory cytokines and impairment of vitamin D have often been blamed. Furthermore, antiretroviral regimens have been associated with reduced bone mineral density, but given the complexity and diversity of the drug regimens used in practice, it is very challenging to establish the exact contribution of each antiretroviral drug. Brown et al. showed that after antiretroviral treatment initiation, BMD loss follows, a decrease of 2-6% over the first 48 weeks of therapy, irrespective of treatment regimen.⁵

The aim of this study was to evaluate BMD using dual-energy X-ray absorptiometry (DEXA), and the risk factors for osteopenia and osteoporosis among HIV-positive patients attending the National Institute for Infectious Diseases "Prof.Dr. Matei Balș", Bucharest, Romania.

Methods

We performed a cross-sectional study that enrolled 60 patients with HIV infection recruited from the National Institute for Infectious Diseases "Prof.Dr. Matei Balș", in Bucharest Romania, from June 2014 to April 2015. The study was conducted in compliance with ethical and moral principles stated in the "Declaration on Human Rights" in Helsinki, 1996. The protocol was reviewed and approved by the institute's independent Bioethics Committee and all patients signed an informed consent prior to the enrolment.

All patients in the cohort had received treatment for at least six months at the time of enrollment. A specific questionnaire was filled out to record demographic (date of birth, sex, ethnicity), and lifestyle parameters (smoking, alcohol consumption and physical activity). Additionally, nadir CD4 cell count (/cmm), current CD4 cell count (/cmm), HIV viral load (copies/mL) and history of cART were recorded, including type and duration (years) of specific antiretroviral classes used: nucleoside reverse-transcriptase inhibitors (NRTIs), non-nucleoside reverse-transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and integrase strand transfer inhibitor.

BMI was calculated as weight divided by squared height (kg/sqm). Patients were categorized according to the WHO classification: underweight (BMI below 18.5 kg/sqm), normal weight (BMI of 18.5-24.9 kg/sqm), overweight (BMI of 25-29.9 kg/sqm) and obese (BMI $\geq$ 30 kg/sqm).⁶

We excluded from the study patients with known factors for bone mineral density loss, such as menopause, endocrinopathies (primary hyperparathyroidism, hyperthyroidism, hypothyroidism), congenital bone disease, autoimmune diseases, malnutrition, vitamin D deficiency, chronic kidney disease (GFR $<$ 30 mL/minute/1.73 sqm), history of malignancy, hematologic diseases that alter bone metabolism, cortisone therapy for more than three months, patients receiving treatment against osteoporosis, or prolonged immobilization.

Bone mineral density assessment

BMD was measured by DEXA on specific anatomic sites: the lumbar spine (L1-L4), allowing the evaluation of trabecular bone tissue, and the hips (femoral neck, trochanter and Ward's area) allowing the assessment of the cortical bone tissue. Also DEXA measurement on the total body was made to explore bone mass. GE Lunar DPX-NT (General Electric Company, New York, Connecticut, USA) was used to determine BMD for all the patients included in the study cohort and the machine was calibrated daily. Bone mineral density was reported as T and Z scores. The World Health Organization (WHO) classification for bone demineralization was used for diagnosis purposes.^{7,9}

For postmenopausal women and men aged 50 years or more, WHO recommends the use of T scores for reporting BMD. In premenopausal women, men less than 50 years of age and children, Z-scores should be used. A Z-score of -2.0 or lower is defined as 'low bone mineral density for chronological age' or 'below the expected range for age' and the significance of Z-score above -2.0 is 'within the expected range for age'.^{9,10}

We chose to use for statistical analysis the T and Z scores recorded at the left hip DEXA assessment, on the grounds that these results were lower compared to those shown at the right hip DEXA.

Statistics

All statistical analyses were done using SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, NY, USA).

Descriptive statistics was performed, using mean ($\pm$ standard deviation – SD) to describe samples with parametric distribution or medians (interquartile range – IQR) for non-normally distributed data and when indicated as an absolute number and percentages for the qualitative (categorical) variables.

For assessing the influence of the factors in our study upon the dependent variables (T and

Z scores), different statistical tests were used, as appropriate: the statistical significant difference between score means was studied using Student's t test for independent samples (for two groups) or analysis of variance – ANOVA (for more than two groups), Mann-Whitney, respectively, for non-normally distributed continuous data. The statistically significant correlation between two quantitative variables was analyzed using Pearson's r correlation coefficient. A p-value less than 0.05 was considered significant.

Results

A total of 60 patients (mean age 34.6 years, range 22-49) were included in the study, 35 men (58.3%, mean age 37.1, range 24-49) and 25 women (41.7%, mean age 31.24, range 22-45).

Descriptive data on the study group are presented in Table 1.

The study patients were divided into age groups: the 20-29 years group (with 20 patients, 33.3%), the 30-39 years group (with 18 patients, 30%) and the 40-49 years group (with 22 subjects, 36.7%).

The mean BMI of the study patients was 23.0 kg/sqm. Overall, 23.3% of the patients were overweight, 5% were obese and 13.3% of the patients corresponded to the underweight category.

Out of the enrolled patients 38.3% were active smokers, 21.7% were former smokers and 40.0% were nonsmoking individuals.

Nadir CD4 cell count was <50 in 16 patients (26.7%); also in 16 patients (26.7%) nadir CD4 cell count was 50-199, in 23 patients (38.3%) nadir CD4 cell count was 200-499, and 5 patients (8.3%) had nadir CD4 cell count over 500 cells/cmm. Out of the enrolled patients, 38 (60.3%) had AIDS stage according to the US Centers for Disease Control and Prevention (CDC) classification. At the time of blood sampling, 33.3% of the patients had serum detectable HIV viral load and 66.7% had undetectable HIV viral load (<20 copies/mL).

Table 1. Characteristics of the study participants

Characteristics	Mean±SD or median (interquartile range)	Minimum	Maximum
Age±SD, years	34.6±8.02	22	49
BMI±SD, kg/sqm	23±4.1	16	36.9
Median nadir CD4 cell count (IQR), cells/cmm	202.5 (48.7-337)	7	926
Median current CD4 cell count (IQR), cells/cmm	521.5 (355.2-71.4)	26	1280
Median HIV viral load (IQR), copies/mL	0 (0-115)	23	10000000
Median time since HIV diagnosis (IQR), years	4 (2-12)	0.5	20
Median duration of antiretroviral therapy (IQR), years	4 (2-9.7)	0.5	20
T score total body±SD	-0.36±1.18	-2.6	1.9
Z score total body±SD	-0.1±1.06	-2.1	2.6
T score lumbar spine (L1-L4) ±SD	-0.93±1.03	-2.9	1.5
Z score lumbar spine (L1-L4) ±SD	-0.69±1.11	-2.7	1.9
T score total hip±SD	-0.61±0.88	-2	1.7
Z score total hip±SD	-0.34±0.85	-1.8	2.1
T score total left hip±SD	-0.6±0.89	-2	1.5
Z score total left hip±SD	-0.37±0.86	-1.8	2.1
T score left femoral neck±SD	-0.56±1	-2.1	1.7
Z score left femoral neck±SD	-0.27±0.9	-1.9	1.8
T score left femoral trochanter±SD	-0.74±0.93	-2.5	1.6
Z score left femoral trochanter	-0.52±0.91	-2.8	1.8
T score left Ward's area±SD	-83±1.15	-2.6	2.2
Z score left Ward's area±SD	-0.43±1.05	-2.3	2.3

BMI – body mass index; **CD4** – cluster of differentiation 4; **HIV** – human immunodeficiency virus; **IQR** – interquartile range; **SD** – standard deviation.

At the time of enrollment all the patients had been treated with antiretroviral drugs in their medical history; 100% were treated with NRTI-based HAART; 81.7% (49 patients) were patients that had received PIs, 21.7% (13) had NNRTIs included in the treatment regimen and 13.3% (eight patients) were patients treated with integrase strand transfer inhibitor containing cART (Table 1).

BMD assessment results

According to Z scores, 13 (21.7%) of the research patients had BMD below the expected range for age at the lumbar spine (L1-L4) and small percentages of patients had BMD below the expected range for age at the femoral trochanter

(four patients, 6.7%) and Ward's area (three patients, 5%). One patient had Z-score ≤ 2 at the total body DEXA assessment and there were no patients with low BMD for chronological age at the femoral neck, total left hip or total hip.

DEXA evaluation reported as T scores showed that lumbar spine BMD and Ward's area BMD were lowest. At lumbar spine (L1-L4) 26 (43.3%) of the patients had osteopenia and three (5%) had osteoporosis. At the left femoral Ward's area BMD was decreased in similar percentage: 26 (43.3%) of the patients had osteopenia and four (6.7%) had osteoporosis (Table 2 and Table 3).

At the lumbar spine measurement, we observed that BMD was reduced significantly at

lumbar vertebra L3 (osteoporosis was found in five (8.3%) patients, osteopenia in 21 (35%) patients) and in lumbar vertebra L4 (five patients – 8.3% – presented osteoporosis and 28 – 46.7% – presented osteopenia), while in L1 and L2 vertebrae reduced BMD was found in a smaller number of patients.

Table 2. Prevalence of low bone mass in the study participants

Patients (n=60)	Normal, n (%)	Low BMD for chronological age ^a , n (%)
Total body	59 (98%)	1 (1.7%)
Lumbar spine (L1-L4)	47 (78.3%)	13 (21.7%)
Total hip	60 (100%)	0
Total left hip	60 (100%)	0
Left femoral neck	60 (100%)	0
Left femoral trochanter	56 (93.3%)	4 (6.7%)
Left Ward's area	57 (95%)	3 (5%)

^aDiagnosed using the World Health Organization (WHO) T-score criteria (osteopenia: T-score ranges between -1 and -2.5 SD; osteoporosis: T-score less than -2.5 SD)^{10,11}

Table 3. Prevalence of osteopenia or osteoporosis in the study participants

Patients (n=60)	Normal n (%)	Osteopenia ^a	Osteoporosis ^b
Total body	39 (65%)	19 (31.7%)	2 (3.3%)
Lumbar spine (L1-L4)	31 (51.7%)	26 (43.3%)	3 (5%)
Total hip	35 (58.3)	25 (41.7%)	0
Total left hip	36 (60%)	24 (40%)	0
Left femoral neck	38 (63.3%)	22 (36.7%)	0
Left femoral trochanter	37 (61.7%)	22 (36.7%)	1 (1.7%)
Left Ward's area	30 (50%)	26 (43.3%)	4 (6.7%)

^{a,b}Diagnosed using the World Health Organization (WHO) T-score criteria (osteopenia: T-score ranges between -1 and -2.5 SD; osteoporosis, T-score less than -2.5 SD)^{10,11}

We used the WHO classification criteria for BMD reporting in individuals younger than age 50 (low bone mineral density for patients with Z-score $\leq$ 2.0) and also the WHO classification according to T score values to define osteopenia and osteoporosis. Low BMD data defined in categories of osteopenia and osteoporosis by T scores values (BMD assessment at lumbar spine and left femoral Ward's area) were combined in further analyses, as well as the Z score values.

We presented both T and Z scores to follow the correspondence between reduced bone mineral density using T-score and Z score, given that a significant number of the patients in our study is composed of young adults (reference population for T score is healthy, young, 25-35 years, gender- and ethnicity-matched population).¹⁰

In the present study, DEXA evaluation showed, as a global result of the bone abnormalities, an overall prevalence of osteoporosis of 16.66% (ten patients) and an overall prevalence of osteopenia of 48.33% (29 patients). In order to estimate these numbers, each patient was counted once taking into consideration his lowest T score. Our data support previous estimates of low bone mineral density in HIV-infected individuals.¹²⁻¹⁵

The overall prevalence of low bone mass according to Z score was 21.7% (13 patients) in our study cohort. This is a higher prevalence than the prevalence reported in the literature (16.7% in a recent report in the Korean HIV-positive population).¹⁶

In our research, BMD evaluated in HIV-positive patients at lumbar spine, total left hip and left hip specific areas showed osteopenia rates ranging from 36.7% (femoral neck and femoral trochanter) to 43.3% (lumbar spine and Ward's area). The numbers of osteoporosis varied: 5% (three patients) at lumbar spine, 6.7% (four patients) at Ward's area, 1.7% (one patient) at the femoral trochanter and there was no patient with osteoporosis in the femoral neck.

At the left hip Ward's area BMD T scores were significantly associated with age, showing that aging is a risk factor for decreased BMD. On the other hand, BMD T scores at the lumbar

spine showed no significant difference stratifying patients according to group age ($p=0.745$).

To explore further correlations between HIV infection and factors significantly associated with the diagnosis of decreased bone mass, patients were divided into groups according to gender (female group: 25 patients and male group: 35 patients). Nineteen of the 35 male patients (54.28%) had osteopenia, eight (22.85%) of the men had osteoporosis. In the women group, ten patients (40%) had osteopenia and two (8%) had osteoporosis. We noticed that patients in the male group had significantly higher rates of low BMD at lumbar spine. At lumbar spine DEXA measurement, there was a statistically significant difference between average T scores in the male and female groups, identifying male gender as an associated factor for reduced BMD ($p=0.002$). Likewise, a statistically significant difference between average Z scores in the male and female groups was found ($p=0.002$) (Table 4).

Table 4. Statistically significant correlations between lumbar spine DEXA measurements and patient characteristics

Groups/ risk factor for osteopenia/ osteoporosis	T/Z score	Statistical test	P
Male			
BMI	T	$r = 0.324$	0.034*
Cigarettes smoking	T	$F = 3.304$	0.041*
Female			
NNRTI	T	$t = -2.269$	0.033*
PI	T	$t = 2.718$	0.012*
PI	Z score	$t = 2.086$	0.048*
All patients			
Male gender	T	$t = -3.243$	0.002**
Male gender	Z score	$t = -3.166$	0.002**
NNRTI	T	$t = -2.502$	0.015*

BMI – body mass index; DEXA – Dual-energy X-ray absorptiometry, NNRTI – non-nucleoside reverse-transcriptase inhibitors; PI – protease inhibitors.

*Significant at the 0.05 level

**Significant at the 0.01 level

Evaluating BMI as a risk factor in our study participants, a significant relationship was found

between low BMI and decreased BMD measured at lumbar spine (in terms of osteopenia/osteoporosis) in men ($p=0.034$), while in women no significant association was found for lumbar spine or left hip areas.

Concerning tobacco consumption in relation to bone mass density, at lumbar spine measurement, osteopenia/osteoporosis was significantly more prevalent in the male group ($p=0.041$), observation explained by the higher rates of tobacco consumption among men in comparison to women.

Risk factors attached to HIV infection that were analyzed for statistical association with osteoporosis/osteopenia included AIDS clinical stage, HIV plasma viral load, nadir CD4 lymphocyte count, current CD4 lymphocyte count, time since HIV diagnosis, duration of cART treatment.

In our study, there were no correlations between HIV plasma viral load, CD4 lymphocyte count nadir or CD4 lymphocyte current count and BMD (T scores lumbar spine and left hip Ward's area) neither in women nor in men.

In the present study, among both men and women no relation was established between AIDS clinical stage and the presence of reduced BMD expressed as T score in the lumbar spine or at the left hip Ward's area, a finding that agrees with some previous reports related to this topic.¹⁷

In relation to the potential role of the antiretroviral treatment on bone mineral density, controversial data still persist. All the patients in this research used NRTI based regimens combined with one of the following: PI, NNRTI, integrase inhibitor. The duration of exposure to cART classes in relation to BMD T scores was evaluated to explore whether there is an association between low BMD and cART use.

In our study no correlation was seen between BMD T scores at the lumbar spine or at the left hip Ward's area and duration of integrase inhibitor treatment (raltegravir) in neither men nor women.

The type of antiretroviral whose presence was found to be statistically correlated to low BMD of the study group, expressed as T scores at lumbar spine, was the NNRTI class ($p=0.015$). Out of the enrolled patients, 21.7% (13) had NNRTIs

included in the treatment scheme, with 3.29 years median time (range 1-6 years) of exposure to NNRTI. The NNRTI type used was etravirine for 58.43% (eight) patients and efavirenz for 41.66% (five) of the patients who had exposure to NNRTIs. This finding (Table 5), although it is not a common one and requires confirmation in follow-up researches, is similar to other reports that indicated that patients with more prolonged exposure to NNRTI are at higher risk of osteopenia/osteoporosis.¹⁸

Table 5. Significant correlations for left hip Ward's area DEXA

Groups/ risk factor for osteopenia/ osteoporosis	T/Z score	Statistical test	p
Male (n=35)	No correlation was found		
Female (n=25)			
PI	T score	t=2.141	0.043*
PI	Z score	t=2.459	0.022*
All patients			
Age	T score	r=-0.330	0.01*
Age (categories)	Z score	F=3.313	0.044*

DEXA – Dual-energy X-ray absorptiometry; PI – protease inhibitors.

*Significant at the 0.05 level

Measurements and patient's data

Another association in our research was found in the female group, between low BMD T score at the lumbar spine ($p=0.012$) as well as low BMD at the left femoral Ward's area and treatment containing PIs ($p=0.043$). In the women group, 76% of the patients had PIs included in the treatment. The median time of PIs exposure in the women group was 2.92 years (range 0.5-8). The PIs used by the patients in our study were lopinavir/ritonavir, saquinavir, darunavir and atazanavir.

BMD assessment at lumbar spine and left femoral Ward's area reported as Z score was not correlated with patient's age, BMI, AIDS clinical stage, HIV plasma viral load, nadir CD4 lymphocyte count, current CD4 lymphocyte

count, time since HIV diagnosis. The association between BMD below the expected range for age and time spent using cART didn't reach statistical significance among HIV male patients from our cohort, neither at the lumbar spine nor at the left hip Ward's area. Still, in HIV-positive women BMD Z scores at the lumbar spine and the left hip Ward's area were significantly associated with time elapsed using PIs ($p=0.022$).

Discussion

Many studies have investigated the prevalence of osteoporosis and osteopenia in HIV-infected cohorts and have reported widely variable rates in relation to age group and gender. Osteopenia ranges between 22%¹² and 71%¹³ and osteoporosis rates were found varying from 3%¹⁴ to 33%.¹⁵

Our study showed important rates for low BMD among young HIV-infected patients. These numbers were much higher than expected in a relatively young population such as our research cohort, similar to the prevalence reported in other studies, supporting that bone mineral density loss may be the result of complex interactions between HIV infection and/or cART and patient lifestyle related factors. Similar to data derived from our research, several studies found higher rates of reduced BMD among male HIV-infected patients.^{15,19}

For men, in the general population, osteoporosis is predominant in the cortical bone tissue, while for women postmenopausal osteoporosis affects preferably trabecular bone, involving especially the lumbar spine. The studies that have described bone disease in HIV-infected patients have found that bone demineralization is predominant in the trabecular bone.¹⁵ An interesting finding of our study is that in men the preferred sites of bone demineralization were both the hip area (the cortical bone) and the lumbar spine area (the trabecular bone). The increased frequency of traditional factors known to cause osteoporosis (such as low body weight, smoking, older age) could have contributed to the high prevalence of reduced BMD in men from the cohort. The important reduction of BMD at both anatomic

sites studied (hip and spine) may have resulted from the cumulated exposure to the virus itself, to the treatment and the presence of traditional risk factors for osteoporosis or osteopenia (which were proven to be statistically associated in the male participants).

Reduced BMD was significantly associated with low BMI in trials evaluating bone disorders in HIV-infected populations.^{15,20} This conclusion is supported by the fact that in HIV-infected groups BMI is lower than in HIV-negative control groups and the weight loss may be due to multiple causes: the chronic illness and the advancing HIV infection, malabsorption, poor nutrition, the higher prevalence of smoking and toxic substances for the metabolism.

Several reports have drawn attention to the habit of smoking, a traditional known risk factor for osteoporosis, even more important in the HIV-positive population, and have shown many causes responsible for the decreased BMD, both related to lifestyle and the presence of HIV infection.^{21,22}

HIV-related risk factors for osteoporosis such as duration of HIV infection, current or nadir CD4 cell count or viral load have been previously studied.

While several researches have failed to establish that nadir CD4 cell count is an associated factor for bone mass loss, Cazanave et al. showed that low nadir CD4 lymphocyte count is independently associated with osteoporosis/osteopenia in women.¹⁵ This finding supports the hypothesis that the severe and prolonged immunosuppression (suggested by the low nadir CD4 cell count) determines bone tissue metabolism alterations inducing early bone demineralization. Another viewpoint of this association is that low nadir CD4 cell count is noticed in individuals who have been treated for a longer period with antiretroviral drugs and this may indirectly support the negative influence of the antiretroviral therapy on bone mineralization.

We have investigated the potential association between reduced BMD and HIV viral load plasma levels. Our research does not agree with several previous reports, which showed that high viral load level was statistically

higher in HIV patients with osteopenia/osteoporosis. This may indicate a potential role of the virus itself.^{21,23,24} The association between persistent chronic inflammation and low BMD is well established. HIV can infect both activated T cells and macrophages; activated T cells and macrophages produce the receptor activator of NFκB ligand (RANKL), which stimulates the activity of osteoclasts (bone resorbing cells). Likewise, studies have reported that HIV proteins have the property of increasing osteoclast activity as well as the capacity to attenuate bone forming via suppression of osteoblasts, and to augment their apoptosis.²⁵ On the other hand, Cazanave et al. in their study on a large number of patients (492 HIV-infected patients) didn't agree with other published reports. They showed that in men low HIV plasma viral load is related to decreased bone mineral density (osteoporosis/osteopenia). A possible explanation is that the study cohort included patients with low plasma viral load as a consequence of successful reduction of viral replication with antiretroviral treatment.¹⁵ This would indicate an indirect harmful effect of the treatment itself on the bone.

Several authors have demonstrated in their research the contribution of antiretroviral therapy to bone demineralization.

It was demonstrated that NRTIs through their mitochondrial toxicity may either directly or indirectly induce BMD loss. Tenofovir, a widely used NRTI in cART regimens is known to cause minimal mitochondrial dysfunction. Also it has been demonstrated that it affects phosphate reabsorption in the proximal renal tubule, leading to phosphate wasting, hypophosphatemia and increased bone-turnover.^{2,26}

Regarding the contribution of PIs to BMD decrease, not all authors agree on the involvement of PIs in bone loss through increased osteoclast activity (ritonavir and saquinavir). However, several studies found a higher prevalence of reduced BMD in patients under PIs treatment. Tebas et al. reported in a group of patients using PIs that 50% had osteopenia and 21% of them had osteoporosis.²⁷ The positive effect of PIs on bone mineralization

has also been shown, as there are some reports in which PIs-containing treatment has been found to increase BMD.²⁸

Studies examining the effect of raltegravir (RAL), an integrase inhibitor, on bone metabolism are limited, so the effects of RAL on BMD in people living with HIV have not been well defined. Recent evidence suggests that the loss of BMD was less important in participants treated with raltegravir when it was compared to NRTI based regimen.²⁸ A study performed in 74 virologically-controlled patients who either switched from a ritonavir- boosted protease inhibitor (PI/r) to RAL or continued with PI/r, reported that switching to RAL led to a significant increase in femoral neck BMD when comparing between groups.²⁹

Some studies have focused their attention on the impact of HAART regimens on bone mineral density. With initiation of cART, regardless of the antiretroviral category, an important BMD loss follows (2-6%) within the first two years.⁵

Our study has a few limitations. First, because it has a cross-sectional design, we could not establish a causal relation between HIV infection and reduced BMD. Also, its statistical power is not very high because of the small number of patients and the low heterogeneity of the enrolled patients.

However, this is an original investigation in terms of searching for bone demineralization at lumbar spine (L1-L4) as well as at the three anatomic sites of the femur (femoral neck, femoral trochanter, Ward's area) and through total body DEXA scan in Romanian HIV-positive individuals. Reporting the associations between risk factors for decreased BMD stratified according to gender led to a better characterization of our study patients. The differences between men and women, considering lifestyle-related factors as well as the hormonal differences between the genders, argue for the separation on groups of gender.

Conclusion

This study shows a high frequency of osteopenia and osteoporosis among a relatively young group of HIV-infected individuals,

although patients with factors known to affect bone mineral density were not included in the cohort study. We used the International Society for Clinical Densitometry (ISCD) and WHO classification criteria to report BMD in individuals younger than 50 years, using Z-score (low bone mineral density for patients with Z-scores ≤ -2.0) and also the classification according to T score values to define osteopenia and osteoporosis. Our results showed that in the male group, smoking and low BMI were significantly associated with reduced BMD at the lumbar spine. The decrease in lumbar spine BMD was significantly higher in the male group. The reduced BMD at the left hip Ward's area was associated with aging as a risk factor. The antiretrovirals (NNRTI, PI) were found to have important role for BMD loss.

Considering the high prevalence of diagnosed BMD alterations, it would be useful to perform osteodensitometry measurement for HIV-infected patients. In this population, both traditional risk factors for reduced BMD and specific risk factors related to HIV infection itself have high significance and require increased attention.

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