

The Role of HIV and Antiretroviral Therapy in Bone Disease

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Abstract

Current knowledge suggests that both HIV and antiretroviral drugs are likely to contribute to bone disorders in patients with HIV infection. This article includes a review and update on the part played by the virus and the drugs in the low bone mineral density of HIV-infected patients, and a discussion about their implications in clinical practice. HIV viral proteins may affect osteoblast and osteoclast function, and many clinical studies have shown that during antiretroviral therapy, especially at the beginning, there is an accelerated bone mineral loss associated with bone resorption markers, which may be of differing intensity depending on the HIV drugs used. Vitamin D insufficiency/deficiency is highly prevalent and in some investigations it has been associated with antiretroviral therapy, more often with regimens based on efavirenz. Recent data suggest that immune reconstitution may play a major role in early antiretroviral therapy-related bone loss. Given the complex interaction between HIV and drugs in causing low bone mineral density, optimization of antiretroviral therapy and preemptive strategies aimed to prevent bone loss during therapy may be of paramount importance. (AIDS Rev. 2011;13:109-18)

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Key words

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Introduction

Investigations conducted over the past decade confirmed that osteopenia and/or osteoporosis are more common in patients with HIV infection than in the general population¹. Furthermore, numerous cases of osteonecrosis have been reported in HIV-infected patients²⁻⁵ and recent epidemiological data suggest that they may be at a higher risk of fragility fractures compared with the general population⁶⁻⁸, raising concerns on the potential magnitude of these conditions in the future.

As HIV-infected populations age, an increase in morbidity and mortality related with bone diseases is expected. Factors that may predispose HIV-infected patients to low bone mineral density (BMD) have been analyzed in several studies (Table 1). Low BMD has been associated with antiretroviral therapy (ART) and HIV infection itself. Specific associations described in epidemiological studies or clinical trials include ART with protease inhibitors (PI)^{9,10}, nucleoside/nucleotide reverse transcriptase inhibitors (NRTI)¹⁰⁻¹⁶, low body mass index (BMI) or weight^{11,17,18}, advanced age¹⁷, male gender¹⁷, smoking¹⁸, lipoatrophy¹¹, coinfection with hepatitis virus¹⁹, longer duration of HIV infection since diagnosis^{11,17,18}, and low CD4 cell count¹⁷.

A controversial issue is how much of the bone mineral loss found in HIV-infected patients is due to the HIV infection itself, and how much is due to antiretroviral drugs. In this article, the currently available data on the part played by HIV infection and ART in accelerating bone mineral loss and their implications in clinical practice are reviewed.

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Table 1. Risk factors for osteopenia/osteoporosis identified in epidemiological studies carried out in HIV-infected patients and in the general population

Risk factors identified in HIV-infected patients	
–	Long duration of HIV infection
–	Low nadir CD4 lymphocyte count
–	Older age
–	Non-black race
–	Male gender
–	Low body mass index
–	Antiretroviral therapy with protease inhibitors
–	Antiretroviral therapy with nucleoside/nucleotide reverse transcriptase inhibitors
–	Cigarette smoking
–	Opiate exposure including methadone
–	Lipoatrophy
–	Coinfection with hepatitis viruses (in women)
Additional risk factors (identified in the general population)	
–	Menopause
–	Physical inactivity
–	Heavy alcohol consumption
–	Nutritional deficiencies
–	Hypogonadism
–	Corticosteroids

Contribution of HIV to bone demineralization

Data from experimental studies suggest that HIV infection *per se* and/or the associated proinflammatory and immune activation state may accelerate bone mineral loss. *In vitro* experiments have shown that HIV viral proteins may have an inhibitory effect on osteoblast function^{20,21} and may also affect osteoclastogenesis²² (Table 2).

HIV gp120 increased the rate of apoptosis in primary osteoblasts²⁰ and reduced calcium deposition, alkaline phosphatase activity and bone-specific Runt-related transcription factor 2 (RUNX-2) expression²¹. An HIV-induced impairment of mesenchymal cell differentiation towards osteoblasts may also contribute to

downregulating osteoblast activity^{21,23}. In addition to inhibiting RUNX-2 protein, HIV gp120 is able to trigger the activation of peroxisome proliferator-activated receptor gamma, both determining a preferential shift of mesenchymal cell differentiation from osteoblasts to adipocytes^{21,23}.

HIV gp120 may also modulate the function and development of osteoclasts. This protein is known to activate expression of different molecules in peripheral blood mononuclear cells such as receptor activator for nuclear factor κB ligand (RANKL), which plays a pivotal role in increasing osteoclastic activity²². Also, HIV viral protein of regulation (Vpr) increased RANKL expression in peripheral blood mononuclear cells²⁴. In addition to upregulating RANKL, HIV infection of macrophages induces a significant increase in macrophage colony stimulating factor (M-CSF) production and secretion²⁵, a cytokine that elicits osteoclast differentiation.

There is also evidence that systemic inflammation in HIV infection may be associated with changes in bone formation and resorption^{26,27}. It has been shown that in addition to RANKL and M-CSF, other cytokines such as the tumor necrosis factor (TNF)-α may induce an increase of osteoclast differentiation and activity, which may favor bone resorption²⁸. The TNF-α also induces apoptosis activation in primary osteoblasts by paracrine/autocrine mechanisms²⁰. Some investigators have found a correlation between HIV plasma viral load and TNF-α concentrations²⁹, and between bone resorption markers and TNF-α activation²⁶.

In some clinical investigations, a higher prevalence of osteopenia and/or osteoporosis was seen in HIV-infected patients not treated with antiretrovirals than in a control group of non HIV-infected patients^{17,30,31}, and in some studies the duration of HIV infection was correlated with bone demineralization^{17,31}. Although these data suggest that HIV infection itself may accelerate bone demineralization, the small sample size of most studies did not allow adjusting for traditional risk factors for low BMD.

Table 2. Possible effects of HIV infection on bone cellular compartment

	Osteoblasts	Osteoclasts
HIV viral proteins	Decrease osteoblast function and increase apoptosis. Reduce cellular differentiation towards osteoblasts.	Increase osteoclastic activity.
Proinflammatory and immune activation state	TNF-α may induce osteoblast apoptosis.	RANKL, M-CSF, and TNF-α may increase osteoclast differentiation and activity.

TNF: tumor necrosis factor; RANKL: receptor activator for nuclear factor κB ligand; M-CSF: macrophage colony stimulating factor.

Many of these factors, such as low body weight, physical inactivity, reduced consumption of calcium and vitamin D, alcohol consumption, cigarette smoking, and opiate exposure, are more prevalent in HIV-infected patients than in the general population.

The baseline measurement of BMD in over 600 ART-naive patients who participated in the clinical trial GS 903 provided information of great interest to determine the contribution of HIV and ART to bone mineralization³². In this study, a prevalence of osteopenia and osteoporosis of 26 and 3%, respectively, was found (mean age of patients, 36 years)³², a significantly higher figure than that expected in the general population. Lower baseline BMD was associated with lower body weight, male gender, and older age at enrolment³².

In summary, there is growing evidence indicating that HIV infection plays a significant role in the bone demineralization seen in HIV-infected patients, which may be more intense in subjects with certain risk factors. Although HIV itself seems to play a role, the proinflammatory and immune activation state associated with uncontrolled HIV infection might also contribute. This situation is associated with an increased production of cytokines, which interact with osteoclasts and/or osteoblasts, affecting bone mineralization.

Contribution of antiretroviral drugs to bone demineralization

The contribution of antiretroviral drugs to bone demineralization in HIV-infected patients continues to be a controversial topic. Antiretroviral therapy has been associated with low BMD in several cross-sectional studies, and analyzed in various review articles^{1,33}. In the meta-analysis carried out by Brown and Qaqish, the odds of osteoporosis was 2.4-times higher in ART-treated patients compared with ART-naive individuals¹. An association has been found with both PI⁹ and NRTI¹¹. The longitudinal epidemiological studies and clinical trials conducted so far also suggest that ART is associated with bone demineralization^{10,12,13,15,16,34-36}. The analysis of changes in BMD in a large sample of patients participating in the INSIGHT Strategies for Management of Antiretroviral Therapy (SMART) study³⁷, an international randomized trial comparing intermittent CD4 lymphocyte count-guided ART with continuous ART, supported this hypothesis. In this study, hip and spine BMD were investigated by annual DEXA in 214 patients over 2.4 years. Continuous ART was associated with a decline in BMD, relative to intermittent, CD4 cell count-guided ART. In all the 5,473 patients participating in

the SMART study, a total of 12 fractures were reported, 10 of them in the continuous ART group (RR: 4.9; 95% CI: 1.1-22.5)³⁷.

Recent data from the iPrEx study, an international randomized, double-blind, placebo-controlled trial evaluating tenofovir/emtricitabine pre-exposure prophylaxis among men who have sex with men also suggest an effect of ART on bone mass in the absence of HIV infection³⁸. In this trial, significant decreases in BMD were observed in those randomized to tenofovir/emtricitabine relative to placebo. The BMD tended to increase in the placebo arm and decreased in the tenofovir/emtricitabine arm, resulting in modest (-0.7 to -1.0%) but statistically significant differences between the groups by week 24³⁸.

Currently, it is agreed that during ART, especially at the beginning, there is an accelerated bone mineral loss, which may be of differing intensity depending on the HIV drugs used. However, the association between the different types of ART or individual HIV drugs and osteopenia/osteoporosis has not been consistent through the studies. The involvement of PI, suggested in some cross-sectional studies, has not been confirmed in most prospective investigations, in which contradictory results have been obtained^{18,34,39-41}, ranging from an increase to a decrease in BMD in patients receiving ART regimens that included these drugs. In some clinical trials evaluating the effect on bone of substituting PI by other types of antiretrovirals, no changes were seen in bone mineralization either^{42,43}.

The changes in BMD in HIV-infected patients starting ART have been analyzed in detail in some randomized clinical trials performed in recent years (Table 3). Although some investigations³⁵, including the SMART study³⁷, did not find an association between bone mass loss and ART composition, other researchers have found significant differences in the intensity of bone demineralization, depending on the ART regimen used¹³. In the MEDICLAS (Metabolic Effects of Different Classes of Antiretrovirals) study, bone demineralization was greater with zidovudine/lamivudine and lopinavir/ritonavir than with nevirapine and lopinavir/ritonavir, which suggests that NRTI may contribute to bone demineralization¹³. The results of the Gilead GS 903 study, a clinical trial comparing tenofovir versus stavudine, indicate that the loss of bone mass after initiating ART may be of differing magnitudes depending on the NRTI regimen used³². Furthermore, the study suggests that bone demineralization occurs mainly during the first months of ART and then tends to become

Table 3. Main randomized clinical trials analyzing changes in bone mass with different antiretroviral treatment regimens

Trial/Reference	Type of patient/ sample size	Regimens compared	Duration of follow-up	Loss of bone mass	Prevalence of osteopenia/ osteoporosis at end of follow-up	Incidence of fractures
Gilead 903 ¹²	Naive/n = 600	TDF+3TC+EFV vs. D4T+3TC+EFV	3 years	TDF+3TC+EFV > D4T+3TC+EFV (femur, 2.8 vs. 2.4%; spine, 2.2 vs. 1%)	No difference	No difference
ANRS 121 sub-study ⁴⁰	Naive/n = 71	PI/r+NNRTI vs. PI/r+NRTI vs. NNRTI+NRTI	1 year	PI/r+NNRTI and PI/r+NRTI > NNRTI+NRTI (lumbar spine, PI/r+NNRTI 4.4%; PI/r+NRTI 5.8%; NNRTI+NRTI 1.5%)	No difference	No difference
Abbott 613 ³⁵	Naive/n = 106	AZI+3TC+EFV vs. AZI+3TC+LPV/r followed by LPV/r	2 years	2.5%; no differences between the groups	No difference	No difference
MEDICLAS ¹³	Naive/n = 50	AZI+3TC+LPV/r vs. NVP+LPV/r	2 years	AZI+3TC+LPV/r > NVP+LPV/r (femur, 6.3 vs. 2.3%; lumbar spine, 5.1 vs. 2.6%)	No difference	No difference
ASSERT ¹⁵	Naive/n = 385	TDF+FTC vs. ABC+3TC in combination with EFV	1 year	TDF+FTC > ABC+3TC (femur, 3.6 vs. 1.9%; lumbar spine, 2.4 vs. 1.6%)	No difference	No difference
STEAL ¹⁶	Pretreated/n = 357	TDF+FTC vs. ABC+3TC	2 years	TDF+3TC > ABC+3TC (mean difference in T score at femur, 0.16; 95% CI: 0.08-0.23)	No difference	No difference
ACTG 5224s ¹⁰	Naive/n = 269	TDF+FTC vs. ABC+3TC in combination with EFV or ATV/r	2 years	TDF+FTC > ABC+3TC (femur, 3.9 vs. 2.6%; p = 0.025; lumbar spine, 3 vs. 1.3%; p = 0.4) ATV/r > EFV (lumbar spine, 3.2 vs. 1.7%; p = 0.035; femur, 3.4 vs. 3.1%; p = 0.59)	No difference	No difference
ACTG 5142 ³⁶	Naive/n = 503	AZI+3TC vs. D4T+3TC vs. TDF+3TC in combination with LPV/r or EFV vs. LPV/r+EFV	2 years	TDF regimens (total body BMD, 2.31-3.98%) > other NRTI (total body BMD, 1.59-2.35%) A trend to greater loss with LPV/r than EFV (2.61 vs. 1.89%)	No difference	No difference

AZI: zidovudine; 3TC: lamivudine; DDI: didanosine; D4T: stavudine; EFV: efavirenz; PI/r: boosted protease inhibitors; NNRTI: nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitors; LPV/r: lopinavir/ritonavir; NVP: nevirapine; TDF: tenofovir; ABC: abacavir; ATV/r: atazanavir/ritonavir.

stable, is quantitatively limited, and, at least in the short term, is of little clinical significance. In this trial, after three years' treatment, a greater decrease in BMD was seen in the tenofovir group than in the stavudine group³². During the open-label extension phase of this trial, an additional decrease in BMD was found at the end of the six years' follow-up in patients who changed from stavudine to tenofovir⁴⁴.

The results at 48 weeks of the ASSERT study, a phase IV clinical trial in which 385 patients with no prior ART were randomized to receive abacavir/lamivudine or tenofovir/emtricitabine, also show that the intensity of bone mass loss after initiating ART differs depending on the NRTI regimen used¹⁵. In this trial, BMD decreased slightly in both treatment arms, but the loss of bone mass was significantly greater in those receiving tenofovir/emtricitabine. Similar results were obtained in the STEAL study, a clinical trial in which patients on stable ART, and virologically suppressed, were randomized to receive abacavir/lamivudine or tenofovir/emtricitabine for two years¹⁶. In this trial, a greater loss in bone mass was also found in those treated with tenofovir, although no differences were found in the incidence of fractures during follow-up.

The ACTG 5142 trial has also provided important information on the impact of different antiretroviral combinations upon BMD. In this study, treatment-naïve subjects were randomized equally to efavirenz plus two NRTI versus lopinavir/ritonavir plus two NRTI versus lopinavir/ritonavir plus efavirenz (no NRTI). Zidovudine, stavudine, or tenofovir (with lamivudine) was selected prior to randomization. There were significant declines in BMD at week 48 that persisted at week 96 among subjects starting each of the regimens. Among the NRTI-containing arms, NRTI selection, especially the use of tenofovir, had a greater impact on BMD than other regimens³⁶.

Recent data from the ACTG A5224s, a substudy of ACTG A5202, a prospective, randomized, partially blinded phase III trial of abacavir/lamivudine or tenofovir/emtricitabine with efavirenz or atazanavir/ritonavir for initial treatment of HIV infection¹⁰, have clarified further the contribution of different ART regimens to the loss of bone mass. In this study, all regimens appeared to produce an initial bone loss, with subsequent stabilization after week 48, but tenofovir/emtricitabine led to greater BMD loss than abacavir/lamivudine in both hip and lumbar spine, and atazanavir/ritonavir led to greater BMD loss than efavirenz in lumbar spine. The decline in BMD occurred

predominantly within the first 48 weeks. Fractures were similarly distributed among study arms¹⁰.

The above studies confirm that there may be detectable differences in short-term bone loss between different treatment regimens in carefully performed studies, but it remains uncertain if these differences persist and become clinically important with long-term follow-up. Although previous longitudinal data from a US cohort showed that BMD was stable in younger HIV-infected men on established ART¹⁸, recent European data suggest that a proportion of those subjects might indeed progress to osteoporosis in the long term^{45,46}.

In summary, the information currently available suggests that ART favors bone demineralization. The loss of bone appears soon after initiation of ART and tends to stabilize after 1-2 years. The magnitude is in the range of 2-6% and may be greater with some ART regimens than with others. Although the association of different ART regimens and/or HIV drugs with bone demineralization has not been shown to be consistent, most randomized clinical trials have found that regimens that include tenofovir lead to greater bone loss than comparators, suggesting that this drug may play a more important role than others. Protease inhibitors may also contribute to bone demineralization, although their role remains controversial.

Antiretroviral-related mechanisms of bone demineralization

Antiretroviral therapy may cause osteopenia to develop by different mechanisms, including direct action of the drugs on the cells that intervene in bone remodeling, or indirectly by increasing renal loss of phosphate or modifying vitamin D or parathyroid hormone (PTH) metabolism (Table 4). It has also been suggested that osteopenia might in some cases be the expression of mitochondrial toxicity associated with nucleoside analogs or the result of calcium mobilization to compensate hyperlactacidemia. Finally, ART might also favor bone demineralization through changes associated with immune reconstitution.

Effects of antiretroviral drugs on bone cellular compartment

A number of experimental studies have shown that some antiretroviral drugs can slow down *in vitro* bone formation by means of an inhibitory effect on osteoblastic function, and some of them may also have a direct

Table 4. Possible effects of antiretroviral drugs on bone cellular compartment and vitamin D or parathyroid hormone metabolism and phosphate balance

	Bone cellular components		Vitamin D or parathyroid hormone metabolism and phosphate balance
	Osteoblasts	Osteoclasts	
Protease inhibitors	Decrease osteoblast activity (indinavir, ritonavir).	Increase osteoclastic activity (saquinavir, ritonavir, nelfinavir, indinavir).	Inhibition of 25-hydroxylase and 1 α -hydroxylase enzymes (ritonavir, indinavir, nelfinavir).
Nucleos(t)ide reverse transcriptase inhibitors		Increase osteoclastic activity (zidovudine, didanosine, lamivudine).	Renal phosphate wasting and reduction in intestinal absorption of phosphate (tenofovir). Elevation in parathyroid hormone plasma concentrations (tenofovir). Decrease in function of 1 α -hydroxylase related to renal toxicity (tenofovir).
Nonnucleoside reverse transcriptase inhibitors			Increase the metabolism of 25-hydroxy vitamin D through upregulation of the 24-hydroxylase.

effect on osteoclasts (Table 4). *In vitro* bone cell cultures have found that different PI may have heterogeneous effects on bone, and suggest that some PI may lead to bone loss by increasing osteoclast resorption and inhibiting the osteoblast rebuilding function^{22,47,48}. With regard to NRTI, it has been reported that zidovudine, lamivudine, and didanosine may activate osteoclastogenesis⁴⁹. Based on the association found between osteopenia and hyperlactacidemia¹¹, it has been suggested that bone demineralization may in some cases be the expression of mitochondrial toxicity associated with treatment with NRTI, or be the consequence of the buffering of the excess lactic acid produced at other sites (e.g. the liver) by calcium hydroxyapatite of the bone, which is subsequently excreted in urine¹¹.

Effects of antiretroviral drugs on vitamin D or parathyroid hormone metabolism and phosphate balance

Vitamin D₃ is a potent calcitropic hormone, essential for the maintenance of a normal bone structure increasing the phosphate bone bioavailability. The biological effects on bone remodeling are exerted by 1,25-dihydroxyvitamin D₃ (calcitriol). Activation of vitamin D to calcitriol involves 25-hydroxylation in the liver, followed by 1 α -hydroxylation of 25-hydroxyvitamin D₃ in the renal proximal tubular cells, whereas vitamin D catabolism is mainly determined by 24-hydroxylase. The vitamin D deficit can progressively determine osteomalacia due to the reduction of phosphates available to bone⁵⁰.

Vitamin D deficiency, usually defined as serum 25-hydroxy vitamin D 25(OH)D < 20 ng/ml (50 nmol/l) or vitamin D insufficiency/deficiency, usually defined as serum 25(OH)D < 30 ng/ml (75 nmol/l), occurs in approximately 60-90% of HIV-infected patients depending on race/ethnicity and season⁵¹⁻⁵⁷. In some epidemiological studies, vitamin D insufficiency/deficiency has been associated with ART^{53,54,58}, suggesting a role for HIV drugs in the metabolism of vitamin D. There are data indicating that nonnucleoside reverse transcriptase inhibitors (NNRTI), tenofovir, and PI may have effects on vitamin D and/or PTH metabolism and/or phosphate balance.

Nonnucleoside reverse transcriptase inhibitors

Efavirenz and other NNRTI can induce cytochrome P450 enzymes and, like anti-epileptics, they might increase the metabolism of 25(OH)D to inactive compounds through upregulation of the 24-hydroxylase⁵⁹. Brown and McComsey⁶⁰ found that ART initiation with efavirenz was associated with significant decreases in 25(OH)D and an increased risk of hypovitaminosis D compared with non-efavirenz regimens. This effect remained significant even after adjustment for baseline 25(OH)D, race, and season of sampling⁶⁰. Mueller, et al.⁵⁶, in a dataset from the Swiss Cohort Study, also found that subjects starting ART with NNRTI-containing regimens (89% on efavirenz) had a greater decrease in 25(OH)D levels than subjects on PI-containing regimens after 12 months⁵⁶. Exposure to efavirenz was also independently associated with vitamin D insufficiency

or deficiency in the cross-sectional analysis of baseline data from the SUN study⁵⁷.

Further information linking efavirenz use with vitamin D insufficiency or deficiency was obtained from the MONET study, a clinical trial that enrolled patients virologically suppressed while taking either NNRTI- or PI-based ART who were switched to darunavir/ritonavir⁶¹. At screening, lower vitamin D levels were associated with season, race, and use of efavirenz and/or zidovudine. Switching from efavirenz and/or zidovudine to darunavir/ritonavir during the trial led to increases in vitamin D levels⁶¹. The results of the ECHO study, a double-blind randomized trial in HIV-1-infected treatment-naive adults comparing rilpivirine with efavirenz, indicate that the effect of NNRTI other than efavirenz on vitamin D metabolism may be different. In this trial, a significant decrease in 25(OH)D levels over 48 weeks was observed with efavirenz but not with rilpivirine. Moreover, patients with 25(OH)D insufficiency at baseline had a significantly lower risk of developing severe 25(OH)D deficiency with rilpivirine than with efavirenz⁶².

Taken together, these data suggest that efavirenz could be associated with a higher risk of vitamin D insufficiency/deficiency, but the exact mechanism whereby the drug may interfere on 25(OH)D metabolism and its long-term clinical impact, if any, still have to be elucidated.

Tenofovir

An impaired phosphorus balance and vitamin D metabolism related to renal toxicity is considered the most likely mechanism of tenofovir-associated decreases in BMD and osteomalacia⁶³⁻⁶⁷. Osteomalacia has been reported in some HIV-infected patients receiving tenofovir, many of them showing nephrotoxicity with hypophosphatemia^{63,65,68,69}. Tenofovir-related renal toxicity may present with tubular dysfunction due to proximal tubular epithelial cell damage, leading to the appearance of hypophosphatemia, and with an impaired glomerular filtration rate. Since the conversion of 25(OH)D to 1,25(OH)2D by 1 α -hydroxylase occurs primarily in the proximal tubule, tenofovir-induced proximal tubule dysfunction might also affect vitamin D activation by reducing renal hydroxylation. In a recent investigation, however, tenofovir use was not associated with lower 1 α -hydroxylation rates⁵⁶.

Antiretroviral therapy with tenofovir regimens has recently been associated with an elevation in PTH plasma concentrations, particularly in patients with suboptimal vitamin D levels⁷⁰⁻⁷². The increase in PTH

occurs early after starting therapy and might therefore play a role in the decrease of BMD associated with the initiation of ART containing tenofovir. Thus, the mechanism of tenofovir-associated bone loss may be more complicated and not entirely due to proximal tubular toxicity leading to phosphate wasting and decreased vitamin D metabolites.

Protease inhibitors

Since 1 α -hydroxylase and 25-hydroxylase enzymes are cytochrome P450 monooxygenases, and PI are potent inhibitors of human hepatic cytochrome P450 enzymes, interference in the bioactivation of vitamin D₃ could be anticipated. Indeed, *in vitro*, ritonavir, indinavir, and nelfinavir decreased hepatic 25-hydroxylase and macrophage 1 α -hydroxylase activity, and were associated with calcitriol degradation, resulting in decreased vitamin D activity⁷³. However, clinical data linking PI with impairment of vitamin D metabolism are scarce and controversial. Some studies have reported an association between ritonavir exposure and lower odds of vitamin D insufficiency or deficiency⁵⁷ and others have found higher levels of 25(OH)D in HIV-infected persons exposed to PI compared with those exposed to NNRTI or naive patients⁷⁴. It has been suggested that a preferential inhibition by PI of the 1 α -hydroxylase, responsible for the conversion of 25(OH)D to 1,25(OH)2D in the kidney, might lead to accumulation of 25(OH)D despite low levels of 1,25(OH)2D⁵⁷.

Effects of immune reconstitution

In HIV-infected patients receiving ART, immune reconstitution is initially rapid, representing redistribution of existing CD4 T-cells with the release of functional memory CD4 T-cells from lymphoid tissues. T-cell recovery with ART reaches a significant magnitude within 12 weeks after initiation of therapy, a timeframe coinciding with the maximum ART-related bone loss. During immune reconstitution, changes take place in circulating cytokine concentrations, including RANKL, which may exert different regulatory effects on bone formation and resorption²⁷. Therefore, a potential role for T-cell reconstitution in ART-related bone loss has been proposed. Recent results from a clinical and experimental study conducted by Oforokun, et al.⁷⁵ suggest that immune reconstitution may indeed play a major role in ART-related bone loss. In this study, following ART initiation in treatment-naive HIV patients, there was an

early increase in bone resorption, peaking at week 12 and remaining significantly elevated at week 24, accompanied by an elevation of RANKL. Interestingly, by means of T-cell adoptive transfer into T-cell null T-cell receptor- β knockout mice, they detected an increase in bone resorption concurrent with pronounced loss of BMD. This was associated with elevated RANKL and TNF- α within the same 12-week window in which resorption peaks in humans initiating ART⁷⁵.

Implications for clinical practice

Given the complex interaction between HIV and drugs causing low BMD, optimization of ART and preemptive strategies to prevent bone loss during therapy may be of paramount importance. From the point of view of prevention, it is advisable to act on factors that may favor osteoporosis, such as physical inactivity, smoking and alcohol consumption, nutritional deficiencies, and hypogonadism, all of which are more common in HIV-infected patients than in the general population. In addition, it should be borne in mind that certain drugs used to treat conditions associated with HIV infection, such as methadone, corticoids, anticonvulsant drugs, and pentamidine, may also favor bone demineralization. A sufficient intake of calcium is essential for an adequate BMD and it may be advisable to administer calcium supplements in some cases, especially when the patient's diet is low in calcium or there is malabsorption.

Since the absorption of calcium depends on adequate levels of vitamin D, and given the high prevalence of vitamin D insufficiency/deficiency found in several clinical studies, it may be reasonable to screen for vitamin D circulating levels in all HIV-infected patients to detect and treat vitamin D deficiency early, as promoted by the European AIDS Clinical Society⁷⁶, and to provide treatment/supplementation to reach levels of 25(OH) vitamin D in the range of 40-60 ng/ml. The evaluation of vitamin D levels is especially advisable in efavirenz-treated and dark-skinned HIV-infected individuals, to detect and treat vitamin D deficiency early.

A recent meta-analysis of the use of calcium in combination with vitamin D supplementation to prevent fractures and bone loss in HIV-uninfected people aged 50 years and older showed a 12% reduction in the risk of fractures in both females and males; the risk reduction increased to 24% in those reporting good adherence and receiving daily doses of at least 1,200 mg of calcium and 800 IU of vitamin D⁷⁷.

To date there is no evidence that the presence of risk factors of bone demineralization or a reduced BMD should determine which ART is chosen, although in these cases the use of tenofovir should probably be avoided, especially in children. Vitamin D₃ supplementation has recently been associated with significant decreases in PTH in HIV-infected youths on tenofovir-containing ART in a 12-week randomized, double-blind, placebo-controlled study, regardless of baseline vitamin D status, and might therefore be a potentially useful strategy to prevent tenofovir-related bone loss⁷⁸.

Conclusions and future prospects

In summary, *in vitro* experiments and clinical studies support that both HIV infection and ART may accelerate bone mineral loss. Antiretroviral therapy may cause osteopenia by direct action of the drugs on bone cell components, or indirectly by increasing renal loss of phosphate or modifying vitamin D metabolism, but the exact mechanisms still have to be worked out. The loss of bone associated with ART appears soon after initiation therapy and tends to stabilize after 1-2 years. Recent data suggest that immune reconstitution may be a driving force behind early ART-related bone loss. Most randomized clinical trials have found that regimens that include tenofovir lead to greater bone loss than comparators, suggesting that this drug may play a more important role than others. Tenofovir has been associated with renal phosphate wasting and elevation of PTH. Protease inhibitors may also contribute to bone demineralization, although their role is less clear. Whether the greater bone loss associated with specific ART regimens will translate into a higher incidence of fragility fractures in the long term remains uncertain. Vitamin D insufficiency/deficiency appears to be highly prevalent in HIV-infected patients and in some investigations it has been associated with ART, more often with regimens based on efavirenz. However, the long-term impact of the different ART regimens on vitamin D levels and its clinical consequences also remain unclear.

Currently, there is no evidence that changing ART improves outcomes of HIV-patients with bone diseases, although the use of regimens associated with bone demineralization should be avoided in patients with significant bone mass loss or high risk of fragility fractures. Since ART-related bone loss begins early during therapy, strategies aimed to block skeletal decline, including vitamin D₃ supplementation and preemptive

antiresorptive therapy, could potentially be useful in patients initiating ART and should be evaluated further in properly designed clinical trials.

Basic knowledge of pathogenetic mechanisms of bone diseases, along with data on long-term safety and efficacy of different therapeutic strategies, will be essential for establishing specific recommendations for preventing and treating bone diseases in this population.

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Disclosure statement

The authors declare no conflicts of interests.

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