Contents available at **PubMed**
www.aidsreviews.com

PERMANYER

Check for updates

AIDS Rev. 2023;25:67-76

HIV patients' bone loss before and after antiretroviral treatment and its possible mechanisms

Pei-min Zheng[1,2,3], Yu-qing Xie[1,4], Shi-fan Lin[1,2], Le Zou[1,2], Zhi-hua Huang[3,5], and Zhi-ping Zhang[1,2,3]**

[1]Nanchang Key Laboratory of Orthopaedics, The Third Affiliated Hospital of Nanchang University; [2]Department of Orthopedics, The First Hospital of Nanchang, The Third Affiliated Hospital of Nanchang University; [3]Huankui Academy, Nanchang University; [4]School of Public Health, Nanchang University; [5]Department of Radiology, The Ninth Hospital of Nanchang City, Nanchang, Jiangxi

Abstract

HIV infection has been reported to cause bone loss and a higher risk of fracture. Under normal conditions, bone metabolism is regulated by mesenchymal cells, osteoclasts differentiated from mononuclear macrophages, osteoblasts, and their expression of regulatory factors, such as receptor activator of nuclear factor-kappa B ligand (RANKL), M-SCF, and transforming growth factor-beta. The balance between bone resorption and osteogenesis depends on the balance between osteoclasts and osteoblasts. In addition, some immune cells, such as B-cells, T-cells, and other non-immune cells expressing RANKL, can contribute to osteoporosis under inflammatory conditions. HIV proteins consist of three types: regulatory proteins, accessory proteins, and structural proteins, which contribute to HIV-mediated bone loss partly by upregulating NF-κB expression, tumor necrosis factor alpha content, and release of inflammatory cytokines. Even worse, although antiretroviral therapy has reduced HIV infection mortality and successfully transformed acquired immunodeficiency syndrome into a chronic disease, its impact on bone loss should not be overlooked, especially when the drug contains tenofovir. This review analyzes some reports focusing on the overall osteolytic situation due to imbalances in osteogenesis and bone resorption due to HIV infection and antiviral therapy. The intrinsic mechanism of bone loss provides a reference for researchers to analyze the risk factors for HIV patients complicated with bone loss and helps clinicians to provide ideas for the intervention and prevention of bone loss during clinical treatment and chronic disease management of HIV patients.

Keywords

Osteoporosis. Antiretroviral therapy. Immune system. Bone inflammation.

***Correspondence to:**
Zhi-hua Huang
E-mail: 8278424@qq.com

Zhi-ping Zhang
E-mail: ndsfy001425@ncu.edu.cn

Received in original form: 08-11-2022
Accepted in final form: 26-04-2023
DOI: 10.24875/AIDSRev.22000028

Introduction

After HIV infection, the patient's immune function is partially lost, and when it deteriorates into AIDS, the number of CD4+ cells is reduced, and opportunistic infections and tumors occur. In terms of geographic distribution, people living with HIV/AIDS are more common in undeveloped countries[1]. Most HIV-positive patients progress to AIDS within 10 years, and those who do not start antiretroviral therapy (ART) have only 2 years of survival on average[2]. When ART can continue to suppress the virus and help the body rebuild its immune system, HIV has become a lifelong chronic disease[3,4]. Chronic disease is often accompanied by other complications including osteolysis or even fracture risk. Osteolysis and fracture are caused by abnormal bone metabolism. Maintaining a healthy bone system balance depends on a balanced relationship between bone deposition and bone resorption mediated by osteoclasts and osteoblasts, with osteoclasts responsible for most of the osteoclastic effects of bone metabolism, especially osteoclast precursor cells that have the potential to differentiate into mature osteoclasts. As osteoporosis and osteonecrosis become the most common skeletal diseases in HIV-infected populations[5], there is an increased likelihood of fractures. Considering the high fracture risk and prevalence of osteoporosis in elderly patients, physical therapists should be more aware of their potential risk of falls and bone demineralization and routinely evaluate these phenomena[6]. Several common factors can lead to increased fracture risk including aging, smoking, and alcohol abuse. It is worth noting that in recent years, ART has also been reported to reduce bone mineral density (BMD)[7,8], and some reports suggest that ART may reduce immune system activity in the short term[9,10]. Therefore, it is important to identify the underlying factors that exacerbate bone resorption by ART. What specific factors lead to the deterioration of the health status of these patients and the mechanism of infection are still unclear, but it is believed to be multifactorial. This article mainly focuses on the direct or indirect disruption of the balance between osteogenesis and osteoclasts by viral proteins and activation of the immune system and summarizes mechanisms at the cellular and molecular levels of bone loss and the impact of antiretroviral drugs on bone health in HIV-infected patients.

Increased fracture prevalence in HIV-infected individuals

As HIV infection progresses into a chronic disease and the application of dual-energy X-ray absorptiometry (DXA) in bone assessment in HIV-infected patients, BMD decreasing issues, which increase the prevalence of fracture, have been reported by other researchers. There are increased circulating markers of bone formation and resorption in HIV-infected patients due to a more active state of bone turnover, resulting in a period called the "catabolic window" in which both osteoclast function and bone resorption are increased first, followed by osteoblast-mediated bone formation, and this time delay in bone metabolism will lead to early bone loss[11]. This notion is reinforced by several recent studies demonstrating that bone loss and low BMD rate are more common in individuals not receiving ART after HIV infection[12-16]. Severe bone loss in the body hip, lumbar spine, and wrist contributes to the elevated prevalence of fragility fractures[17], especially in gerontic patients.

For ART-treated individuals, there was a 35 enrolled randomized controlled trial to determine the significant BMD reduction in the mean total hip and the mean femoral neck in the TDF-treated group[18]. This reduction in BMD in individuals treated with TDF was also confirmed by another randomized controlled trial involving 328 HIV-infected, treatment-naive participants[19].

BMD reduction in anatomical sites such as the vertebrae, total hip, and wrist may translate into an increased prevalence of fracture or osteoporosis, as shown in figure 1A. A comparison of HIV- and HIV+ patients, in total or stratified by gender and race, showed a significantly higher prevalence of bone disease in HIV-infected patients, and the same results were observed in both male and female subunits. The fracture prevalence was also determined according to race in both males and females, as shown in figure 1B (figure created by author from authorized data of Triant et al.[20]). Significantly higher fracture rates are presented among HIV-infected patients, regardless of whether they receive ART. The mechanical-specific factors leading to the deterioration of the bone health status of these patients are still unclear, but it is believed to be multifactorial. By reading and arranging, we summarized the following possible mechanisms that may play an important role in bone health deterioration in HIV.

Direct effects of HIV-induced bone loss

HIV is an RNA virus, and its target cells in the body are mainly CD4+ T-cells, which attack the host's immune system and cause serious defects in cellular

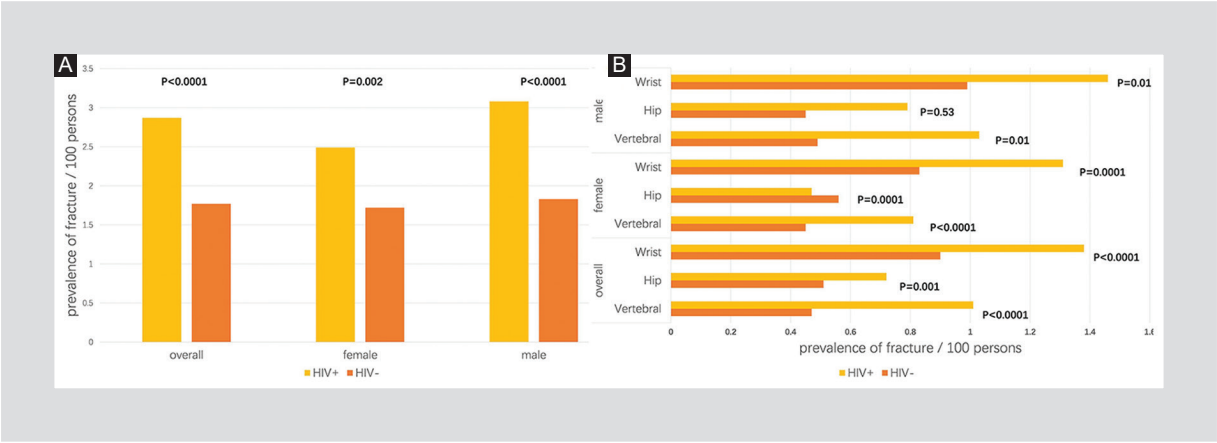

Figure 1. *Prevalence of fracture comparison between HIV+ (HIV-infected) and HIV- (non-HIV-infected patients). Prevalence of fracture comparison between HIV+ (HIV-infected) and HIV– (non-HIV-infected patients).* **A:** *comparison of fracture prevalence in HIV+ and HIV - patients by gender.* **B:** *comparison of fracture prevalence in HIV+ and HIV– patients by race.* **A** *and* **B:** *dark bars represent HIV-infected patients, and light bars represent non-HIV-infected patients. Figure 1 was created by the authors from the data in a population-based study conducted at a large U.S. health-care system in 2008.*

immunity. The virus is spherical in shape and its outer shell contains a lipid bilayer derived from the host cell membrane and the spike glycoprotein composed of two proteins, gp120 and gp41[21].

Viral structural proteins include surface glycoprotein (gp120) and group-specific antigen (p55-gag multimer protein). Regulatory proteins include HIV transactivator (Tat) and virion protein expression regulator (Rev). HIV assistive proteins including Negative Factors (Nef) perform many functions in viral pathogenesis: they help the virus invade the immune response against the host, help establish long-term evasion of immune surveillance, and their high-level expression during the viral life cycle keep a high viral load in persistent infection[22]. Three viral proteins, structural proteins, regulatory proteins, and accessory proteins play important roles in bone loss.

Structural proteins

Gp120

The HIV protein gp120 is one of the glycoprotein spikes on the viral coat that mediates binding to the CD4 receptor, and the interaction of gp120 with the cellular receptor CD4 initiates the process of HIV-1 invading host cells.

Direct infection of HIV protein gp120 can affect the tumor necrosis factor (TNF)-TNF receptor pathway and lead to increased TNF content[23], thereby affecting osteoclast maturation and receptor activator of nuclear factor-kappa B ligand (RANKL) expression. In a published report, researchers exposed CD3+ T-cells to gp120 and discovered that a nuclear factor that helps induce osteoclast maturation-the expression of RANKL-is increased. In addition, gp120 also exhibits the ability to upregulate MSC CXCR expression, which may lead to a change in the differentiation tropism of MSC cells in chronic HIV infection toward promoting the maturation of osteoclasts[22]. It is speculated that gp120 promotes osteoclastogenesis by upregulating the expression of CXCR[24], resulting in bone loss.

Normal bone rebuilding depends on the coupling of two tightly regulated processes, osteoblastic bone formation, and osteoclast bone resorption. Human osteoblasts (HOBs) treated with HIV protein gp120 showed slowed cell proliferation, decreased cell viability, a time-dependent increase in apoptosis within 48 h, and decreased intracellular β-catenin staining in HOBs, indicating that the Wnt/β-catenin signaling pathway was significantly inhibited[25]. The Wnt/β-catenin signaling pathway plays a key role in the increase and maintenance of bone mass and bone remodeling[26], which may be a potential target for reducing HIV infection-associated bone abnormalities[27]. In addition, a study found that HIV-1 gp120-induced apoptosis was effectively inhibited when naive osteoblasts were treated with anti-TNF-α polyclonal antibody[21], suggesting that the apoptosis-inducing effect of gp120 on osteoblasts may be related to HIV-1 gp120 dependent regulation of TNF-α, which plays an important role in osteoclast maturation at the same time. By breaking the coupling balance between osteogenesis and

osteoclastogenesis *in vivo*, gp120 makes human bone metabolism progress toward the osteoclast direction, thus causing visible bone loss in HIV-infected patients.

P55-gag

HIV protein P-55 gag, a precursor protein of the HIV matrix, reduces the osteogenic activity of mesenchymal stem cells[28]. Specifically, the HIV structural protein p55-gag regulates the differentiation of MSCs toward an osteoclastogenic phenotype[22], reduces the expression level of osteocalcin in osteoblast in bone calcium metabolism[29], and induces early senescence of bone marrow mesenchymal stem cells[30]. The differentiation of bone marrow mesenchymal stem cells is one of the sources of osteoblasts in the body. From this perspective, the osteogenic process is inhibited by the reduction in the number of osteoblasts and the lack of factors that promote bone growth. Paradoxically, some scholars have also found that P55-gag and Rev exhibit opposite effects in regulating this differentiation process. P55-gag often inhibits CTGF levels, BMP-2 secretion, and RUNX-2 activity (three significant molecules and proteins in the osteogenic process of differentiated MSCs) to inhibit osteogenesis, while Rev can increase CTGT levels and total mineralization rate at most concentrations to present a bone deposition situation[31]. More molecular experiments should be focused on the effect of HIV Rev on various sites of the body according to infection duration. Under the influence of p55-gag, the process of bone deposition was inhibited due to the decrease in osteocalcin levels, and the decreased osteoblasts could not be replenished in time; thus, the phenomenon of bone loss became more serious.

Regulatory proteins and auxiliary proteins

Tat

Tat is involved in the regulation of reverse transcription of the viral genome and plays a role in HIV-mediated bone loss. In the process of viral infection, the HIV protein Tat is increased and released into the extracellular matrix and surrounding uninfected cells. HIV-infected cells can absorb these tat proteins, resulting in the high expression of inflammatory factors and the activation of related signaling pathways. These pathways have a huge possibility of leading to the occurrence of bone loss.

At present, there is little research on the effect of HIV Tat on the bone mass of HIV patients. However, data are showing that the regulatory protein Tat can enhance the differentiation and activity of peripheral blood monocyte-derived osteoclasts, thereby increasing the mRNA transcription level of some specific osteoclast differentiation markers representing osteoclast-producing signals, including cathepsin K, calcitonin receptor, and matrix metalloprotease 9 (MMP9), and upregulating the expression and activity of tartrate-resistant acid phosphatase (TRAP)[32]. In short, these results suggest that Tat is an important viral factor for bone resorption and plays an important role in HIV-induced bone loss in patients, indicating that peripheral blood monocytes serve as a potential target in treating HIV patients' bone loss.

Rev

Rev is another regulatory protein responsible for the synthesis of major viral proteins, with the ability to promote the differentiation of monocytes into osteoclasts. Rev can enhance the bone resorption capacity of osteoclasts by increasing the production of reactive oxygen species (ROS) and TNF-α in osteoclast precursors to further induce the maturation of osteoclasts and bone resorption[29]. Tat and rev are both soluble proteins of HIV, and when researchers treat mouse osteoclast precursor cells with Tat and Rev, respectively, the rate of osteoclastogenesis increased compared to the control group (zoledronate) 70% and 26%, and researchers found that the level of TNF-α cytokine production by osteoclast precursors had significantly increased[33], indicating that the combined treatment of Tat and Rev proteins not only increased the number of osteoclasts formed but also enhanced the bone resorption activity of osteoclasts, showing a biological effect of driving the osteoclast process, suggesting that the bone loss in HIV-infected individuals may be related to the water-soluble protein of HIV.

Nef

Nef is a small myristoylated protein with a mass of approximately 27-35 kilodaltons and is a negative regulator, capable of manipulating host cell machinery so that the virus can infect, survive, and replicate continuously. It is believed to be a protein necessary for the progression from HIV infection to AIDS.

The HIV protein Nef is involved in almost all HIV-1-induced effects. Nef induces osteolytic effects with HIV infection itself partly by increasing the size and activity of the sealing zone (a structure that attaches osteoclasts to the bone surface and creates a suitable

environment for bone resorption, SZ) by increasing podosome expression to form larger F-actin and enhancing the activity of the Src kinase family to maintain the stability of SZ[32]. In addition, bone marrow mesenchymal cells (BM-MSCs) are also targets of Nef. In an experiment aimed at determining the effects of Nef and Tat on the senescence of human BM-MSCs, researchers found that MSCs treated with Tat and/or Nef for up to 30 days showed reduced cell proliferative activity and exhibited early senescence, which might be associated with mitochondrial dysfunction[34], resulting in a diminished potential of MSCs to differentiate into osteoblasts, eventually leading to bone loss.

Indirect effects of HIV-induced bone loss

HIV infection induces immune activation leading to bone loss

The RANK/RANKL/osteoprotegerin (OPG) pathway is a main regulatory pathway of osteoclast bone resorption[35]. RANKL is expressed on the surface of osteoblasts, T-cells, and B cells[36] and is an important regulator of osteoclast precursor differentiation and function[37,38] as well as T-cell growth[39]. RANK is expressed on the surface of osteoclast precursor cells, and after combining with RANKL, osteoclast maturation is elevated, which disrupts the balance between bone formation and bone resorption, and ultimately leads to bone loss.

B-cells

OPG plays a key role in regulating bone turnover[40]. Most OPG is produced by B-cells, and it can compete with RANK for binding to RANKL, thereby inhibiting osteoclast maturation and reducing bone loss. Shortly after infection with HIV, B cells switch from an OPG-producing type to a RANKL-producing type[41], showing diametrically opposite effects on the balance between bone formation and resorption (preferring bone resorption). In addition to phenotypic changes in B-cell surface receptors caused by HIV infection, the number of RANKL+ B-cell subsets was significantly increased. In addition, it was also found that RANKL produced by B-cells was increased and OPG was significantly decreased in HIV transgenic mice[42]. After osteoclast precursors and B-cells bind with RANKL and RANK, the maturation of osteoclasts is accelerated, and thus, bone metabolism exhibits higher osteolytic activity.

A recent study found an expansion of immature/transitional B-cells in severely infected CD4+ T-lymphopenic patients, which may also be associated with HIV-induced bone loss, as higher RANKL/OPG ratios were found in B-cells and elevated plasma interleukin (IL)-7 in HIV-infected patients[43], which may ultimately lead to higher osteolytic levels through the RANKL pathway of bone resorption.

T-cells

Since ART could rebuild the immune system, the repopulation of T-cells may explain the bone loss just after ART begins. To verify this hypothesis, one experiment focused on the role of T cells in ART initiation, using an immunodeficient mouse model engrafted with T-cells to mimic ART-induced T-cell expansion to study whether T-cell repopulation causes bone loss alone (without HIV infection): After CD3+ T-cells were successfully engrafted into genetically identical syngeneic T-cell-deficient TCR-β KO (knockout) mice and reconstituted in the spleen and bone marrow as expected, researchers observed a significant deterioration in cumulative BMD of the whole body, lumbar spine, femur, and tibia in reconstituted mice compared with untransplanted TCRβ mice[44], indicating a crucial influence of T-cell repopulation on bone metabolism. Another study further discovered that the reconstitution of CD4+ T-cells and CD8+ T-cells also cause significant cortical and trabecular bone loss[45]. The results above suggest that although T-cell reconstitution is a key part of ART, paradoxically, its repopulation aggravates the process of bone loss, probably leading to bone loss in bone inflammation, in which the immune system plays a crucial role in a cytokine storm.

Both experiments demonstrated that T-cell repopulation had a detrimental effect on bone loss at the onset of ART, possibly due to their coproduction of TNF with B-cells and induction of RANKL production.

The T-cell RANKL/OPG ratio correlates with a BMD decrease

Whether it is the change in the number of T-cells or the change in the expressed protein, the RANKL/OPG ratio on the surface of T-cells plays an important role in the body's tendency toward osteoclast or osteogenic metabolism.

More intuitively, studies have found that the T-cell RANKL/OPG ratio is associated with the hip, lumbar spine, and femoral neck BMD-derived z-scores in HIV-infected individuals, and the imbalance between the

two factors may explain why T-cell repairment experimentally or ART-induced leads to HIV induction of bone loss, as mentioned above[46]. In an animal experiment, researchers found higher levels of RANKL mRNA in HIV-1 transgenic rats, and correspondingly, in these transgenic rats, the OPG that reduced this RANKL/OPG ratio was not significantly changed[47].

Although the previously mentioned, bone loss is associated with disruption of the immune-skeletal interface, it can be avoided using the anti-resorptive drug prophylaxis with zoledronic acid in animals during T-cell repopulation[44].

Another feature of untreated HIV infection is the massive production of pro-inflammatory cytokines[48]. Since T-cells and B-cells link HIV-induced bone loss with immune activation through the RANK/RANKL/OPG pathway, many other cell factors also appear in HIV-infected patients in the immune process, and recent experiments have shown that bone loss in HIV-infected/AIDS patients is also related to certain cytokines[49]. One study showed that osteoclast precursor cells from HIV-1 transgenic mice expressed higher levels of suppressor of cytokine signaling-1 (SOCS-1) and TNF receptor-related factor 6 (TRAF6) and resistance to the ability of interferon-γ (IFN-γ) to inhibit osteoclast differentiation, resulting in increased osteoclastogenesis and greater bone loss[47]. Furthermore, other inflammatory cytokines appear in the majority of viruses/bacterial infection-related bone inflammation such as IL-1, IL-6, IL-7, IL-17, M-CSF, TNF-α, and IL-1β that can promote the production of RANKL or inhibit the production of OPG to promote osteoporosis or downregulate activation of the immune system[41], especially IL-6, which has been shown to promote thymic regression, resulting in a reduction in the number of circulating naive T-cells[50]. In bone inflammation, proinflammatory cytokines and molecular modulators also contribute to osteoclastogenesis. In a report investigating the role of osteocytes in inflammatory bone loss, proinflammatory cytokines were classified as Th1, while anti-inflammatory cytokines were classified as Th2[51]. A review of established RA-induced inflammatory bone loss models reported that IL-32γ, IL-19, IL-20, IL-21, and IL-22 all exhibited the ability to promote bone loss[52]. In addition to the cytokines mentioned above, some studies suggest that some cytokines that inhibit bone loss, such as IFN-γ, IL-1, IL-4, IL-13, GM-CSF, and IL-33, belong to the Th2 group. IL-4 and IL-1 exert their anti-osteoclastogenic effect by blocking the activation of the NF-kβ pathway induced by RANKL, and when they act together with IL-13, they can increase the expression of OPG mRNA[52,53]. Table 1 summarizes the osteoclastic effects of some cytokines in bone inflammation. Since HIV patients also cause the body to produce proinflammatory factors before treatment, there may be a connection and similarities between HIV infection and normal bone inflammation.

In addition to immune activation, most patients take ART after diagnosis, and some antiretroviral drugs play an important role in osteopenia/osteoporosis in HIV patients.

Direct infection of osteoclasts

In addition, a recent experiment found that infected osteoclasts were observed in the bone of HIV-1-infected humanized mice and in human synovial explants exposed to HIV infection[32], revealing a new pathogenic pathway for the virus to directly affect bone metabolism in patients: HIV-1 infection increases the number of human osteoclasts by altering the structure and function of the sealing zone to increase the adhesion and osteolytic activity of human osteoclasts. Since HIV can infect osteoclast precursor cells at different stages of osteoclast maturation, these infected osteoclast precursor cells will all serve as reservoirs for HIV, providing greater capacity for HIV replication, and enhancing its ability to recruit and aggregate, whereby the virus alters the level of bone resorption.

The role of ART drugs in bone loss

CD4+ T-cells are the main target of HIV, and their reconstruction has become an important part of ART. Although morbidity and mortality directly attributable to HIV infection have been significantly reduced in patients receiving ART in recent years[54], studies have found that treatment (including treatment medications used in the regimen) also negatively influences bone health. Data suggest that bone loss is already observed at the beginning stage of ART[55], and this phenomenon was further demonstrated in a meta-analysis that found that the prevalence of osteopenia/osteoporosis in both the HIV infection and ART treatment groups was more than twice that in the control group[56]. Using dual-energy DXA, regardless of the amount of tenofovir disoproxil fumarate (TDF) exposure, there was aggravated spinal loss associated with phosphaturia and increased hip bone loss associated with CD4 recovery in HIV-infected women receiving TDF-containing ART[8].

In conclusion, ART does lead to loss of spinal or cortical and trabecular bone, causing a greater fracture risk in HIV-infected patients. This risk of a decrease

Table 1. Several cytokines in bone inflammatory

Cytokines/molecules	Effects on skeletal health without HIV infection
TNF-α	– Increase the production of RANKL in osteoblast. – Induces osteoblasts apoptosis[53]. – Inhibit osteoblast differentiation, possibly through the downregulation of POEM by TNF dependent on NF-κB[62]. – Upregulate the expression of Dickkopf-related-1 (DKK-1) which inhibits the Wnt pathway together with sclerostin[63].
IL-6	– Increase the production of RANKL. – A predictor of bone loss in postmenopausal osteoporosis[64].
IL-17	– Sensitize osteoclast precursors to RANKL thus indirectly enhancing osteoclast maturation, and enhancing osteoclastogenesis through increasing the number of osteoclasts[52,53].
ACPA	– Bind directly to citrullinated proteins on the surface of osteoclast precursors and directly increase bone resorption[65], possibly through increasing the production of TNF by monocytes[66].
RANKL	– Stimulate osteoclastogenesis.
M-CSF	– Involved in osteoclasts differentiation and survival. Important for monocyte maturation.
IL-7	– Osteoclastogenic effects.
IL-20	– Osteoclastogenic effects.
IL-17A	– Induces the expression of IL-6, IL-1β, and IL-8 in macrophages and bone cells[67]. – IL-17A mediated the inhibition of Wnt signaling in osteoblasts and osteocytes, as well as bone formation *in vivo*[68].

RANKL: receptor activator of nuclear factor-kappa B ligand; IL-7: Interleukin-7; ACPA: anti-citrullinated peptide antibodies; TNF-α: tumor necrosis factor-alpha; NF-κB: nuclear factor-kappa B.

in BMD during ART is also associated with a person's PA (physical activity) (one cross-sectional study reported that patients with low PA levels were more likely to have decreased BMD compared with patients with high PA levels[57]), considering the reduction in PA after the start of ART in patients. PA levels should be included in further controlled trials.

While the bone loss is indeed prevalent in ART, treatment with specific regimens, such as TDF, has been one of the most commonly used drugs for many years for its virological efficacy and few side effects, but in several controlled trials, TDF has been observed to induce greater bone loss and higher prevalence of fracture[58]. In this experiment, patients showed increased levels of bone turnover markers soon after initiation of TDF-containing ART, such as higher PTH levels at the beginning of ART treatment, which translated to higher levels of BMD loss after 24 weeks.

Interestingly, most of the bone loss reported in the above studies occurred when these antiretroviral treatments contained TDF regimens.

By comparing the effects of TDF and tenofovir alafenamide (TAF) on bone metabolism in patients, we propose several possible mechanisms for how TDF use induces bone loss: influence on gene expression of osteoblasts and osteoclasts in cell signaling and metabolism, direct toxicity to bone effect, subclinical proximal tubular dysfunction, and increased PTH-driven bone resorption, as elevated levels of β2-microglobulin, a marker of proximal tubular dysfunction, were found in ART experiments that included TDF[59]. In addition, strong supporting evidence was provided by a study letter that primarily assessed the dynamics of PTH and ALP in patients who switched from TDF to TAF from June 1, 2016, to March 1, 2018, and found that both PTH and ALP had decreased from above-limit levels to normal levels, especially PTH, in contrast, half of the patients on TDF had PTH levels above 6.9 p mol/L[60]. Appropriate low doses of PTH help to stimulate the development of osteoblasts, while high levels of PTH promote the transformation of monocytes to osteoclasts, correspondingly inhibiting the

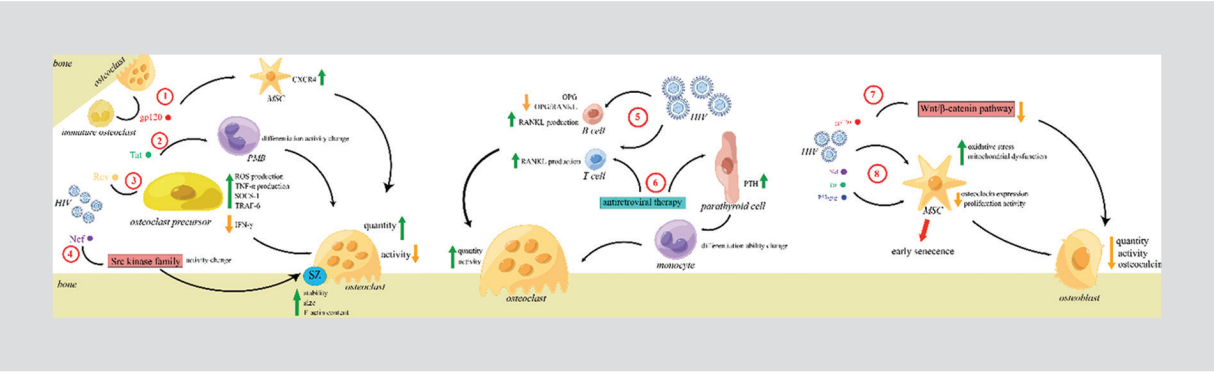

Figure 2. *HIV and antiretroviral therapy (ART) interaction with bone metabolism balance.*
HIV and ART interaction with bone metabolism balance. **1:** *HIV proteins infect osteoclasts through infected osteoclast precursors, peripheral blood monocytes, and MSCs. HIV gp120 increases CXCR4 expression in mesenchymal cells as a larger source of osteoclasts. In addition, gp120 accelerates the transfer from immature osteoclasts to osteoclasts, stimulating osteoclastogenesis.* **2:** *HIV tat induces a differentiation potential change in peripheral blood cells and increases the number of osteoclasts.* **3:** *HIV Rev induces osteoclastogenesis by increasing ROS and TNF-alpha production and upregulating SOCS-1 and TRAF-6 secretion by osteoclast precursor cells.* **4:** *HIV and Nef increase the stability, size, and F-actin content of the sealing zone partly through activation of the Src kinase family, which increases the number and osteolytic effects of osteoclasts.* **5:** *HIV infection induces the production of RANKL in B-cells and T-cells while decreasing the osteoprotegerin (OPG)-expression and OPG/RANKL ratio of B-cells, resulting in osteoclastogenesis.* **6:** *the intervention of antiretroviral therapy further upregulates T-cell RANKL expression and increases parathyroid cell parathyroid hormone. PTH stimulates a change in the differentiation potential of monocytes. Effects of ART and HIV on the immune interface and endocrine downregulate osteoclastogenesis.* **7:** *HIV gp120 downregulates the Wnt/β-catenin pathway to inhibit the maintenance of bone mass and bone remodeling, leading to a reduction in the number of osteoblasts.* **8:** *the HIV proteins Nef, Tat, and p55-gag reduce the number of osteoblasts by inducing MSC early senescence. In addition, both Nef and Tat reduce the cell proliferative activity of bone marrow MSCs and correlate with mitochondrial dysfunction and elevated oxidative stress. p55-gag also downregulates osteocalcin expression levels in osteoblasts (Figure 2 is created by Figdraw).*

development of osteoblasts, and ultimately improving blood calcium levels by increasing the degree of bone resorption. Although a decrease in PTH has been observed following the conversion of TDF to TAF, it is unclear whether TDF-mediated bone loss occurs through an increase in PTH. Future studies should focus on the changes in bone turnover markers with changes in PTH levels (Fig. 2).

Using TAF/elvitegravir (EVG)/cobicistat (COBI)/emdtricitabine, compared to patients who continued their baseline regimen in the lumbar spine and hip compared with TDF, BMD was found to increase by 1.6%, indicating that TAF may be superior to TDF in terms of bone safety[61]. Considering the high risk of fracture in patients, it is recommended that TDF use should be changed to TAF.

Discussion

In this review, we analyzed the possible mechanisms of HIV-induced bone loss from the direct effect of HIV proteins, the indirect effect of HIV-induced immune activation, and the direct effect of HIV infection on osteoclasts. We also highlighted a new way of direct infection of osteoclasts discovered in recent studies, pointing out that osteoclasts as newly discovered target cells for HIV infection became a reservoir for virus replication. Given this, an intervention targeting osteoclast precursors is a feasible way to simultaneously attenuate viral reproduction and osteolytic effects. Furthermore, since the RANKL/OPG ratio in B-cells and T-cells plays an important role in determining the balance between bone formation and bone resorption, and since B-cells undergo class switching when exposed to HIV infection, it is more difficult to change the ratio reversing this process. The change from TDF to TAF resulted in lower levels of bone loss during ART; therefore, TAF/EVG/COBI/FCT should be considered more in clinical use than TDF. Additional mechanisms in HIV infection-associated bone loss should be further investigated. In summary, HIV infection-induced bone loss involves a variety of cells and cytokines, and there must be several common factors that are associated with normal bone inflammation. It is hoped that this will provide new approaches for treating HIV-induced bone loss.

Funding

Health Commission of Jiangxi Province (grant numbers 202211611 to Z.P.Z); Nanchang Science and Technology Bureau (grant numbers 2019-258-5 and 2021-129-4 to Z.P.Z).

Conflicts of interest

None.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

References

1. Le NT, Nguyen TV, Nguyen HT, Ikeda S. Mortality due to HIV/AIDS in Vietnam: time trend and related socio-economic status in some populations and periods from 2005 to 2014. AIDS Care. 2020;32:843-9.
2. Poorolajal J, Hooshmand E, Mahjub H, Esmailnasab N, Jenabi E. Survival rate of AIDS disease and mortality in HIV-infected patients: a meta-analysis. Public Health. 2016;139:3-12.
3. Bolland MJ, Grey A, Reid IR. Skeletal health in adults with HIV infection. Lancet Diabetes Endocrinol. 2015;3:63-74.
4. Yoshimura K. Current status of HIV/AIDS in the ART era. J Infect Chemother. 2017;23:12-6.
5. Glesby MJ. Bone disorders in human immunodeficiency virus infection. Clin Infect Dis. 2003;37 Suppl 2:S91-5.
6. Charumbira MY, Berner K, Louw Q. Physiotherapists' awareness of risk of bone demineralisation and falls in people living with HIV: a qualitative study. BMC Health Serv Res. 2021;21:333.
7. Hileman C, Overton ET, McComsey GA. Vitamin D and bone loss in HIV. Curr Opin HIV AIDS. 2016;11:277-84.
8. Kalayjian RC, Albert JM, Cremers S, Gupta SK, McComsey GA, Klingman KL, et al. Women have enhanced bone loss associated with phosphaturia and CD4+ cell restoration during initial antiretroviral therapy. AIDS. 2018;32:2517-24.
9. Keating SM, Golub ET, Nowicki M, Young M, Anastos K, Crystal H, et al. The effect of HIV infection and HAART on inflammatory biomarkers in a population-based cohort of women. AIDS. 2011;25:1823-32.
10. Andersson J, Fehniger TE, Patterson BK, Pottage J, Agnoli M, Jones P, et al. Early reduction of immune activation in lymphoid tissue following highly active HIV therapy. AIDS. 1998;12:F123-9.
11. Mallon PW. Aging with HIV: osteoporosis and fractures. Curr Opin HIV AIDS. 2014;9:428-35.
12. Alvarez-Barco E, Mallon PW. What's new in bone disease and fractures in HIV? Curr Opin HIV AIDS. 2021;16:186-91.
13. Birabaharan M, Kaelber DC, Karris MY. Bone mineral density screening among people with HIV: a population-based analysis in the United States. Open Forum Infect Dis. 2021;8:ofab081.
14. Pramukti I, Lindayani L, Chen YC, Yeh CY, Tai TW, Fetzer S, et al. Bone fracture among people living with HIV: a systematic review and meta-regression of prevalence, incidence, and risk factors. PLoS One. 2020;15:e0233501.
15. Paccou J, Viget N, Drumez E, Cortet B, Robineau O. Prevalence and risk factors for low bone mineral density in antiretroviral therapy-naive HIV-infected young men. Med Mal Infect. 2018;48:442-8.
16. Wattanachanya L, Jantrapakde J, Avihingsanon A, Ramautarsing R, Kerr S, Trachunthong D, et al. Antiretroviral-naïve HIV-infected patients had lower bone formation markers than HIV-uninfected adults. AIDS Care. 2020;32:984-93.
17. Chang CJ, Chan YL, Pramukti I, Ko NY, Tai TW. People with HIV infection had lower bone mineral density and increased fracture risk: a meta-analysis. Arch Osteoporos. 2021;16:47.
18. Cook PP, Stang AT, Walker LR, Akula SM, Cook FJ. Bone mineral density and Vitamin D levels in HIV treatment-naïve African American individuals randomized to receive HIV drug regimens. South Med J. 2016;109:712-7.
19. Brown TT, Moser C, Currier JS, Ribaudo HJ, Rothenberg J, Kelesidis T, et al. Changes in bone mineral density after initiation of antiretroviral treatment with tenofovir disoproxil fumarate/emtricitabine plus atazanavir/ritonavir, darunavir/ritonavir, or raltegravir. J Infect Dis. 2015;212:1241-9.
20. Triant VA, Brown TT, Lee H, Grinspoon SK. Fracture prevalence among human immunodeficiency virus (HIV)-infected versus non-HIV-infected patients in a large U.S. healthcare system. J Clin Endocrinol Metab. 2008;93:3499-504.
21. Gibellini D, De Crignis E, Ponti C, Cimatti L, Borderi M, Tschon M, et al. HIV-1 triggers apoptosis in primary osteoblasts and HOBIT cells through TNFalpha activation. J Med Virol. 2008;80:1507-14.
22. Li L, Lim RZ, Lee LS, Chew NS. HIV glycoprotein gp120 enhances mesenchymal stem cell migration by upregulating CXCR4 expression. Biochim Biophys Acta Gen Subj. 2018;1862:1790-800.
23. Fakruddin JM, Laurence J. Interactions among human immunodeficiency virus (HIV)-1, interferon-gamma and receptor of activated NF-kappa B ligand (RANKL): implications for HIV pathogenesis. Clin Exp Immunol. 2004;137:538-45.
24. Liao T, Chen W, Sun J, Zhang Y, Hu X, Yang S, et al. CXCR4 accelerates osteoclastogenesis induced by non-small cell lung carcinoma cells through self-potentiation and VCAM1 secretion. Cell Physiol Biochem. 2018;50:1084-99.
25. Butler JS, Dunning EC, Murray DW, Doran PP, O'Byrne JM. HIV-1 protein induced modulation of primary human osteoblast differentiation and function via a Wnt/β-catenin-dependent mechanism. J Orthop Res. 2013;31:218-26.
26. Santiago F, Oguma J, Brown AM, Laurence J. Noncanonical Wnt signaling promotes osteoclast differentiation and is facilitated by the human immunodeficiency virus protease inhibitor ritonavir. Biochem Biophys Res Commun. 2012;417:223-30.
27. Erlandson KM, O'Riordan M, Hileman CO, Rapaport E, Labbato D, Campbell TB, et al. Plasma sclerostin in HIV-infected adults on effective antiretroviral therapy. AIDS Res Hum Retroviruses. 2015;31:731-8.
28. Ahmad AN, Ahmad SN, Ahmad N. HIV infection and bone abnormalities. Open Orthop J. 2017;11:777-84.
29. Delpino MV, Quarleri J. Influence of HIV Infection and antiretroviral therapy on bone homeostasis. Front Endocrinol (Lausanne). 2020;11:502.
30. Yuan YH, Zhao SS, Wang XL, Teng ZP, Li DS, Zeng Y. HIV-1 p55-gag protein induces senescence of human bone marrow mesenchymal stem cells and reduces their capacity to support expansion of hematopoietic stem cells *in vitro*. Cell Biol Int. 2017;41:969-81.
31. Cotter EJ, Ip HS, Powderly WG, Doran PP. Mechanism of HIV protein induced modulation of mesenchymal stem cell osteogenic differentiation. BMC Musculoskelet Disord. 2008;9:33.
32. Raynaud-Messina B, Bracq L, Dupont M, Souriant S, Usmani SM, Proag A, et al. Bone degradation machinery of osteoclasts: an HIV-1 target that contributes to bone loss. Proc Natl Acad Sci U S A. 2018;115:E2556-65.
33. Chew N, Tan E, Li L, Lim R. HIV-1 tat and rev upregulates osteoclast bone resorption. J Int AIDS Soc. 2014;17:19724.
34. Beaupere C, Garcia M, Larghero J, Fève B, Capeau J, Lagathu C. The HIV proteins Tat and Nef promote human bone marrow mesenchymal stem cell senescence and alter osteoblastic differentiation. Aging Cell. 2015;14:534-46.
35. Vega D, Maalouf NM, Sakhaee K. CLINICAL Review #: the role of receptor activator of nuclear factor-kappaB (RANK)/RANK ligand/osteoprotegerin: clinical implications. J Clin Endocrinol Metab. 2007;92:4514-21.
36. Han X, Lin X, Seliger AR, Eastcott J, Kawai T, Taubman MA. Expression of receptor activator of nuclear factor-kappaB ligand by B cells in response to oral bacteria. Oral Microbiol Immunol. 2009;24:190-6.
37. Adamopoulos IE. Inflammation in bone physiology and pathology. Curr Opin Rheumatol. 2018;30:59-64.
38. Braun T, Zwerina J. Positive regulators of osteoclastogenesis and bone resorption in rheumatoid arthritis. Arthritis Res Ther. 2011;13:235.
39. Rothman MS, Bessesen MT. HIV infection and osteoporosis: pathophysiology, diagnosis, and treatment options. Curr Osteoporos Rep. 2012;10:270-7.
40. Christoph F, König F, Lebentrau S, Jandrig B, Krause H, Strenziok R, et al. RANKL/RANK/OPG cytokine receptor system: MRNA expression pattern in BPH, primary and metastatic prostate cancer disease. World J Urol. 2018;36:187-92.
41. Moran CA, Weitzmann MN, Ofotokun I. Bone loss in HIV infection. Curr Treat Options Infect Dis. 2017;9:52-67.
42. Titanji K, Vunnava A, Sheth AN, Delille C, Lennox JL, Sanford SE, et al. Dysregulated B cell expression of RANKL and OPG correlates with loss of bone mineral density in HIV infection. PLoS Pathog. 2014;10:e1004497.
43. Titanji K, Ofotokun I, Weitzmann MN. Immature/transitional B-cell expansion is associated with bone loss in HIV-infected individuals with severe CD4+ T-cell lymphopenia. AIDS. 2020;34:1475-83.
44. Ofotokun I, Titanji K, Vikulina T, Roser-Page S, Yamaguchi M, Zayzafoon M, et al. Role of T-cell reconstitution in HIV-1 antiretroviral therapy-induced bone loss. Nat Commun. 2015;6:8282.
45. Weitzmann MN, Vikulina T, Roser-Page S, Yamaguchi M, Ofotokun I. Homeostatic expansion of CD4+ T cells promotes cortical and trabecular bone loss, whereas CD8+ T cells induce trabecular bone loss only. J Infect Dis. 2017;216:1070-9.

46. Titanji K, Vunnava A, Foster A, Sheth AN, Lennox JL, Knezevic A, et al. T-cell receptor activator of nuclear factor-κB ligand/osteoprotegerin imbalance is associated with HIV-induced bone loss in patients with higher CD4+ T-cell counts. AIDS. 2018;32:885-94.
47. Lafferty MK, Fantry L, Bryant J, Jones O, Hammoud D, Weitzmann MN, et al. Elevated suppressor of cytokine signaling-1 (SOCS-1): a mechanism for dysregulated osteoclastogenesis in HIV transgenic rats. Pathog Dis. 2014;71:81-9.
48. Nasi M, De Biasi S, Gibellini L, Bianchini E, Pecorini S, Bacca V, et al. Ageing and inflammation in patients with HIV infection. Clin Exp Immunol. 2017;187:44-52.
49. Stacey AR, Norris PJ, Qin L, Haygreen EA, Taylor E, Heitman J, et al. Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus Type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. J Virol. 2009;83:3719-33.
50. Freeman ML, Shive CL, Nguyen TP, Younes SA, Panigrahi S, Lederman MM. Cytokines and T-Cell homeostasis in HIV infection. J Infect Dis. 2016;214 Suppl 2:S51-7.
51. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: at the crossroads of cell signalling and inflammatory disease. Biochim Biophys Acta. 2014;1843:2563-82.
52. Braun T, Schett G. Pathways for bone loss in inflammatory disease. Curr Osteoporos Rep. 2012;10:101-8.
53. Metzger CE, Narayanan SA. The role of osteocytes in inflammatory bone loss. Front Endocrinol (Lausanne). 2019;10:285.
54. Mondy K, Tebas P. Emerging bone problems in patients infected with human immunodeficiency virus. Clin Infect Dis. 2003;36:S101-5.
55. Carr A, Grund B, Schwartz AV, Avihingsanon A, Badal-Faesen S, Bernadino JI, et al. The rate of bone loss slows after 1-2 years of initial antiretroviral therapy: final results of the Strategic Timing of Antiretroviral Therapy (START) bone mineral density sub study. HIV Med. 2020; 21:64-70.
56. Goh SSL, Lai PSM, Tan ATB, Ponnampalavanar S. Reduced bone mineral density in human immunodeficiency virus-infected individuals: a meta-analysis of its prevalence and risk factors. Osteoporos Int. 2018;29:595-613.
57. Chisati EM, Constantinou D, Lampiao F. Reduced bone mineral density among HIV infected patients on anti-retroviral therapy in Blantyre, Malawi: prevalence and associated factors. PLoS One. 2020;15:e0227893.
58. Bedimo R, Maalouf NM, Zhang S, Drechsler H, Tebas P. Osteoporotic fracture risk associated with cumulative exposure to tenofovir and other antiretroviral agents. AIDS. 2012;26:825-31.
59. Grant PM, Cotter AG. Tenofovir and bone health. Curr Opin HIV AIDS. 2016;11:326-32.
60. Van Welzen BJ, Thielen MA, Mudrikova T, Arends JE, Hoepelman AI. Switching tenofovir disoproxil fumarate to tenofovir alafenamide results in a significant decline in parathyroid hormone levels: uncovering the mechanism of tenofovir disoproxil fumarate-related bone loss? AIDS. 2019;33:1531-4.
61. Brown TT, Hoy J, Borderi M, Guaraldi G, Renjifo B, Vescini F, et al. Recommendations for evaluation and management of bone disease in HIV. Clin Infect Dis. 2015;60:1242-51.
62. Tsukasaki M, Yamada A, Suzuki D, Aizawa R, Miyazono A, Miyamoto Y, et al. Expression of POEM, a positive regulator of osteoblast differentiation, is suppressed by TNF-α. Biochem Biophys Res Commun. 2011;410:766-70.
63. Heiland GR, Zwerina K, Baum W, Kireva T, Distler JH, Grisanti M, et al. Neutralisation of Dkk-1 protects from systemic bone loss during inflammation and reduces sclerostin expression. Ann Rheum Dis. 2010;69:2152-9.
64. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: an inflammatory tale. J Clin Invest. 2006;116:1186-94.
65. Coury F, Peyruchaud O, Machuca-Gayet I. Osteoimmunology of bone loss in inflammatory rheumatic diseases. Front Immunol. 2019;10:679.
66. Lu MC, Lai NS, Yu HC, Huang HB, Hsieh SC, Yu CL. Anti-citrullinated protein antibodies bind surface-expressed citrullinated Grp78 on monocyte/macrophages and stimulate tumor necrosis factor alpha production. Arthritis Rheum. 2010;62:1213-23.
67. Amarasekara DS, Yu J, Rho J. Bone loss triggered by the cytokine network in inflammatory autoimmune diseases. J Immunol Res. 2015;2015:832127.
68. Uluçkan Ö, Jimenez M, Karbach S, Jeschke A, Graña O, Keller J, et al. Chronic skin inflammation leads to bone loss by IL-17-mediated inhibition of Wnt signaling in osteoblasts. Sci Transl Med. 2016;8:330ra37.