Pathogenesis of bone disorders in HIV infection

Iatrogenic long-term complications of antiretroviral therapy have become a focus of intense research in patients with HIV infection. Antiretroviral therapy has almost normalized the survival of newly infected and drug-adherent individuals, but skeletal complications are of growing concern in the aging population. It has become clear that the prevalence of osteoporosis and related fractures in HIV-infected subjects has increased. Risk factors are multifactorial and include a low body mass index, smoking and possibly viral replication itself and antiretroviral treatment. Treatment with tenofovir in particular may induce osteomalacia in some patients. HIV infection is also complicated by osteonecrosis, which is approximately 100-fold more prevalent than in the general population. Biphosphonates represent the first-line therapy for osteoporosis in HIV-infected patients, but their effects on fractures have not yet been evaluated.

KEYWORDS: AIDS ▪ antiretroviral therapy ▪ bone disease ▪ HIV ▪ NRTI ▪ nucleoside analog reverse transcriptase inhibitor ▪ osteomalacia ▪ osteonecrosis ▪ osteopenia ▪ osteoporosis ▪ protease inhibitor

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Since 1996, HIV-infected patients have been typically treated with an antiretroviral combination therapy (ART) consisting of three different substances that are chosen from different classes of antiretrovirals, including nucleoside analog reverse transcriptase inhibitors (NRTIs), non-nucleoside analog reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). After the widespread implementation of ART, AIDS-defining complications and malignancies have declined steeply. However, with prolonged patient survival, the long-term effects of chronic HIV infection and its treatment have become an area of intensive research. An increased prevalence of bone demineralization and other osseous complications has been observed among HIV-infected subjects and have recently been the subject of more detailed studies [1–3]. This review examines the epidemiology, pathogenesis and management of osteoporosis and osteonecrosis – the most common bone disorders in HIV-infected subjects.

Normal regulation of bone turnover

Bone tissue undergoes a continuous remodeling process consisting of resorption and formation of its calcified matrix. Osteoblasts generate osteoid and are normally in synchronized balance with the number and activity of osteoclasts. This equilibrium of bone remodeling is crucial in maintaining calcium homeostasis, as well as maintaining the biomechanical properties of the osseous architecture. Disproportional increases in bone resorption result in lower bone mineral density (BMD), microarchitectural deterioration and predisposition to fracture. Osteoblasts derive from mesenchymal stem cells while osteoclasts originate from hematopoietic cells similar to monocytes and macrophages. On a molecular basis, the bone remodeling process is governed by complex intercellular signaling. Osteoblast function and development is regulated by bone morphogenetic proteins (BMPs) and the activation of the transcription factor RUNX-2 [4].

Osteoclastogenesis, on the other hand, is predominantly regulated by the OPG/RANKL/RANK system. The receptor activator of NF-κB ligand (RANKL) is expressed by osteoblasts and activated T cells and acts as the key mediator of osteoclastogenesis by promoting the formation and functional development of osteoclasts, thus enhancing bone resorption [5,6]. RANKL binds to its receptor RANK on osteoclast precursors [6]. RANK signaling then triggers the activation of NF-κB, its translocation to the nucleus and transcription of osteoclastic genes. The RANKL–RANK interaction is blocked by osteoprotegerin (OPG), a bone-protecting member of the TNF-receptor family that binds to RANKL, thereby acting as its soluble decoy receptor and inhibiting osteoclast differentiation [7]. Bone metabolism is also tightly regulated by hormones, including parathyroid hormone, 1,25-dihydroxy-vitamin D_3, calcitonin, estrogens and androgens. Furthermore, local factors such as IL-1 and IL-6, prostaglandins,

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TNF-α, prostaglandins, TGF, colony stimulating factors (CSFs), and IFN-γ, play an important role. TNF-α in particular augments osteoclast proliferation and survival [6].

HIV effects on bone turnover

Several viral components appear to directly regulate the activity of cultured osteoblasts, osteoclasts and macrophages with respect to bone turnover (Table 1). HIV-1 appears not to induce a cytopathic effect [8] and not to directly infect cultured osteoblasts [9], but HIV-1 glycoproteins (p55-gag and gp120) have been seen to reduce calcium deposition, alkaline phosphatase activity, RANKL secretion, BMP levels and expression and activity of RUNX-2 in human osteoblasts [10,11]. p55-gag also had similar effects in human mesenchymal stem cells [10,11]. Furthermore, gp120 was found to induce RANKL in T cells [12]. On the contrary, HIV-1 rev, a regulatory protein essential for viral replication, appears to enhance the mineralization rate, RUNX-2 activity and secretion of BMP-2 [11], while the HIV-1 accessory viral protein fosters the production of RANKL by osteoblasts [13]. In infected macrophages, HIV-1 induces macrophage CSF (M-CSF), and thus promotes macrophage proliferation and differentiation [14–16]. RANKL and M-CSF bind to their respective receptors on osteoclastic precursor cells and induce osteoclast proliferation and differentiation [14]. M-CSF also enhances osteoclastogenesis by downregulating the secretion of OPG in bone marrow macrophages [17].

Several cytokines that are upregulated in HIV-infection (IFN-γ, TNF-α, IL-1, -3 and -4) also foster M-CSF production [18]. IL-1, TNF-α and TNF-β are also direct stimulators of osteoclastogenesis [6,19,20]. Recent *in vitro* findings indicate that HIV-1 may also impair bone homeostasis by inducing TNF-α-mediated osteoblast apoptosis [21].

Thus, *in vitro* work has identified a plethora of mechanisms by which HIV infection may enhance bone loss. The following section will examine the evidence for HIV effects on bone *in vivo*.

Osteopenia & osteoporosis in HIV-infected individuals

In clinical practice, bone loss is usually measured by dual energy x-ray absorptiometry (DEXA). DEXA assesses the BMD as the amount of matter per cm^3 of bone in g/cm^3. In individuals, BMD results can also be expressed as a T-score, which refers to the number of standard deviations above or below the mean BMD of a population of healthy sex-matched adults at the age of their peak bone density (30 years). The Z-score refers to the number of standard deviations above or below the mean for the patient's age and sex. The WHO defines osteopenia as a T-score between -1 and -2.5, and osteoporosis as a T-score below -2.5 [22].

Prior to the era of potent antiretroviral therapy, investigators noted several abnormalities in the bone metabolism of HIV-infected patients.

Table 1. Effects of HIV-1 on osteoblasts and osteoclasts *in vitro* and animal models.

Study	Model	Main findings	Ref.
Viral factors			
Mellert *et al.* (1990)	hOB-like cells	HIV-1 does not induce a cytopathic effect	[8]
Nacher *et al.* (2001)	hOBs from HIV-infected patients	hOBs do not express CD4; HIV is not detected in osteoblast cultures from HIV-infected patients	[9]
Fakkrudin *et al.* (2003)	Human PBMCs	HIV gp120 induce RANKL secretion by PBMCs and increase osteoclastic activity	[12]
Fakkrudin *et al.* (2005)	Human PBMCs	HIV Vpr increases RANKL expression in PBMCs in a glucocorticosteroid-receptor-dependent mechanism. Vpr acts synergistically with exogenous glucocorticosteroids	[13]
Cotter *et al.* (2007)	Human MSCs hOBs	HIV p55-gag and gp120 reduce calcium deposition, ALP activity and lower levels of BMP, RANKL and RUNX-2. HIV components impair MSC differentiation into osteoblasts	[10]
Gibellini *et al.* (2008)	HOBIT Primary hOBs	HIV induces TNF-α and triggers apoptosis via an interaction between gp120 and cell membranes	[21]
Cotter *et al.* (2008)	Human MSCs	HIV rev enhances mineralization, RUNX-2 activity and BMP-2 secretion. HIV p55-gag has opposite effects and also reduces alkaline phosphatase activity. The effects are dependent on MSC differentiation status	[11]

ALP: Alkaline phosphatase ; BMP: Bone morphogenetic protein; gp: Glycoprotein; hOB: Human osteoblast; HOBIT: Human osteoblast-like initial transfectant – osteoblast derived cell line; MSC: Mesenchymal stem cell; PBMC: Peripheral blood mononuclear cell; RUNX: Runt-related transcription factor; Vpr: Viral protein of regulation.

Table 2. Bone mineral density in HIV-infected patients: clinical studies.

Study	n HIV (n control)	% male (% control)	Study design	Main findings	Risk factors for low BMD	Ref.
Bruera *et al.* (2003)	111 (31)	80 (77)	Cross-sectional; HIV infected versus uninfected	BMD was lower and prevalence of osteopenia and osteoporosis was higher in HIV-infected patients compared with healthy controls. HIV-infection time correlated with BMD. HIV RNA and $CD4^+$ count were not associated with osteoporosis	–	[28]
Mondy *et al.* (2003)	128	86%	Longitudinal (72-week follow-up)	Lumbar and spinal BMD increased during the observation period. Duration of HIV infection associated with osteopenia	Low BMI	[26]
Amiel *et al.* (2004)	148 (81)	100 (100)	Cross-sectional; HIV infected versus uninfected	Z-scores were lower in HIV-infected patients at lumbar spine and femoral neck. The prevalence of osteoporosis was 16% in HIV-positive individuals and 4% in controls. HIV patients had lower bone alkaline phosphatase and higher urinary cross-laps/Cr	Low BMI	[47]
Brown *et al.* (2004)	51 (22)	86 (82)	Cross-sectional; HIV infected versus uninfected	63% of HIV-infected patients had a T-score of less than -1.0 at the spine, hip and/or forearm compared with 32% in the control group.	Dyslipidemia	[27]
Dolan *et al.* (2004)	84 (63)	0 (0)	Cross-sectional; HIV infected versus uninfected	Lumbar spine and total hip BMD were reduced in HIV-infected women. The prevalence of osteopenia was 54% (vs 30%).	Low BMI and urinary *N*-telopeptides type 1 collagen	[49]
Yin *et al.* (2005)	31 (186)	0 (0)	Cross-sectional; Postmenopausal HIV infected versus uninfected	BMD was lower at lumbar spine and hip in HIV-infected patients. Osteoporosis was more prevalent at lumbar spine (42 vs 23%) and hip (10 vs 1%). AIDS diagnosis and $CD4^+$ nadir were not predictors of BMD.	Low BMI	[89]
Arnsten *et al.* (2006)	263 (232)	0 (0)	Cross-sectional; over 40 years of age HIV infected versus uninfected	Femoral neck and lumbar spine BMDs were reduced in women with HIV compared with HIV-negative controls. HIV infection was associated with BMD only in non-Black women.	Low body weight	[90]
Bolland *et al.* (2006)	59 (118)	100 (100)	Cross-sectional; HIV infected versus uninfected	BMD at hip but not spine was lower in HIV-infected subjects but HIV infection was not an independent BMD predictor at any site.	Low BMI	[91]
Dolan *et al.* (2006)	100 (100)	100 (100)	Longitudinal (2-year follow-up)	BMD was reduced at the spine, hip and femoral neck among women with HIV. No further bone loss during follow-up. Duration of HIV infection was a risk factor for bone loss.	Low BMI	[29]
Dolan *et al.* (2007)	152 (100)	0 (0)	Cross-sectional; HIV infected versus uninfected	BMD was significantly lower at lumbar spine, total hip and femoral neck in HIV-infected patients compared with controls.	Low BMI and low lean body mass	[34]
Arnsten *et al.* (2007)	328 (231)	100 (100)	Longitudinal; HIV infected versus uninfected	Femoral neck and lumbar BMD was significantly lower in HIV-infected patients compared with controls. Low BMD was associated with incident fractures.	Low BMI, no methadone therapy	[40]
Prior *et al.* (2007)	138 (402)	0 (0)	Cross-sectional; HIV infected versus uninfected	BMD in spine and femur was similar in HIV-infected patients and controls. The prevalence of lifetime fragility fractures was higher in HIV-infected patients versus controls (26 vs 17%).	–	[39]
Gibellini *et al.* (2007)	31 (30)	100 (100)	Cross-sectional	Osteoprotgerin and RANKL increased in the plasma of HIV-infected, ART-naive individuals compared with healthy blood donors. RANKL correlated with HIV RNA. Osteopenia or osteoporosis in 40% of HIV-infected patients.	–	[92]
Cazanave *et al.* (2008)	492 (0)	73 (0)	Cross-sectional	The prevalence of osteopenia was 54%, the prevalence of osteoporosis was 27%. Osteoporosis predominated at femoral neck for men and for women. Low plasma HIV load was associated with BMD loss in men. Low $CD4^+$ lymphocyte nadir was associated with low BMD in women.	Low BMI, homosexual transmission	[23]

ART: Antiretroviral combination therapy; BMD: Bone mineral density; BMI: Body mass index; CR: Creatinine.

Bone histomorphometry revealed diminished bone remodeling in comparison with HIV-uninfected controls [1]. Serum osteocalcin was lower in patients with advanced HIV and correlated positively with the number of $CD4^+$ T lymphocytes [1].

Several cohort studies have compared the effects of HIV on bone and found a substantially lower BMD in both men and women (Table 2).

In the largest of these cohorts (492 HIV-infected French patients), osteopenia was found in 55% of men and 51% of women, and osteoporosis was observed in 34% of men and 8% of women [23]. The frequency of osteoporosis was higher compared with other studies, which reported prevalence rates ranging from 0 to 22% [23–27]. This was explained by the absence of French references for the T-score for men. An American database had to be used for comparison and perhaps led to an overestimation of the prevalence of male osteoporosis.

The notion that the HIV infection itself contributes to bone loss is also supported by studies that associated osteopenia and osteoporosis with higher HIV RNA levels [24], as well as with time of HIV infection [26,28,29].

The finding of a higher prevalence of bone loss with low plasma viral load in one study [23] is, at first glance, contradictory to other reports that hypothesized a role of HIV itself. This finding may result from the harmful effects of ART on bone (see below).

One study randomized 600 ART-naive patients (mean age of 36 years) to one of two different antiretroviral combination regimens [30]. The prevalence of osteopenia at baseline as measured by DEXA was approximately 25%, a figure that was significantly higher than the national prevalence among US adults.

A recent meta-analysis has confirmed an increased prevalence of osteoporosis (15%) in HIV-infected individuals [3]. Older age, homosexual transmission, low body mass index (BMI) and low HIV-plasma viral load were independently associated with low bone mineralization in men, whereas older age and a low $CD4^+$ lymphocyte nadir were associated with bone loss in women [23].

Established risk factors for bone loss independent from HIV infection [31] also contribute to the risk of osteoporosis in HIV-infected subjects (Table 3) [24]. In the HIV-infected population, low body weight, physical inactivity, alcohol use, cigarette smoking and vitamin D deficiency may in fact be particularly prevalent [26,32]. Furthermore, in typical also for the HIV-uninfected population, lumbar spine T-scores correlated positively with body weight, and inversely with increased age; high T-scores were associated with male gender. A meta-analysis of ten studies reporting BMD and BMI in HIV-infected adults revealed that the body weight of HIV-infected patients is on average 5.1 kg lower than that of controls [33]. Other investigators found an increased prevalence of low spinal and hip bone densities in association with low body weight, oligomenorrhea and reduced serum testosterone among HIV-infected women [34]. Androgen deficiency is common among HIV-infected women and may contribute to bone loss [35]. Furthermore, low albumin, catabolic steroid use and menopause may accelerate bone loss [36].

In summary, the exact underlying reasons for low BMD in the HIV-infected population are still unclear but probably multifactorial [2,3,26,36,37]. Therefore, owing to the lack of adjustment for body weight in most studies, it cannot be confidently concluded that the HIV infection itself is a risk factor for bone demineralization. HIV infection may no longer be an independent predictor of BMD after adjusting for body weight differences [33]. Therefore, a definite role of HIV in the pathogenesis of bone loss remains to be proven.

Fractures in HIV-infected individuals

Fracture risk in HIV infection has only recently been evaluated. In these studies, HIV-infected subjects had an increased risk of vertebral, hip and wrist fractures compared with HIV-uninfected individuals [38]. In another cohort of 492 HIV-infected patients, 50 patients (10.2%) had at least one pathological fracture [23]. In a Canadian study, 138 HIV-infected women reported a significantly higher incidence of lifetime fragility fractures compared with 402 female controls (26.1 vs 7.3%) [39]. Both groups were matched for the most important risk factors for osteoporosis, but the HIV-infected women had a higher prevalence of smoking and intravenous drug use (53%), were more often treated with glucocorticosteroids, had oligomenorrhea and reported weight cycling. Interestingly, no significant differences were found in the BMD between both groups [39]. Changes in bone microarchitecture or an increased frequency of falls in HIV-infected women represent a possible explanation for the increased fracture rate in the absence of BMD differences. However, other investigators found an increased risk of fractures in association with a low BMD in the population of HIV-infected patients [40].

Table 3. Risk factors for bone demineralization and studies indicating increased prevalence in HIV-infected patients.

Variable	Risk factors for osteopenia/osteoporosis*	HIV-infected patients ref.
Nutrition, lifestyle	Vitamin D deficiency	[32,93,94]
	Low dietary calcium intake	–
	Cigarette smoking	[26,29,48]
	Consumption of more than 16 g alcohol per day	–
	Immobilization	[36]
	Lack of strength training	[36]
	Low body weight/BMI	See Table 2
	Diabetes mellitus	[27]
Demographic attributes	Gender	[24,27]
	Race	[40,90]
	Increased age	[23,24,40,90]
Others	Family history of osteoporosis or hip fracture	–
	Hormonal	–
Hormonal	Renal insufficiency	–
	Hyperparathyroidism	[32,94]
	Hyperthyroidism	–
	Low free testosterone	[29,34,40]
	Oligomenorrhea	[34]
	Postmenopausal status	[29,36,89]
Chronic inflammation	Rheumatoid arthritis, other conditions	–
Medication	Corticosteroids	[26,36]
	Anticonvulsants	–
	Opiates	[40,90,95]

BMI: Body mass index.
**Data taken from* [31].

Influence of antiretroviral therapy on bone metabolism

In HIV-infected individuals, RANKL serum levels were significantly higher in patients treated with ART than those not receiving ART [41]. RANKL serum levels were inversely correlated with lumbar spine BMD and positively correlated with urinary deoxypyridinoline excretion as a marker of bone resorption [41]. In HIV-infected patients exposed to long-term ART, a low BMD was associated with a high bone turnover and high levels of OPG [32]. In analogy with other conditions of high bone turnover, the elevated OPG levels may represent a protective response to counteract bone loss [42]. However, another study by Seminari *et al*. did not demonstrate elevated RANKL serum levels in osteopenic patients, nor did it find a difference in the OPG:RANKL ratio between osteopenic and nonosteopenic subjects with HIV infection [32]. Although the plasma levels of RANKL and OPG are generally poorly correlated with bone status, there appears to be a clinical correlation since a meta-analysis confirmed an increase of the odds of osteoporosis in ART-treated compared with ART-naive subjects (odds ratio: 2.4) [3]. We will now review the effects of the individual components of the antiretroviral combination regimens on osteoporosis. An overview of the effects of ART on bone is given in Tables 4 & 5 for the preclinical and clinical studies, respectively.

Effects of protease inhibitors

In vitro experiments suggest that some PIs induce the expression of proinflammatory chemokines in primary human osteoblasts, an effect that could contribute to the development of decreased BMD in HIV patients [43]. There is also evidence that some PIs alter the

Table 4. Effects of antiretroviral drugs on osteoblasts and osteoclasts *in vitro* and preclinical models.

Study	Model	Main findings	Ref.
Jain *et al.* (2002)	Rat	Nelfinavir, indinavir, saquinavir and ritonavir, but not lopinavir and amprenavir, increase osteoclast activity in neonatal calvaria. Unlike the other PIs, lopinavir and nelfinavir decrease osteoblast activity	[46]
Wang *et al.* (2002)	Mouse	Indinavir decreases vertebral and peripheral BMD. Indinavir reduces cortical and trabecular bone mass and volume. Osteoclast and osteoblast remain unchanged	[96]
Fakkrudin *et al.* (2003)	Murine osteoclast precursors; human PBMCs	Ritonavir and saquinavir, but not indinavir or nelfinavir, enhance osteoclast activity	[12]
Wang *et al.* (2004)	Murine osteoclast precursors	Ritonavir inhibits osteoclast differentiation downstream of RANKL signaling	[97]
Pan *et al.* (2004)	Murine osteoclast precursors	Zidovudine stimulates osteoclastogenesis by upregulating NF-κB downstream of RANKL	[51]
Pan *et al.* (2004)	Mouse	Zidovudine lowers BMD by inducing osteoclastogenesis	[51]
Pan *et al.* (2006)	Murine osteoclast precursors; human PBMCs	Zidovudine, didanosine and lamivudine induce RANKL-mediated osteoclastogenesis. This effect is not regulated by TNF-α.	[98]
Pan *et al.* (2006)	Mouse	Zidovudine, didanosine and lamivudine induce RANKL-dependent osteoclastogenesis and osteopenia. No additive or synergistic effects by NRTI combinations	[98]

BMD: Bone mineral density; NRTI: Nucleoside analog reverse transcriptase inhibitor; PI: Protease inhibitor; PMBC: Peripheral blood mononuclear cell.

physiological regulation of the RANKL/RANK balance. Osteoclast differentiation promoted by RANKL is physiologically inhibited by IFN-γ [44,45]. In precursor cells of murine osteoclasts and primary human osteoclasts, the PIs ritonavir and saquinavir suppress the physiological block of IFN-γ on the effects of RANKL, resulting in increased osteoclast formation [12]. Other PIs, such as indinavir and nelfinavir, had no impact on this system. However, there are conflicting reports concerning the effects of particular PI on osteoblasts, osteoclasts and bone formation. In a rat model, ritonavir, nelfinavir, indinavir and saquinavir, but not lopinavir or amprenavir, were associated with increased osteoclast activity [46].

The first larger clinical study that examined the effects of ART on bone was published in 2000 [2]. In this cross-sectional study, DEXA scans were performed in 112 men. A total of 60 out of 95 HIV-infected patients received PI, while 17 men were HIV-uninfected controls. The prevalence of osteopenia or osteoporosis was significantly higher in subjects receiving PI (50%) compared with those not receiving PI (23%) and controls (29%). However, several subsequent longitudinal studies have not confirmed the association between bone loss and PI treatment [23,26,28,40,47].

It seems that not all PIs are associated with bone demineralization. In 54 patients studied for 1 year, a low pretreatment BMI was associated with a low BMD at baseline, whereas treatment with the PI indinavir or nelfinavir was associated with stable or even increasing BMD at follow-up [25]. This effect of nelfinavir was also found on extremity bone mineral content. However, treatment with other PIs was paralleled by decreases in bone mineral content [48,49].

A meta-analysis reviewed 14 studies that investigated the effects of PI on BMD and confirmed slightly increased odds of osteoporosis in HIV-infected patients receiving a PI compared with those not treated with a PI [3]. When the meta-analysis was restricted to studies that had been adjusted for potential confounders, the odds remained similar [3].

A special situation results from the pharmacological interference of ritonavir with the activity of the hepatic cytochrome P450 3A4, which is responsible for the metabolism of corticosteroids and many other drugs. After fluticasone inhalation for asthma, six HIV-infected and PI-treated patients developed Cushing syndrome [50]. Three of these cases were complicated by osteoporosis and one by a fracture [50].

Effects of NRTIs

In vitro, the NRTI zidovudine enhances the osteoclastogenesis of bone marrow osteoclast precursors in the presence of RANKL [51]. Spine sections of zidovudine-treated mice showed osteopenia with markedly increased osteoclast and unchanged osteoblast numbers, suggesting that the bone loss is also caused by increased osteoclastogenesis *in vivo* [51]. In addition to zidovudine, two other NRTIs (didanosine and lamivudine) induced RANKL-dependent osteoclastogenesis *in vitro* and osteopenia in mice [52]. However, the combinations of these NRTIs did not cause additive or synergistic effects in bone, unlike their additive or synergistic toxicity in other tissues [53,54].

A French cohort did not find a general association between exposure to the NRTI class of antiretrovirals and BMD loss [23]. However, the evaluation of a possible effect of single NRTIs

and their association with BMD showed different results. In patients on zidovudine, for example, the mean annual decrease of extremity BMD was 1.2%, while patients on stavudine showed less decrease (-0.39%) [48]. The location of the DEXA scan also appears to be relevant since in the same study patients on stavudine had increases in trunk BMC. Another prospective study in men using stavudine calculated a 0.3% gain of total body BMD during the 3-year observation period [36]. In conclusion, current data do not indicate that NRTIs lower BMD in patients, but individual NRTIs may have a significant impact on bone metabolism. Two further interesting candidates seem to be tenofovir and didanosine as participants treated with one of these substances had greater decreases in total BMD over time compared with patients on ART without tenofovir or didanosine. The calculated annual loss of total BMD in men on didanosine was 0.84% after 1 year compared with an annual loss of 1.37% after 3 years, indicating a possible delayed onset of bone toxicity. However, the highest annual BMD loss was noted for tenofovir (2.04%) [36].

Tenofovir

Tenofovir is an NRTI with a favorable safety profile with respect to most organ systems [30]. However, preclinical studies in rhesus monkey and other vertebrates have demonstrated that tenofovir inhibits cortical bone mineralization [55,56]. In animals and humans, the use of tenofovir is associated with renal phosphate loss due to Fanconi syndrome, particularly at supratherapeutic dosing and with prolonged drug exposure [57,101]. A clinical trial has demonstrated diminished tubular phosphate reabsorption and an elevated alkaline phosphatase level after only 12 weeks of tenofovir treatment [58]. Tenofovir is taken up into the proximal renal tubules by organic anion transporters. Within the renal tubule, high intracellular drug concentrations are likely to induce a functionally relevant loss of mitochondrial DNA copy numbers [59], leading to mitochondrial toxicity and proximal tubular dysfunction, possibly with renal phosphate loss. Lesions similar to hypophosphatemic rickets and osteomalacia were found as a consequence of impaired osteoid mineralization that developed from tenofovir-induced chronic hypophospatemia in macaques [55].

The Gilead Study 903, which followed ART-naive patients for a 144-week, double-blind period after randomization to either tenofovir and lamivudine plus efavirenz, or the combination of stavudine and lamivudine plus efavirenz, demonstrated larger decreases of spinal BMD in the tenofovir arm (2.2%), compared with the stavudine arm (1%). However, BMD in the hip decreased to a similar extent (2.8% in the tenofovir arm and 2.4% in the stavudine arm) [30]. The majority of the changes were observed after the first 24–48 weeks and were not progressive until the final 144-week follow-up visit. A total of 86 HIV-infected patients that were followed in the ongoing additional open-label extension phase of Study 903 did not exhibit an additional bone loss between week 144 and week 288, but the participants were supplemented with vitamin D_3 (400 IU) and calcium citrate (630 mg) after week 144 [60].

A longitudinal study in children demonstrated a significantly decreased BMD during a 48-week course of tenofovir exposure [61]. A recent report described two HIV-infected children who developed hypophosphatemia and osteopenia associated with limb pain while being treated with tenofovir for 12–18 months. Switching to a tenofovir-free regimen was paralleled by clinical and laboratory improvement in both cases [62]. Symptomatic osteomalacia due to tenofovir nephrotoxicity has been described in further cases [63,64]. Osteomalacia should be suspected in the setting of hypophosphatemia and elevated alkaline phosphatase. Skeletal scintigraphy may reveal pseudofractures (Looser's zones), which are not evident in routine radiographs [Walker UA, Basel University, Switzerland, unpublished observation]. Observations with adefovir, another nucleotide analog with the same mechanism and side-effect profile, indicate that osteomalacia may improve during the course of a few weeks when the nucleotide analog is discontinued and phosphate is substituted [65].

Effects of NNRTIs

The influence of NNRTI-based regimens on BMD has been evaluated in 42 NNRTI-treated patients and compared with 47 ART-naive subjects. In the study group, 39% of patients had osteopenia and 12% had osteoporosis. No significant differences were found between the two groups [37]. Predictors of bone loss were older age and low BMI. Consideration of only NNRTI-treated subjects, older age, low BMI and more prolonged exposure to NNRTI was predictive of bone loss. The French cohort and other studies also failed to find a significant association between low BMD and NNRTI [23,49].

Table 5. Effects of antiretroviral combination therapy on bone mineral density in clinical studies.

Study	n HIV (n control)	% male (% control)	Study design	Main findings	Ref.
Tebas *et al.* (2000)	95 (17)	100 (100)	Cross-sectional	PI treatment was univariately associated with bone loss and central adiposity. No correlations between T- or Z-scores and central fat gain (lipodystrophy)	[2]
Bruera *et al.* (2003)	111 (31)	80 (77)	Cross-sectional	No association between BMD and ART status or PI use	[28]
Mondy *et al.* (2003)	128	86	Prospective (72 week)	PI use was not an independent risk factor for bone loss	[26]
Amiel *et al.* (2004)	148	100	Cross-sectional	No significant BMD difference between treated and untreated HIV-patients. TNF-α was increased in treated subjects but not correlated with age-matched BMD. No association between BMD and lipodystrophy status	[47]
Dolan *et al.* (2004)	84	0	Cross-sectional	No influence of PI, NRTI or NNRTI on BMD	[49]
Gallant *et al.* (2004)	299 301	74 75	Prospective (144 weeks)	A greater mean percentage decrease from baseline was observed in BMD at the lumbar spine in the tenofovir- compared with the stavudine-treated group (-2.2 vs -1.0%, respectively), similar changes were observed at the hip (-2.8 vs -2.4%)	[30]
Yin *et al.* (2005)	31	0	Cross-sectional; Postmenopausal	Duration or class of ART was not predictive of bone loss	[89]
Konishi *et al.* (2005)	39	100	Cross-sectional	Serum RANKL was higher with ART than without ART. Serum RANKL correlated inversely with BMD and positively with urinary deoxypyridinoline	[41]
Seminari *et al.* (2005)	68	81	Cross-sectional	RANKL levels or the OPG:RANKL ratio was not associated with bone loss in heavily pretreated HIV-infected subjects	[32]
Bongiovanni *et al.* (2006)	89	63	Prospective First-line NNRTI versus ART naive	Osteopenia/osteoporosis was no more frequent in NNRTI-treated versus NNRTI-naive patients. Time on NNRTI, predicts bone loss	[37]
Arnsten *et al.* (2006)	263 (232)	0 (0)	Cross-sectional; over 40 years of age	The use of ART and PI was not associated with low BMD	[90]
Arnsten *et al.* (2007)	328 (231)	100 (100)	Prospective	The durations of ART or PI use were not associated with BMD	[40]
Cassetti *et al.* (2007)	86	–	Prospective (288 weeks)	Tenofovir was associated with an initial drop of vertebral and hip BMD	[60]
Jacobson *et al.* (2008)	379	75	Prospective (median 2.5 years)	BMD loss was independently associated with tenofovir use and longer duration of didanosine. Long duration of stavudine prevented or mitigated bone loss. BMD loss was associated with low albumin and low BMI	[36]
Cazanave *et al.* (2008)	492 (0)	73 (0)	Cross-sectional	Cumulative exposure to any antiretroviral drug, cumulative exposure to HAART and naive versus ART-experienced status was not independently associated with osteoporosis. Low HIV viral load was an independent predictor of low BMD	[23]

ART: Antiretroviral combination therapy; BMD: Bone mineral density; BMI: Body mass index; HAART: Highly active antiretroviral therapy; NNRTI: Nonnucleoside analog reverse transcriptase inhibitor; NRTI: Nucleoside analog reverse transcriptase inhibitor; OPG: Osteoprotegerin; PI: Protease inhibitor.

Treatment of bone loss in HIV-infected patients

Prevention and treatment strategies for bone loss, such as weight-bearing exercises, smoking cessation, lowering alcohol consumption and optimization of vitamin D and calcium intake, should be implemented in HIV-infected patients similar to HIV-uninfected individuals [66]. Since a low BMI represents an explicit risk factor for osteoporosis in HIV-infected patients, it is important that an adequate body weight and a balanced nutrition are maintained. Strength training was found to protect against bone demineralization in HIV-infected individuals [36].

Biphosphonates reduce bone resorption and increase bone mass in HIV-infected individuals [67–70]. Compared with the supplementation of calcium and vitamin D alone, alendronate was well-tolerated and resulted in significant increases of bone density at the lumbar spine, total hip and femoral neck in both men and women [71]. Similar results were also obtained for an annual infusion of zoledronate (4 mg) [70]. In a 2-year randomized, placebo-controlled study of 43 HIV-infected men, zoledronate resulted in significant increases of the BMD in the lumbar spine (8.9 vs 2.6%) and in the total hip (3.8 vs 0.8%) [70]. Despite this promising data, whether fractures can be prevented by biphosphonates in the setting of HIV-infection has not been investigated.

Raloxifene is a bone-selective estrogen-receptor agonist approved for the treatment of postmenopausal osteoporosis in women [72]. However, raloxifene should be used with caution in HIV-infected women because this substance inhibits cytochrome P450 3A4 [73], a biotransformation system also required for the inactivation of PI. Other interventions, such as teriparatide [74], calcitonin and hormone replacement with progesterone or estrogen, have not been specifically studied in HIV-infected populations and, therefore, cannot be recommended as standard therapy for osteoporosis.

Osteonecrosis

Osteonecrosis results from an avascular ischemic death of the cellular components in bone. Triggers of osteonecrosis include exogenous or endogenous corticosteroid exposure, alcoholism, hypertrigliceridemia, sickle cell and Gaucher's disease [75]. Studies have also associated osteonecrosis with mutations in several genes including factor V Leiden and plasminogen-activating inhibitor-1 [75]. The risk of osteonecrosis in HIV-infected patients is elevated approximately 100-fold in both adults and children compared with the general population [76,77]. The first case reports and case series were reported in the 1990s [78,79]. At the hip, which is the most frequent localization of osteonecrosis, the minimal incidence rate of symptomatic osteonecrosis was 0.05% in a large cohort [80]. Another study used MRI for the detection of osteonecrosis and calculated the annual incidences of asymptomatic and symptomatic osteonecrosis of the femoral head as 0.7% and 0.3%, respectively [76]. Multivariate analysis identified prior AIDS-defining illnesses, the $CD4^+$ cell nadir and exposure to ART as risk factors. The prevalence of symptomatic hip necrosis was estimated as 4.4% by MRI [81]. Hip osteonecrosis is frequently bilateral and may also be associated with avascular necrosis of other bones. HIV-infected patients were also suggested to be at a higher risk for osteonecrosis in the jaw [82]. Chronic inflammation, corticosteroids in the setting of immune reconstitution and anticardiolipin autoantibodies have been suggested as risk factors by some, but not all investigators [76,80,81,83,84]. Osteonecrosis was also attributed to hypertriglyceridemia secondary to PI treatment [80,85], but not all studies could confirm the association between PI-induced metabolic complications and osteonecrosis [86,87].

Similar to the situation in HIV-uninfected subjects, no conservative treatment was found to be effective in arresting or slowing down the progression of osteonecrosis. Approximately 11% of the initially asymptomatic patients with hip osteonecrosis were found to eventually require hip replacement [76].

Future perspective

It is important to gain more certainty regarding the impact of HIV infection and individual ART components on BMD in order to rule out a future epidemic of fragility fractures. Most of the available data are subject to confounders owing to the absence of randomized, controlled trials. It is also unclear if BMD can serve as a predictor of future fracture risk in HIV-infected patients because the osseous microarchitecture has not been studied in detail in HIV-infected patients and studies lacked fractures as a clinical end point. Guidelines for the screening of HIV-infected patients for osteoporosis, its prophylaxis and treatment need to be established, and more data on HIV-infected women and children are required. Based on the growing understanding of the pathogenesis of bone

loss, many novel interventions [88] are currently being developed and need to be evaluated in the setting of HIV infection.

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Executive summary

HIV effects on bone turnover

- HIV envelope glycoproteins and viral protein of regulation promote osteoclastogenesis *in vitro*. Enhanced production of RANKL, macrophage colony-stimulating factor and lowered secretion of osteoprotegerin result in bone demineralization. Important stimulators of bone resorption in the setting of inflammation are IL-1 and TNF-α.

Osteopenia & osteoporosis in HIV-infected individuals

- HIV-infected patients without prior antiretroviral combination therapy have lower BMD than HIV-uninfected controls. The exact underlying mechanisms of bone demineralization in the HIV-infected population are still unclear but the contributors are probably multifactorial since many of the traditional risk factors for osteoporosis are also highly prevalent in the HIV-infected population.
- Fracture risk is enhanced in HIV-infected patients.

Influence of antiretroviral combination therapy on bone mass

- It is still unclear whether antiretroviral combination therapy truly influences bone mass regulation. There is evidence that tenofovir induces hypophosphatemia and osteomalacia in some patients. With regard to protease inhibitors, some appear to be linked to osteopenia, while others do not.

Treatment of bone loss in HIV-infected patients

- Given the paucity of data, most prevention and treatment strategies for bone loss are currently extrapolated from the general population. Biphosphonates were found to be safe and effective in HIV-infected patients with osteoporosis. Raloxifene should be avoided in the setting of protease inhibitor treatment.

Osteonecrosis

- The risk of osteonecrosis is approximately 100-fold higher in HIV-infected patients than in the general population. MRI is the gold standard for its early diagnosis. Suspected risk factors are inflammation, corticosteroids and anticardiolipin antibodies. Data concerning an association between the use of protease inhibitors and osteonecrosis are controversial.

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