AN OVERVIEW OF BONE DISEASE IN HIV-INFECTED PATIENTS

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ABSTRACT

Introduction: *Reduced bone mineral density in HIV-infected (Human Immunodeficiency Virus) patients is a growing issue in recent areas of research linked to the arrival of highly active antiretroviral therapy (HAART), which improved life expectancy for most HIV-infected patients.*

Material and method*: We carried out a literature search on PubMed, Google Scholar and Springer Link databases using appropriate terms linked to bone disease in HIV infection and we analyzed the most recent data, providing an overview on the bone disorders connected to HIV infection.*

Results*: Several causes contribute to the complex mechanism of bone loss associated with HIV infection: the virus itself, the specific antiretroviral therapy factors, as well as the traditional determinants of bone demineralization. Multiple cohort studies have reported an increased prevalence of bone demineralization among HIV-infected individuals. Bone fragility resulting from osteoporosis may lead to high fracture rates, as this population continues to grow old, therefore representing an important morbidity factor and also a serious challenge in the care of HIV-infected patients.*

Conclusion*: In HIV-infected persons, osteoporosis is probably underdiagnosed. Since osteoporosis is often an asymptomatic condition, with a long latency until fracture occurs, prevention of fractures through targeted screening and early treatment is a reasonable strategy. Further research is needed to clarify the algorithms for the screening, diagnosis, monitoring and management of bone disease in this category.*

Key words*: HIV, bone density, osteoporosis/osteopenia, fracture, antiretroviral therapy.*

Received *January 30, 2014;* ***Accepted*** *March 30, 2015*

Introduction

Reduced bone mineral density (BMD) in HIV infected patients is a growing issue in recent areas of research linked to the arrival of highly active antiretroviral therapy (HAART) which has transformed HIV infection from a fatal illness into a manageable chronic condition. The use of HAART has improved life expectancy for most HIV-infected patients, but soon followed concerns about the higher prevalence of comorbid conditions known to increase by aging. In addition to the immune system, HIV virus also targets several organs and tissues, causing impairment in the central nervous system, bones, cardiovascular and renal systems[1, 2].

The etiology of low BMD in HIV positive individuals is multifactorial and it represents a complex interaction among traditional osteoporosis risk factors (such as smoking, alcohol abuse, low body weight, physical inactivity, opiate use, hypogonadism, decreased intake of calcium and Vitamin D deficiency), the effect of viral infection itself and antiretroviral therapy (ART) exposure[3].

Osteoporosis is a systemic skeletal disorder characterized by reduced bone mineralization and impairment of the skeletal microarchitecture, consequently resulting increased bone fragility and fractures[4]. The diagnosis of reduced BMD is based on the results of Dual Energy-X Ray Absorptiometry (DEXA) measurement at the hip or spine.

World Health Organization (WHO) defines reduced BMD categories as standard deviation (SD) below the mean BMD for a young (25-35 years), healthy, ethnicity, sex-matched population (T score). According to WHO criteria osteoporosis is characterized by T-score less than -2.5 SD, and osteopenia is defined by T-score ranges between -1 and -2.5 SD[4, 5].

Several studies that analyzed bone mineral density in HIV infected patients have described a higher prevalence of osteopenia and osteoporosis among HIV-positive individuals. The prevalence of low BMD in these individuals was reported as osteopenia in 22.0% to 67.5% and as osteoporosis in 1% to 26.8%[3, 6-9].

It is estimated that HIV-infected adults have a 6.4-fold increased odds of reduced BMD and a 3.7 fold increased odds of osteoporosis compared to their uninfected counterparts[10].

Median time of progression to osteopenia in HIV infected individuals with normal baseline BMD was found to be 6.7 years, but in those patients with baseline BMD considered at "high risk", the progression was much faster (1.7 years). The "high risk" category included patients with T score ranging from -1 to -0.6[11].

Many of these studies have shown that low bone mineral density is more frequent in HIV positive male patients[12]. In fact, there is evidence that HIV-infected males have a significantly higher risk of reduced BMD compared to HIV-infected women, with a male/female reported ratio of 3:1 for osteopenia and 11:1 for osteoporosis[8].

Data referring to bone demineralization rates in HIV positive women shows that they have twice more a higher rate of bone loss when compared with age-, weight-, race-matched HIV negative women.

The presence of HIV virus has been linked to an increased risk of fragility fracture. Recent reports acknowledged that HIV infection remains associated with an increased fracture risk [HR: 1.24 (95% CI: 1.11, 1.39)] even after adjusting for demographics, comorbid disease, smoking and alcohol abuse[13].

The aim of this review is to summarize recent literature knowledge concerning HIV-related bone disease. The review discusses the epidemiology, pathophysiology and risk factors for low bone mass in HIV-infected patients. Clinical evidence of bone demineralization during HIV infection, as well as recommendations for screening and treatment were revised.

Material and method

For the accomplishment of this narrative review, we carried out a literature search using the keywords: "HIV", "bone density", "osteoporosis", "osteopenia", "fracture", "antiretroviral therapy". We searched PubMed, Google Scholar and Springer Link databases for English language articles from 2000 through January 31, 2015. We have selected for our analysis original articles as well as review articles and we identified in the search 126 papers. We considered more appropriate for the aim of our review 84 papers. Articles presenting data on BMD in HIV positive adults participants (>18 years), clinical implications, pathogenic mechanisms, data regarding risk factors for low bone mineral density and fractures in HIV infection were eligible for inclusion. We also included in our analysis references and recommendations for the evaluation and management of bone disease in HIV infection. Studies related to the connection between vitamin D deficiency and HIV infection were excluded if data regarding the impact of hypovitaminosis D bone health were not provided.

Results and discussions

Evidence of low BMD in HIV infection

Brown et al. in a meta-analysis of 12 cross-sectional studies (performed between 2000 and 2005) showed that 67% of patients with HIV infection (n=884) had reduced BMD (osteopenia and osteoporosis) and 15% had osteoporosis. Compared with seronegative control subjects (n=654) the prevalence of osteoporosis (patients who had T-score less than -2.5) was more than three times higher in HIV infection[10].

A recent analysis regarding osteoporosis in a cohort of HIV-infected patients in Rome reported data that confirm a high prevalence of low BMD in young HIV-positive patients, with antiretroviral treatment playing a crucial role. In the group of patients on first-line HAART (median age 45.6 (IQR 24.1-68.3)) 42.9% had low BMD of lumbar spine and 7.1% had osteoporosis. In the multi-experienced group of patients, at the lumbar spine osteopenia was observed in 36.6% of patients and 15.5% of them had osteoporosis (49.8 years was median age of the group (IQR 44.2-54.0)[14].

In a cohort survey of 492 French patients that had their BMD measured by DEXA, Cazanave et al. have found osteopenia in 54.6% of men (95% CI,

49.4-59.7) and 51.1% of women (95% CI, 42.6-59.6) and osteoporosis in 33.7% of men (95% CI, 28.8-38.6) and 8.3% of women (95% CI, 3.6-13.9)[8].

Information on prevalence of osteopenia and osteoporosis in HIV-infected patients in selected cross-sectional studies is presented in Table 1.

Author	No. patients	Sex	Age (median)	ART	Osteopenia (T score<-1 and ≥-2.5 SD)	Osteoporosis (T score<-2.5 SD)	Factors associated with low BMD
D'Avino et al.(12)	208 HIV +	71% male	49	100%	LS 42.9% (first HAART regimen) LS 36.6% (multi-experienced HAART)	LS 7.1% (first line HAART) LS 15.5% (multi-experienced HAART)	MSM patients, non-Caucasian race
Yin et al.(13)	100 HIV +	100% female	55.9	59%	LS 5% FN 9%	LS 9% FN 0%	HIV infection status, low BMI, alcohol use
	68 HIV-		59.6		LS 6% FN 7%	LS 0% FN 0%	
Calmy et al.(14)	153 HIV +	98% male	48	100%	36%	4%	Boosted IP
Cazanave et al.(9)	492 HIV +	73% male	43	93%	54% (men)	33.% (men)	Age, low BMI, low HIV plasma VL, homosexual transmission
		27% female			51.1% (female)	8.3% (female)	
Dolan et al.(15)	100HIV+	100% female	41		41%	7%	Low weight, duration of HIV, increased bone turnover
	100 HIV-						

Table 1: Prevalence of osteopenia and osteoporosis in HIV-infected patients.
HIV-human immunodeficiency virus, ART-antiretroviral therapy, BMD-bone mineral density, LS- lumbar spine, FN-femoral neck, BMI-body mass index, MSM-men who have sex with men, PI-protease inhibitor, VL-viral load

Fracture risk

Although both detrimental effects of HIV infection and ART on bone metabolism have been previously established, data on their impact on fracture risk is controversial[18, 19]. The cohort study conducted by Güerri-Fernandez on 2489 Spanish subjects with diagnosis of HIV/AIDS, established a powerful association between HIV infection and hip fracture incidence. HIV infected patients had an almost five fold increased risk of fracture, independent of sex, age, smoking, alcohol drinking, and comorbidities. They also concluded that patients with diagnosed HIV infection have a 75% higher risk of all clinical fractures and a 60% increase in risk of non-hip clinical fractures[17]. Other studies analyzed fracture risk and ART exposure and identified a significantly higher risk of fragility fracture in patients receiving ART in comparison to general population, even after adjusting for the comorbidity index. Risk of non-fragility fractures was not proven to be correlated to ART exposure[20].

When analyzing the exposure to specific ART, an association was reported between the increased incidence of osteoporotic fracture and the presence of tenofovir (TDF) or PIs[21]. In addition, Triant et al. in a population-based study in the US healthcare system showed an increased prevalence of fractures in HIV-infected patients (overall fracture prevalence was 2.87 vs. 1.77 patients with fractures per one hundred persons having HIV infection towards HIV uninfected patients). A higher fracture prevalence per 100 persons was reported in the male group, for any fracture (vertebral, wrist, hip fractures)[22].

Some studies failed to find an increased risk of fracture in HIV positive individuals. A recent meta-analysis comprising thirteen studies investigating the incidence of fracture (both overall and fragility) reported a modest increase for all fractures risk in HIV infection and/or ART use[23].

Bone turnover in patients with HIV infection

Normally bone is constantly undergoing remodeling, a process representing a balance between bone resorption and bone formation. In healthy individuals, bone remodeling is determined by the two major bone cells types: osteoblasts- bone forming cells and osteoclasts- bone resorbing cells. Osteoblasts are originated from mesenchymal stem cells and determine the constitution of extracellular bone matrix. Osteoclasts differentiate from monocyte or macrophage under the influence of macrophage colony stimulating factor (M-CSF) and receptor activator for nuclear factor kB-ligand (RANKL)[24]. The balance between these two type cells activity is the crucial determinant of bone mass.

Bone-forming cells express RANKL interacting with a specific protein existing on the surface of osteoclast precursors, called RANK protein. Following this process, the result is differentiation and proliferation of osteoclasts[2]. Osteoblasts also

have a control mechanism of osteoclast activation. They produce and they release a protein called osteoprotegerin (OPG) which binds to RANKL and prevents osteoclast activation[10]. Researchers have observed in HIV infection an uncoupling of the bone resorption and the bone formation and also increased markers of bone resorption and decreased or stable markers of bone formation[10].

A study, whose aim was to define the role of RANKL in the development of HIV-related osteopenia/osteoporosis, found serum RANKL levels inversely proportional to the BMD of the lumbar spine[25]. Another research reported also a significant increase in plasma RANKL levels with an impairment of RANKL/OPG ratio in a group of ART naive HIV-positive men. Bone resorption could be increased when the OPG/RANKL ratio is low, preventing OPG, which constantly acts as a decoy receptor to inhibit the activity of RANKL. Correlations between the RANKL high levels and high plasma viral RNA load were also found, indicating a direct relation between HIV infection status and RANKL synthesis[26].

In a 72-week longitudinal evaluation of BMD research, Mondy et al. found that bone resorption and bone formation markers levels remained high throughout the study period, suggesting a high bone turnover state. Bone turnover markers remained increased, regardless of patient's immune status or type of ART used, while over the period of the study BMD had a modest increase. The authors underlined that generally known risk factors as well as HIV viral infection itself are crucial to the pathogenesis of osteopenia and osteoporosis.

Bone formation and bone resorption are closely linked and synchronized[27, 28]. So, a disruption of the osteoblast interactions due to pathological states such as immune pathologies, infection and alterations of metabolism conspire to the damage of bone tissue and increased risk of fragility fractures[24].

Etiology of excess bone loss

The pathogenesis of reduced BMD associated with HIV is likely multifactorial. Bone loss is the result of various interactions between viral factors, ART and patient related causes[10] (Table 2).

The role of HIV infection

Some data suggested the hypothesis that HIV virus can infect bone marrow stromal cells or that human osteoblasts may be a target for HIV infection, leading to BMD loss through a direct viral mechanism. Other studies conducted on the subject could not agree on the matter. No further evidence could support these findings[22, 27].

HIV-related factors
Elevated RANKL
Low CD4 count
HIV viral proteins
Chronic inflammation
Antiretroviral medication
Protease inhibitors
Tenofovir
Efavirenz
Patient risk factors
Low body weight
Inadequate physical activity
Smoking
Alcohol use
Opiate use
Steroid exposure
Hypogonadism
Vitamin D deficiency
HCV coinfection

Table 2: Risk factors for BMD loss in patients with HIV infection.
HIV-human immunodeficiency virus, HCV-hepatitis C virus, RANKL- receptor to activate nuclear factor kappa B ligand, CD4-cluster of differentiation 4

Recent evidence has argued the concept that the immune and skeletal systems interact and the result of a centralization of common cell types and cytokine mediators, described as the "immune-skeletal interface" impairs bone turnover in the chronic inflammation status[29]. Increased cytokine activation associated with high levels of TNF in advanced HIV disease may be a possible mechanism for increased bone resorption. This is because advanced HIV infection (defined as a mean CD4 count of 20 cells/mL) is associated with markers of bone resorption that positively correlate with TNF activation[27]. Successively, activation of TNF adjusts other pathways that lead to osteoclast differentiation and bone resorption[30].

B cells are a source of OPG, thereupon B cells act as stabilizers of peak BMD in vivo. B-cell production of osteoprotegerin is sustained by interac-

tions with T cells through CD 40 ligand co-stimulation. In HIV infection, the presence of the virus disrupts the communication between T cells and B cells leading to increased levels of RANKL and low OPG production by the B cells[29]. Evidence of HIV disruption of the immune-skeletal interface has been demonstrated using a HIV-1 transgenic rat model. The HIV-1 transgenic rat model suffered skeletal changes and severe loss of BMD similar to those observed in human HIV infection. The increased osteoclast number histologically found was associated with altered-B cell function, which determined diminished production of bone-sparing OPG, increased expression of the osteoclastogenetic cytokine RANKL and accordingly increased number of osteoclast precursors[29].

In addition, there are researches that argued the direct viral effects on bone turnover through a possible role of HIV proteins on bone cells. In vitro studies have shown that HIV proteins gp120 and Vpr have the property of increasing osteoclast activity[31, 32]. HIV protein p55-gag was shown to determine bone resorption via suppression of osteoblasts and augmenting apoptosis of osteoblasts[33, 34].

In a meta-analysis that included a large number of subjects (796 HIV positive) from 3 studies of ART initiation, Philip Grant et al. evaluated the effects of baseline CD4+ and found a strong association (even after adjusting for potential confounders such as BMI) between low baseline CD4+ count (<50 cells/μL) and total BMD loss in the first two years of treatment. This findings support the hypothesis of a potential role of the immune system in skeletal balance. Furthermore, older age, lower BMI, higher HIV-1 RNA levels and PI and TDF use were also associated with increased demineralization[35].

Patient related factors

Low body weight

As in the general population, body weight is an important predictor of bone mineral density among HIV-positive persons. Cross sectional studies have identified the association between low body weight and reduced bone mineral density among HIV infected persons[8, 15, 36, 37].

The meta-analysis conducted by Bolland et al. found that HIV-infected patients are lighter than control subjects and suggested that low BMI could be considered a mediator in the relationship HIV–low bone mineral density. They concluded that low body weight can largely be responsible for the higher prevalence of reduced BMD found in HIV infected patients[38].

Fausto et al. tried to identify potential predictive factors of osteoporosis in their research including one hundred sixty-one HIV-infected. They reported a 49.7% prevalence of osteopenia/osteoporosis and identified independent predictors of low BMD: female gender, older age, low body mass index (BMI) and higher HIV-RNA levels at the moment of DEXA scan, this last factor suggesting that HIV virus itself has a direct role in the appearance of bone disease[39].

Age and gender

In general population the average rate of bone loss is considered 0.5%/year as they become older of age, in both women and men, but at the moment of menopause, women are continuously losing bone mass at a higher rate (1%/year)[40, 41].

Epidemiological and clinical data of several studies confirm a higher incidence of osteopenia and osteoporosis in HIV-infected males than in HIV-infected women.

HIV-infected men in the cross-sectional study (n=148 patients) realized by Amiel et al. had significantly lower BMD. This research reported osteoporosis in 16% of the patients and osteopenia in 66% of all HIV-positive men, among the highest reported prevalence data reported so far[42].

The prevalence of reduced BMD (T score less than -1 SD) in a cross sectional study of 328 male HIV-positive was slightly higher: 55% versus 51% in the control cohort (n=231), but similar to national estimated numbers in USA. Nevertheless, the percentage of men having osteoporosis was substantially higher in this study (14%) than in the general population[36].

Changes in bone mineral density in HIV-infected women have also been assessed in studies. In a prospective cohort study in which BMD was measured by DEXA in 100 HIV-infected females and 100 healthy controls, those with HIV had significantly lower bone density at the lumbar spine (1.01 ± 0.01 vs. 1.07 ± 0.01 g/cm2, P = 0.001), hip (0.94 ± 0.02 vs. 0.98 ± 0.01 g/cm2, P = 0.02) and femoral neck (0.83 ± 0.01 vs.0.87 ± 0.01 g/cm2, P = 0.02), compared with the age-, race-, and weight-matched control subjects. Osteopenia was diagnosed in 41% of the HIV-infected women (hip, femoral neck, or spine) and 7% demonstrated osteoporosis[17].

The concern of most studies was inclined towards younger HIV-infected individuals and only a few studies reported the skeletal status of middle-aged men and women. A prospective cohort study

performed by Yin et al. enrolled 95 HIV-uninfected and 92 HIV-infected postmenopausal women and it demonstrated a significantly higher prevalence of reduced BMD (T scores below-1.0) in HIV-infected women at the lumbar spine (78 vs. 64%), at total hip (45 vs. 29%) and at the femoral neck (64 vs. 46%), although HIV-positive women were younger (56±1 vs. 60±1 years). These results were comparable to those reported in a previous research by Dolan et al. HIV positive status remained an independent predictor of spine and hip reduced BMD after the adjustment for factors traditionally associated with BMD loss (age, BMI, race/ethnicity, alcohol use)[37].

The longitudinal analysis of BMD changes including a cohort of postmenopausal women demonstrated higher rates of bone loss adjusted for baseline BMD in HIV-positive women. The annualized rate of bone loss was 2.4-fold higher at the lumbar spine and 3.7-fold higher at the 1/3 radius in HIV[43].

The number of older HIV-infected individuals has increased as a result of widespread use of HAART. Considering how the demographics of HIV positive population is evolving, the concern regarding early onset of aging complications, such as neurocognitive decline, osteoporosis, fractures and impaired physical function has emerged[44].

For the Cohort of HIV-at risk Aging Men's Prospective Study (CHAMPS), a cross-sectional study conducted to investigate changes among 230 HIV-infected men and 159-uninfected men aged more than 49, BMD measurement showed that HIV-infected men had lower BMD at the femoral neck, (0.98 vs. 1.02 g/cm2, p= .02), total hip (1.01 vs. 1.06 g/cm2, p< .01) and lumbar spine (1.15 vs. 1.19 g/cm2, p= .03) when compared with HIV-uninfected controls[45].

Likewise, a cross-sectional study performed among 57 HIV-infected and 47 HIV-negative subjects over age 55 showed that HIV-infected persons had higher demineralization (prevalence of reduced BMD was 59 vs. 26% at the total hip, 67 vs. 39% at the lumbar spine). Turnover markers did not differ between the two groups[46].

Comorbidities

There are multiple diseases causing or exacerbating osteoporosis in both general population and HIV-infected persons. Bone loss may result from nutritional and hormonal changes commonly associated with advanced HIV infection: immobility, malnutrition, malabsorption, hypogonadism and estrogen deficiency. Also endocrine disorders, that affect bone density, such as vitamin D deficiency, hyperthyroidism, hyperparathyroidism, Cushing syndrome, have been reported as secondary osteoporosis associated conditions and can be suspected in selected HIV-infected population[1].

Lipodystrophy or abnormal fat distribution, with central adiposity and subcutaneous fat atrophy are usual findings among HIV-positive patients. Additionally, some studies reported association between changes in body composition and reduced BMD. Bone loss was independently associated with central lipohypertrophy and also with subcutaneous fat atrophy[10].

Another factor found to be associated with **low** BMD is hepatitis C co-infection. Several studies have reported more than two fold increased risk of reduced bone mass and fracture among HIV and C hepatitis virus co-infected individuals[47,48].

Lifestyle factors

Some factors related to lifestyle, factors that are being considered traditional risk factors for bone loss are more prevalent in HIV infected population. These are physical inactivity, smoking, alcohol abuse, opiate use. Therefore, the counseling to modify them should not be forgotten in any treatment strategy targeting bone disease in HIV infection[49].

Concomitant medications

Multiple medications have been proven to reduce bone mineralization in general population, most frequently glucocorticoids. They decrease osteoblast activity and increase osteoclast activation, thus being responsible of bone turnover uncoupling.

Corticoid use has been identified as risk factor associated with low BMD in HIV-infected persons in several studies[50]. In addition, some ART used in HIV infection are known to produce pharmacological inhibition of Cytochrome P4503A4 (for example ritonavir) and thus interfere with the metabolism of corticosteroids, leading to increased exposure to steroids. These drug interactions can lead to systemic side effects (also low bone mineralization) even if small doses are used or if the steroids are given by routs not typically associated with systemic side effects[49,51].

Vitamin D deficiency

Chronic HIV infection causes an increased risk of low bone mineral density and osteoporosis, increased systemic inflammation and dysfunction of

the immune system. Vitamin D has been known to play an important role in modulating the immune system. Therefore, abnormalities in vitamin D metabolism and how much 25-hydroxy vitamin D (25(OH)D (the most stable form of vitamin D and the best indicator of an individual's vitamin D status) constitutes an adequate concentration of the vitamin in circulation represent serious concerns in HIV infection[52-54].

Vitamin D deficiency in HIV-infected patients is extensive, some cross sectional and prospective studies have showed increased prevalence among HIV infected individuals (rates ranging between 36% to 86%, using vitamin D deficiency as 25(OH)D<20 g/mL). Some studies suggested that the HIV-infected individuals have vitamin D levels similar to control groups[52].

There is evidence that HIV-specific factors substantially complicate vitamin D deficiency, along with traditional risk factors. Traditional risk factors playing a role in vitamin D deficiency (such as non-white race, insufficient intake of vitamin D, lack of exposure to sunlight, lack of physical exercise, female gender, hypertension, older age, smoking and increased BMI), have a major influence on vitamin D levels also in people with HIV[50, 55].

Both vitamin D and ART (non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (IPs)) metabolism rely heavily on cytochrome P450 enzymes, thus research data indicate that exposure to ART is a risk factor for inadequate concentration of vitamin D.

More accurate, efavirenz (EFV) is the antiretroviral drug that seems to be seriously affecting the metabolism of vitamin D[56, 57]. EFV seems to accelerate the catabolism of the 25(OH)D by increasing the 24-hydroxylase, the enzyme which breaks down the 25(OH)D, or by suppressing the 25-hydroxylase enzyme that converts vitamin D2 and D3 in the 25(OH)D. Vitamin D deficiency caused by EFV is particularly important in patients receiving concomitant medications which use the way of the CYP450 enzyme complex, eg corticosteroids, antiepileptics or antituberculosis drugs[52, 58, 59].

In vitro studies have showed that IP, ritonavir, indinavir, nelfinavir cause a strong inhibition of the 1 alpha-hydroxylase and 25-hydroxilase and also a slight inhibition of the 24-hydroxylase, effects leading to the decrease of the active form of vitamin D (1,25(OH)2D)[52, 53, 60]. Dao et al. have explored serum concentrations of 25-hydroxy vitamin D (25(OH)D) and the risk factors for vitamin D deficiency among HIV-infected individuals (672 participants) and have compared 25(OH)D levels with those in the general population in US. They concluded that there is an association between ritonavir- and EFV-containing regimens exposure and vitamin D deficiency, suggesting an underlying action of ritonavir on vitamin D metabolism[55].

In spite of that, several studies have failed to prove a correlation between PIs use and vitamin D deficiency. They have found that PIs were associated with increased plasma concentrations of 25(OH)D, while BMD was decreased[55, 61]. These apparently conflicting data might be explained by PIs effects on key enzymes that sustain vitamin D metabolism, thus converting 25(OH)D into inactive compounds and leading to accumulation of 25(OH)D precursors[50, 52]. Many studies have shown the association between ART and reduced BMD, but only few of them have also evaluated vitamin D concentrations, BMD and bone turnover markers, so skepticism about the involvement of vitamin D deficiency in ART-related bone loss is yet legitimate. For instance, Welz et al. evaluated 1077 HIV-infected patients and identified that exposure to EFV and TDF is an independent risk factor for increased bone turnover among patients receiving ART, but not for severe vitamin D deficiency[62]. Also, Fausto et al. assessed bone markers level and vitamin D metabolites in a 48 ART-naive group compared to 113 subjects on ART. They found similar levels of bone metabolism markers in both groups and no prediction value for neither 25(OH)D nor 1,25(OH)2D[39].

Bone loss induced by HAART

Increasing access to antiretroviral therapy has led to substantial improvements in terms of life quality and life expectancy, but also evidence continues to emerge that HIV infection and antiretroviral therapy are established independent risk factors for bone demineralization[10].

Usually HAART regimens include two nucleoside (tide) reverse transcriptase inhibitors (NRTIs) plus a boosted protease inhibitor (PI boosted by ritonavir) or a non-nucleoside (tide) reverse transcriptase inhibitor (NNRTI). Other regimens, less common recommended, consist of two PIs or more than three antiretroviral drug types[3].

There are also researchers who focused their attention on changes in bone density over time in association with ART exposure. Some HAART regimens were found to influence more bone loss, but given the complexity and diversity of the drug regi-

mens used in practice, the contribution of each antiretroviral drug is very challenging to establish[63].

In a meta-analysis performed on 11 cross-sectional studies Brown et al. showed that HIV patients receiving antiretroviral treatment have a 2.5 fold increased prevalence of reduced BMD in comparison to those who are HAART-naive[10].

Some studies have focused their attention on impact of HAART regimens on bone mineral density. Brown et al. showed that after antiretroviral treatment initiation, BMD loss follows, a decrease of 2-6% over the first weeks of therapy[63-65]. This decrease was found to be independent of the ART type used and is similar with the bone loss sustained in women aged 50-59 over 2 years of menopause[66].

Protease inhibitors

Some antiretroviral drugs have been found to have more influence on the consistency of bone demineralization, such as PIs.

The interaction between PIs and bone cells has been analyzed in several in vitro studies, which have shown that some PIs (indinavir, ritonavir) cause bone demineralization by decreasing osteoblast activity, whereas other reports have suggested PIs involvement in bone loss through increased osteoclast activity (ritonavir and saquinavir)[31, 67].

Tebas et al. conducted one of the first studies and a higher prevalence of reduced BMD in patients under PIs treatment was reported. 50% of the patients had osteopenia and 21% of them had osteoporosis[68]. Similarly, Duvivier et al. reported a significant decrease in BMD after 1 year of treatment, in patients receiving one of the PI-containing regimen compared with those who received a combination of NNRTI and NRTIs[65]. But not all authors agree on the negative effect of PIs on bone loss. In some reports, regimens with PIs containing have been found to increase BMD[69].

Nucleoside reverse transcriptase inhibitors

Aiming for the viral reverse transcriptase enzyme, NRTIs determine the premature termination of cDNA chain. It was demonstrated that NRTIs through their mitochondrial toxicity may either directly or indirectly induce BMD loss. (10) In particular, zidovudine was able to intensify RANKL-mediated osteoclastogenesis (in vitro)[2].

Tenofovir

Another NRTI widely used in ART regimens, TDF, was strongly associated with bone demineralization[1]. The mechanism that elucidates the effect of TDF on bone metabolism is not clearly established. TDF causes minimal mitochondrial dysfunction, but it affects phosphate reabsorption in the proximal renal tubule, leading to phosphate wasting, hypophosphatemia and increased bone-turnover[60].
In experimental models, TDF alters the expression of several genes implied in synthesis of proteins with the role of signal transmission, suggesting a TDF-dependent osteoblast dysfunction causes a decrease in BMD[24].

The ASSERT study, that compared the impact of commonly used first-line ART, demonstrated that both groups had bone mineral density loss (more than 6% both at the hip and at the spine). The study enrolled 385 HIV positive subjects, ART-naive, who received 2 fixed-dose combinations: abacavir-lamivudine (ABC-3TC) or tenofovir-emtricitabine (TDF-FTC) with EFV. After the first 24 weeks, it was noticed that bone turnover markers increased in both treatment groups, but higher growth in all the marker levels was found in (TDF-FTC) group[70].
Another report demonstrated that antiretroviral regimens containing (TDF-FTC) (in the spine but also at the hip) and atazanavir plus ritonavir lead to significantly greater BMD reduction at week 96 than (ABC-3TC) or EFV-based antiretroviral combination[71].

Furthermore, Bedimo et al. found an increased risk of incident fracture (HR, 1.16; 95% CI, 1.08–1.24; P<.0001) in the study of the Veterans Affairs Clinical Case Registry which aimed to clarify the consequences of the cumulative treatment containing TDF on the risk of fragility fractures in HIV positive individuals both in the pre-effective ART (1988-1995) and effective ART (1996-2009) yeras[72].

A small retrospective study conducted by Horizon et al. suggested that HIV infected patients on TDF have a higher incidence of foot fractures than in non-TDF treated HIV-positive individuals. The median plasma concentrations of alkaline phosphatase, parathyroid hormone, 25-hydroxyvitamin D were higher in the TDF treated group, and they were found to have a lower median white blood cell count. Median time of TDF exposure was 2.57 years until fracture occurred[73].

Prospective cohort studies of bone mineralization change among HIV-infected persons have reported mixed results. Whereas numerous studies reported the association between HIV infection and bone mineral density, some researchers concluded that BMD is stable or increasing over time. Bolland

et al. meta-analysis investigated how BMD changes and if there are differences according to HAART status at baseline. Their conclusion was that cohorts initiating HAART have a short term increased BMD loss, followed by a longer period of time when BMD is stable or improves[74].

Screening for bone disease in HIV infection

The Clinician's Guide to Prevention and Treatment of Osteoporosis developed by the National Osteoporosis Foundation (NOF) experts recommends DEXA screening for all women aged more than 65 and men aged more than 70 years, regardless of the presence of clinical risk factors. In postmenopausal women and men of 50-69 years of age, DEXA screening should be performed if they have an additional risk factor. Likewise, screening is recommended for younger post-menopausal women and men over age of 50 who have suffered an adult age fracture[75].

Among many factors, diseases and medications known to have been associated with osteoporosis and increased risk of osteoporosis-related fracture, the latest Clinician's Guide to Prevention and Treatment of Osteoporosis (2014), also listed HIV infection/AIDS[80].

McComsey et al. in their review concerning low bone mineral density in HIV infected subjects, emphasize that HIV infection should be considered as a risk factor for bone disease. Taking into consideration that clinical risk factors that contribute to osteoporosis are so prevalent among HIV-infected patients, they have a more aggressive position and suggest DEXA screening for all HIV-infected postmenopausal women and men over age 50 and in all patients who suffered fragility fractures, irrespective of their age[76].

In a recent guide whose aim was to offer guidance on the management of bone disease in HIV infected patients, Brown et al. suggested a detailed algorithm for the screening and the assessment of bone disease in HIV infection. Their recommendations do not entirely overlap with NOF of USA clinician's guide or with European AIDS Clinical Society (EACS) guidelines.

It is recommended that the risk of fragility fracture and low bone mineral density should be appraised in all HIV infected adults. In particular, DEXA screening is indicated for patients considered at high risk for fragility fracture, namely patients with a history of atraumatic fracture, those who have had more than three months glucocorticoid exposure and patients who are at risk of suffering falls.

For those patients who are not at high risk of fragility fracture, men of 40-49 years of age and premenopausal women aged more than 40, the use of FRAX algorithm is suggested. The Fracture Risk Assessment Tool (FRAX score) is a screening tool designed to assess the 10-year probability that the patient would suffer a major fracture (at the hip, spine, forearm, humerus). FRAX is the WHO equation that includes classical risk factors such as age, gender, prior fragility fracture, long-term use of glucocorticoids, tobacco smoking, and history of parental hip fracture[77].

However, there is evidence that FRAX algorithm underestimates the percentage of subjects with reduced bone mineral density, even considering some experts recommendation to include HIV infection as a secondary cause of osteoporosis[78, 79].

Regarding the evaluation with DEXA scan, HIV specialists who contributed to the recent recommendation guide mentioned above, advise screening for: 1) all post-menopausal women, 2) all men above age 50, 3) premenopausal women ≥40 years and for men of 40-49 years of age if an intermediate or high risk is established by FRAX equation (more than 10% the 10-year risk of major osteoporotic fracture) and 4) for patients considered at high risk for fragility fracture irrespective of age[78, 79]. DEXA assessment should be interpreted using T-score when postmenopausal women and men≥50 years of age are screened and Z-scores should be used if subjects younger than 50 years undergo DEXA scan[79].

Treatment considerations

As reduced BMD is a frequent finding for HIV-infected patients, management strategies for patients at high risk for reduced BMD and fragility fracture should be followed. Non-pharmacological recommendations include adequate nutrition (especially calcium and vitamin D intake) and lifestyle changes. Guidelines suggest regular weight-bearing (including walking or jogging) thirty minutes at least 3 days a week and muscle strengthening exercises to reduce the risk of fracture and to improve BMD. Smoking cessation and quitting alcohol abuse are also advised[80].

As a first-line approach calcium intake from the diet should be increased and calcium supplements should be received if dietary calcium is insufficient. It is recommended that HIV-infected subjects receive 1000-1500 mg of calcium and 800-1000 UI of Vitamin D daily[1]. Some experts suggest the target

level for vitamin D to be 30-50 ng/mL, but for the optimal vitamin D replacement doses, further clarifications are necessary[1].

After appropriate evaluation and treatment of secondary causes generating reduced BMD and osteoporosis, pharmacological treatment should be initiated for postmenopausal women and men age 50 and older, presenting a hip or vertebral fracture (clinically found or revealed by vertebral imaging), or a T-score at the lumbar spine, femoral neck, total hip ≤2.5. Treatment is also indicated when low BMD is identified (T-score between -1.0 and -2.5 at the femoral neck or lumbar spine) and FRAX algorithm predict a 10-year probability of a hip fracture ≥3% or a 10-year probability of a major osteoporosis-related fracture≥20%[79, 80].

As a first-line pharmacologic intervention, bisphosphonates are the most commonly used antiresorptive agents[3]. Their mechanism is to inhibit osteoclast-mediated bone resorption and they can be given either weekly (alendronate) or once a month (risendronate or ibandronate). For those who cannot tolerate oral bisphosphonates intravenous dosage forms could be prescribed (ibandronate, zoledronic acid)[76]. In many randomized placebo-controlled trials bisphosphonates have proven effective in reducing fracture in general population, but not many researches have demonstrated bisphosphonates efficiency in HIV positive patients[79]. Guidelines recommend that HIV positive patients with osteoporosis receive alendronate 70 mg once weekly (with calcium carbonate 1000 mg/vitamin D 400 IU per day) or as an alternative to alendronate intravenous zoledronic acid 5 mg[79].

Because of concerns for their long-term safety (there have been reports of osteonecrosis of the jaw and atypical femoral fractures) experts recommend reassessment and cease of bisphosphonates treatment after 3 to 5 years[76, 79].

Second-line therapies such as the selective estrogen receptor modulator raloxifen may be a reasonable alternative to bisphosphonates for postmenopausal women category, but evaluation studies in HIV positive subjects are not available.

Teriparatide, an analogue of PTH (human parathyroid hormone), that has the ability to stimulate the number and/or activity of bone-forming osteoblasts is indicated to be used in cases of very high fracture risk or in cases of failure to the bisphosphonates therapy. After the terparatide therapy (two years usually) rapid decrease was observed in bone mineralization, so antiresorbtive therapy is advised after finishing teriparatide treatment[10, 76]. The safety data of teriparatide administration is however deficient in HIV-positive patients.

Denosumab, a human monoclonal antibody against RANKL, proved to decrease activation of RANKL, therefore osteoclastogenesis, may be indicated in patients with fragility fractures or intolerant to other osteoporosis treatment strategies. However, the safety and efficacy of denosumab have not been yet evaluated in HIV-infected individuals[79].

Considering the role of certain ART regimens in BMD changes, Brown et al., in their recent published guideline, make specific recommendations regarding the avoidance of ART therapies that have been shown to alter skeletal health, including TDF and boosted PIs, in patients at risk for osteoporosis and frailty fractures[79].

Conclusions

Effective antiretroviral therapy has led to an enhanced number of aging HIV-infected individuals, therefore to increased concerns about aging-related comorbidities. Low BMD was shown in several studies to be prevalent among patients with HIV infection. Still, the pathogenesis of bone impairment is partially elucidated, appearing to be multifactorial, caused by established risk factors as well as the presence of HIV itself and HAART therapy. Which are the best and how aggressive the recommendation for screening, diagnosis, monitoring and management of bone disease should be, is yet a field of controversy and uncertainty. Most definitely, the concern for HIV positive patients should be elaborated, considering all the age-related challenges, including modifying risk factors for low BMD or fractures to achieve optimal skeletal health.

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Acknowledgement

This paper is supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/132395

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