Bone alterations during HIV infection

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SUMMARY

Osteopenia and osteoporosis are common in HIV-1-infected individuals and represent a challenge in clinical and therapeutic management. Since the mechanisms underlying this degenerative process are largely unsettled and it has not yet been determined whether bone dysfunction is linked to HIV-1-mediated direct and/or indirect effects on osteoblasts/osteoclasts cross-talk regulation, this brief review analyzes an array of mechanisms that could account for the dramatic bone derangement (bone loss and osteopenia/osteoporosis) during the course of HIV-1 infection.

KEY WORDS: HIV, Bone lesion, Osteoblasts, Osteoclasts

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The advent of HAART has significantly extended the life-span of patients living with AIDS. The increase in average life expectancy has given rise to the long-term complications of HIV such as metabolic complications (Samaras *et al.,* 2005), cardiovascular disease and osteoporosis.

Bone is a connective tissue characterized by hardness and plasticity, consisting of cells dipped in a mineralized extracellular matrix. This matrix is composed of a net of collagen and glycoproteins which serves as a support for the deposition of calcium phosphate, in the form of hydroxyapatite crystals. Cells forming bone tissue belong to two main populations: osteoblasts and osteoclasts. Osteoblasts are cube-shaped cells characterized by the ability to secrete bone matrix (Harada & Rodan, 2003). They derive from mesenchymal stem cells and at the end of their life are trapped in the matrix and become osteocytes. Osteocytes are connected to each other via long cytoplasmic extensions and form a physical and functional network able to sense mechanical stress and to trigger adaptation of bone (Melissa *et al.,* 2004). The other cell population present in bone is represented by osteoclasts that arise from a bifurcation on the monocyte/macrophage lineage evolving from the common myeloid progenitor. These cells are able to resorb bone by the creation of an acid environment and the secretion of several lytic enzymes (Boyle *et al.,* 2003).

Despite its strength, bone is subjected to a continuous remodelling process necessary both to maintain calcium and phosphates homeostasis and to promote body adaptation to external tensional forces. The bone renewal process is called remodelling and consists of continuous cycles of resorption and deposition of bone matrix. It begins with the proliferation and activation of osteoclasts on the bone surface where they create a lacuna that is subsequently filled by osteoblasts, attracted by molecules released by degradation of bone matrix (Kong & Penninger, 2000). This process is controlled by several mechanisms including systemic hormones, such as calcitonin,

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parathormone or estrogens, and local factors, most of them involved in inflammatory responses such as IL-1, IL-6, TNF-α and prostaglandins. Several studies have demonstrated that the OPG/RANKL/RANK system is the most important system responsible for bone remodelling. RANKL is a soluble factor capable of differentiating and activating osteoclasts after binding with its receptor RANK. OPG, released by osteoblasts, can bind RANKL preventing its interaction with RANK and consequently inhibiting bone resorption (Boyce and Xing, 2007).

Remodelling rates of bone tissue can be monitored measuring molecular markers of bone remodelling. In particular, serum osteocalcin and alcaline phosphatase activity reflect bone formation, whereas products of matrix catabolism, such as cross-linked N-telopeptides of type I collagen (NTx) or C-telopeptides, are indicative of rates of bone resorption (Camozzi *et al.,* 2007).

The status of bone can alternatively be evaluated using dual-energy x-ray absorptiometry (DXA) to determine the bone mineral density (BMD), an areal assessment of mineralized tissue. DXA results are expressed in terms of T-score, that is the number of standard deviations below or above the mean of BMD of a population matched for race and sex (Cummings *et al.,* 2002).

Bone mass is achieved during the first three decades of life after which there is a progressive reduction of the matrix, due to the uncoupling of the processes of deposition and resorption. This phenomenon can evolve to a situation called osteoporosis, a skeletal disease characterized by low bone mass and bone micro-architectural deterioration with a consistent increase in bone fragility and fracture susceptibility. The most common kind of osteoporosis, called primary osteoporosis, is connected to age and is mostly found in postmenopausal women. Conversely, when osteoporosis is the effect of other disorders, such as gastrointestinal diseases (Stazi *et al.*, 2008; Rothfuss *et al.,* 2006), hypogonadism, or diabetes (Strotmeyer & Cauley, 2007), it is defined as secondary osteoporosis. According to WHO osteoporosis is characterized by a T-score value lower than -2.5, whereas a T-score between -2.5 and -1 indicates osteopenia and normality is defined as T-score above -1 (WHO, 1994; Johnell *et al.,* 2005). Clinically osteoporosis is often not detectable until the occurrence of low-trauma fractures, defined as fragility fractures (Becker, 2006; Kanis et al., 2008). Fragility fractures mainly involve vertebras or hips and have significant effects on patients' quality of life, leading to pain and physical incapacity. This severe clinical evolution coupled with the high incidence of osteoporosis in the population indicates a central role of this disease in public health.

HIV-1 AND BONE LESIONS

The upgrading of management and antiretroviral therapy (HAART) of HIV-infected patients have determined a significant increase in life expectancy for these subjects. The extended course of viral infection and prolonged HAART treatment have displayed several degenerative aspects of HIV-related disease regarding different cell lineages. HIV infection *per se* and HAART therapy can affect the homeostasis of several cell types and tissues as well as hormonal status, metabolism and cytokine networks.

A growing body of evidence demonstrates a decreased bone density in HIV positive patients (Loiseau-Peres *et al.,* 2002; Annapoorna *et al.,* 2004; Amorosa & Tebas, 2003, 2006). Serrano *et al.* first carried out an epidemiological study on bone derangement in HIV-1 positive patients. The study involved 21 males and found a small but significant decrease of BMD in HIV positive subjects. Interestingly they performed a histomorphometrical study reporting a marked decrease in bone turnover and reduced bone formation which were more evident in patients with greater disease severity (Serrano *et al.,* 1995). These data were confirmed by several studies where the BMD decrease was observed in larger HIV-1 naïve patient groups (Thomas & Doherty, 2003; Bruera *et al.,* 2003; Amiel *et al.,* 2004). Data concerning bone turnover markers agree with BMD results, generally reporting lower osteocalcin levels and increases in matrix degradation products (Aukrust *et al.,* 1999; Teichmann *et al.,* 2000; Mondy *et al.*, 2003). Altogether these data demonstrated a correlation between HIV-1 infection and bone loss.

Since some observations displayed a BMD decrease in HIV seropositive patients, several groups have analyzed the HAART/bone interaction to determine whether the multidrug treat-

ment is involved in BMD decrease (Carr *et al.*, 2001; Dube *et al.*, 2002; Bongiovanni *et al.*, 2005; Vescini *et al.*, 2005; Garcia-Aparicio *et al.*, 2006; Fernandez-Rivera *et al.*, 2003). The results showed a high variability related to specific drug class and treatment length. Some studies reported a pivotal role of HAART, especially when treatment included a protease inhibitor (PI) (Tebas *et al.*, 2000). These data were also supported by in vitro analysis (Jain & Lenhard, 2002; Fakruddin & Laurence, 2003).

Some cross-sectional studies described a significant decrease of BMD by DXA analysis (Knobel *et al.*, 2001; Mallon *et al.*, 2003; Gallant *et al.*, 2004) especially with the initiation of therapy. On the other hand, other reports failed to reveal any influence of HAART on bone condition, finding no differences in BMD reduction between naive and treated patients (Bruera *et al.*, 2003; Lawal *et al.*, 2001; Dube *et al.*, 2002; Landonio *et al.*, 2004; Amiel *et al.*, 2004). Bolland and coworkers performed a longitudinal study on 23 HIV-infected HAART-treated men and 26 uninfected controls, evaluating BMD and bone turnover markers over 2 years. At the end of follow-up BMD was stable or increased with respect to baseline and with no significant differences compared to a control group (Bolland *et al.*, 2007). This apparent paradox could be explained by the hypothesis of a double effect derived from HAART. Initially drugs exacerbate abnormalities in bone homeostasis associated with HIV infection (Mallon *et al.*, 2003). Then HAART improves the general condition of the patient and restores some cytokine networks allowing normalization of the bone remodelling process (Nolan *et al.*, 2001). In addition, it is noteworthy that the composition of the cocktail may be pivotal for the induction of bone mass loss suggesting a complex and still not elucidated interaction between HAART and bone structure.

Brown and Qaqish performed a meta-analysis reviewing papers published from 1994 to 2005, concerning epidemiological studies on skeletal disorders in HIV positive patients. Their work estimated a threefold higher prevalence of osteoporosis in HIV-1 seropositive patients and a relative risk of 6.5 related to HIV infection (Brown & Qaqish, 2006). Although these findings have been partly criticized for the poor attention paid to confounding factors (Berg *et al.*, 2007; Brown & Qaqish, 2007), the association between HIV-seropositive status and decrease in BMD was ascertained. A recent study that enrolled 492 patients belonging to the Aquitaine Cohort reported osteopenia in 50% and osteoporosis in 30% of cases. The bone abnormalities mostly involved the trabecular bone in women whereas the cortical bone area was mainly affected in men. This anatomical localization is not specific for HIV-positive patients because even the HIV-negative osteopenic patients showed the same gender-dependent bone lesions (Cazanave *et al.*, 2008).

In the HIV seronegative population osteopenia and osteoporosis are mostly observed among women (Bonnick, 2006; Dennison *et al.*, 2006). Bone metabolism in women is related to hormonal status because of the protective role of estrogens which basically consists in downregulating the production of pro-inflammatory cytokines such as IL-1, IL6 and RANKL thereby inhibiting osteoclast differentiation (Sorensen *et al.*, 2006). There are fewer data on BMD in HIV seropositive women with respect to men, but they clearly identify an impairment of skeletal condition associated with infection and HAART (Huang *et al.*, 2001; Teichmann *et al.*, 2003; Yin *et al.*, 2005; Dolan *et al.*, 2006).

A recent work compared bone mineral density between 152 HIV-seropositive and 100 healthy women. Results disclosed a highly significant ($p<0.0001$) decrease in mineralization in HIV subjects, which remained significant also after controlling for race. This work also identified low weight ($p=0.014$), presence of oligomenorrhea ($p=0.0006$) and reduced testosterone levels ($p=0.0007$) as contributors to bone loss among the HIV population (Dolan *et al.*, 2007).

High decreases in BMD lead to a high risk of fragility fractures, especially in aging people (Guaraldi *et al.*, 2001; Arnsten *et al.*, 2007; Mackey *et al.*, 2007). Prior *et al.* carried out a study on 138 HIV+ and HIV- women, median age 38 years, belonging to the Canadian Multicentre Study for Osteoporosis. They reported higher incidences of fractures in HIV patients than in the control group (26.1% vs 17.7%), but no difference in BMD. The authors suggest as a possible explanation that HIV exerts its influence on bone structure rather than bone mineralization (Prior *et al.*, 2007).

Childhood is a critical period for skeletal forma-

tion because more than 50% of ultimate adult mineral content is gained over this time. In children alterations connected to infection can lead to growth delay, and all these factors can impair bone modelling (Bianchi, 2007). To date longitudinal and cross-sectional studies reported low BMD, low BMD accrual and biochemical evidence of enhanced bone turnover in HIV vertically infected children (O' Brien *et al.,* 2001; Arpadi, 2000; Arpadi *et al.,* 2002; Mora *et al.,* 2001, 2004; Jacobson *et al.*, 2005; Verweel *et al.,* 2007). The most recent study published was performed on 27 children (aged 5-17 years) and reported a significant difference in bone mineralization between HIV positive children and the control group. Analysis of turnover markers confirmed the evidence of bone derangement. thereby avoiding doubts related to densitometry measurements in children (Binkley *et al.,* 2008; Pitukcheewanont *et al.,* 2005). Indeed bone resorption, measured as bone collagen equivalent/NTx ratio was markedly increased in HIV seropositive children ($p<0.00001$) and the difference remained highly significant also after controlling for age, gender and height (Mora *et al.,* 2007).

PATHOGENESIS

The role of HIV-1 infection

The pathogenesis of bone mass loss during HIV infection is still not elucidated. Early studies on HIV/osteoblasts interaction to determine whether HIV is able to infect osteoblasts yielded controversial results. Although several studies in the 1990s indicated the susceptibility of osteoblasts to HIV infection (Mellert *et al*., 1990; Campbell *et al.*, 1996), the most recent report on this issue failed to confirm this finding (Nacher *et al.,* 2001). Recent observations have reported that several HIV proteins may affect the functionality and maturation of osteoblasts. Indeed it has been reported that HIV can induce apoptotic stimuli in mesenchymal stem cells which are precursors of osteoblasts (Wang *et al.,* 2002). Moreover Cotter *et al.* demonstrated that HIV-1-gp120 and p55 gag can reduce bone alkaline phosphatase activity and calcium deposition by osteoblasts and that Rev and p55gag, but not gp120, affect the differentiation of mesenchymal stem cells towards the osteoblastic lineage (Cotter *et al.,* 2007).

We recently demonstated (Gibellini *et al.,* 2008 manuscript in preparation) that HIV-1, heat-inactivated HIV-1 and recombinant gp120 triggered apoptosis in primary osteoblasts and HOBIT cells, suggesting that HIV-1 does not infect osteoblasts and the apoptosis induction is related to gp120/cell membrane interaction, resembling a mechanism similar to that already demonstrated in derangement of progenitor cells (CD34+) (Re *et al.,* 1994, Gibellini *et al.,* 2007).

Besides the HIV effects on osteoblasts, several studies have focused on HIV activity either on osteoclast or osteoblast/osteoclast cross-talk. As cited above, the most important regulative mechanism of osteoclast/osteoblast activity is the OPG/RANKL/RANK system (Boyce & Xing, 2007). Since RANKL and OPG are yielded by lymphocytes (Chakravarti *et al.,* 2007; Li *et al.,* 2007) other than osteoblasts, increased levels of this protein are common in pathologies characterized by persistent immune activation (Ueland *et al.*, 2001) that can determine bone loss (Clowes *et al.,* 2005). The production of both proteins is altered in HIV infected individuals (Seminari *et al.,* 2005; Gibellini *et al.,* 2007; Mora *et al.,* 2007) and their levels are correlated with bone status (Konishi *et al.*, 2005) and infection parameters (Gibellini *et al.,* 2007). Interestingly, RANKL can also be upregulated by viral proteins. As demonstrated by Fakruddin and coworkers, gp120 and Vpr can both induce secretion of biologically active RANKL after contact with lymphocytes (Fakruddin & Laurence, 2003, 2004, 2005) suggesting an osteoclastic hyperactivation in the bone compartment with bone homeostasis imbalance. Osteoclasts can also differentiate under the influence of TNF-α, that is a cytokine commonly increased in the serum of HIV patients (Breen, 2002). In the study performed by Aukrust *et al.* TNF-α levels correlated negatively with osteocalcin and positively with c-telopeptide at baseline and all three molecules were in the normal range after treatment, suggesting an etiological role for TNF-α a in bone loss (Aukrust *et al.,* 1999).

HIV is associated with a number of conditions which, in turn, represent risk factors for osteoporosis (Tomazic *et al.,* 2007). Undernutrition and adsorption pathologies which can influence levels of vitamin D and calcium often go noticed in HIV seropositive patients (Cashman, 2007), as do

endocrine complications such as androgen and estrogen deficiency (Madeddu *et al.,* 2004). Moreover in this group some specific habits like smoking, drug abuse or alcohol consumption all associated with an increased risk of bone loss are common (Cooper *et al.,* 2003; Cummings & Melton, 2002). Fausto *et al.* (2006) built a model based on observations on 161 HIV-1 positive patients to estimate the importance of classical risk factors in HIV associated osteopenia and found that this condition as well as HIV infection can be considered predictive factors of bone loss.

The role of HAART

The real impact of HIV-1 infection and/or HAART therapy in the assessment of bone loss remains largely unsettled even though some observations suggested a direct pathogenetic role of therapy (Klotsas & Klotsas, 2007; Brown & Qaqish, 2006; Bongiovanni *et al.,* 2003). In spite of controversy over the real in vivo effects of HAART on bone structure, several studies on specific antiretroviral compounds were performed.

The association between osteopenia and PI was disclosed by several in vitro models and seems to have a different etiology depending on the specific molecule (Pan *et al.,* 2004, 2006). Malizia *et al.* (2007) performed a large microarray analysis on osteoblasts and demonstrated an increase in the transcription of several inflammation related genes, such as MCP-1 and IL-8, after contact with Nelfinavir, Saquinavir or Ritonavir. Similar results are the outcome of a previous study carried out by Jain *et al.* where Nelfinavir, but not Ritonavir and Saquinavir, inhibited osteoblastic differentiation of mesenchymal stem cells and decreased osteoblast functionality (Jain & Lenhard, 2002).

Although therapy cocktails always contain one or more trascriptase inhibitors, there are no specific epidemiological data available regarding their action. However some in vitro experiments demonstrated that they might induce the differentiation of osteoclasts (Pan *et al.,* 2006).

Bone metabolism alterations can be determined by functional damage of other organs such as liver (Collier, 2007) or kidney (Moe *et al.,* 2006) involved in bone homestasis. In addition, PIs can impair vitamin D synthesis in vitro, by suppressing the activity of several enzymes involved in its synthesis and catabolism (Cozzolino *et al.,* 2003) inhibiting osteoblast anabolic activity.

BONE LOSS TREATMENT IN PATIENTS WITH HIV

Several published trials examined pharmacological protocols to avoid bone loss in HIV seropositive patients. Most of them concerned the use of biphosphonates, in particular alendronate, to increase bone mass of HIV patients (Mondy *et al.,* 2005; McComsey *et al.,* 2007; Lin & Rieder, 2008; Guaraldi *et al.,* 2004). McConsey *et al.* compared the effects of alendronate daily intake versus placebo in a diet supplemented with calcium and vitamin D. Although they could not assess the effect on risk fractures, they found a significant increase in BMD, comparable to that observed in the non-HIV population and did not notice major adverse effects (McComsey *et al.,* 2007). Only one study examined the role of testosterone but some positive effects of this hormone on BMD have been hampered by several adverse effects (Fairfield *et al.,* 2001).

In spite of the availability of effective drugs the most important question in the clinical management of HIV-associated bone loss is how to assess the need for treatment. To find a subset of higher risk population it is conceivable to support BMD evaluation with other information on patients such as smoking, drug abuse and previous reported traumas. Moreover, before starting a therapy, it may be appropriate to try vitamin D and calcium supplementation and recommend smoking cessation, decreased alcohol consumption and physical exercise.

CONCLUSIONS

Osteopenia and osteoporosis are described in a higher proportion of HIV-infected patients than healthy individuals (Paton *et al.,* 1997; Annapoorna *et al.,* 2004; Glesby, 2003; Amorosa & Tebas, 2006; Martin *et al.*, 2004). Although several general mechanisms could be involved in bone derangement during HIV-1 infection (physical inactivity, low body weight, decreased intake of calcium and vitamin D and so on) and may contribute to decreased bone mineral density in HIV-infected patients, the role of increased levels of pro-inflammatory cytokines associated with HIV infection, and HIV itself, may also contribute directly to accelerated bone loss (Glesby, 2003;

Gibellini *et al.,* 2007). Moreover even though a high incidence of osteopenia/osteoporosis has been associated with both protease inhibitor (PI) and nucleoside/nucleotide reverse transcriptase inhibitor (NRTI)-based treatments (Pan *et al.,* 2006) the observations on HIV-1 naïve patients suggested a consistent direct pathogenetic role of HIV-1 (Bruera *et al.*, 2003; Gibellini *et al.,* 2007) eliciting the derangement of bone homeostasis. Overall, these studies, performed on the relationship between HAART, HIV and osteopenia/osteoporosis, show the clinical growing importance of bone damage in the course of HIV infection and the need to disclose the pathogenesis mechanisms of bone derangement. Hence, the management of bone damage in HIV-infected patients represents a major concern and must be solved in the near future for a useful follow-up of HIV seropositive patients.

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