Infect Dis Trop Med 2017; 3 (4): e425

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

F. Del Puente[1], D. Gibellini[3], M. Borderi[2], R. Prinapori[4], A. Di Biagio[4,5]

[1]Infectious Diseases Unit, University of Genoa-DISSAL, IRCCS San Martino-IST Hospital, Genoa, Italy
[2]Department of Experimental, Diagnostic and Specialty Medicine (DIMES), Microbiology Section, University of Bologna, Bologna, Italy
[3]Department of Pathology and Diagnostic, Microbiology and Virology Unit, University of Verona, Verona, Italy
[4]Infectious Disease Unit, Sant'Andrea Hospital, La Spezia, Italy
[5]Infectious Diseases Unit, IRCCS San Martino-IST Hospital, Genoa, Italy

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.

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 osteoporosis[1-5]. 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 fracture[5,6]. Moreover, previous studies indicated that RANKL/OPG ratio resulted elevated during HIV infection for increased bone

Corresponding Author: Filippo Del Puente, MD; e-mail: filippodelpuente@gmail.com

remodeling, particularly in cART-naïve patient[7]. 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 osteoporosis[8].

Cross-sectional and longitudinal studies demonstrated the value of phalangeal quantitative ultrasound (QUS) technology in identifying bone changes in children with pathological conditions[9-16]. 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-related[17-19]. 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 size[22]. BTT measures transmission time in bone excluding soft tissues bias, showing a close relationship with cortical bone thickness[17]. BTT is also related to bone age, independently of chronological age[23]. Measurements were performed by trained observers (repeatability: mean CV%: 0.96; reproducibility: mean CV%: 0.96). The Italian Phalangeal Quantitative Ultrasound Group reference was used[24]. AD-SoS and BTT were standardized using the Least Mean Squares method.

Growth measurements

Height was determined with Harpenden stadiometer and weight with electronic scale. Height and BMI-SDS were calculated using WHO growth charts[25]. Pubertal development was evaluated by Tanner's criteria[26]. Bone age was assessed according to Tanner-Whitehouse method (third version)[27]. The difference between bone and chronological age was expressed as SDS. For each subject, at least two estimates were available. Intermediate "bone ages" were obtained by linear interpolation.

Clinical and laboratory data

The following parameters were collected: CD4 T-lymphocytes count and HIV-RNA. Virological failure was defined by inability to achieve viral suppression (HIV-RNA <50 copies/mL) after 24 weeks from treatment start, or two consecutive measurements of viral replication during cART.

At last observation, OPG and RANKL were measured by Human OPG Duo ELISA kit (R&D System, Minneapolis, MN, USA) and Ampli sRANKL kit (Biomedica, Wien, Austria). Informed consent was obtained. The local Ethics Committee approved the study.

Statistical Analysis

Statistical analysis was performed with STATA statistical software for Windows (release 7; 2001). The parameters of bone quality were initially compared using a paired Student's *t*-test. Longitudinal profiles of QUS variables were obtained from each measurement per patient by third degree polynomial regression. To test the association between QUS parameters with age, height, weight, virological load and lymphocyte count, the mixed model approach for repeated measurements over time was alternatively used for continuous and for categorical variables. p-values < 0.05 were considered statistically significant.

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.

AD-SoS Z-score					
Effect	**Effect estimate**	**Prob level**	**Effect**	**Effect estimate**	**Prob level**
Intercept	-0.7157±0.4640	<0.0001	Intercept	-1.0098±0.4971	0.0431
No virological failure	0.0161±0.6618	<0.0001	CD4 ≥500 cell/ml	0.5917±0.6656	<0.0001
Time	-0.0922±0.0528	0.0809	Time	-0.0951±0.0642	0.1389
Interaction no virological failure*time	0.0178±0.0789	<0.0001	Interaction CD4≥500 cell/ml*time	0.0040±0.0811	<0.0001
BTT Z-score					
Effect	**Effect estimate**	**Prob level**	**Effect**	**Effect estimate**	**Prob level**
Intercept	-0.2240±0.1742	0.2611	Intercept	0.0004±0.4494	0.9994
No virological failure	-0.0833±0.0000	1.0000	CD4 ≥500 cell/ml	-0.4564±0.6560	<0.0001
Time	0.0064±0.0430	0.8819	Time	-0.0532±0.0529	0.3148
Interaction no virological failure*time	-0.0785±0.0528	0.0528	Interaction CD4≥500 cell/ml*time	0.0469±0.0685	<0.0001

(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<0.001). 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 studies[28,29]. 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 reports[30]. 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 sarcopenia[31]. On this basis, it is reasonable to assume that AD-SoS may be more suitable than BTT to evaluate the viral/host/therapy 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

1. Warriner A, Mugavero M, Overton E. Bone alterations associated with HIV. Curr HIV/AIDS Rep 2014; 11: 233-240.
2. Labarga, P. Bone mineral density loss, osteoporosis, and fractures in HIV. AIDS Rev 2013; 15: 189-190.
3. Puthanakit T, Siberry G. Bone health in children and adolescents with perinatal HIV infection. J Int AIDS Soc 2013; 16: 18575.
4. Rosso R, Vignolo M, Parodi A, Di Biagio A, Sormani M, Bassetti M, Aicardi G, Bassetti D. Bone quality in perinatally HIV-infected children: role of age, sex, growth, HIV infection, and antiretroviral therapy. AIDS Res Hum Retroviruses 2005; 21: 927-932.
5. Jacobson D, Spiegelman D, Duggan C, Weinberg G, Bechard L, Furuta L, Nicchitta J, Gorbach S, Miller T. Predictors of bone mineral density in human immunodeficiency virus-1 infected children. J Pediatr Gastroenterol Nutr 2005; 41: 339-346.
6. Rosso R, Vignolo M, Parodi A, Di Biagio A, Sormani M, Bassetti M, Aicardi G, Bassetti D. Bone quality in perinatally HIV-infected children: role of age, sex, growth, HIV infection, and antiretroviral therapy. AIDS Res Hum Retroviruses 2005; 21: 927-932.
7. Gibellini D, Borderi M, De Crignis E, Cicola R, Vescini F, Caudarella R, Chiodo F, Re M. RANKL/OPG/TRAIL plasma levels and bone mass loss evaluation in antiretroviral naive HIV-1-positive men. J Med Virol 2007; 79: 1446-1454.
8. Baroncelli G, Federico G, Bertelloni S, De Terlizzi F, Cadossi R, Saggese G. Bone quality assessment by quantitative ultrasound of proximal phalanxes of the hand in healthy subjects aged 3-21 years. Pediatr Res 2001; 49: 713-718.
9. Pedrera J, López M, Canal M, Costa C, Mañas P, Hernández E, Rico H. Quantitative phalangeal bone ultrasound is normal after long-term gluten-free diet in young coeliac patients. Eur J Gastroenterol Hepatol 2001; 13: 1169-1173.
10. Pluskiewicz W, Halaba Z, Chelmecka L, Drozdzowska B, Sońta-Jakimczyk D, Karasek D. Skeletal status in survivors of acute lymphoblastic leukemia assessed by quantitative ultrasound at the hand phalanges: a longitudinal study. Ultrasound Med Biol 2004; 30: 893-898.
11. Pluskiewicz W, Pyrkosz A, Drozdzowska B, Halaba Z. Quantitative ultrasound of the hand phalanges in patients with genetic disorders: a pilot case-control study. Osteoporos Int 2003; 14: 787-792.
12. Pluskiewicz W, Adamczyk P, Drozdzowska B, Szprynger K, Szczepańska M, Halaba Z, Karasek D. Skeletal status in adolescents with end-stage renal failure: a longitudinal study. Osteoporos Int 2004; 16: 289-295.
13. Valerio G, Puente A, Buono P, Esposito A, Zanatta M, Mozzillo E, Moretto E, Mastidoro L, Franzese A. Quantitative ultrasound of proximal phalanxes in patients with type 1 diabetes mellitus. Diabetes Res Clin Pract 2004; 64: 161-166.
14. Damilakis J, Galanakis E, Mamoulakis D, Sbyrakis S, Gourtsoyiannis N. Quantitative ultrasound measurements in children and adolescents with: type 1 diabetes. Calcif Tissue Int 2004; 74: 424-428.
15. Baroncelli G, Federico G, Bertelloni S, Sodini F, De Terlizzi F, Cadossi R, Saggese G. Assessment of bone quality by quantitative ultrasound of proximal phalanges of the hand and fracture rate in children and adolescents with bone and mineral disorders. Pediatr Res 2003; 54: 125-136.
16. Stagi S, Cavalli L, Signorini C, Bertini F, Cerinic M, Brandi M, Falcini F. Bone mass and quality in patients with juvenile idiopathic arthritis: longitudinal evaluation of bone-mass determinants by using dual-energy x-ray absorptiometry, peripheral quantitative computed tomography, and quantitative ultrasonography. Arthritis Res Ther 2014; 16: R83. doi: 10.1186/ar4525.
17. Barkmann R, Rohrschneider W, Vierling M, Tröger J, de TF, Cadossi R, Heller M, Glüer CC. German pediatric reference data for quantitative transverse transmission ultrasound of finger phalanges. Osteoporos Int 2002; 13: 55-61.
18. Halaba Z, Pluskiewicz W. Quantitative ultrasound in the assessment of skeletal status in children and adolescents. Ultrasound Med Biol 2004; 30: 239-243.
19. Fricke O, Tutlewski B, Schwahn B, Schoenau E. Speed of sound: relation to geometric characteristics of bone in children, adolescents, and adults. J Pediatr 2005; 146: 764-768.
20. Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. https://aidsinfo.nih.gov/guidelines/html/2/pediatric-arv/0
21. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. https://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-arv/0
22. Gluer C, Wu C, Jergas M, Goldstein S, Genant H. Three quantitative ultrasound parameters reflect bone structure. Calcif Tissue Int 1994; 55: 46-52.
23. Vignolo M, Torrisi C, Parodi A, Becchetti F, De Terlizzi F, Aicardi G. Skeletal age as a determinant of phalangeal quantitative ultrasound measures of bone quality in children and adolescents. Open Bone J 2010; 2: 38-42.
24. Baroncelli G, Federico G, Vignolo M, Valerio G, del Puente A, Maghnie M, Baserga M, Farello G, Saggese G. Cross-sectional reference data for phalangeal quantitative ultrasound from early childhood to young-adulthood according to gender, age, skeletal growth, and pubertal development. Bone 2006; 39: 159-173.
25. WHO multicentre growth reference study group. WHO child growth standards based on length/height, weight and age. Acta Paediatr 2006; Suppl 450: 76-85.
26. Tanner J, Whitehouse R. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976; 51: 170-179.
27. Tanner JM, Healy MJR, Goldstein H, Cameron N. (2001). Assessment of skeletal maturity and prediction of adult height (TW3 method). Saunders Ltd; 3rd edition, 2001. ISBN: 978-0702025112
28. Vignolo M, Brignone A, Mascagni A, Ravera G, Biasotti B, Aicardi, G. (2003). Influence of age, sex, and growth variables on phalangeal quantitative ultrasound measures: a study in healthy children and adolescents. Calcif Tissue Int 2003; 72: 681-688.
29. Vignolo M, Rosso R, Parodi A, Viscoli C. Quantitative ultrasound and bone density in vertically HIV-infected children. Clin Infect Dis 2006; 43: 112-113.
30. Isanaka S, Duggan C, Fawzi W. Patterns of postnatal growth in HIV-infected and HIV-exposed children. Nutr Rev 2009; 67: 343-359.
31. Gowda C, Brown T, Compher C, Forde K, Kostman J, Shaw P, Tien P, Lo Re V. Prevalence and predictors of low muscle mass in HIV/viral hepatitis coinfection. AIDS 2016; 30: 2519-2528.