Longitudinal bone evaluation in HIV-1 vertically infected patients. A study from childhood to early adult age

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INTRODUCTION

Since the introduction of combined antiretroviral (cART), life expectancy of HIV-1 infected patients increased up to be comparable to non-HIV-1 infected population. Several studies reported early occurrence of aging-related effects in HIV-1 infected patient such as reduced bone quality and osteoporosis1-5. This condition is currently considered the result of the interplay among host, HIV-related chronic inflammation, and antiretroviral therapy, and may correspond to an increased risk from bone fracture5,6. Moreover, previous studies indicated that RANKL/OPG ratio resulted elevated during HIV infection for increased bone

ABSTRACT:

Introduction: The present study aimed at evaluating the role of HIV infection and antiretroviral treatment on bone status through a longitudinal observational study based on a confrontation between two cohorts of HIV-1 vertically infected patients followed from childhood to adulthood.

Materials and Methods: We enrolled all patients with vertical HIV-1 infection who attended our hospital. Every patient was taking cART throughout the observation period. QUS variables AD-SoS and BTT were used to assess bone status. RANKL and OPG measurements were performed during treatment. Height, weight, BMI, pubertal stages and bone age were also determined. Longitudinal trend of HIV-RNA and CD4 count and percentage were evaluated. 26 patients have a history of more than one virological failure, 21 patients have at most one virological failure.

Results: 47 patients were studied (23 males). Observations per patient ranged from 3 to 10 (123 observations for females and 122 for males). At baseline, mean age and SD were 10.9±4.6 years in females and 11.3±4.4 years in males. QUS values were reduced in HIV-infected youths, mainly in females. Mean ADSoS and BTT Z-scores were lower in patients with a history of more than one virological failure than in subjects with sustained viral load suppression. ADSoS, but not BTT mean values, were significantly lower in subjects with absolute CD4 lymphocyte count persistently <500 cells/ml than in subjects with CD4 T-lymphocyte count >500 cells/ml. RANKL/OPG ratio, AD-SoS SDS and BTT SDS were not related.

Conclusions: This longitudinal study provides the opportunity to evaluate the effects of HIV vertical infection on bone status. A non-invasive technique like QUS allowed to follow bone mass acquisition during growth.

Keywords: Quantitative ultrasound, Bone status, HIV infection, Growth, Inflammation.
remodeling, particularly in cART-naïve patient7. HIV infection effects on bone are of particular concern in vertically infected youths as the achievement of a low peak bone mass at adolescence may result with precocious osteoporosis8.

Cross-sectional and longitudinal studies demonstrated the value of phalangeal quantitative ultrasound (QUS) technology in identifying bone changes in children with pathological conditions9-16. This method is based on the transmission of ultrasound (US) through the hand proximal phalangeal diaphysis. The trace obtained reflects the characteristics of the signal generated by US after going through soft tissues, cortical bone and trabecular bone. QUS variables are used to obtain information on bone size, bone mass and architectural features and are age and growth-related17-19. The aim of this longitudinal observational study was to evaluate the disease effects on bone status using QUS in HIV vertically infected patients, followed from childhood to early adult age. We also investigated the influences of growth on QUS and the role of RANKL/OPG ratio.

**METHODS**

We enrolled all patients with vertically transmitted HIV-1 infection who attended a regional reference hospital in Liguria. The analysis was performed during follow-up visits (February 2002 -June 2014). All patients received cART according to international guidelines (20,21). No patient had suffered of bone or joint disease during the observation period or had hand-wrist deformities, diseases known to influence growth, soft tissue swelling, chronic use of medication including steroids, growth hormone or anticonvulsants.

**Bone quality assessment**

Two QUS variables were measured by the DBM Sonic BP IGEA (Italy): amplitude-dependent speed of sound (AD-SoS, m/s) and bone transmission time (BTT, m/s). AD-SoS measured the speed of sound emitted through cortical and trabecular bone giving information about bone mass and size22. BTT measures transmission time in bone excluding soft tissues bias, showing a close relationship with cortical bone thickness23. BTT is also related to bone age, independently of chronological age23. Measurements were performed by trained observers (repeatability: mean CV%: 0.96; reproducibility: mean CV%: 0.96). The Italian Phalangeal Quantitative Ultrasound Group reference was used24. AD-SoS and BTT were standardized using the Least Mean Squares method.

**RESULTS**

47 HIV-infected patients were studied (23 males, 48.9%). Observations per patient ranged from 3 to 10 with a median of 5 (123 observations for females and 122 for males). At baseline, mean age and SD were 10.9±4.6 years in females and 11.3±4.4 years in males; at last observation, they were 19.3±8.4 years and 18.9±3.8 years. Median observation period duration was 9.8 years (range 2.2-10.4 years). Their BMI and BMI-SDS were comparable to the average reference values. 33 patients completed growth at last observation (18 females, mean age 18.3±2.0 years; 14 males, mean age 19.3±1.4 years). At baseline, mean and SD of bone age resulted -0.82±1.08 in males and 0.07±0.97 in females. Bone age was retarded by more than 1 SDS in 12/23 males and 3/24 females. ADSoS Z-score mean values were significantly lower than 0 in the entire period of follow-up (-1.13±1.23 SD for males, -0.90±1.42 SD for females, p<0.0001) (Figure 1). The same was observed for BTT Z-score mean values (-0.75±1.25 SD for males, -0.68±1.26 SD for females, p<0.0001) (Figure 2). Significant correlations of AD-SoS Z-score were also found with height Z-score (p<0.01), BMI (p<0.05), bone age...
Table 1. Results of mixed effect model approach for ADSoS and BTT Z-scores in patients with and without virological failure and in patients with CD4≥500 cell/ml and <500 cell/ml, respectively.

<table>
<thead>
<tr>
<th>AD-SoS Z-score</th>
<th>Effect</th>
<th>Effect estimate</th>
<th>Prob level</th>
<th>Effect</th>
<th>Effect estimate</th>
<th>Prob level</th>
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<td>CD4 ≥500 cell/ml</td>
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<tr>
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<td>0.0809</td>
<td></td>
<td>Time</td>
<td>-0.0951±0.0642</td>
<td>0.1389</td>
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<tr>
<td>Interaction no virological failure*time</td>
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<td>&lt;0.0001</td>
<td></td>
<td>Interaction CD4≥500 cell/ml*time</td>
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<table>
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<th>Effect</th>
<th>Effect estimate</th>
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<td>Interaction CD4≥500 cell/ml*time</td>
<td>0.0469±0.0685</td>
<td>&lt;0.0001</td>
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(p<0.001), in both sexes. The BTT-Z-score was significantly associated with height Z-score (p<0.0001). Both AD-SoS and BTT Z-scores were related to bone age and bone age Z-scores in both sexes (p<0.0001). Correlations were higher for BTT Z-score (r = 0.56) than for AD-SoS Z-score (r = 0.45). When QUS variables were examined as a function of pubertal stages for ages >9 years in girls and >10 years in males, a tendency was found toward low-values of AD SoS and BTT Z-scores, which were found significantly lower than zero in each pubertal stage group (p<0.05) (Figures 3 and 4). In young adult patients AD-SoS Z-score mean and SD were -1.26± 1.13 in females and -0.57±1.17 in males. BTT Z-score Mean and SD were -0.57±1.17 in females and 0.07±1.38 in males. AD-SoS and BTT Z-scores mean values resulted lower in patients with at least one virological failure during the period of observation than in subjects with a sustained viral load suppression (p<0.001). AD-SoS, but not BTT mean values, were significantly lower in subjects with absolute CD4 lymphocyte count persistently < 500 cells/ml than in subjects with CD4 T lymphocyte count > 500 cells/ml (p<0.001) (Table 1). RANKL/OPG ratio and concomitant AD-SoS SDS and BTT SDS performed at last observation were not related.

**DISCUSSION**

We confirmed that bone mass is reduced in HIV-infected youths as reported in cross-sectional studies28,29. However, this may also result from the maturation delay observed, especially in male patients, via evaluation of bone age, as it has been stated in previous reports30. This potential bias, however, cannot be interpreted as the sole reason of reduction of bone quality per age, since bone age and bone age-SDS were significantly related to AD-SoS and BTT, compared according to the growth chart, thus reducing the effects of maturation delay on bone measurements.

BTT, a marker of cortical bone tissue thickness, was more related to pubertal stage than AD-SoS, as cortical bone is more dependent to bone age; however, it was not related to BMI-SDS, as it is not influenced by soft-tissue thickness. In fact, AD-SoS measurements, less influenced by pubertal stage and bone age, represented a more specific marker of bone status in this particular setting. It is worth of note that AD-SoS, but not BTT, was significantly reduced not only in patients with history of virological failure, but even in patients whose CD4 count resulted persistently below 500 cells/mmc. These data, in the light of data previously stated in the literature, confirm that the process of inflammation induced by HIV involves not only a reduced bone mass but, to a greater extent, to higher tendency to sarcopenia31. On this basis, it is reasonable to assume that AD-SoS may be more suitable than BTT to evaluate the viral/host/therapies interplay effects on bone tissue and soft tissue. The relationship between QUS and clinical features of HIV infection showed that HIV status had significant effects on bone. In particular, patients whose HIV-RNA remained <50 copies/mL and those with higher CD4+ cell count have better bone status than those with poor HIV control. In this cohort RANKL/OPG ratio was not a proper indicator of bone status. This may be linked to the characteristics of our population, composed by patients with a long history of cART. Since RANKL/OPG ratio seems to be influenced by the state of HIV replication at the measurement time, it is conceivable that patients with uncontrolled HIV-RNA may have reduced bone quality despite having normal RANKL/OPG ratio, as long as their current virological status is controlled by adequate cART. The strengths of this study are its longitudinal design and the large number of serial observations. Furthermore, few studies are available examining the effects of growth timing on bone status. The main limitations of the study are the irregular intervals between observations.
CONCLUSIONS

HIV vertical transmission has decreased in recent years. It is important to remain aware of bone changes in HIV-1 infected youths and of the importance of a long-term follow-up. A non-invasive technique like QUS allows to follow bone status longitudinally and to evaluate conditions interfering with bone mass acquisition during growth and the aging process.

CONFLICTS OF INTEREST:
The authors declare that they have no conflict of interest.

REFERENCES
