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Pediatric HIV infection and bone health: an emerging challenge

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TABLE OF CONTENTS

1. ABSTRACT

The available data indicate that HIV-infected children and adolescents have reduced bone mass compared to healthy peers. The increased survival due to the control of HIV infection by potent antiretroviral treatment, exposes patients to the achievement of a reduced peak bone mass and to an increased fracture risk during adult life. Reduced bone mass in HIV-infected children is the result of altered bone metabolism, showing significantly increased bone resorption rate. Both infection *per se* and the use of certain antiretroviral compounds seem to contribute to the altered metabolism. Preventative measures to improve bone health are thus necessary in all young patients that exhibit low bone mass measurements and altered bone metabolism.

2. INTRODUCTION

The interest in children's bone health has been expanding over the last decade, following the evidence for a role of bone accrual in the determination of fracture susceptibility in adult life (1). In addition, the application of bone mass measurements to the pediatric population unraveled an ever expanding number of chronic diseases and behavioral derangements associated to important reduction of bone mass (2-5). Low bone mineral density (BMD) is a recently recognized metabolic complication of human immunodeficiency virus (HIV) infection and its treatment, even in children and adolescents (6-8). The decreased morbidity and mortality rates of HIV-infected youths attributable to more effective antiretroviral therapy has contributed to an increased survival well into

adulthood. Bone health is therefore becoming an emerging issue in these patients, and a precocious screening of patients at higher risk is mandatory to assure an optimal bone mass accrual.

In the following paragraphs we will review the physiology of bone mass accrual, the issues regarding the measurements of bone mass and bone metabolism in children and adolescents, and the results obtained in the studies that considered HIV-infected youths. Possible pathophysiologic mechanisms responsible for the bone findings will be also discussed.

3. PEDIATRIC BONE HEALTH

Bone mass increases considerably during growth, reaches peak values in young adult life, and declines with age both in men and women (9,10). The rate of gain in bone mass during childhood and adolescence is very rapid, about 4 to 8% per year (11,12), and the maximum accrual is reached during the 2-year period across peak height velocity (13). The exact age at which values for bone mass reach their peak at various skeletal sites has not yet been determined with certainty. It is likely that the timing of peak values differs between the axial and the appendicular skeletons, and between men and women. The differences among studies are likely to be a reflection of the different modalities used for measuring bone mass. Peak bone mass (PBM) is determined by several factors. The most important of all is heredity, which accounts for up to 60-80% of its variance (14-16). The genetic potential is achieved only if environmental modifiable factors are optimal. Of fundamental importance in this issue are loading or weight bearing physical activity, nutrition, and a correct hormonal status. Several hereditary or acquired disorders may compromise children's bone health. The list of conditions leading to secondary osteoporosis in children is constantly expanding (2-5). In many cases low bone mass is the result of different causes, such as decreased mobility, unbalanced nutrient intake, increased cytokine production, and alteration of hormonal function, acting at the same time. Furthermore, medications that are often used to treat many chronic diseases play an important role in the determination of low bone mass. The effect of each disease or medication on individual patient is unpredictable. For this reason it is important to seek reliable diagnostic tools to identify patients at risk of bone fragility. The understanding of the factors that contribute to bone strength should help to identify the best screening tools.

Bone mass is not the sole determinant of whole bone structural strength (17): bone size and the distribution of bone mineral within the bone greatly influence the resistance of bone to fracture. Given two bones of equal true mineral density, the larger bone will be less likely to fracture. In addition, resistance of bone to torsion increases with the distance of bone mass from the center of the bone. The ideal technique for bone measurements should be able to measure all the parameters simultaneously with little side effects at a reasonable cost. However, currently available non-invasive techniques vary considerably in their ability to quantify the true density and the geometric properties of bone.

4. NON-INVASIVE BONE MASS MEASUREMENTS IN CHILDREN

The development of precise non-invasive methods for measuring bone mineral content has considerably improved our ability to study the changes in bone mass occurring in various physiological and pathological settings. A variety of non-invasive methods are available to assess the peripheral, central or entire skeleton, and each is more sensitive than conventional radiography for detecting deficits in bone mineral. However, all available techniques have known limitations that may affect the interpretation of bone mass measurements.

4.1. Dual-energy -x ray absorptiometry

Dual-energy x-ray absorptiometry (DXA) has become the most commonly employed method for the assessment of the bone mineral content (BMC) worldwide (18). X-rays emitted at two energy levels are attenuated during the passage through the body, and the attenuation values are converted into measurements of mass of mineral with the use of calibration materials, and the results are expressed in g. BMC values are frequently divided by the projected area of the bone analyzed, and the resulting measurements are conventionally referred to as bone mineral density (BMD expressed in g/cm^2). DXA measurements are considerably influenced by the size of the bones measurements, and by the amount of soft tissue surrounding the bones. Because bones with equal true mineral density but different dimensions give different results with DXA (19), the influence of bone size on these measurements needs to be accounted for. DXA values are also influenced by the unknown composition of soft tissues in the beam path of the region of interest. Because corrections for the soft tissues are based on a homogeneous distribution of fat around the bone, changes in DXA measurements are observed if fat is distributed inhomogeneously around the bone measured (20). Although this is not a limitation when studying subjects whose weight and body size remains fairly constant, DXA bone measurements in growing individuals reflect a large number of biological parameters. DXA technique is the most used in pediatric studies because of its widespread availability, rapidity, negligible radiation exposure, and low cost. However, several important issues should be considered in the evaluation of pediatric DXA studies. DXA measurements are heavily size-dependent: the evaluation of growing individuals is therefore very challenging (21). The limitations include technical issues related to the acquisition of data, and issues relating to the use of the data that are generated.

4.2. Quantitative computed tomography

Quantitative computed tomography (QCT) allows for the independent study of the marked alterations that occur in the size and the shape of the skeleton during growth, as well as the concomitant changes in the bone volume and bone density, without the influence of the surrounding soft tissues (22-24). QCT measurements of bone are able to separately assess the density of cortical and trabecular bones. Moreover, the scan section has a definite

thickness, which varies according to the region examined. Bone density measurements are expressed as grams per cubic centimeters, and are therefore true volumetric measurements. Analysis of bone mineral density and bone size by computed tomography requires the availability of a CT scanner and a proper monitored setting. Recently, smaller, portable, and less expensive QCT devices have been introduced in the market (25). These scanners are not designed for axial skeleton examination, but they assess the bones of the appendicular skeleton: for this reason the technique has been defined as peripheral QCT (pQCT). The major advantage of QCT and pQCT used in children is the ability to assess both the dimensions and the true density of bone. Unfortunately, the cost and accessibility of CT scanners have limited its use for bone measurements in children. The radiation exposure is relatively low and localized in the appendicular or axial skeleton. The effective dose of radiation varies from 3 to less than 30 μSV and these figures include the radiation associated with screening digital radiographs used to localize the site of measurement (21). Therefore, bone measurement determinations using QCT, such as those using DXA, do not expose children to amounts of ionizing radiation that deviate from the amount that constitutes part of their normal life experience (the average natural background irradiation is 200 μSv/month).

4.3. Quantitative ultrasonography

Ultrasound measurements have been recently applied for the study of bone (quantitative ultrasound, or QUS). Measurements of the changes occurring in the velocity and energy of ultrasound waves passing through bone are the basis of QUS. In general, QUS devices measure one or two parameters: the speed of sound (SOS), and the broadband ultrasound attenuation (BUA). The former is obtained by dividing the width of the region of interest by the transit time and is expressed in meters per second. The loss of acoustic energy that occurs when the ultrasound wave is absorbed or scattered by the medium through which is propagated results in a reduction of the amplitude of the wave (BUA). BUA measurements are expressed in decibel per megahertz. The number, thickness, and mineral content of bone trabeculae, as well as their three-dimensional arrangement influence ultrasound measurements. For this reason, QUS measurements reflect bone geometry, and not only bone mass properties. SOS and BUA are measured in the peripheral skeleton to overcome interferences due to excessive soft tissue. The sites where QUS measurements are currently performed are the heel, the tibia, the distal forearm, the phalanges, and the patella. The use of ultrasonography in the assessment of bone health in children is increasing because of its low cost, ease of use, and absence of ionizing radiations. Several issues should however be considered in the applicability of this method for obtaining bone measurements. Positioning is a key issue in QUS measurements: small changes in the position of the transducers leads to markedly different results, regardless of the skeletal site considered. This problem obviously affects negatively reproducibility. Moreover, the sites where QUS measurements are performed do not represent accurately the properties of the entire skeleton, thus limiting the applicability of the method. A source of major inaccuracy of QUS technique employed in pediatrics is represented by the size of the ultrasound transducers. As for the other techniques, QUS devices were designed and realized for adults. The transducers' size is thus often inappropriate for smaller subjects, leading to marked errors in the QUS measurements. Some manufacturers offer now transducers of various sizes, suitable for the use in infants and children.

5. BONE METABOLISM IN CHILDREN

Growth of the skeleton and increase of bone mass are achieved by the coordinated activity of two distinct bone cell types: osteoblasts synthesize new bone matrix and promote therefore bone formation, while osteoclasts operate bone resorption (26). Bone metabolism in children is the result of different physiologic processes occurring at the same time in different portions of the skeleton: bone elongation, increase in bone circumference, and bone remodeling. Elongation occurs by endochondral bone formation that takes place in the growth plates. Increase of bone circumference is the result of a process called bone modeling (27), which involves the presence of osteoblasts and osteoclasts on opposite sites of a given bone segment. Osteoblasts are active incessantly during bone modeling, promoting rapid increases in the amount of bone tissue. During this process osteoclasts usually remove less bone tissue than is deposited by osteoblasts. Consequently, modeling usually leads to a net increase in bone mass. The bone tissue that is created is constantly turned over in a process called remodeling (28). Remodeling takes place in the whole skeleton in discrete sites called basic multicellular units (BMU), in which the action of osteoclasts precedes and is coupled with that of osteoblasts that synthesize new bone tissue.

Readily available to physicians and researchers are reliable biochemical tests to be performed on blood or urine samples, which mirror the ongoing bone metabolic processes (29). These tests are based on the measurements of either an enzymatic activity characteristic of the bone forming or resorbing cells, or bone matrix components released into the circulation during bone apposition or resorption. The bone formation markers most frequently used in children and adolescents are bone-specific alkaline phosphatase (BALP or BAP), osteocalcin (OC), and the C- and N-terminal propeptides of collagen type I (PICP and PINP). Among others, the bone resorption markers that are frequently employed in pediatric studies are deoxypyridinoline (DPD), and the C- and N-terminal telopeptides (ICTP, CTX, NTx) of type I collagen.

The concentration of bone metabolism markers is higher in children than in adults, because of the intensive growth, modeling and remodeling activities. Moreover, the serum and urine levels change markedly as a function of age and pubertal stage. Maximum levels are observed in infancy and during the pubertal period, when skeletal growth is more rapid (30,31).

6. BONE MASS AND METABOLISM IN HIV-INFECTED CHILDREN

HIV infects many cell types in the body, leading to diverse immunologic and metabolic effects. To date, much attention has been deserved to the complications related to the use of potent antiretroviral treatment in HIV-infected patients. The main focus has been on metabolic disorders associated with cardiovascular disease such as insulin resistance, hyperlipidemia, and fat redistribution. However, with the increasing life expectancy related to the new therapies, disease of bone and mineral metabolism are becoming increasingly apparent.

Several factors may negatively affect bone and mineral metabolism: direct interaction of HIV with cells of the bone and bone microenvironment, chronic T-cell activation, abnormal cytokine production affecting osteoblast and osteoclast functions, disturbances of calcium homeostasis, parathyroid hormone function, vitamin D metabolism, opportunistic or neoplastic diseases, and adverse effects of drugs. Considerable attention has been placed on the effect of highly active antiretroviral treatment (HAART) on bone health, and in particular on the adverse effect of protease inhibitors (PIs).

The available data on skeletal health of HIV-infected children and adolescents indicate a reduced bone mass and an altered bone metabolism rate. The causes of impaired skeletal health are still largely unclear.

6.1. Bone mineral measurements

Many studies on bone mineral measurements in HIV-infected children indicate an important reduction of bone mineral content, bone mineral density or QUS parameters expressed both as absolute values or as standard deviation scores for the mean of age- and sex-matched healthy individuals (z scores).

O'Brien an collaborators measured total body bone mineral content by DXA in 19 young girls (mean age 9.2 years) with vertical HIV infection (6). Total body BMC was on average 2.7 z scores below age-and race-matched values reported in non HIV-infected healthy girls.

A cross-sectional analysis of total body mineral content (TBBMC) in 51 (26 males) HIV-infected prepubertal children and 262 healthy children aged 4.2 to 14.7 years (7) showed that TBBMC was lower in HIV-infected children than in healthy controls; the reduction in TBBMC remained significant after adjusting for age, sex, race, weight and height. In the HIV-infected group, no associations were observed between TBBMC and use of PIs, duration of treatment with antiretroviral drugs, viral load or CD4+ cells count.

In another study, 35 HAART-treated HIV-infected children and 314 healthy controls have been included (8). All the patients were in good general health, with well controlled infection. All patients were receiving PIs as part of antiretroviral treatment. Spine and total body BMD were significantly lower in HAART-treated children than in healthy children. The differences in bone mineral measurements, obtained with DXA, could not be attributed to differences in body size or to delayed maturation, because all patients had normal anthropometric measurements and pubertal development.

Fifty patients studied with DXA receiving HAART have been studied in a cross-sectional manner (32). All but 3 patients received PIs as part of treatment. Low BMD values were found in 40% of the patients, and the mean lumbar spine z score was -0.66.

Calcaneal BUA measurements were obtained in a group of 44 HIV-infected children (33). About half of them had no or mild symptoms, and the others had severe clinical symptoms. The patients of the latter group had lower height and bone age standard deviation scores compared to patients with mild symptoms. QUS measurements expressed as z scores were significantly lower in the group of patients with clinical symptoms, compared to the group of patients with mild symptoms.

Differences in skeletal maturation and stature have been reported in an other study in which QUS parameters were measured at the distal phalanges in 44 HIV-infected patient (mean age was 10.4 years) (34). Lower QUS measurements were observed in the group of patients compared with those obtained in a large sample of healthy children. The authors compared the QUS variables after adjusting for skeletal age retardation, and they found that the difference between patients and controls remained significant.

Bone mass measurements indicate that low values are found both in children with growth retardation and in children with no signs of skeletal age or pubertal delay. Nevertheless, some concern has been expressed by the use of techniques that are greatly influenced by skeletal size. A recent study compared the results obtained using DXA with those of QCT in a group of 58 HIV-infected children (35). They were matched with 58 healthy controls, and several variables were compared. Vertebral bone area, BMC, BMD, and their z scores measured by DXA were significantly lower in patients compared to healthy controls. Similar results were found when the entire skeleton was studied. In contrast, the results obtained with QCT indicated that vertebral bone density and z scores were similar in both groups. HIV-infected children were also found to be shorter and with a retarded pubertal development compared to matched controls. The authors conclude that the findings obtained by DXA in HIV-infected children reflect the smaller bone and body size rather than true decreased bone mass measurements. This study does not however clarify the low measurements observed in HIV-infected children who were in good general health, and showed normal growth and pubertal development.

Longitudinal studies indicate that bone mineral accrual of HAART-treated children is impaired, compared to healthy children (36,37). In a 12-months prospective study, The BMD accrual of HAART-treated patients was comparable to that of healthy control patients at the

vertebral site, but it was lower than that of controls in the whole skeleton (36). In another study, 60% of the patients had no change or decreased BMD z scores (37).

The development of new potent antiretroviral drugs that change drastically the daily schedule of assumption, increased dramatically the compliance to the therapy and also improved the quality of life of HIV-infected patients. The use of tenofovir (one of the new molecules) has however been linked to a reduction of bone mineral measurements in primates (38) and adult patients (39). The available data in children are still scarce. To date only two longitudinal studies have been published (40,41). One study found that children switched to an antiretroviral regimen containing tenofovir showed an unaltered bone mineral accrual over a follow-up period of 12 months (40). On the other hand, when tenofovir was used as a salvage therapy in children not responding to more classical HAART, a decrease in BMD was observed after 24 and 48 weeks of the new regimen (41). The apparently divergent results are probably due to the different cohorts of patients studied, and the diverse doses of tenofovir employed in the two studies. Larger studies are needed to understand the effect of new drugs on bone mineral accrual in children.

6.2. Bone metabolism

Alterations of bone metabolism have been reported in HIV-infected children. Increased serum concentration of 1,25-$(OH)_2$-vitamin D_3 and parathyroid hormone were described in young girls (6). Elevated urinary calcium excretion, normalized for creatinine excretion, were also found (6). Urine NTx (a marker of bone resorption) positively correlated to 1,25-$(OH)_2$-vitamin D_3 concentration. These data indicate a calcium insufficiency that promotes an imbalance of bone metabolism.

HAART-treated children showed higher BALP and PINP (markers of bone formation) and NTx (marker of bone resorption) levels as compared to antiretroviral naïve children and controls (8). In addition, a longitudinal study indicated that BALP and NTx concentrations were significantly higher compared to controls both at baseline and after a 12-month follow-up (36).

Children not receiving protease inhibitors showed reduced serum concentration of osteocalcin, and high levels of urinary NTx (42). Serum concentration of insulin-like growth factor-I (IGF-I), and insulin-like growth factor binding protein-3 (IGFBP3) measured in these patients were comparable to those of healthy controls. Nevertheless, some patients had IGF-I concentration below normal, and their values were correlated to low bone mineral measurements. In another study, HIV-infected patients with severe symptoms showed significantly lower osteocalcin concentration, compared to patients with mild symptoms and healthy controls (33). Bone resorption, assessed by urine measurements of DPD, was also depressed in patients with severe symptoms. This group of patients showed reduced serum free IGF-I and total IGF-I levels, and increased interleukin-6 (IL-6) concentration. Severely affected children may thus have an impairment of growth factors secretion (IGF-I), possibly due to overproduction of IL-6.

6.3. Pathogenesis of skeletal health impairment

A still open issue is the role of antiretroviral treatment or infection *per se* in the genesis of poor bone health in HIV-infected youths. Most of the studies have been performed in children who were receiving different antiretroviral drugs. The cohorts studied are extremely heterogeneous in terms of treatment regimens employed, and thus it is not possible to delineate definitive conclusions on the role of different classes of drugs on skeletal health. On the other hand, two studies reported results on HIV-infected untreated children (8, 43). Five vertically infected patients, classified as long-term non progressor, were examined, and their DXA measurements were compared to those of treated patients and healthy controls (8). Vertebral and whole body BMD values were found to be significantly higher to those of HAART-treated patients, and comparable to those of healthy children.

Horizontally infected children have been studied before starting antiretroviral treatment in another study (43). The vertebral and whole body BMC values of the 16 HIV-infected children, did not differ from those of healthy controls. To account for anthropometric differences, a subgroup of 13 patients was matched with pair-matched by anthropometric measures, sex, and age with healthy children. No differences in BMC values between the two groups were found. These data seem to indicate that infection *per se* may not play an important role in the determination of impaired bone health in children. Nevertheless, more data are needed to clarify this issue.

The processes of bone modeling ad remodeling are regulated by many systemic and local factors that assure an optimal balance between resorption of old and production of new bone. Bone resorption is made possible by the recruitment of osteoclast progenitors present in the bone marrow (44,45). Stromal cells and osteoblasts, following mechanical or humoral stimuli, produce a cytokine (RANK-ligand, or RANKL) that by binding on a specific membrane receptor (receptor activator of nuclear factors kappa B, or RANK) expressed on osteoclast precursors induces the fusion and the differentiation, and promotes the activity of mature osteoclasts (46). Stromal cells and osteoblasts produce also a soluble decoy receptor, named osteoprotegerin (OPG) that binds to RANKL, regulating its activity. The production of RANKL and OPG are stimulated by several different cytokines and hormones acting in concert to control osteoclasts (47). Circulating concentration of OPG and RANKL can be accurately measured, and may have a role in monitoring metabolic bone diseases (48).

Bone metabolism derangements are the likely cause of impaired bone mass observed in HIV-infected children. Accelerated bone resorption has been observed even in children with very well controlled viral replication and no symptoms (6,8,36). *In vitro* experiments documented the induction of RANKL in T cells exposed to soluble HIV-1 envelope glycoprotein gp120 (49).

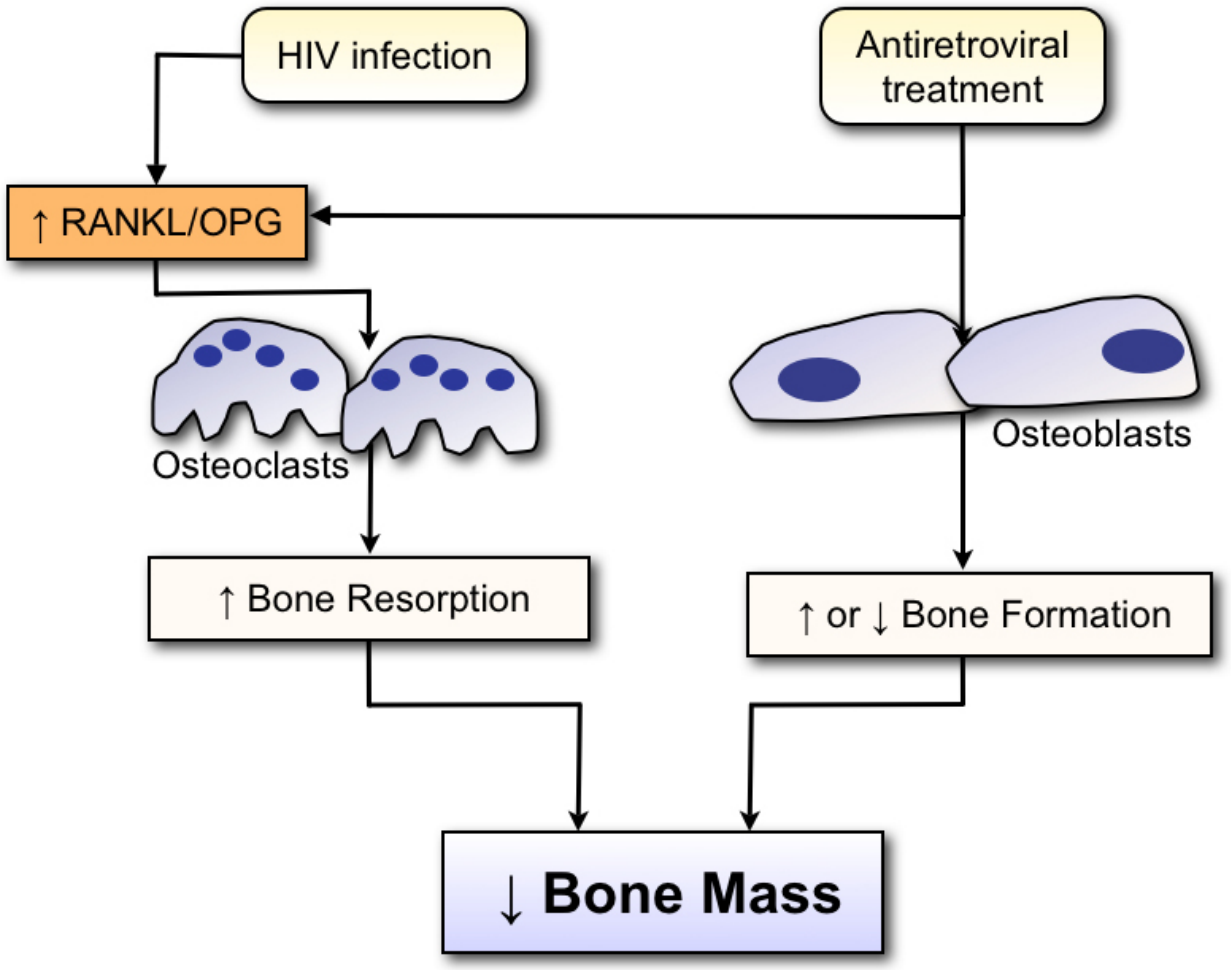

Figure 1. The observed reduced bone mass in HIV-infected children is the result of altered bone metabolism rate. Available data indicate that children receiving potent antiretroviral treatment (HAART) have increased bone formation and bone resorption rate, while children receiving different therapeutic regimens have increased bone resorption and decreased bone formation. Both infection *per se*, and certain antiretroviral compounds act on bone cells, promoting osteoclastogenesis via amplification of the RANKL pathway, and altering osteoblast activity.

Moreover, pharmacologic concentrations of some PIs used in cell culture have been found to abrogate a physiologic block to RANKL activity, thus promoting the differentiation of osteoclastic precursors (49). These data indicate that infection per se may be responsible for the increased bone resorption activity, and that some antiretroviral drugs may potentiate such effect. A recent study performed in HIV-infected children revealed increased serum concentration of both RANKL and OPG (50). More importantly, the study showed an increased RANKL/OPG ratio in HIV-infected youths compared to healthy subjects. The imbalance between the two factors indicates a predominance of RANKL, which in turn may be responsible for an increased osteoclast activation, leading to a decreased bone mineral mass. To study the influence of PIs on RANKL and OPG production, the patients were categorized according the PI they were receiving: lower RANKL serum concentration and RANKL/OPG ratio in patients receiving ritonavir were observed, but the difference with the other patients receiving different PIs was not significant (50). The small number of subjects in each group certainly limited the power of the statistical test.

The available data do not offer a clear picture of the pathogenesis of skeletal alterations observed in HIV-infected youths. *In vitro* evidence indicate a direct role of viral infection for increased bone resorption rate (49). Moreover, certain antiretroviral compounds may increase bone resorption, or alter normal osteoblast function (51). Altered mechanisms of osteoclastogenesis, found in antiretroviral experienced children (50), offer a possible key to interpret bone mass measurements and bone metabolism data. The effects of HIV infection on the skeleton are therefore the results of complex interactions between cytokines, immune cells, antiretroviral drugs, and bone cells, with a net effect of increased bone metabolic activity, leading to a net decrease of bone mass in HIV-infected children (Figure).

7. THERAPEUTIC INTERVENTION FOR HIV-RELATED OSTEOPATHY

Although the symptomatic manifestations of reduced bone density are currently rare, therapeutic attempts to prevent bone loss or to promote recovery of bone density warrants attention.

The first level of intervention includes the action on environmental factors that influence the gain of bone mass during childhood and adolescence and the maintenance of bone mass during adulthood. These factors include a balanced diet that provides optimal calcium and vitamin D intake, and a regular weight-bearing activity. Adequate sunlight exposure is also important for the conversion of vitamin D into its active form.

Endocrine disorders are frequent among HIV-infected individuals. In particular, disturbances of sexual hormones are deleterious for bone mass. Correction of endocrine dysfunction ameliorates the bone abnormalities, and in children usually corrects the bone density deficits.

Chronic viral and opportunistic infections are another cause of bone mineral disturbance. Prevention and timely cure of such complications is important for the prevention of bone loss.

When all the strategies to protect the skeleton are not enough to prevent decrements of bone mass, specific therapy with anti-osteoporotic agents should be considered. Current treatment of pediatric osteoporosis to date includes the use of antiresorptive drugs, and in particular, bisphosphonates (52). These compounds selectively inhibit bone resorption and thus contribute to enhance bone density. Although their use is not approved for children, bisphosphonates have shown benefit in children with a variety of metabolic bone diseases (52). However, there have been concerns about whether the use of prolonged high doses of bisphosphonates may impair bone turnover to such an extent to impair bone strength: case reports of induction of osteoporosis-like lesions in children who are treated with high doses of pamidronate have been published (53). Moreover, a recent study showed provided direct evidence of long-term release of a bisphosphonate, and showed that the drug can persist in the body of children for many years after the cessation of treatment (54). All these data suggest the need for caution in the selection of patients to be treated with antiresorptive drugs, and the necessity to keep treatment time as short as possible.

8. PERSPECTIVE

The available data indicate that bone metabolism derangement is present in HIV-infected children, and adolescents, and that such alterations might be the cause of the detected low bone mass measurements. The study of bone mass and bone metabolism in HIV-infected patients is very important, because of the improved life expectancy due to the new treatment regimens. This is of particular relevance in HIV-infected youths: impairments in obtaining an optimal bone mass should be identified during childhood to avoid future complications. Therefore, bone mass assessments should be performed in all HIV-infected patients to monitor their bone mineral status. We suggest obtaining bone mass measurements yearly in patients with poor control of viral infection, or treated with antiviral drugs associated to bone mass decrement. Measurements of biochemical indexes of bone metabolism may help in monitoring bone health in young patients. However, due to the paucity of available data, and the known limitations of the biochemical assays, it is not currently recommended to measure serum vitamin D concentration routinely.

The role of antiretroviral treatment in the genesis of low bone mass is not clear, and therefore more longitudinal studies are required to establish conclusively whether any HIV drug contribute to reduced bone density. Clinical trials comparing different therapeutic regimens are needed. Moreover, an improved understanding of the pathogenesis of the bone disorders in HIV-infected individuals should result in better preventative and therapeutic measures.

A major limitation of several published studies was the inclusion of patients with many risk factors for the development of reduced bone mass. This markedly limits our ability to establish a role of the infection *per se* in the development of low bone mass. Therefore, future studies should include patients who are early in the course of HIV infection, who are naïve to antiretroviral therapy, and who are free of known risk factors.

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