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HIV and Bone Metabolism

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Abstract

The skeleton is an organ whose integrity is maintained by constant lifelong renewal involving coordinated removal of worn bone by osteoclasts and resynthesis of new bone by osteoblasts. In young adult humans and animals this process is homeostatic with no net gain or loss of bone mass. With natural aging and exacerbated by numerous pathological conditions, bone removal exceeds bone formation, disrupting homeostasis and resulting in bone loss. Over time, skeletal decline reaches clinical significance with development of osteopenia and eventually osteoporosis, conditions that dramatically increase bone fragility and the risk of fracture. Bone fractures can be devastating with significant morbidity and mortality. Over the last decade, it has become clear that skeletal renewal is strongly influenced by the immune system, a consequence of deep integration and centralization of common cell types and cytokine mediators, which we have termed the "immuno-skeletal interface." Consequently, dysregulated skeletal renewal and bone loss is a common feature of inflammatory conditions associated with immune activation. Interestingly, bone loss is also associated with conditions of immunodeficiency, including infection by the human immunodeficiency virus (HIV) that leads to acquired immunodeficiency syndrome (AIDS). Disruptions to the immuno-skeletal interface drive skeletal deterioration contributing to a high rate of bone fracture in HIV infection. This review examines current knowledge concerning the prevalence and etiology of skeletal complications in HIV infection, the effect of antiretroviral therapies (ART) on the skeleton, and how disruption of the immuno-skeletal interface may underlie bone loss in HIV infection and ART.

Introduction

The skeleton is formed through "bone modeling" which involves the deposition of bone matrix (predominantly collagen) and its mineralization (predominantly calcium phosphate) by osteoblasts. The skeleton achieves its shape and size through the coordinated deposition and removal of bone by osteoblasts and osteoclasts, respectively. Once formed, adult bone is rejuvenated throughout life by the process of "bone remodeling" involving removal of worn bone by osteoclasts, and resynthesis by osteoblasts. As the magnitude of bone replaced is quantitatively similar to that removed, there is no net gain or loss of bone mineral density (BMD). Homeostasis is short lived however, and by the forth decade of life the rate of resorption outpaces that of formation leading to a slow decline in BMD. Bone loss is

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accelerated for 5-10 years after menopause in women while men undergo a slow but linear decline in BMD. Because the bones of women are typically smaller and thinner than men, the incidence of bone fracture is 2 to 3 fold higher in women (Manolagas, 2000; Melton *et al.*, 1998; Orwoll and Klein, 1995).

Modeling and remodeling are complex processes and it has long been understood that bone turnover is extremely responsive to changes in multiple metabolic and biochemical systems in the body. Consequently, numerous diverse pathologies may impact bone turnover. However, it is only comparatively recently that the degree of integration between the immune and skeletal systems has begun to be appreciated (Clowes *et al.*, 2005; Weitzmann and Pacifici, 2007). Not only are osteoclasts derived from cells of monocytic origin, but their differentiation and activity is regulated primarily by receptor activator of NF-κB ligand (RANKL), a cytokine first identified as a mediator of T-cell growth and dendritic cell function (Anderson *et al.*, 1997) and now also recognized to play roles in controlling autoimmunity and immune responses in the skin (Leibbrandt and Penninger, 2010). RANKL-induced osteoclastogenesis is controlled by osteoprotegerin (OPG), a RANKL-decoy receptor that prevents RANKL from binding to its receptor (RANK) on osteoclast lineage cells (Simonet *et al.*, 1997). Historically, osteoclastogenesis has been considered to be regulated by RANKL and OPG produced by cells of the osteoblast lineage (Hofbauer *et al.*, 1999). Recently, however, our group demonstrated in mice that B-lineage cells are critical regulators of basal osteoclastogenesis and are important suppressors of bone breakdown by virtue of copious OPG production (Li *et al.*, 2007). Interestingly, T-cells collaborate with B-cells to stabilize bone resorption, by stimulating B-cell OPG production through co-stimulatory interactions (Li *et al.*, 2007; Yun *et al.*, 1998).

In contrast to the basal production of bone sparing OPG, under inflammatory conditions, activated T- and B-cells are a significant additional source of the osteoclastogenic cytokine RANKL. In fact, lymphocytes have been implicated in RANKL production and the bone loss associated with pathologies as diverse as postmenopausal osteoporosis (Eghbali-Fatourechi *et al.*, 2003), the inflammatory autoimmune disease rheumatoid arthritis (Kong *et al.*, 1999), and in alveolar bone loss associated with periodontal infection (Han *et al.*, 2006). Interestingly, HIV-infection is another disease that has long been associated with high rates of osteopenia and osteoporosis (Ofotokun and Weitzmann, 2010). However, because HIV/AIDS leads to immunodeficiency, in contrast to other infectious and inflammatory states that tend to activate the immune system, explaining this bone loss association has been challenging. This review is dedicated to examining the prevalence and etiology of skeletal complications in HIV-infection, the effect of antiretroviral therapies on bone turnover, and how disruption of the immuno-skeletal interface may underlie bone loss in HIV-infection.

The Aging HIV/AIDS Population

With successful antiretroviral therapy (ART), HIV-infected individuals are living into old age (Mocroft *et al.*, 1998; Mugyenyi *et al.*, 2010; Palella *et al.*, 1998) and life-expectancy in excess of 35 years from the time of diagnosis is now common (Lohse *et al.*, 2007). As a consequence, a gradual shift in the age distribution of the HIV-infected population from the younger to the older aged group is underway (Mack and Ory, 2003; Ofotokun and Weitzmann, 2010). While AIDS incidence appears to be leveling off in the general population, older people as a group are now representing a substantial share of new cases (Mack and Ory, 2003; Ofotokun and Weitzmann, 2010).

Aging, in its broadest sense, is a progressive and irreversible loss of function accompanied by increasing mortality (Demetrius, 2006). Two key manifestations of aging are accelerated bone resorption leading to osteoporosis, and progressive loss of immune function (Weyand

et al., 1998). This is not simply coincidental and it is now becoming clear that the immune and skeletal systems are deeply intertwined as a consequence of a centralization of common cell types and cytokine mediators that we have termed the immuno-skeletal interface (Li *et al.*, 2007; Vikulina *et al.*, 2010; Weitzmann and Pacifici, 2005). Interestingly, both osteopenia/osteoporosis and loss of immunocompetence coexist in the context of HIV-infection, giving rise to speculation that HIV/AIDS may lead to accelerated aging (Deeks, 2009). Importantly, such alterations may also exacerbate the natural process of skeletal aging, leading to an epidemic of osteoporosis and fragility fractures in the aging HIV/AIDS population (Amorosa and Tebas, 2006). The individual and societal financial cost of fractures is tremendous once lost productivity and health care costs are factored in (Burge *et al.*, 2007). Furthermore, most hip fractures are often complicated by prolonged or permanent disability. Management of hip fracture almost always require major surgical intervention and mortality rates can be as high as 30% in the first year alone (Lewis *et al.*, 2006), Furthermore, up to three-quarters of survivors may be confined to extensive rehabilitation in long-term care facilities (Brainsky *et al.*, 1997). In addition, osteoporosis can lead to vertebral deformity and chronic back pain (Black *et al.*, 1995). Consequently the early identification and treatment of osteoporosis is critical in protecting the future health of HIV/AIDS patients.

Diagnosis of Osteopenia and Osteoporosis

Osteopenia and osteoporosis are diagnosed using bone densitometry, a low intensity X-ray technology referred to as dual-energy X-ray absorptiometry (DXA or DEXA). DXA provides an estimate of BMD (the amount of mineral per cm^2) computed for skeleton sites prone to fracture such as femoral neck and lumbar vertebrae. Bone status is defined by a "T score," which entails a comparison of BMD of the subject to that of a theoretical optimal condition (peak BMD in the average healthy young population) and is generally further normalized for sex and race. Osteopenia, or low BMD, is defined as a BMD falling between 1 and 2.5 standard deviations below the optimal peak BMD reference range while 2.5 standard deviations or less is defined as osteoporosis, and generally warrants pharmacological antiresorptive or anabolic intervention. (Karaguzel and Holick, 2010). While DXA is non-invasive and convenient, BMD, however, is not an accurate measurement of bone condition and does not provide data on bone structure and quality, which is an important determinant of load bearing strength. Consequently, DXA can grossly underestimate fracture risk. This concept is illustrated by the fact that 50% of postmenopausal women sustaining a fracture do not meet the clinical definition of osteoporosis by DXA (Nguyen *et al.*, 2007).

HIV Infection and the Skeleton

It is now established that the prevalence of bone abnormalities in HIV-infected patients is several fold higher than that in age, race, and sex matched control groups (Mallon, 2010; Ofotokun and Weitzmann, 2010; Stone *et al.*, 2010; Tebas *et al.*, 2000). The extent of this problem has been highlighted in numerous reports including a recent meta-analysis of 11 studies encompassing 884 HIV-infected patients and 654 controls which concluded that rates of osteopenia and osteoporosis were as high as 67% and 15%, respectively, among the HIV-infected groups. The magnitude of BMD reduction was 6.4-fold higher and that of osteoporosis 3.7-fold higher in HIV-infected groups (Brown and Qaqish, 2006). In a recent publication, BMD in 671 HIV-infected patients revealed that the osteopenia and osteoporosis rates were 47.5% and 23%, respectively, with a progressive loss of BMD in 28% of the subjects over 2.5 years of follow-up (Bonjoch *et al.*, 2010). In another recent observational study consisting of 230 HIV-seropositive men and 159 controls, osteopenia and osteoporosis rates of 42% and 12%, respectively, were documented. The degree of

osteopenia was almost 3 fold higher in the HIV-infected subjects with 12% progressing to osteoporosis (Sharma *et al.*, 2010).

Taken together, these findings validate earlier observations of the existence of a high prevalence of osteopenia and osteoporosis in HIV patients at the time of diagnosis.

Skeletal Deterioration Associated with HIV Infection and the Role of the Immuno-skeletal Interface

The underlying pathologies leading to skeletal deterioration in the setting of HIV infection are poorly understood and traditional risk factors for osteoporosis likely play a role. These include low body weight and life-style factors such as smoking, alcohol, and substance abuse. Furthermore, indirect HIV/AIDS-associated pathologies such as muscular degeneration, kidney disease, and imbalances in sex steroids and calcetropic hormones, such as parathyroid hormone (PTH) and vitamin D, likely also contribute to low bone mass at some level with different combinations of these factors affecting bone mass in different patients (Bolland and Grey, 2011; Mondy *et al.*, 2003). However, the role of the immune system and disruptions to the immuno-skeletal interface in the bone loss associated with HIV-infection has only recently been explored.

Because the production and the regulation of key osteoclastogenic cytokines such as RANKL and OPG are under the influence of key cells of the immune system that are specifically targeted by HIV infection (T-cells), and cells regulated by T-cells (namely, B-cells and monocytes), we speculated that skeletal abnormalities encountered in HIV infection may be mediated via this dysregulation of the immune system.

Given the extreme complexity and co-existing confounders associated with human patients, we explored the impact of immune disruption on the skeletons of an animal model of chronic HIV-1 infection, the HIV-1 transgenic rat. In this model a replication defective HIV-1 viral genome is integrated into the rat DNA leading to constitutive expression of HIV-1 viral proteins. This animal replicates many of the immunological and metabolic pathologies synonymous with human HIV-infection/AIDS (Reid *et al.*, 2001).

Detailed analysis of the rat skeleton using DXA revealed that similar to human HIV/AIDS, HIV-1 transgenic rats undergo severe loss of BMD. Employing micro-computed tomography we were able to further catalog significant deteriorations in bone architecture and structure that would suggest a significant predisposition to diminished load bearing capacity, synonymous with enhanced fracture risk. Histological analyses of bones from these rats revealed enhanced numbers of osteoclasts, consistent with evidence of elevated serum markers of *in vivo* bone breakdown. Additional studies revealed that increased osteoclastogenesis was a consequence of an alteration in B-cell function leading to elevated RANKL and diminished OPG expression (Vikulina *et al.*, 2010). These data are consistent with the fact that B-cells are in large measure regulated by interactions with T-cells, and with the fact that severe perturbations of the B-cell linage are mediated by HIV-1 infection, through direct effects of viral infection and/or indirectly though disruption of costimulatory signals from T cells and other disrupted immune components (Moir and Fauci, 2009; Moir *et al.*, 2008).

In addition to the switch from production of bone sparing OPG by B-cells to production of bone destroying RANKL, we further documented a dramatic increase in the number of osteoclast precursors (defined as monocytes expressing the RANKL receptor RANK) (Vikulina *et al.*, 2010). Taken together these data provided the first evidence of alterations in

the immuno-skeletal interface favorable to accelerated bone resorption and loss of bone mass in the context of HIV-1-infection.

Although studies to validate this model in human HIV infection are underway, it is worth noting that low serum OPG concentrations have previously been reported in HIV/AIDS patients (Chakravarti *et al.*, 2008). Furthermore, human peripheral blood T-cells have also been implicated as a potential source of OPG with decline in T-cell OPG expression following *in vitro* treatment of circulating CD4 T-cells with gp120, an HIV envelope protein (Chakravarti *et al.*, 2008).

ART and Bone Loss

While it is now clear that bone loss is common in HIV-infected patients, another perplexing issue is why bone loss undergoes further rapid decline following initiation of ART. Unlike its impact on many other HIV-related pathologies, ART worsens, rather than ameliorates, bone loss. This paradoxical effect of ART is concerning particularly for a population whose skeletal integrity is already seriously compromised by direct HIV-induced assault and AIDS associated pathologies and complications including immuno-skeletal perturbations.

A study by Brown and Qaqish (2006) noted that the prevalence of reduced BMD and of osteoporosis was significantly higher in ART-treated subjects.

Furthermore, continuous exposure to ART was associated with a profound decline in BMD compared with intermittent ART exposure (Grund *et al.*, 2009), an observation recapitulated in other recent studies (Brown *et al.*, 2009; Cassetti *et al.*, 2007; Rivas *et al.*, 2008).

Despite these data, several unresolved controversies remain regarding ART-related bone loss. For example, are certain ART co-formulations, or specific drugs, more detrimental to the skeleton than others? How early does ART-related bone loss begin? Does it occur in a defined window or is it a chronic effect? Answers to these questions are critical to inform strategies for maintaining bone health in the HIV/AIDS population who are chronically exposed to ART.

A major obstacle to elucidating the effects of ART on the skeleton is the fact that ART consists of multiple classes of antiretroviral agents and with multiple drug variants within each class. Among the first class of antiretroviral agents to be developed were the nucleoside analog reverse transcriptase inhibitors (NRTIs), such as zidovudine, the first approved agent for treatment of HIV infection. Since then non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) have been developed and are now in routine use. In addition, newer generation antiretroviral agents including chemokine receptor antagonists that block viral entry into the cell (fusion inhibitors) (Biswas *et al.*, 2007) and drugs that block viral integrase activity (Prada and Markowitz, 2010) are now in clinical use, with yet other novel agents under development. Furthermore, ART almost always comprises multiple agents from multiple classes of compounds. Dissecting out the individual contributions and specific effects of individual agents in their classes and co-formulations is daunting, and not surprisingly the data regarding the effects of different drugs and drug categories on bone turnover is contentious.

Among the NRTIs, tenofovir has been associated with bone loss the most frequently (Castillo *et al.*, 2002; Gafni *et al.*, 2006; Gallant *et al.*, 2004; Purdy *et al.*, 2008; Van Rompay *et al.*, 2004; Wactawski-Wende *et al.*, 1996). In one study a comparison of tenofovir-containing and non-containing regimens revealed increased bone resorption and loss in the tenofovir-containing arm compared to patients receiving an alternate NRTI (stavudine), at both lumbar spine (−3.3% vs. −2.0%) and hip (−3.2% vs. −1.8%).

Importantly, the majority of bone loss was observed within the first 24 to 48 weeks of the study. Thereafter, bone loss declined, but BMD did not recover over the 144 weeks of the study (Gallant *et al.*, 2004).

Similarly, a study comparing tenofovir to an alternate NRTI (abacavir) revealed a greater loss of BMD at total hip (−3.6% *vs.* −1.9%) and lumbar spine (−2.4% *vs.* −1.6%) with patients receiving tenofovir. Once again bone loss occurred relatively soon after initiation of therapy and was maximal in the spine at 24 weeks and in the hip at 48 weeks (Stellbrink *et al.*, 2010).

In addition to NRTIs, PIs have also been associated with skeletal deterioration (Bonjoch *et al.*, 2010; Briot *et al.*, 2011; Brown and Qaqish, 2006; Fernandez-Rivera *et al.*, 2003; Malizia *et al.*, 2007). In a recently study, the effect of PI-based, NNRTI-based, or combined PI/NNRTI regimens on BMD was evaluated. After 48 weeks of ART, a 4.1% BMD decline in the lumbar spine and a 2.8% decline in hip BMD were observed. Although BMD loss occurred in all arms, the loss of BMD in the spine (but not hip) was significantly greater in the PI-containing regimens (Duvivier *et al.*, 2009).

By contrast, Hanson *et al.* (2011) reported significant BMD loss of approximately 5-6% at the femoral neck by 48 weeks of ART in patients that received neither NRTI nor PI. In all cases there was no additional bone loss evident between weeks 48 and 144.

Although the skeletal effects of the newer antiretroviral classes including the integrase and entry inhibitors remain to be evaluated, the findings of existing studies on ART-skeletal integrity would seem to suggest that the phenomenon of skeletal decline is most likely a universal property of all ART regimens. While the degree of bone loss may vary somewhat between regimens, almost all evaluated ART regimens exhibit a significant loss of BMD in the order of 2% - 6% at key fracture prone anatomic sites over time. Furthermore, the preponderance of BMD loss following ART initiation appears to occur within 24 to 48 weeks; however, once lost, BMD never recovers to pre-treatment baseline levels, let alone to normal BMD levels characteristic of uninfected individuals of similar age. In the HIV/AIDS population where the majority of individuals are already moving into their fourth or fifth decade of life, a time at which bone density has already long since peaked and the skeleton is in a steady state of both age- and HIV-associated deterioration, these findings may portend a looming epidemic of early osteoporosis and fragility fractures.

Bone Fractures in the Setting of HIV Infection

It is generally now agreed upon that both HIV infection and ART are independent predictors of osteoporosis. Opinions are however divided on the public health implications of reduced BMD in the setting of HIV infection. Current data on HIV fracture risk appear conflicting with some studies reporting no association, while others have observed strong association between HIV infection and fracture risk (Arnsten *et al.*, 2007; Calmy *et al.*, 2009; Collin *et al.*, 2009; Guaraldi *et al.*, 2001). Of note, several of these studies are limited by small sample size and retrospective or observational nature of the study design, that are often not amenable to control for biases and confounding risk factors for osteoporosis that abound among HIV-infected individuals. Despite these limitations, in three of the four recent large observational cohort series, a strong association between HIV infection and the risk of fractures was observed. In the landmark population-based study by Triant *et al.* (2008), data were collected from 8,525 HIV-infected and 2,208,792 non-HIV-infected patients in a large U.S. health care system. Compared with HIV-seronegative patients, fracture prevalence was highly significant and 3.5-fold higher in the HIV-infected patients, and this trend was accentuated by increasing age in both men and women.

Recently, the risk of fragility fracture was evaluated in 119,318 men, 33% of whom were HIV infected, and was found to be significantly higher among HIV infected men even after adjusting for other risk factors (Womack *et al.*, 2011). Another large fracture study involving 8,456 HIV-infected patients revealed that fracture rates were 2 to 4 times higher among HIV-infected persons than in the general U.S. population (Young *et al.*, 2011).

Lastly, in another small Canadian study of 138 HIV-infected and 402 non-HIV-infected women, lifetime fragility fracture was found to be significantly higher for HIV-infected compared with non-HIV-infected subjects, although BMD did not differ by HIV-serostatus (Prior *et al.*, 2007). The findings of this study reiterate the pitfall of over reliance on BMD as a predictor of fracture.

In contrast to the positive outcomes described above, in the Women's Interagency HIV Study (WIHS), HIV-serostatus was not found to be a predictor of fracture incidence among 1,728 HIV-infected women (mean age 40 years) compared to 663 HIV-uninfected controls (mean age 36 years). Participants were evaluated for time to first new fracture at any site with a median follow-up period of 5.4 years (Yin *et al.*, 2010).

While not all recent studies have found increased fracture risk, taken together, the emerging data overwhelmingly support a conclusion of significantly increased fracture prevalence in HIV/AIDS populations.

The Mechanisms of Action of ART-induced Bone Loss

The mechanisms by which ART induce bone loss remain contentious, and are likely a consequence of either direct effects on bone cells, realignment of HIV-associated pathologies resulting from disease reversal, or a combination of these factors.

Until recently, direct effects of specific ART components on bone cells have been favored as the most likely scenario. However, teasing out specific effects of individual agents in complex drug formulations *in vivo* has been challenging. While it is clear that antiretroviral drugs do have effects on osteoclasts and osteoblasts *in vitro*, and in animal models *in vivo*, the actions for the most part have not been as expected and generally have failed to recapitulate *in vivo* effects observed in humans. For example, the PI ritonavir, long considered a major protagonist of bone loss in humans, has been reported to suppress osteoclastogenesis and osteoclast function *in vitro* and *in vivo* by impairing RANKL-induced signaling. By contrast, the related PI indinavir had no effect on osteoclastogenesis (Wang *et al.*, 2004). In another study the PI fosamprenavir elicited a significant increase in OPG and a decrease in RANKL production *in vitro*. This reduced RANKL/OPG ratio if recapitulated *in vivo* would be expected to favor protection of bone mass rather than bone loss observed empirically. Other PIs including atazanavir, saquinavir, and indinavir failed to impact the OPG/RANKL ratio (Gibellini *et al.*, 2010).

NRTIs are another class of antiretroviral implicated in bone loss in humans; however, preliminary studies *in vitro* found no effect of several NRTIs on osteoclastogenesis but instead found suppression of osteoblast activity (Taylor and Rogers, 2010).

Consequently, opinions still differ widely among investigators as to the direct effects of ART or their components on bone cells, or their mechanisms of action on the skeleton. Based on accumulating new data, consensus is beginning to emerge that all ART formulations may be inherently detrimental to the skeleton as bone loss following ART appears to be a general phenomenon observed regardless of the regimen type (Brown *et al.*, 2009; Bruera *et al.*, 2003).

The data presented above demonstrating that the preponderance of BMD decline occurs relatively early in the course of ART initiation (typically within the first 24 to 48 weeks) (Gallant *et al.*, 2004; Stellbrink *et al.*, 2010) and at a time of heightened immune restoration (Franco *et al.*, 2002); it lends support to the hypothesis that bone loss might be driven by a mechanism aligned with HIV-disease reversal, in particular immune regeneration. We speculate that regeneration of the immune system may once again lead to a disruption of the delicate immuno-skeletal interface, initiating a new wave of bone resorption and loss of BMD. Studies to address this hypothesis are currently in progress.

Conclusion

With up to two thirds of HIV-infected patients exhibiting osteopenia or osteoporosis at time of diagnosis, coupled with an additional significant loss of BMD in the first 1 to 2 years of ART, it is not surprising that fracture prevalence for AIDS patients is considerably higher than in the general population. Furthermore, there is little doubt that the combined assault of HIV infection, ART, and other risk factors for osteoporosis, coupled with the natural effect of aging on the skeletons of HIV-infected individuals, forewarn of a looming epidemic of osteoporosis and fragility fractures. While the causes of skeletal deterioration in this population are multi-factorial, viral induced disruption of the immuno-skeletal interface appears to play a key role. The alignment of skeletal decline with early events of HIV-disease reversal following ART initiation further suggests a putative role for immune reconstitution in the pathogenesis of bone loss in this context.

Management strategies for osteoporosis in the setting of HIV-infection are still evolving (McComsey *et al.*, 2010); however, the repeated observation that 2% to 6% loss in BMD attributed to ART occurs within the defined window of 24 to 48 weeks following initiation of therapy would seem to offer a unique window of opportunity for therapeutic intervention. This could, and probably should, be exploited for pre-emptive intervention to block further skeletal decline in a population with an already vulnerable skeletal profile caused by chronic HIV infection.

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