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Does Systemic Inflammation and immune Activation Contribute to Fracture Risk in HIV?

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Abstract

Purpose of Review—There is increasing evidence pointing towards an important role of heightened immune activation and inflammation in PLWH contributing to the development of non-AIDS co-morbidities. This review aims to explore low BMD in HIV with a focus on the underlying mechanisms and relationships between the immune and skeletal systems.

Recent Findings—Baseline immune activation and inflammation negatively impact BMD at ART initiation. B- and T-cell alterations in HIV lead to an imbalance in the osteoblastic (OPG) and osteoclastic (RANKL) cytokines which favours osteoclastogenesis and bone resorption. These findings suggest an important role for immune mediated mechanisms in the pathogenesis of low BMD in HIV.

Summary—Bone homeostasis is in part regulated by cells of the immune system through complex interactions with the RANK/RANKL/OPG axis. Disturbances in the normal functioning of T-, B-cells and monocytes in HIV and the resulting pro-inflammatory state may contribute to dysregulation of this finely controlled balance leading to increased bone loss. Pre-ART levels of immune activation and inflammation have a consistently negative effect on BMD and further suggests the immunocentric basis of bone loss in HIV alongside supporting the benefits of earlier

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ART initiation. Further longitudinal studies will help determine the effect this will have on fracture risk in PLWH.

Keywords

Osteoimmunology; B-cells; T-cells; HIV; Immune activation; BMD

Introduction

The advent of successful antiretroviral therapy (ART) for those with HIV infection has led to significant gains in life expectancy [1]. However, as the HIV-infected population ages, the burden of morbidity and mortality related to ‘non-AIDS’ co-morbidities will increase. Low bone mineral density (BMD) and consequent fragility fractures are over-represented in people living with HIV (PLWH), with studies reporting an almost 4-fold increase in prevalence of osteoporosis [2] and a 35% increased prevalence in fragility fractures compared to the general population [3–5].

Although traditional risk factors for low BMD are over-represented in PLWH alongside increased prevalence of the secondary causes of osteoporosis [6–8], HIV itself has been shown to be independently associated with low BMD, even after correction for these risk factors [9]. Moreover, HIV-related factors such as ART exposure, CD4⁺ T-cell counts, duration of HIV disease and active HIV viraemia have all been implicated as playing a role in bone disease [9, 10].

Studies investigating the complex interactions between the skeletal and immune systems have significantly enhanced our understanding of mechanisms through which bone loss in HIV infection is potentially mediated. This review aims to discuss current research with a focus on the role of the immuno-skeletal interface and its dysregulation, as well as the effects of chronic inflammation in the development of bone disease in HIV.

The immunoskeletal interface and its role in bone health

Bone remodelling and BMD homeostasis is a continuous process balancing bone resorption by osteoclasts alongside bone formation by osteoblasts in a process known as coupling [11]. Osteoclasts derive from precursors within monocyte lineage. Osteoclast precursors express receptor activator NF- κ B (RANK) when stimulated by macrophage colony stimulating factor (M-CSF) [12]. RANK binds with receptor activator of NF- κ B ligand (RANKL), a member of the TNF cytokine family expressed by osteoblasts [13]. Osteoclast differentiation and activity, known as osteoclastogenesis, is driven by this interaction between RANK and RANKL. The osteoclastogenic activity of RANKL is balanced by osteoprotegerin (OPG), expressed by osteoblasts. OPG acts as a decoy receptor for RANKL, hence downregulating osteoclastogenesis. The relative abundance of RANKL and OPG is thought to be integral to the maintenance of bone mass [14].

In pro-inflammatory conditions, RANK, RANKL, and OPG can also be produced by cells of the immune system [14–18], potentially affecting RANKL:OPG ratio. Indeed, chronic inflammation is shown to be involved in the pathogenesis of osteoporosis and fragility

fractures in conditions such as rheumatoid arthritis (RA), periodontal disease and perimenopausal osteoporosis [14, 15, 18–20].

Proinflammatory cytokines are known to be potent stimulators of bone resorption via osteoclast signalling [18, 21]. In postmenopausal osteoporosis, the archetypal disease of bone, serum concentrations of interleukin 6 (IL-6) predict extent of bone loss [18]. Importantly, HIV-infected persons have 40–60% increased circulating IL-6 levels [22] and baseline IL-6 levels are independently associated with progression to osteopenia or osteoporosis in HIV-infected, ART-naïve patients [23]. IL-6 has also been shown to be strongly associated with all-cause mortality in PLWH in the Strategies for Management of Antiretroviral Therapy (SMART) study [24]. This study randomised HIV infected patients to CD4+ T-cell count-directed intermittent ART (i.e. stopping or deferring ART until CD4+ count <350cells/mm³) versus continuous ART. Although implicated in all-cause mortality, a SMART substudy did not find any association between IL-6 with BMD loss at hip or spine. Therefore, the role of IL-6 in bone remodeling in HIV-infected individuals remains unclear.

A number of cytokines have functions that have been implicated in bone remodelling is shown in Table 1.[16, 18, 22, 23, 25–47] Several of these cytokines are involved in regulation of osteoclastogenesis via upregulation of RANK expression or RANKL signalling [19, 23]. The pro-inflammatory cytokines TNF α and IL-1 β have long been implicated in inflammatory conditions, such as postmenopausal osteoporosis and RA, where bone loss and destruction is a feature [33, 48–50]. In the SMART study, higher TNF α levels were associated with loss of spine BMD [31]. Another pro-inflammatory cytokine, IL-17, produced by Th17 T-cells, directly acts on stromal cells and osteoblasts to stimulate RANKL production and upregulate the expression of the proinflammatory cytokines TNF α and IL-1 [47]. The role of Th17 cells in the context of HIV-associated bone loss merits further investigation as IL-17 levels in those with HIV infection are elevated compared to non-infected subjects.[43]

Immune activation and inflammation in HIV: Cross talk between B-, T-cells and the RANK/ RANKL/OPG axis

HIV infection is characterized by gut microbial translocation, thought to result from dysfunction in the gut barrier which leads to subsequent activation of both innate and adaptive immune pathways via monocyte activation [51]. Indeed, elevated levels of systemic bacterial cell wall product lipopolysaccharide (LPS) have been reported in those infected with HIV [51]. LPS was the first microbial product shown to induce bone resorption *in vitro* [52], and to increase the number of osteoclast precursors *in vivo* [53]. LPS, a potent activator of innate immune cells [51], also promotes the expression of RANKL by osteoblasts *in vitro*, stimulating osteoclast differentiation [54]. Furthermore, injection of LPS into T- and B-cell deficient mice can induce significant bone loss [55]. Whilst higher circulating levels of LPS and monocyte activation markers such as sCD14 and sCD163 have been reported in HIV-infected patients [38, 39, 41, 56, 57], their impact on bone loss remains to be fully determined.

HIV infection is associated with an increase in the frequency of activated and exhausted CD4⁺ and CD8⁺ T-cells, an increase in monocyte activation markers [58], a reduction in B-

cell numbers, an increase in the frequency of immature/transitional B-cells, and increased circulating levels of inflammatory mediators [23]. This state of hyper-immune activation and inflammation favours RANKL expression, reflected in higher circulating RANKL in those with HIV infection [37, 40, 59, 60] while circulating OPG levels have been reported to be unchanged or lower in HIV-infected subjects [31, 37–40]. This imbalance may potentially bring about a shift in the RANKL:OPG ratio, which would favour osteoclastogenesis in HIV-infected individuals.

As major producers of OPG, B-cells are recognised as important stabilisers of peak bone mass [14–16]. CD40-ligand, expressed by activated T-cells, can upregulate B-cell OPG production via activation of the CD40 co-stimulatory receptor [14]. Indeed, T-cell deficiency or loss of the CD40/CD40L co-stimulatory interaction in mice results in increased bone resorption and decreased bone mass [14]. In humans, osteopenia and fracture are prominent features of hyper IgM syndrome in which *CD40L* gene expression is altered.[61]. Figure 1: outlines the immune-skeletal interface in HIV as described above.

In a recent cross-sectional study, Titanji et al. examined OPG and RANKL expression by B-cells in both ART-naïve HIV-infected and HIV-uninfected individuals, and found a 60% increase in the frequency of RANKL producing B-cells and a 20% decrease in the frequency of OPG expressing B-Cells in HIV-infected persons compared with matched controls [37]. An altered expression of RANKL and OPG by B-cells in HIV-infected individuals was associated with a loss of BMD. There was a significant correlation between RANKL:OPG ratio, BMD, and T-scores (derived from BMD, measuring the difference in standard deviations of an individual's BMD to that of a Caucasian female with peak bone mass) or Z-scores (representing differences from an age-matched reference population) in the femoral neck and hip in HIV infected patients only. This might be due to the fact that RANKL expression in B-cells is not a feature of normal B-cells in uninfected subjects [37].

HIV+ individuals were found to exhibit higher proportions of RANKL expressing B-cells with an activated and exhausted tissue like memory phenotype [37]. In contrast, OPG expression by activated and exhausted B-cells, was lower in HIV+ individuals [37]. These data support an immunocentric basis for HIV-associated bone loss with dysregulation of the OPG/RANKL axis towards osteoclastogenesis, driven by a significant imbalance in circulating B-cell RANKL and OPG expression. The impact of initiation of ART on these disturbances and whether these changes may ultimately contribute to the higher fracture rates seen in HIV-infected patients remains to be determined.

Activated T-cells also play an important role in osteoclastogenesis by producing cytokines such as TNF α , IL-1, IL-6, IL-17 and RANKL. To study the relationship between T-cell activation and BMD, Gazzola et al. assessed 78 HIV-infected patients [60] and found that those with low BMD had higher levels of T-cell activation ((CD4+, HLA-DR+) and (CD8+, HLA-DR+) T-cell subsets). This activated T-cell phenotype was an independent predictor of low BMD. Furthermore, an increase in the frequency of activated T-cell population (CD28⁺/CD4⁺) was also independently associated with low BMD in HIV⁺ patients [60]. Interestingly, in a recent study of a mouse model of RA, the administration with CTLA-4Ig, an inhibitor of CD80/CD28 costimulation and therefore a downregulator of T-cell activation,

resulted in significant gains in bone volume [62]. The drug has recently been approved for use in immune-mediated conditions such as RA. It will be interesting to study how this inhibition of CD80/CD28 might affect bone volume in PLWH.

ART-associated bone loss – the role of immune reconstitution and ongoing immune activation and inflammation

The role of HIV as an independent predictor for low BMD and a risk factor for increased rate of fractures has been well documented [2, 9, 63–66]. Additionally, multiple studies have shown a 2–6% loss in BMD in the first 2 years post ART initiation [41, 67–69]. Increased levels of bone turnover markers (BTM), such as the bone resorption markers osteocalcin and serum type 1 procollagen (P1NP), have been consistently observed with ART initiation in ART-naïve patients and have been associated with increased TNF α levels at ART initiation [9, 39, 70]. Likewise, pre-ART HIV disease severity, measured by absolute CD4 $^{+}$ T-cell counts and HIV RNA, have been associated with a greater subsequent loss of BMD at ART initiation [41, 71]. In a large longitudinal study including 796 patients initiating ART, lower baseline CD4 $^{+}$ T-cell count was strongly associated with greater loss of BMD over 96 weeks. Initiating ART at a CD4 $^{+}$ count of <50 cells/ μ L resulted in 3% more BMD loss than those who initiated ART at a CD4 $^{+}$ count of $\geq$ 500 cells/ μ L [71]. Lower baseline CD4 $^{+}$ T-cell counts have also been associated with increased fracture prevalence in several HIV cohorts [71–74]. These data suggest an important role for the degree of baseline immune activation and inflammation in bone loss and fracture risk in HIV and supports the benefits of earlier ART initiation.

Further support for the role of T-cells in regulation of bone mass was reported by Ofotokun et al. They reconstituted T-cell deficient, TCR-beta knockout (KO) mice with syngeneic CD3 $^{+}$ T-cells and found a significant increase in bone resorption and a decline in BMD following T-cell reconstitution [59]. The re-populated T-cells produced increased levels of the pro-resorptive cytokines RANKL and TNF α , while OPG levels did not differ significantly, shifting the RANKL:OPG ratio towards one favouring bone loss. Finally, administering zoledronic acid (an antiresorptive bisphosphonate) at the time of T-cell transfer protected the TCR-beta KO mice from BMD loss at all sites [59]. While this work provides important insight into the complex relationship between T-cells and osteoclastic bone loss and simulates re-expansion of the T-cell compartment seen at ART initiation, HIV infection itself has complex host interactions and HIV viral proteins can also directly affect osteoclasts. This, as well as the direct effects of ART or varying regimes, were not explored in this model and require further investigation.

The effect of short course anti-resorptive therapy in preventing bone loss in this study was encouraging as it could be used to prevent this irrecoverable bone loss in patients initiating ART. Two randomised control trials of bisphosphonates in preventing bone loss at ART initiation are currently underway; one using oral alendronate (the APART study) (<https://clinicaltrials.gov/show/NCT02322099>) and another, using intravenous zoledronate (<https://clinicaltrials.gov/show/NCT01228318>). These studies will also examine the effects of such strategies on immune activation which will add to the reported work by Natsag et al. on the

interaction between bisphosphonates and the immunoskeletal interface in improving BMD and decreasing fracture risk [75].

Further analyses of the effects of ART on immune activation, inflammation and bone loss were recently reported by Brown et al[38]. In this study, changes in BMD with initiation of ART with a nucleotide reverse transcriptase inhibitor (NRTI) backbone including tenofovir disoproxil fumarate (TDF), combined with either protease inhibitors (either ritonavir-boosted atazanavir or darunavir) or the integrase strand transfer inhibitor (INSTI) raltegravir were examined. BMD declined significantly in all 3 treatment arms although BMD loss in the pooled protease inhibitor arms was greater than the raltegravir arm in both sites. Higher baseline sCD14, IL-6 and hs-CRP were all associated with greater bone loss over the 96 weeks at total hip. In addition, increases in both activated (CD4+, CD38+, HLA-DR+) and senescent CD4+ T-cells (CD4+, CD28⁻, CD57+) were also associated with bone loss at 96 weeks at the lumbar spine. These data suggest that the degree of underlying immune activation and inflammation at least in part determines extent of bone loss after ART initiation, independent of absolute CD4+ T-cell counts or HIV RNA levels [38].

A further study exploring the relationship between BMD and inflammation at ART initiation in 106 ART naïve patients, independent of regimen used (patients were randomised to either efavirenz or ritonavir boosted lopinavir in combination with zidovudine/lamivudine NRTI backbone for 24–48 weeks), reported that, although the inflammatory markers soluble tumor necrosis factor receptor 1 (sTNFR1) and sTNFR2, were suppressed through 24 weeks and sTNFR2 levels remained low through 96 weeks post initiation [41], there was a modest association between sTNFR2 and BMD loss at 96 weeks, which was no longer present when adjusted for baseline CD4+ T-cell count. Data on bone biomarkers or other inflammatory cytokines were not available in this study, making it difficult to speculate on potential pathways through which BMD loss may have been mediated.

The NEAT001/ANRS study groups reported on changes in BMD, alongside inflammatory and bone biomarkers, after 96 weeks of therapy with ritonavir boosted darunavir, in combination with either TDF / emtricitabine (standard therapy) or raltegravir (NRTI-sparing)[56]. The study reported a significantly greater loss in BMD at all sites in those receiving standard therapy. Levels of the bone turnover markers [osteocalcin, Bone-Specific Alkaline Phosphatase (BSAP) and P1NP], as well as RANKL were all increased at baseline in both treatment groups. There was a reduction, but no significant between-group difference, in RANKL at 48 weeks. However, those on NRTI-based therapy had significantly lower OPG levels. The resulting imbalance in the RANKL:OPG ratio therefore potentially contributing to the greater BMD loss seen in the standard group. The ACTG study A5303, which examined BMD changes in 259 ART naïve patients commencing therapy, comparing maraviroc with TDF [57] recently completed work on the immunology substudy and did not detect any significant associations between changes in immunological markers (measured at baseline and week 48) and percent change in hip BMD. The completed results of this study are to be presented at the 2016 Conference on Retroviruses and Opportunistic Infections in Boston, MA.

Pre-ART levels of inflammation have been shown to impact the degree of post-viral suppression, immune activation, and the development of serious non-AIDS co-morbidities [22, 76–78]. However, while emerging evidence suggest that earlier ART initiation is beneficial, more studies are needed to examine the effects of persisting inflammation on bone health in those on long-term suppressive ART. The SATURN-HIV trial aimed to investigate the effect of low dose rosuvastatin on bone health in persons on stable ART [79]. Rosuvastatin decreases monocyte and lymphocyte activation in HIV [80]. Those randomised to rosuvastatin had a small but significant gain in hip BMD which was strongly associated with baseline and week 48 sTNFR2 levels supporting a potential beneficial effect on BMD mediated through modulation of inflammation [79]. In a cross-sectional analysis of this parent study looking at the relationships between immune activation, inflammation and bone health, including 142 participants with median CD4+ T-cell counts of 604 cells/mm³ and median duration of ART of 56 months, 23% had low BMD at hip and 21% at lumbar spine. Those with low BMD at hip had unexpectedly lower IL-6 levels compared to those with normal BMD, while markers of CD4+ T-cell activation were higher in those with low BMD at lumbar spine only. There were no significant correlations between BMD and BTMs with markers of T-cell activation, monocyte activation or inflammatory cytokines [81]. Similarly 96 week data from the study did not show any significant associations with inflammatory markers or T-cell activation markers and BMD[82]. However numbers included were small and therefore it may not have been adequately powered to detect such differences.

Conclusion

HIV infection, even in the setting of effective ART, is a state of immune activation and inflammation that can affect the expression and regulation of key immunological factors involved in bone remodelling. This complex interaction is likely influenced by other parameters including traditional risk factors for low BMD and fragility fracture. Whilst there are data supporting the interplay between immune activation and chronic inflammation and the development of low BMD in HIV, longitudinal studies are needed to establish the role this may have in the pathogenesis of fragility fractures in PLWH.

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* =of interest

** = of considerable interest

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Key Points

There is a high prevalence of low BMD and fractures in PLWH, with initiation of ART significantly contributing to BMD losses.

Important cross talk between the skeletal and immune systems underpins the maintenance of bone mass.

In PLWH, T-cell lymphopenia (including lower CD4+ T-cell counts) and activation alongside B-cell dysfunction are increasingly implicated in the pathogenesis of low BMD in HIV.

Increased inflammation is recognised in HIV infection, with markers of inflammation associated with development of low BMD and osteoporotic fractures in the general population.

Table 1

Cytokine	Effects on the Immunoskeletal Interface	Effects in HIV-related bone loss
INF- γ	<ul style="list-style-type: none"> Produced by activated T cells; activates Antigen Presenting cells (APCs) such as macrophages increasing T-cell activation Shown to stimulate osteoclast formation and bone loss[25] Also shown to suppress osteoclast differentiation by interfering with the RANKL–RANK signalling pathway[26] Polymorphism in INF-γ gene shown to associate with bone loss in elderly women[27] 	<ul style="list-style-type: none"> Downregulated in HIV infection in favour of Th2 (TNF-α) cytokine pathway. Impaired signalling between INF-γ and osteoblasts which normally downregulate RANKL expression[26]
IL-6	<ul style="list-style-type: none"> Produced by immune cells and osteoblasts Estrogen deficiency potentiates secretion of IL-6[29] Strong predictor of bone loss in postmenopausal osteoporosis[18] Polymorphisms in IL-6 gene which causes increased IL-6 levels associated with increased risk of osteoporotic fracture[30] 	<ul style="list-style-type: none"> Higher IL-6 levels in HIV-infected persons[22] Variable results in relation to bone loss in studies: Baseline IL-6 associated with progression to osteopaenia/osteoporosis[23] IL-6 levels however not directly associated with BMD in SMART[31]
IL-7	<ul style="list-style-type: none"> Key role in T- and B-cell homeostasis[32] Promotes RANKL production by T cells[33] Involved in ovariectomy-induced bone loss in mice[34] Mediates T-cell re-expansion post ART initiation[35] 	<ul style="list-style-type: none"> High circulating levels in HIV infection Effects on bone undetermined
M-CSF	<ul style="list-style-type: none"> Key cytokine involved in survival and differentiation of osteoclasts Required for monocyte/macrophage maturation 	<ul style="list-style-type: none"> <i>M-CSF</i> gene expression was higher in a rat model of HIV infection.[16] No human studies to date
OPG	<ul style="list-style-type: none"> Decoy receptor for RANKL 	<ul style="list-style-type: none"> Expressed at lower levels in B cells in HIV+ people[37] Changes in plasma OPG did not correlate with bone resorption in this study Lower plasma levels reported in several studies in HIV infected patients[31, 37–40]
RANKL	<ul style="list-style-type: none"> Key osteoclastogenic cytokine 	<ul style="list-style-type: none"> Expressed at higher levels in B cells in HIV+ people[37] Changes in plasma RANKL did not correlate with bone resorption in this study Higher baseline RANKL levels in HIV infected ART naïve patients.[42,43]

Cytokine	Effects on the Immunosteletal Interface	Effects in HIV-related bone loss
TNF α	<ul style="list-style-type: none"> osteoclastogenic cytokine promotes RANKL production inhibits osteoblast differentiation and activity key inflammatory cytokine 	<ul style="list-style-type: none"> Increased in ART naïve HIV infected patients In the SMART study higher TNF-α levels were associated with loss of BMD at LS[31] Surrogate markers such as sTNF-α and 2 levels have not been significantly associated with BMD[41]
IL-17	<ul style="list-style-type: none"> Elevated levels in synovial fluids of RA patients Stimulates Osteoclastogenesis[42] Enhances expression of inflammatory cytokines TNF-α and IL-1[47] Prominent bone loss in murine model mediated via upregulation of IL-17 and RANKL[44] 	<ul style="list-style-type: none"> Higher circulating levels in HIV-infected persons.[43] No studies on effects on bone directly but those with lower BMI have significantly higher levels of IL-17 in HIV infected patients.[43]
IL-1 β	<ul style="list-style-type: none"> First cytokine shown to stimulate bone loss via stimulation of osteoclasts and inhibiting osteoblasts[45] Associated with postmenopausal osteoporosis[46] 	<ul style="list-style-type: none"> Undetermined effects on bone in HIV