European Review for Medical and Pharmacological Sciences 2015; 19: 4576-4589

Different impact of NNRTI and PI-including HAART on bone mineral density loss in HIV-infected patients

G. MADEDDU[1], A. SPANU[2], P. SOLINAS[2], S. BABUDIERI[1], G.M. CALIA[1], C. LOVIGU[1], M. MANNAZZU[1], S. NUVOLI[2], B. PIRAS[2], P. BAGELLA[1], M.S. MURA[1], G. MADEDDU[2]

[1]Unit of Infectious Diseases, Department of Clinical and Experimental Medicine, University of Sassari, Sassari, Italy
[2]Unit of Nuclear Medicine, Department of Clinical and Experimental Medicine, University of Sassari, Sassari, Italy

Abstract. – OBJECTIVE: To evaluate the changes in Bone Mineral Density (BMD) and bone remodelling markers in a group of HIV patients treated with HAART and controlled in a long follow up and to identify possible risk factors for accelerated bone mass loss.

PATIENTS AND METHODS: In a series of 172 HIV patients treated with HAART a total of 67 patients (44 males and 33 females) underwent repeated bone mineral density measurement by DEXA in lumbar spine and in femur; the patients were classified according to T-score WHO criteria. Serum bone alkaline phosphatase (BAP), by IRMA, and urine pyridinoline/deoxypyridinoline (PYD&DPD), by EIA, were also assayed in all cases.

RESULTS: At baseline, 62/67 patients were on HAART, while 5 were naïve; 44.8% were previous intravenous drug users (IVDU), 46.3% heterosexual and 8.9% homosexual, mean age being 40.2 ± 6.5 years, and 23.9% had previous AIDS diagnosis. Fifteen/67 (22.4%) of treated patients had osteoporosis and 25/67 (37.3%) osteopenia in spine and/or femur including 3 naïve, 27/67 (40.3%), including 2 naïve, had normal BMD in both sites. Fifty-one/67 patients were monitored during follow up (56.8 ± 5.3 months); 27 (52.9%) of these (Group 1), received protease inhibitors (PI) and 24 (47.1%), including naïve, (Group 2) received not nucleoside reverse transcriptase inhibitors (NNRTI) for > 50% of follow up period. In Group 1 patients, BMD reduction was observed after follow up in respect of basal condition in both spine and femur, but significantly ($p = 0.011$) only for the latter. However, mean BMD values remained stable in both sites in Group 2 patients. Basal BAP and PYD&DPD levels were higher in Group 1 than Group 2, but not significantly. Moreover, only PYD&DPD levels at the follow up evaluation were significantly (p = 0.031) higher in Group 1 than Group 2. Of the remaining 16/67 patients with osteoporosis/osteopenia, 10 received PI and 6 NNRTI and were treated with therapies that could increase bone density, in particular, 9 with Alendronate/Vitamin D/Calcium and 7 with only vitamin D/calcium; these patients were excluded from statistical analysis of 51 Group 1/Group 2 cases. In the 16 patients, after these specific treatments, mean spine and femur BMD increased over time, but significantly only in those cases including alendronate in their protocol.

CONCLUSIONS: The study showed that in HIV patients on HAART BMD decrease, even osteoporosis, can be present persisting over time, particularly in PI in respect of NNRTI treated patients. The pathogenesis is probably multifactorial, the different antiviral drugs seeming to differently affect bone metabolism. Alendronate/Vitamin D/Calcium therapy can be useful to slow down bone mass loss and also improve osteoporosis/osteopenia conditions, thus, reducing fracture risk also continuing HAART.

Key Words:
Bone mineral density, HAART, Protease inhibitors, Non nucleoside reverse transcriptase inhibitors, HIV.

Introduction

Since the introduction of highly active antiretroviral therapy (HAART), a significant improvement of AIDS related morbidity and mortality with prolonged survival has been observed[1-3]. However, the risk of non-HIV-related morbidity and mortality, including cardiovascular and bone disease, neurocognitive impairment and malignancies, has markedly increased in recent years[4-24]. In particular, HIV patients receiving HAART may develop metabolic and mor-

Corresponding Author: Giordano Madeddu, MD; e-mail: giordano.madeddu@uniss.it

phologic abnormalities including low bone mineral density and several studies have evaluated the presence of risk factors for bone mineral loss in HIV-infected patients. In particular, a meta-analysis including 20 cross-sectional studies have suggested a more increased risk of developing both osteopenia and osteoporosis in HIV-infected patients in respect of HIV non-infected individuals and in HAART treated patients in respect of naive cases[25].

Moreover, HIV patients receiving protease inhibitors (PI) showed a more increased risk of developing osteopenia/osteoporosis when compared with those receiving HAART not including PI[25-31], the former drugs also affecting adolescents[32], and even more so when the patients are affected by lipodystrophy in both adults[33] and children[34,35].

However, the role of both HIV infection and HAART in the development and the progression of bone mineral loss is still controversial and some authors, unlike the results obtained in several other studies, reported a not significant decrease in bone mineral density (BMD) values in HIV patients treated with HAART[36] remaining stable over time or, according to other studies, BMD decrease has been described to be restricted to the first period after HAART initiation followed by stabilization independent of different antiretroviral drugs[37]. According to other authors, no accelerated bone loss in HIV patients treated with HAART, including PI, has been found[38] as well as a beneficial effect on bone remodelling from this treatment has even been suggested in both adults[39] and children[40]. On the other hand, divergent metabolic effect on bone by the individual PI drugs have been hypothesized based on "*in vitro*" studies[41]. Moreover, in the patients with no evidence of accelerated bone loss over many years a routine monitoring of BMD is not considered necessary by some authors[42] over the short/medium term.

The aetiology of low bone density seems to be multifactorial. Regarding to the role of HIV infection itself, it is possible to be deduced from the studies of some authors who observed low mineral density, reduction of bone formation and increase of bone resorption markers in naive patients[28,39,43,44]. HAART could affect bone metabolism trough both a direct and an indirect (i.e. immune activation and cytokine pathways) effect[28]. In particular, PI have been observed to inhibit "*in vitro*" osteoblastogenesis and to increase osteoclast activity**,** while nucleoside reverse transcriptase inhibitors (NRTI) proved to enhance bone demineralisation and promote calcium hydroxyapatite release from bone, as a consequence of lactic acidosis[45]. An indirect action of HAART on vitamin D metabolism has also been hypothesized probably suppressing the activity of 25 and 1α-hydroxylase which is critical in the synthesis of $1,25(OH)_2D$[28]. Moreover, a role for the not nucleoside reverse transcriptase inhibitors (NNRTI) efavirenz in accelerating catabolism of vitamin D trough the upregulation of 25-hydroxivitamin D3-24-hydroxilase (CYP 24) gene expression with hypovitaminosis D has also been suggested[28,46-48] or in interfering with cytochromes involved in the vitamin D metabolism[49].

Other authors in experimental models have observed that PI and tenofovir adversely affects bone metabolism through its action on renal phosphate handling with the result of accelerated bone turnover and osteomalacia[50-52]. Tenofovir effect on bone could be worsened when an hypovitaminosis D exists[53]. In another study, a decrease of BMD and an increase of bone turnover markers after abacavir/lamivudine (ABC/3TC) or tenofovir/emtricitabine (TDF/FTC) with no major difference in renal function were reported[54].

Furthermore, it should be considered that traditional risk factors for bone mineral mass loss, such as low BMI, smoking, alcohol abuse and malnutrition are frequent findings in HIV infected patients.

Finally, most studies investigating the impact of HIV and HAART on bone metabolism are cross-sectional in nature and, therefore, do not allow to link cause and effect.

The aim of the present study, performed in a cohort of HIV infected patients treated with HAART for a long period of time without interruption, was to evaluate the change of BMD and of bone remodelling markers during follow up in respect of basal values and to also ascertain the presence of possible risk factors responsible for accelerated BMD loss as well as to identify possible therapies which can be effective in both limiting BMD loss in progress and preventing future losses in the course of HIV disease continuing HAART.

Patients and Methods

We enrolled 172 HIV infected adult outpatients consecutively attending our referral HIV University Care Centre, 112 males and 60 fe-

males, aged 20 to 61 years and, among these, we could monitor 67 patients in a long follow up continuing HAART, 16 of whom received drugs which could increase bone mineral density.

Demographic (including age and risk factors for HIV infection) clinical (CDC stage) and therapeutic (current HAART regimen and therapeutic history) data of the patients has been collected from clinical records.

Fifty-one of 67 patients, 27 males and 24 females, with a mean age of 39.5 ± 6.7 years, could be rechecked after 48-60 months in a long longitudinal study. At baseline, 25/51 (49.0%) cases were heterosexuals, 21/51 (41.2%) previous intravenous drug users (IVDU) and 5/51 (9.8%) homosexuals; moreover, 16/51 (31.4%) patients had previous AIDS diagnosis. Furthermore, 46/51 (90.1%) patients were HAART experienced, 29 (56.8%) were receiving protease inhibitors (PI) and 17 not nucleoside reverse transcriptase inhibitors (NNRTI) plus nucleoside reverse transcriptase inhibitors (NRTI) and 5 were naïve. Mean CD4 cell count was 597 ± 287 cells/μL and mean HIV-RNA 4.88 ± 5.48 log10 cp/ml. Baseline patient characteristics are summarized in Table I.

Moreover, 8/51 (15.7%) patients had osteoporosis and 16/51 (31.4%) osteopenia in spine and/or femur, while 27/51 (52.9%) had normal BMD in both sites. Three of the 5 naïve patients had osteopenia while 2 had normal BMD.

Table I. Demographic, clinical and therapeutic characteristics in 51 HIV infected patients at enrolment.

Parameter	
Age (years)	39.5 ± 6.7
Male gender	27 (52.9%)
IVDU	21 (41.2%)
Heterosexuals	25 (49.0%)
Homosexuals	5 (9.8%)
CDC stage A	20 (39.2%)
CDC stage B	15 (29.4%)
CDC stage C	16 (31.4%)
CD4 cell count (cells/μL)	597 ± 287
HIV RNA ($\log_{10}$ cp/ml)	4.88 ± 5.48
HCV infection	24 (47.0%)
Current HAART	46 (90.1%)
Current PI	29 (56.8%)
Current NNRTI	17 (33.3%)
Naive to antiretrovirals	5 (9.9%)

IVDU: intravenous drug users; CDC: Center for Disease Control; HAART: highly active antiretroviral therapy; PI: protease inhibitors; NNRTI: non-nucleoside reverse transcriptase inhibitors.

In the follow up, the patients were divided into two groups: 27 patients who received PI plus NRTI (Group 1) and 24 patients who received NNRTI plus NRTI (Group 2), the latter including these 5 naïve, both Groups for > 50% of follow up.

At baseline, the remaining group of 16/67 patients included 10 males and 6 females, with mean age of 40.9 ± 6.4 years; 2 were CDC stage A, 7 in B and 7 in C. Furthermore, regarding to risk factors, 9 patients were previous IVDU, 6 heterosexual and 1 homosexual; 9 had osteopenia and 7 osteoporosis. Seven patients have previous AIDS diagnosis. Ten of these 16 patients were receiving PI and 6 NNRTI therapy. After the first observation, the 16 patients also received specific treatment (alendronate 70 mg/week + vitamin D 500 I/U daily + calcium 1000 mg daily in 9 and only vitamin D 500 I/U daily + calcium 1000 mg daily in 7) and had a follow-up evaluation of BMD at 12 and at 24 months. No fracture was ascertained in these patients before and at our first observation as well as during the follow up. These patients were excluded from statistical analysis of the 51 Group 1/Group 2 cases.

Patients who underwent HIV disease progression or opportunist cancers or infections during follow up or received other drugs affecting bone metabolism were excluded.

The protocol of the present study was in accordance with the Helsinki Doctrine on Human Experimentation and all patients gave written informed consent.

In all cases both at baseline and during at follow up we measured:

- CD4 cell count by flow cytometry (FACScalibur flurimeter, Bekton Dickinson, Erembodigem-Aalst, Belgium) and HIV plasma viral load (HIV-RNA) by Nuclisens NASBA (Organon Teknica, Boxtel, Netherlands). We also investigated both hepatitis C virus (HCV) status, by measuring antibody to HCV in serum by enzyme-linked immunoadsorbent assay (ELISA) (Ortho HCV 3-0 ELISA test, Ortho Clinical Diagnostic, Amersham, Bucks, UK) and by immunoblot assay (SIA, Chiron RIBA, Chiron Corporation, Emeryville, CA, USA) as confirmation test, and hepatitis B virus (HBV) status, by measuring HBsAg by enzyme-linked fluorescent assay (ELFA; Biomerieux, Lyon, France).

- BMD (g/cm^2) by dual energy X-ray absorptiometry (DEXA; QDR 4500 A, Hologic Bedford, MA, USA) with a precision value "*in vivo*" of ≤ 1% in both lumbar spine and in femur; according to T-score WHO criteria the patients

were classified as normal (T-score > -1.0), with osteopenia (T-score ≤ -1.0 and ≥ -2.5) and with osteoporosis (T-score < -2.5).

- Serum bone alkaline phosphatase (BAP, ng/ml) as a marker of bone formation and urine pyridinoline and deoxypyridinoline (PYD&DPD, nM/mM creatinine) as markers of bone resorption were also measured.

BAP was assayed in serum (ng/ml) by an immunoradiometric assay (Tandem-R Ostase; Beckman Coulter, Brea, CA, USA) with a lower detection limit of 1.65 ± 0.63 ng/ml; the interassay CV was 6.21% at 20.3 ng/ml and 5.9% at 84.1 ng/ml and the intraassay CV was 4.26% at 21.3 ng/ml. Normal values ranged from 0 to 20.1 ng/ml for males and from 0 to 21.3 ng/ml for females.

Pyridinoline & deoxypyridinoline (PYD&DPD) was assayed in urine (nM/mM creatinine) by a competitive enzyme immunoassay (Metra PYD EIA kit; Quidel, San Diego, CA, USA), with the results corrected for the urinary concentration of creatinine (Creatinine; Metra Biosystems Inc., Santa Clara, CA, USA) and with a lower detection limit of 4.4 ± 1.3 nM/mM creatinine; the interassay CV was 10.47% at 93.7 nM/mM creatinine and 7.71% at 459.7 nM/mM creatinine and the intraassay CV was 13.54% at 12.8 nM/mM creatinine and 6.99 at 37 nM/mM creatinine. Normal PYD&DPD values ranged from 12.8 to 25.6 nM/mM creatinine for males and from 16 to 37 nM/mM creatinine for females.

Statistical Analysis

The statistical analysis was performed using StatSoft STATISTICA Software, release 6.0 and SPSS 14.0 software Package (SPSS Inc., Chicago, IL, USA).

The difference in categorical variables among groups was evaluated using chi-square or Fisher exact test, when appropriate, whereas Student's *t*-test for independent variables was used to compare continuous variables. Student's test for dependent variables was used when the parameters obtained at the first observation were compared to those evaluated at follow-up.

Multiple linear regression analysis was used to evaluate predictors of percent change from baseline in BMD in both spine and femur.

Statistical significance was considered as $p < 0.05$.

Results

At baseline, in the 51/67 patients no statistical difference between the Group 1 and Group 2 was observed in age, HAART duration, weight, CD4 cell count, HIV RNA, femur and spine BMD, BAP and PYD&DPD (Table II).

The follow up of the 51 patients was in average 56.8 ± 5.3 months; Group 1 patients received lopinavir/r in 13 cases, atazanavir/r in 4, indinavir/r in 4, saquinavir/r in 3, fosamprenavir/r in 2 and nelfinavir in 1 whereas Group 2 patients received nevirapine in 12 cases and efavirenz in the remaining 12.

Table II. Baseline characteristics in HIV patients receiving antiretroviral therapy including PI (Group 1) or NNRTI (Group 2).

Parameters	Group 1 (n = 27)	Group 2 (n = 24)	p
Age (years)	38.9 ± 6.4	40.2 ± 6.9	0.471
Male gender	15 (55.5%)	12 (50%)	0.691
IVDU	14 (51.8%)	7 (29.2%)	0.100
Heterosexuals	12 (44.4%)	13 (54.2%)	0.488
Homosexuals	1 (3.7%)	4 (16.7%)	0.174
Previous AIDS diagnosis	9 (33.3%)	7 (29.2%)	0.749
CD4 cell count (cells/μL)	540 ± 265	661 ± 303	0.133
HIV RNA ($\log_{10}$ cp/ml)	5.12 ± 5.61	4.14 ± 4.54	0.166
HCV infection	15 (55.5%)	9 (37.5%)	0.197
HAART duration (months)	39.6 ± 14.6	35.1 ± 11.8	0.270
PI duration (months)	38.5 ± 15.0	34.9 ± 10.6	0.495
Naive to antiretrovirals	2 (7.4%)	3 (12.5%)	0.661
Spine BMD (g/cm^2)	0.95 ± 0.10	0.99 ± 0.12	0.376
Femur BMD (g/cm^2)	0.86 ± 0.10	0.87 ± 0.12	0.181
BAP (ng/ml)	16.1 ± 7.6	12.5 ± 5.2	0.06
PYD & DPD (nM/mM creatinine)	29.9 ± 15.9	24.4 ± 9.26	0.14

IVDU: intravenous drug users; HCV: hepatitis C virus; PI: protease inhibitors; HAART: highly active antiretroviral therapy; BMD: bone mineral density; BAP: Bone alkaline phosphatase; PYD & DPD: pyridinoline and deoxypyridinoline.

Overall, in the comparison between baseline and follow up values, a reduction in both spine and femur BMD values were observed, but significantly only in the latter ($p = 0.019$). Both BAP and PYD&DPD levels increased, but non significantly.

Sub-dividing the patients on the basis of the type of HAART, as Group 1 and Group 2, no statistically significant difference was observed in the number of months of follow up (57.4 ± 4.8 *vs* 56.1 ± 5.8, $p = 0.396$). When comparing baseline and follow up values, a not significant reduction in BMD was ascertained in lumbar spine in Group 1, while a slight increase was observed in Group 2 patients, but not significantly, as illustrated in Figure 1. Moreover, BMD values had a significant ($p = 0.011$) reduction in femur in Group 1 when compared with basal values, while in Group 2 patients a not significant reduction was ascertained (Figure 1).

Furthermore, BAP mean levels did not significantly change in both Group 1 and Group 2 and PYD&DPD levels further increased in Group 1 in respect of Group 2, but not significantly.

At the follow up evaluation, PI duration was significantly longer in Group 1 ($p < 0.001$) in respect of Group 2 and the months of exposure to tenofovir and thymidine analogues were longer in Group 1, but not significantly (Table III). No statistical difference in CD4 cell count, HIV RNA levels, spine and femur BMD and BAP concentrations was observed. Only PYD&DPD levels were significantly higher ($p = 0.020$) in Group 1 in respect of Group 2 (Table III).

At multivariate linear regression analysis, only PI receipt was negatively associated with the percent changes from baseline in spine BMD (Beta -0.78, $p = 0.053$) and in femur BMD (Beta = -1.37; $p = 0.0003$) whereas age, years of HIV infection duration, CD4 cell count, HIV RNA,

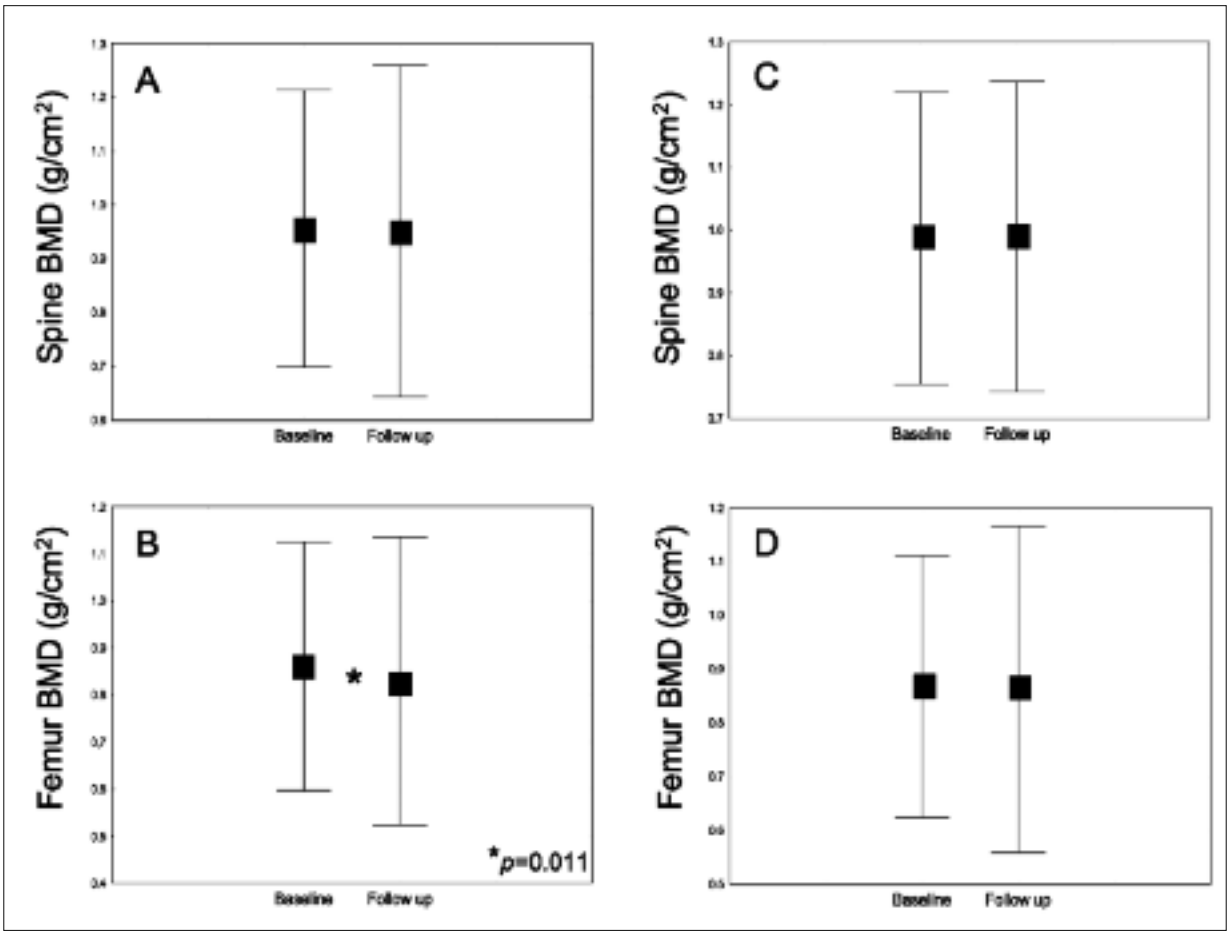

Figure 1. Bone mineral density (BMD, g/cm^2) values at baseline and follow up evaluation in Group 1 ***A,*** and ***B,*** and Group 2 ***C,*** and ***D,*** patients at spine and femur site. Values are expressed as Mean and Standard Deviation.

Table III. Comparison of therapeutic, immuno-virological and bone parameters in 51 HIV patients receiving antiretroviral therapy including PI (Group 1) versus NNRTI or triple NRTI (Group 2) at the follow up evaluation.

Parameters	Group 1 (n = 27)	Group 2 (n = 24)	p
CD4 cell count (cells/μL)	575 ± 195	685 ± 295	0.119
HIV RNA $\log_{10}$ cp/ml)	4.28 ± 4.92	3.77 ± 4.45	0.455
PI duration	48.5 ± 12.1	4.3 ± 8.7	0.000
Thymidine analogues duration	44.5 ± 16.8	36.1 ± 21.1	0.267
Tenofovir duration	7.9 ± 10.5	6.0 ± 9.1	0.505
Spine BMD (g/cm^2)	0.95 ± 0.16	0.99 ± 0.13	0.339
Femur BMD (g/cm^2)	0.83 ± 0.16	0.86 ± 0.15	0.461
BAP (ng/ml)	17.7 ± 17.3	14.4 ± 7.9	0.403
PYD & DPD (nM/mM creatinine)	32.4 ± 17.5	24.7 ± 7.71	0.031

PI: protease inhibitors; NNRTI: non nucleoside reverse transcriptase inhibitors; BMD: bone mineral density; BAP: bone alkaline phosphatase; PYD & DPD: pyridinoline and deoxypyridinoline.

BMI, BAP, PYD&DPD, baseline spine BMD and femur BMD did not show any significant association.

Globally, in remaining 16/67 patients mean BMD values significantly ($p = 0.05$) increased at 12 months in respect of basal values in both spine (0.867 ± 0.099 *vs*. 0.846 ± 0,074 mg/cm^2, $p = 0.05$) and femur (0.756 ± 0.077 *vs*. 0.698 ± 0.078 mg/cm^2, $p = 0.006$), as well as BMD significantly increased at 24 months in both spine (0.878 ± 0.098 *vs*. 0. 846 ± 0.074 mg/cm^2, $p = 0.019$) and femur (0.737 ± 0.097 *vs*. 0.698 ± 0.078 mg/cm^2, $p = 0.029$). Regarding to the patients with the protocol including alendronate, spine BMD values increased, in respect of baseline, at both 12 and 24 months, but significantly in the latter control (0.907 ± 0.102 *vs*. 0.874 ± 0.072 mg/cm^2, $p = 0.025$), while the increase of femur BMD was significant both at 12 (0.721 ± 0.117 *vs*. 0.695 ± 0.093 mg/cm^2, $p = 0.0014$) and at 24 months (0.760 ± 0.117 *vs*. 0.695 ± 0.093 mg/cm^2, $p = 0.021$). In the patients with protocol including only vitamin D/calcium, both spine and femur BMD values in the follow up showed a not significant increase in respect of basal values at both 12 and 24 months.

Discussion

The prognosis of patients with HIV infection has dramatically improved in the last decade following the introduction of HAART[31-33]. However, notwithstanding its undoubted benefits, several long-term adverse events have emerged during the years of exposure to HAART[28,58-70]. Among these toxicities, bone metabolism alterations, including both osteopenia and osteoporosis, have been frequently described in naïve, but especially in HAART treated patients[25]. Recently, another factor that could affect bone and that should be carefully considered in upcoming years, is represented by the aging of HIV populations due to both increased survival and older age at first HIV diagnosis. If such trend continues, a prevalence increase of metabolic alterations could occur, including bone disease, in HIV patients, both treated with HAART and naïve.

In the present long longitudinal study, we evaluated the changes of BMD in both spine and femur in 51 HIV infected patients with stable clinical conditions and we also studied bone metabolism trough bone remodelling markers.

At baseline, no statistical difference in BMD values in both spine and femur was observed between patients who received for > 50% of follow up PI + NRTI and those treated with NNRTI + NRTI in their therapeutic protocol. However, when comparing baseline and follow up results, Group 1 patients showed a not significant reduction and Group 2 cases a slight, not significant increase of spine BMD values. Conversely, femur BMD values had a statistically significant reduction in Group1 patients and a slight decrease in Group 2 cases.

The pathogenesis of bone mineral density alterations in HIV infected patients is probably multifactorial and among the responsible factors the exposure to different antiviral drugs seems to differently affect bone metabolism.

It has been hypothesized that PI could affect bone metabolism trough both direct and indirect mechanisms.

A direct effect on bone cells with osteblastogenesis inhibition has been suggested for indinavir and an increased osteoclast activity has been observed using ritonavir, saquinavir, nelfinavir and indinavir[71].

Some PI (indinavir, nelfinavir and ritonavir) have been shown to affect vitamin D metabolism causing a reduction of its active metabolite 1,25 dihydroxy vitamin D and consequent alteration in phosphocalcic metabolism as well as an increase of serum PTH levels was also ascertained in an our previous study hypothesizing a compensatory response to the reduced 1,25 (OH)2 D levels due to drug inhibitory effect and consequently low Ca levels, as in secondary hyperparathyroidism without renal failure[28]. These last data, more recently, were also confirmed by other authors either using the same drugs[72] or NRTIs and NNRTI[73].

In monocyte-macrophages cells-lines, where 1-α hydroxylase is identical to that of renal cells, it was demonstrated a 79.4% reduction of 1,25(OH)2 D formation by ritonavir, 63.4% by indinavir and 31.7% by nelfinavir, whereas efavirenz did not cause a reduction[74].

In the present study, an increase of both bone formation (BAP) and resorption (PYD&DPD) markers was observed during follow up in both Group 1 and 2 and, in particular, Group 1 patients had statistically significant higher levels of PYD&DPD. These data suggest that an increased bone remodelling is present in HIV infected patients, especially bone resorption that is significantly higher in patients receiving PI, thus indicating a persistent accelerated bone turnover.

On the other hand, bone remodelling can also be perturbed by a variety of pathologic conditions including postmenopausal osteoporosis and rheumatoid arthritis in which there is a local and systemic alteration in hormones and pro-inflammatory cytokines, respectively[75].

Regarding to inflammatory factors probably affecting bone metabolism, an "*in vitro*" study[76] showed a selective increase of the pro-inflammatory cytokines monocyte chemoattractant protein (MCP)-1 and interleukin-8 (IL-8) in primary human osteoblast cells following exposure to a pharmacological concentration of ritonavir and nelfinavir, thus suggesting the development of bone loss in HIV patients treated with these protease inhibitors.

The balance in the number and in the activity of osteoblasts and osteoclasts is regulated by the receptor activator of nuclear factor-κB ligand (RANKL)/RANK/osteoprotegerin (OPG) system. RANKL is a type II homotrimeric transmembrane protein that is expressed as a membrane bound and a secreted protein produced by both osteoblasts and stromal cells that regulate differentiation, function and survival of osteoclasts[71,77]. RANK, a type I homotrimeric transmembrane protein which is expressed in osteoclasts, after binding RANKL, recruits an adaptor protein called TRAF6 leading to NF-κB activation and translocation to the nucleus where it increases the expression of c-Fos which interacts with the nuclear factor of activated T cells (NFATc1) to trigger the transcription of osteoclastogenetic genes. OPG is expressed in many tissues and is produced by osteoblasts and binds to RANKL, thus avoiding its binding to RANK with inhibition of bone resorption. At a cellular level, some PI are capable of inhibiting osteogenenesis and OPG increasing osteoclastogenesis and bone resorption[78]. In particular, nelfinavir, indinavir, saquinavir and ritonavir have been found to increase osteoclast activity, whereas nelfinavir and lopinavir could decrease osteoblast alkaline phosphatase enzyme activity and gene expression and diminish calcium deposition and OPG expression[78].

However, it should be considered that in the cases observed in the present study PI have been used in combination with thymidine analogues that have been also shown to alter directly or indirectly bone metabolism. Mitochondrial toxicity induced by thymidine analogues has been reported to cause lactic acidosis that can result in bone demineralization as a compensative mechanism[45]; a direct effect on osteoblast mitochondria has been also hypothesized by the same authors.

In a more recent experimental research, a direct effect of AZT on bone both in vitro and in vivo was reported, AZT has been found to enhance osteoclastogenenesis in the presence of RANKL with a concentration dependent effect[79]. The same group extended the study to ddI and 3TC that have been found to increase TRAP-positive osteoclasts, but only in the presence of RANKL that seems to mediate their osteoclastogenetic effect. Furthermore, AZT effect on bone seems not to be accelerated by HIV infection itself[80]. However, no statistically significant difference in thymidine analogues therapy duration was observed among different groups; thus, tenofovir has been associated with bone mineral loss in randomized clinical trials. In the study 903E, patients who received tenofovir in combination

with lamivudine and efavirenz and completed 144 weeks in the 903 study where were followed for 336 more weeks. In these patients a significant reduction in BMD was evidenced in both lumbar spine (-1.7%, $p = 0.002$) and hip (-3.3%, $p < 0.001$) at week 288 despite calcium citrate plus vitamin D supplementation[81,82].

Some authors[83] reported that the loss of BMD was significantly greater in the patients treated with zidovudine/lamivudine/lopinavir compared to those treated with nevirapine/lopinavir suggesting that zidovudine/lamivudine affect bone metabolism with a yet unclear mechanism.

In a study of other authors[84], a prompt BMD reduction (48 weeks) in both spine and femur independent of HAART type was reported, while a longer follow up (96 weeks) showed a significantly more bone loss in spine and femur with tenofovir-emtricitabine and in spine with atazanavir plus ritonavir than did abacavir-lamivudine or efavirenz.

Furthermore, kidney proximal tubular toxicity with phosphorus loss has been described by some authors in patients receiving tenofovir with consequent hypophosphatemia and osteomalacia[85,86]. However, given the short period of exposure, with no statistical difference in the two groups, tenofovir treatment had a limited contribution to bone loss and no conclusion about the effect of this drug on bone can be drawn from their data.

Taken together, the results of our study seem to suggest that HAART can be associated with bone loss and increased bone resorption particularly in the femur site of patients receiving PI in respect of those receiving NNRTI. The entity of bone mass loss was apparently independent of HAART duration in our cases, but low BMD seemed to persist over time during treatment, as shown when the patients were rechecked during follow up; moreover, the bone remodelling biochemical markers BAP and PYD&DYP remained elevated, especially the latter, thus indicating a persistent accelerated bone turnover. Thus, PI could be an aggravating factor on the bone mass loss due the HIV infection itself. As reported in studies on lipid metabolism[87-89], all different PI have not the same toxicity profile. However, given the small number of patients, it is not possible to analyse the contribution of the single PI to bone loss. Finally, it should be considered that newer PI (such as darunavir), not included in the present study, may have a different impact on bone and their role should be further investigated.

Although limited in the number of patients, our experience seems to confirm that the treatment with alendronate, calcium and vitamin D is able to reduce BMD loss in patients with osteopenia/osteoporosis receiving HAART with also a significant BMD increase in the patients who had alendronate in their protocol in both spine and femur, as observed in our patients already at the first control at 12 months and confirmed at 24 months.

In some studies, a decrease of bone resorption and a preservation of bone mineral density has been reported with a combination of cholecalciferol and calcium in HIV female patients[90] as well as a significant decrease of bone formation and bone resorption markers has been observed with vitamin D treatment alone in both male and female HIV patients suggesting a protective effect on bone structure with vitamin D supplementation[91]. Moreover, it has also been postulated that vitamin D supplementation by different mechanisms may play a role in the reduction of immune activation levels[92].

However, HIV elderly male patients, despite adequate HIV replication control by successful HAART and despite vitamin D supplementation can show trabecular and cortical bone microstructure alterations associated with higher bone resorption[93]. Therefore, calcium and vitamin D treatment in HIV children and adolescent have been reported not to affect bone mass despite a significant increase of serum 25(OH)D levels[94], while in adolescent and young adult HIV patients a long period of vitamin D supplementation could promote an improved antibacterial immunity[95].

With regard to bisphosphonate therapy that has been previously considered as therapy for HIV-associated bone loss, Alendronate has been the most studied and clinically used to increase bone density in both lumbar spine and hip of HIV-infected patients who meet WHO defined criteria for osteopenia and osteoporosis in most studies[96-102]. Furthermore, annual zoledronate has proved to reduce bone resorption and increased bone density in the lumbar spine and hip of HIV-infected men with osteopenia/osteoporosis[103,104].

Thus, patients experiencing bone mineral loss should be evaluated for 10-year fracture risk using available algorithms such as the WHO Fracture Risk Assessment Tool (Frax®) for those > 40 years of age, and risk factors for bone loss should be ascertained in those < 40 years to bet-

ter identify the cases who need to be treated with anti-resorption agents[105].

The cases treated with alendronate in the present study were relatively young and no female were in menopause, all with HIV stable disease and without history of fractures, the latter being absent also in the period before the first 12 months and between these and the 24 months; moreover, no adverse effects by the therapy was ascertained thus proving safe and well tolerated. Moreover, the association of alendronate with vitamin D and calcium proved the most effective tool to limit bone loss, also considering that vitamin D deficiency is very frequent in HIV patients treated with HAART, as reported by several authors[1,5,26] and also by us[28]. Obviously, to this treatment an adequate nutritional intake and physical activity must be followed, also avoiding smoking and excessive alcohol intake. However, the main limitation of these data is represented by the low number of cases and the short duration of follow up as well as by the absence of patients with senile or post-menopause osteoporosis/osteopenia.

Conclusions

The present longitudinal study suggests that in HIV patients receiving HAART a decrease of BMD, even osteoporosis, can occur. Bone mass loss, continuing treatment, may persist over time and further worsen with accelerated turnover, in particular in patients receiving PI in respect of NNRTI-based HAART. The mechanism by which HAART acts on the bone is not yet clear, but some hypotheses can be made concerning its action directly on bone remodelling and/or indirectly on vitamin D metabolism aggravating the bone mass loss due to HIV infection itself. Baseline and follow up evaluation of bone metabolism parameters is suggested in patients on HAART to early identify those cases to be submitted to appropriate preventive treatments to reduce their fracture risk which represents an aggravating factor to HIV infection. Moreover, in patients with ascertained osteoporosis and osteopenia a sequential evaluation of the response to specific treatment for blocking bone mass loss should be considered. In particular, alendronate in association with vitamin D/calcium appears to be the therapy with the best results in HIV patients on HAART with very low BMD, obviously also preventing and limiting all the other general risk factors affecting bone metabolism. However, a larger number of cases is necessary to confirm these suggestions.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998; 338: 853-860.
2) Mocroft A, Ledergerber B, Katlama C, Kirk O, Reiss P, d'Arminio Monforte A, Knysz B, Dietrich M, Phillips AN, Lundgren JD; EuroSIDA study group. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. Lancet 2003; 362: 22-29.
3) Lohse N, Hansen AB, Pedersen G, Kronborg G, Gerstoft J, Sørensen HT, Vaeth M, Obel N. Survival of persons with and without HIV infection in Denmark, 1995-2005. Ann Intern Med 2007; 146: 87-95.
4) Bonfanti P, Ricci E, de Socio G, Zeme D, Carradori S, Penco G, Parruti G, Grosso C, Madeddu G, Vichi F, Bini T, Martinelli C, Melzi S, Quirino T; CISAI Study Group. Metabolic syndrome: a real threat for HIV-positive patients?: Results from the SIMONE study. J Acquir Immune Defic Syndr 2006; 42: 128-131.
5) Bonfanti P, De Socio GL, Marconi P, Franzetti M, Martinelli C, Vichi F, Penco G, Madeddu G, Orofino G, Valsecchi L, Vitiello P, Menzaghi B, Magni C, Ricci E. Is metabolic syndrome associated to HIV infection per se? Results from the HERMES study. Curr HIV Res 2010; 8: 165-171.
6) De Socio GV, Ricci E, Parruti G, Maggi P, Madeddu G, Quirino T, Bonfanti P. Chronological and biological age in HIV infection. J Infect 2010; 61: 428-430.
7) Bonfanti P, De Socio GV, Ricci E, Antinori A, Martinelli C, Vichi F, Penco G, Madeddu G, Orofino G, Valsecchi L, Rusconi S, Menzaghi B, Pocaterra D, Quirino T. The feature of Metabolic Syndrome in HIV naive patients is not the same of those treated: results from a prospective study. Biomed Pharmacother 2012; 66: 348-353.
8) Madeddu G, Fois AG, Calia GM, Babudieri S, Soddu V, Becciu F, Fiori ML, Spada V, Lovigu C, Mannazzu M, Caddeo A, Piras B, Pirina P, Mura MS. Chronic obstructive pulmonary disease: an emerging comorbidity in HIV-infected patients in the HAART era? Infection 2013; 41: 347-353.

9) Schillaci G, Maggi P, Madeddu G, Pucci G, Mazzotta E, Penco G, Orofino G, Menzaghi B, Rusconi S, Carenzi L, Celesia BM, Martinelli C, Bonfanti P, De Socio GV; CISAI Study Group. Symmetric ambulatory arterial stiffness index and 24-h pulse pressure in HIV infection: results of a nationwide cross-sectional study. J Hypertens 2013; 31: 560-567.

10) Zanet E, Berretta M, Martellotta F, Cacopardo B, Fisichella R, Tavio M, Berretta S, Tirelli U. Anal cancer: focus on HIV-positive patients in the HAART-era. Curr HIV Res 2011; 9: 70-81.

11) Pinzone MR, Fiorica F, Di Rosa M, Malaguarnera G, Malaguarnera L, Cacopardo B, Zanghì G, Nunnari G. Non-AIDS-defining cancers among HIV-infected people. Eur Rev Med Pharmacol Sci 2012; 16: 1377-1388.

12) Berretta M, Cinelli R, Martellotta F, Spina M, Vaccher E, Tirelli U. Therapeutic approaches to AIDS-related malignancies. Oncogene 2003; 22: 6646-6659.

13) Martellotta F, Berretta M, Cacopardo B, Fisichella R, Schioppa O, Zanghì A, Spartà D, Cappellani A, Talamini R, Izzi I, Ridolfo A, Torresin A, Fiorica F, Tirelli U. Clinical presentation and outcome of squamous cell carcinoma of the anus in HIV-infected patients in the HAART-era: a GICAT experience. Eur Rev Med Pharmacol Sci 2012; 16: 1283-1291.

14) Simonelli C, Tedeschi R, Gloghini A, Talamini R, Bortolin MT, Berretta M, Spina M, Morassut S, Vaccher E, De Paoli P, Carbone A, Tirelli U. Plasma HHV-8 viral load in HHV-8-related lymphoproliferative disorders associated with HIV infection. J Med Virol 2009; 81: 888-896.

15) Berretta M, Garlassi E, Cacopardo B, Cappellani A, Guaraldi G, Cocchi S, De Paoli P, Lleshi A, Izzi I, Torresin A, Di Gangi P, Pietrangelo A, Ferrari M, Bearz A, Berretta S, Nasti G, Di Benedetto F, Balestreri L, Tirelli U, Ventura P. Hepatocellular carcinoma in HIV-infected patients: check early, treat hard. Oncologist 2011; 16: 1258-1269.

16) Zanet E, Berretta M, Martellotta F, Cacopardo B, Fisichella R, Tavio M, Berretta S, Tirelli U. Anal cancer: Focus on HIV-positive patients in the HAART-era. Curr HIV Res 2011; 9: 70-81.

17) Berretta M, Zanet E, Basile F, Ridolfo AL, Di Benedetto F, Bearz A, Berretta S, Nasti G, Tirelli U. HIV-positive patients with liver metastases from colorectal cancer deserve the same therapeutic approach as the general population. Onkologie 2010; 33: 203-204.

18) Berretta M, Lleshi A, Cappellani A, Bearz A, Spina M, Talamini R, Cacopardo B, Nunnari G, Montesarchio V, Izzi I, Lanzafame M, Nasti G, Basile F, Berretta S, Fisichella R, Schiantarelli C C, Garlassi E, Ridolfo A, Guella L, Tirelli U. Oxaliplatin based chemotherapy and concomitant highly active antiretroviral therapy in the treatment of 24 patients with colorectal cancer and HIV infection. Curr HIV Res 2010; 8: 218-222.

19) Scarpino M, Pinzone MR, Di Rosa M, Madeddu G, Focà E, Martellotta F, Schioppa O, Ceccarelli G, Celesia BM, d'Ettorre G, Vullo V, Berretta S, Cacopardo B, Nunnari G. Kidney disease in HIV-infected patients. Eur Rev Med Pharmacol Sci 2013; 17: 2660-2667.

20) Castronuovo D, Cacopardo B, Pinzone MR, Di Rosa M, Martellotta F, Schioppa O, Moreno S, Nunnari G. Bone disease in the setting of HIV infection: update andreview of the literature. Eur Rev Med Pharmacol Sci 2013; 17: 2413-2419.

21) Nunnari G, Berretta M, Pinzone MR, Di Rosa M, Berretta S, Cunsolo G, Malaguarnera M, Cosentino S, De Paoli P, Schnell JM, Cacopardo B. Hepatocellular carcinoma in HIV positive patients. Eur Rev Med Pharmacol Sci 2012; 16: 1257-1270.

22) Zanet E, Berretta M, Benedetto FD, Talamini R, Ballarin R, Nunnari G, Berretta S, Ridolfo A, Lleshi A, Zanghì A, Cappellani A, Tirelli U. Pancreatic cancer in HIV-positive patients: a clinical case-control study. Pancreas 2012; 41: 1331-1335.

23) Pinzone MR, Di Rosa M, Cacopardo B, Nunnari G. HIV RNA suppression and immune restoration: can we do better? Clin Dev Immunol 2012; 2012: 515962.

24) Pinzone MR, Di Rosa M, Malaguarnera M, Madeddu G, Focà E, Ceccarelli G, d'Ettorre G, Vullo V, Fisichella R, Cacopardo B, Nunnari G. Vitamin D deficiency in HIV infection: an underestimated and undertreated epidemic. Eur Rev Med Pharmacol Sci 2013; 17: 1218-1232.

25) Brown TT, Qaqish RB. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. AIDS 2006; 20: 2165-2174.

26) Tebas P, Powderly WG, Claxston S, Marin D, Tantisiriwat W, Teitelbaum SL, Yarasheski KE. Accelerated bone mineral loss in HIV-infected patients receiving potent antiretroviral therapy. AIDS 2000; 14: F63-F67.

27) Moore A,Vashishe A, Sabin CA, Mocroft A, Madge S, Phillips AN, Studd JW, Johnson MA. Reduced bone density in HIV-positive individuals. AIDS 2001; 15: 1731-1733.

28) Madeddu G, Spanu A, Solinas P, Calia GM, Lovigu C, Chessa F, Mannazzu M, Falchi A, Mura MS, Madeddu G. Bone mass loss and vitamin D metabolism impairment in HIV patients receiving highly active antiretroviral therapy. Q J Nucl Med Mol Imaging 2004; 48: 39-48.

29) Todd TT, Qaqish RB. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis:a meta-analytic review. AIDS 2006; 20: 2165-2174.

30) Ground B, Peng G, Gibert CL, Hoy JF, Isaksson RL, Shlay C, Martinez E, Reiss P; Visnegarwala F, Carr AD for the INSIGHT SMART Body Composition substudy group. Continuous antiretroviral therapy decreases bone mineral density. AIDS 2009; 23: 1519-1529.

31) Bonjoch A, Figueras M, Estany C, Perez-Alvarez N, Rosales J, del Rio L, di Gregorio S, Puig J, Gomez G, Clotet B, Negredo E. The osteporosis Study Group. High prevalence of and progression to low bone mineral density in HIV-infected patients: a longitudinal cohort study. AIDS 2010; 24: 2827-2833.

32) Mulligan K, Harris DR, Emmanuel P, Fielding RA, Worrell C, Kapogiannis BG, Monte D, Sleasman J, Wilson CM, Aldrovandi GM. Low bone mass in behaviorally HIV-infected young men on antiretroviral therapy: adolescent trials network study 021B. Clin Infect Dis 2012; 55: 461-468.

33) Huag JS, Rietschel P, Hadigan CM, Rosenthal DL, Grinspon S. Increased abdominal visceral fat is associated with reduced bone density in HIV-infected men with lipodystrophy. AIDS 2001; 15: 975-982.

34) Mora S, Zamproni I, Beccio S, Bianchi R, Giacomet V, Viganò A. Longitudinal changes of bone mineral density and metabolism in antiretroviral-treated human immunodeficency virus-infected children. J Clin Endocrinol Metab 2004; 89: 24-28.

35) Dimeglio LA, Wang J, Sberry GK, Miller TL, Geffner ME, Hazra R, Borkoswsky W, Chen JS, Dooley L, Patel K, Van Dyke RB, Fielding RA, Gurmu Y, Jacobson DL, and for the Pediatric HIV/AIDS Cohort study. Bone mineral density in children and adolescents with perinatal HIV infection. AIDS 2013; 27: 211-220.

36) Bolland MJ, Grey AB, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Woodhouse AF, Gamble GD, Reid IR. Bone mineral density is not reduced in HIV-infected Caucasian men treated with highly active antiretroviral therapy. Clin Endocrinol 2006; 65: 191-197.

37) Hansen AB, Obel N, Nielsen H, Pedersen C, Gerstoft J. Bone mineral density changes in protease inhibitor-sparing vs. nucleoside reverse transcriptase inhibitor-sparing highly active antiretroviral theraphy: data from a randomized trial. HIV Med 2011; 12: 157-165.

38) Nolan D, Upton R, McKinnon E, John M, James I, Adler B, Roff G, Vasikaran S, Mallal S. Stable or increasing bone mineral density in HIV-infected patients treated with nelfinavir. AIDS 2001; 15: 1275-1280.

39) Aukrust P, Haug CJ, Ueland T, Lien E, Muller F, Espevik T, Bollerslev J, Froland SS. Decreased bone formative and enhanced resorptive markers in human immunodeficiency virus infection: indication of normalization of the bone-remodelling process during highly active antiretroviral therapy. J Clin Endocrinol Metab; 84: 145-150.

40) Bounders MJ, Frinking O, Scherphier HJ, van Arnhem LA, van Eck-Smit BL, Kuijpers TW, Zwinderman AH, Reiss P, Pajkrt D. Bone mineral density increases in HIV-infected children treated with long-term combination antiretroviral therapy. Clin Infect Dis 2013; 56: 583-586.

41) Teitelbaum S. Effect of protease inibitors on osteoblast and ostoclast differentiation and function. Bone Metabolism and HIV Disease. Meeting Report 2000 August 29; Washington DC, USA; 42-47.

42) Bolland MJ, Grey A, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Gamble GD, Reid IR. Stable bone mineral density over 6 years in HIV-infected men with highly active antiretroviral therapy (HAART). Clin Endocrinol 2012; 76: 643-648.

43) Serrano S, Marinoso ML, Soriano JC, Rubies-Prat J, Aubia J, Coll J, Bosh J, Del Rio L, Vila J, Goday A, Nacher M. Bonere modelling in human immunodeficiency virus-1 infected patients. A histomorfometric study. Bone 1995; 16: 185-191.

44) Paton NIJ, Macallan DC, Griffin GE, Pazianas M. Bone mineral density in patients with human immunodeficiency virus infection. Calcified Tissue Int 1997; 61: 30-32.

45) Carr A, Miller J, Eisman JA, Cooper DA. Osteopenia in HIV infected men: association with asymptomatic lactic acidemia and lower weight pre-antiretroviral therapy. AIDS 2001; 15: 703-709.

46) Gyllensten K, Josephson F, Lidman K, Sääf M. Severe vitamin D deficiency diagnosed after introduction of antiretroviral therapy including efavirenz in a patient living at latitude 59 degrees N. AIDS 2006; 20: 1906-1907.

47) Barrett JS, Joshi AS, Chai M, Ludden TM, Fiske WD, Pieniaszek HJ Jr. Population pharmacokinetic meta-analysis with efavirenz. Int J Clin Pharmacol Ther 2002; 40: 507-519.

48) Fabbriciani G, De Socio GVL. Efavirenz and bone health. AIDS 2009; 23: 1181.

49) Conesa-Botella A, Florence E, Lynen L, Colebunders R, Menten J, Moreno-Reyes R. Decrease of vitamin D concentration in patients HIV infection on a non nucleoside reverse transcriptase inhibitor-containig regimen. AIDS Res Ther 2010; 7: 40.

50) Verhelst D, Monge M, Meynard, Fouqueray B, Mougenot B, Girard PM, Ronco P, Rossert J. Fanconi syndrome and renal failure induced by tenofovir: a first case report. Am J Kidney 2002; 40: 1331-1333.

51) Karras A, Lafaurie M, Furco A, Bourgarit A, Droz D, Sereni D, Legendre C, Martinez F, Molina JM. Tenofovir-related nephro toxicity in human immunodeficiency virus-infected patients: three cases of renal failure, Fanconi syndrome, and nephrogenic diabetes insipidus. Clin Infect Dis 2003; 36: 1070-1073.

52) Harris VW, Brown TT. Bone loss in HIV-Infected patient: evidence, clinical implication, and treatment strategies. J Infect Dis 2012; 205 : s391-s398.

53) Childs KE, Fishman SL, Constable C, Gutierrez JA, Wyatt CM, Dieterich DT, Mullen MP, Branch AD. Short comunication: inadeguate vitamin D exacerbates parathyroid hormone elevation in tenofovir users. AIDS Res Hum Retroviruses 2010; 26: 855-859.

54) Rasmussen TA, Jensen D, Tolstrup M, Nielsen US, Erladsen EJ, Birn H, Ostergaard BL, Langdhal BL, Laursen AL. Comparison of bone and renal effects in HIV-infected adults switching to abacavir or tenofovir based therapy in a randomized trial. PLos One 2012; 7: e32445.

55) Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD. Declining morbidity and mortality among pa-

tients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998; 338: 853-860.

56) Moore RD, Chaisson RE. Natural history of HIV infection in the era of combination antiretroviral therapy. AIDS 1999; 13: 1933-1942.

57) Lohse N, Hansen AB, Pedersen G, Kronborg G, Gerstoft J, Sørensen HT, Vaeth M, Obel N. Survival of persons with and without HIV infection in Denmark, 1995-2005. Ann Intern Med 2007; 146: 87-95.

58) Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, Cooper DA. A syndrome of peripheral lipodystrophy hyperlipidemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS 1998; 12: F51-58.

59) Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. Lancet 1999; 353: 2093-2099.

60) Heath K, Hogg RS, Chan KJ Harris M, Montessori V, O'Shaughnessy MV, Montanera JS. Lipodystrophy-associated morphological, cholesterol and triglyceride abnormalities in a population-based HIV/AIDS treatment database. AIDS 2001; 15: 231-239.

61) HIV Lipodystrophy Case Definition Study Group. An objective case definition of lipodystrophy in HIV-infected adults: a case control study. Lancet 2003; 361: 726-735.

62) Riddler SA, Smit E, Cole SR, Li R, Chmiel JS, Dobs A, Palella F, Visscher B, Evans R, Kingsley LA. Impact of HIV infection and HAART on serum lipids in men. JAMA 2003; 289: 2978-2982.

63) Richter A, Pladevall M, Manjunath R, Lafata JE, Xi H, Simpkins J, Brar I, Markowitz N, Iloeje UH, Irish W. Patient characteristics and costs associated with dyslipidaemia and related conditions in HIV-infected patients: a retrospective cohort study. HIV Med 2005; 6: 79-90.

64) Noor MA, Seneviratne T, Aweeka FT, Lo JC, Schwarz JM, Mulligan K, Schambelan M, Grunfeld C. Indinavir acutely inhibits insulin-stimulated glucose disposal in humans: a randomized, placebo-controlled study. AIDS 2002; 16: F1-F8.

65) Murata H, Hruz PW, Mueckler M. The mechanism of insulin resistance caused by HIV protease inhibitor therapy. J Biol Chem 2000; 275: 20251-20254.

66) Hertel J, Struthers H, Horj CB, Hruz PW. A structural basis for the acute effects of HIV protease inhibitors on GLUT4 intrinsic activity. J Biol Chem 2004; 279: 55147-55152.

67) Caron M, Auclair M, Vigouroux C, Glorian M, Forest C, Capeau J. The HIV protease inhibitor indinavir impairs sterol regulatory element-binding protein-1 intranuclear localization, inhibits preadipocyte differentiation and induces insulin resistance. Diabetes 2001; 50: 1378-1388.

68) Tsiodras S, Mantzoros C, Hammer S, Samore M. Effects of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: a 5-year cohort study. Arch Intern Med 2000; 160: 2050-2056.

69) Madeddu G, Spanu A, Chessa F, Calia GM, Solinas P, Mannazzu M, Falchi A, Mura MS, Madeddu G. Thyroid function in human immunodeficency virus patients treated with highly active antiretroviral therapy (HAART): a longitudinal study. Clin Endocrinol (Oxf) 2006; 64: 375-383.

70) Madeddu G, Spanu A, Chessa F, Calia GM, Lovigu C, Mannazzu M, Falchi A, Sanna D, Mura MS, Madeddu G. Serum leptin and bone metabolism in HIV patients treated with highly active antiretroviral therapy. Q J Nucl Med Mol Imaging 2009; 53: 290-301.

71) Pan G, Yang Z, Ballinger SW, McDonald JM. Pathogenesis of osteopenia/osteoporosis induced by highly active anti-retroviral therapy for AIDS. Ann NY Acad Sci 2006; 1068: 297-308.

72) Marques de Menezes Barbosa EG, Albuquerque de Paola FJ, Artioli Machado A, de Assis Pereira F, Barbosa Junior F, Navarro AM. Impact of antiretroviral therapy on metabolism markers in HIV-seropositive patients. Bone 2013; 57: 62-67.

73) Zhang L, Su Y, Hsieh E, Xia W, Xie J, Han Y, Cao Y, Li Y, Song X, Zhu T, Li T, Yu W. Bone turnover and bone mineral density in HIV-1 infected Chinese taking highly active antiretroviral therapy--a prospective observational study. BMC Muscoloskeletal Disord 2013; 14: 224.

74) Cozzolino M, Vidal M, Arcidiacono MV, Tebas P, Yarasheski KE, Dusso AS. HIV protease inhibitor impair vitamin D bioactivation to 1,25-dihydroxyvitamin D. AIDS 2003; 17: 513-520.

75) Boyce BF, Xing L. Biology of RANK, RANKL and osteoprotegerin. Arthritis Res Ther 2007; 9 Suppl 1: S1.

76) Malizia AP, Vioreanu MH, Doran PP, Powderly WG. HIV1 protease inhibitors selectively induce inflammatory chemokine expression in primary human osteoblast. Antiviral Res 2007; 74: 72-76.

77) Ikeda T, Kasai M, Utsuyama M, Hirokawa K. Determination of three isoforms of the receptor activator of nuclear factor-[kappa]B ligand and their differential expression in bone and thymus. Endocrinology 2001; 142: 1419-1426.

78) Jain RG, Lenhard JM. Select HIV protease inhibitors alter bone and fat metabolism ex vivo. J Biol Chem 2002; 277: 19247-19250.

79) Pan G, Wu X, McKenna MA, Feng X, Nagy TR, McDonald JM. AZT enhances osteoclastogenesis and bone loss. AIDS Res Hum Retroviruses 2004; 20: 608-620.

80) Pan G, Kilby M, McDonald JM. Modulation of osteoclastogenesis induced by nucleoside reverse transcriptase inhibitors. AIDS Res Hum Retroviruses 2006; 2: 1-13.

81) Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, Coakley DF, Lu B, Toole JJ, Cheng AK; 903 Study Group. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA 2004; 292: 191-201.

82) Cassetti I, Madruga JVR, Suleiman JMAH, Etzel A, Zhong L, Cheng AK, Enejosa J; Study 903E Team. The safety and efficacy of tenofovir DF in combination with lamivudine and efavirenz through 6 years in antiretroviral-naive HIV-1-infected patients. HIV Clin Trials 2007; 8: 164-172.

83) Van Vonderen MGA, Lips P, van Agtmael MA, Hassink EAM, Brinkman K, Geeling SE, Sutinen J, Ristola M, Danner SA, Reiss P. First line zidovudine/lamivudine/lopinavir/ritonavir leads to greater bone loss compared to nevirapine/ lopinavir/ritonavir. AIDS 2009; 23: 1367-1376.

84) McComsey GA, Kitch MR, D, Dear ES, Tierney C, Jahed NC, Tebas P, Myers L,Melbourne K, Ha B, Sax PE. Bone mineral density and fractures in antiretroviral naive persons randomized to receive abacavir-lamivudine or tenofovir disoproxi fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir, AIDS clinical trial group A5224s, a substudy of ACTG A5202. J Infect Dis 2011; 203: 1791-1801.

85) Parsonage MJ, Wilkins EG, Snowden N, Issa BG, Savage MW. The development of hypophosphataemic osteomalacia with myopathy in two patients with HIV infection receiving tenofovir therapy. HIV Med 2005; 6: 341-346.

86) Perrot S, Aslangul E, Szwebel T, Caillat-Vigneron N, Le Jeunne C. Bone pain due to fractures revealing osteomalacia related to tenofovir-induced proximal renal tubular dysfunction in a human immunodeficiency virus-infected patient. J Clin Rheumatol 2009; 15: 72-74.

87) Arathoon E, Schneider S, Baraldi E, Lim PL, Opravil M, Van De Casteele T, Lavreys L. Effects of once-daily darunavir/ritonavir versus lopinavir/ritonavir on metabolic parameters in treatment-naive HIV-1-infected patients at week 96: ARTEMIS. Int J STD AIDS 2013; 24: 12-17.

88) Orkin C, DeJesus E, Khanlou H, Stoehr A, Supparatpinyo K, Lathouwers E, Lefebvre E, Opsomer M, Van de Casteele T, Tomaka F. Final 192-week efficacy and safety of once-daily darunavir/ritonavir compared with lopinavir/ritonavir in HIV-1-infected treatment-naïve patients in the ARTEMIS trial. HIV Med 2013; 14: 49-59.

89) Molina JM, Andrade-Villanueva J, Echevarria J, Chetchotisakd P, Corral J, David N, Moyle G, Mancini M, Percival L, Yang R, Wirtz V, Lataillade M, Absalon J, McGrath D; CASTLE Study Team Once-daily atazanavir/ritonavir compared with twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naive HIV-1-infected patients: 96-week efficacy and safety results of the CASTLE study. J Acquir Immune Defic Syndr 2010; 53: 323-332.

90) Zhu K, Devine A, Dick IM, Wilson SG, Prince RL. Effects of calcium and vitamin D supplementation on hip bone mineral density and calcium-related analytes in elderly ambulatory Australian women: a five-year randomized controlled trial. J Clin Endocrinol Metab 2008; 183: 743-749.

91) Piso RJ, Rothen M, Rothen JP, Stahl M, Fux C. Per oral substitution with 300000 IU vitamin D (Cholecalciferol) reduces bone turnover markers in HIV-infected patients. BMC Infect Dis 2013; 13: 577.

92) Fabre-Mersseman V, Tubiana R, Papagno L, BayardC, Briceno O, Fastenackels S, Dudoit Y, Rostane H, Salmon D, Costagliola D, Caby F, Sauce D, Viard JP, Appay V. Vitamin D supplementation is associated with reduced immune activation levels in HIV-1-infected patients on suppressive antiretroviral therapy. AIDS 2014; 28: 2677-2682.

93) Biver E, Calmy A, Delhumeau C, Durosier C, Zawadynski S, Rizzoli R. Microstructural alterations of trabecular and cortical bone in long-term HIV-infected elderly men on successful antiretroviral therapy. AIDS 2014; 28: 2417-2427.

94) Arpadi SM, McMahon DJ, Abrams EJ, Bamji M, Purswani M, Engelson ES, Horlick M, Shane E. Effect of supplemantation with cholecalciferol and calcium on 2-y bone mass accrual in hiv-infected children and adolescent: a randomized clinical trial. Am J Clin Nutr 2012; 95: 678-685.

95) Chun RF, Liu NQ, Lee T, Schall JL, Demburg MR, Rutstein RM, Adams JS, Zemel BS, Stallings VA, Hewison M. Vitamin supplementation and antibacterial immune responses in adolescents and young adults with HIV/AIDS. J Steroid Biochem Mol Biol 2015; 148: 290-297.

96) Guaraldi G, Orlando G, Madeddu G, Vescini F, Ventura P, Campostrini S, Mura MS, Parise N, Caudarella R, Esposito R. Alendronate reduces bone resorption in HIV-associated osteopenia/osteoporosis. HIV Clin Trials 2004; 5: 269-277.

97) McComsey GA, Kendall MA, Tebas P, Swindells S, Hogg E, Alston-Smith B, Suckow C, Gopalakrishnan G, Benson C, Wohl DA. Alendronate with calcium and vitamin D supplementation is safe and effective for the treatment of decreased bone mineral density in HIV. AIDS 2007; 21: 2473-2482.

98) Mondy K, Powderly WG, Claxton SA, Yarasheski KH, Royal M, Stoneman JS, Hoffmann ME, Tebas P. Alendronate, vitamin D, and calcium for the treatment of osteopenia/osteoporosis associated with HIV infection. J Acquir Immune Defic Syndr 2005; 38: 426-431.

99) Negredo E, Martinez-Lopez E, Paredes R, Rosales J, Perez-Alvarez N, Holgado S, Gel S, del Rio L, Tena X, Rey-Joly C, Clotet B. Reversal of HIV-1-associated osteoporosis with once-weekly alendronate. AIDS 2005; 19: 343-345.

100) Clay PG, Voss LE, Williams C, Daume EC. Valid treatment options for osteoporosis and osteopenia in HIV-infected persons. Ann Pharmacother 2008; 42: 670-679.

101) Rozemberg S, Lanoy E, Bentata M, Viard JP, Valantin MA, Missy P, Darasteneau I, Roux C, Kolta S, Castagliola D, ANRS 120 Fosivir Study Group. Effect of alendronate on HIV-associated osteoporosis: a randomized, double-blind, placebo-controlled, 96-week trial (ANRS 120). AIDS Res Hum Retroviruses 2012; 28: 972-980.

102) Pinzone MR, Moreno S, Cacopardo B, Nunnari G. Is there enough evidence to use bisphosphonates in HIV-infected patients? A systemic review and meta-analysis. AIDS Rev 2014; 16: 213-222.

103) Bolland MJ, Grey AB, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Woodhouse AF, Gamble GD, Reid IR. Annual zoledronate increases bone density in highly active antiretroviral therapy-treated human immunodeficency virus-infected men: a randomized controlled trial. J Clin Endocrinol Metab 2007; 92: 1283-1288.

104) Huang J, Meixner L, Fernandez S, McCutchan JA. A double-blinded, randomized controlled trial of zoledronate therapy for HIV-associated osteopenia and osteoporosis. AIDS 2009; 23: 51-57.

105) World Health Organization Collaborating Centre for Metabolic Bone Diseases, University of Sheffield, UK. Available online at: http://www.shef.ac.uk/FRAX/