

Phalangeal quantitative ultrasound: cheaper methods for screening and follow-up of bone pathologies in HIV-infected women?

Roberta Prinapori¹, Raffaella Rosso¹, Antonio Di Biagio¹, Laura Nicolini¹, Daniele Roberto Giacobbe¹, Laura De Hoffer¹, Sara Grignolo¹, Francesca De Terlizzi², Marina Vignolo³, Marco Borderi⁴, Giulia Martelli⁴, Leonardo Calza⁴, Pierluigi Viale⁴, Fabio Vescini⁵, Claudio Viscoli¹

¹Department of Internal Medicine and Infectious Diseases, IRCCS A.O.U. San Martino-IST, Genoa, Italy;

²Medical Office, IGEA s.r.l., Modena, Italy;

³Paediatric Clinic, University of Genoa, G. Gaslini Institute;

⁴Department of Medical and Surgical Sciences, Section of Infectious Diseases, University of Bologna, S. Orsola-Malpighi Hospital, Bologna, Italy;

⁵Endocrinology and Metabolism Unit, University-Hospital S. Maria della Misericordia, Udine, Italy

SUMMARY

This study estimated the prevalence of bone pathologies in a cohort of HIV-infected women in comparison with a cohort of HIV-negative women. Bone mineral density was measured by phalangeal quantitative ultrasound (AD-SoS: amplitude-dependent speed of sound; UBPI: ultrasound bone profile index). Risk of fracture, expressed by UBPI, was considered for value <0.39. Comparisons between groups and multivariate analyses were carried out using an ANOVA model. Correlations were evaluated using the Pearson correlation coefficient. Osteopenia and osteoporosis were present in 34.4% and 2% of patients, respectively. UBPI was pathologic in 5.7%. In a multivariate linear regression model significant correlations were found between AD-SoS z-score, duration of HIV-infection and BMI value. We also compared our cohort with 499 HIV-negative women as a historical control group of healthy subjects. AdSoS (2100 versus 2070 m/s) and UBPI (0.89 versus 0.74) were lower in HIV-infected women ($p < 0.001$). Significant differences were also found in T-score values ($p = 0.0013$). These data show a high prevalence of bone diseases in women with HIV infection, correlated with duration of HIV-infection and BMI values. This non-invasive technique opens up new interesting perspectives, suggesting a possible use for bone mass screening in HIV-infected women.

KEY WORDS: Quantitative ultrasound, HIV infection, Women, Bone diseases, Osteopenia, Fracture.

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INTRODUCTION

Low bone mass is a well-recognized metabolic complication associated with HIV infection in men and women (Walker Harris and Brown, 2012). As for the other toxicities related to HIV and combined antiretroviral therapy (cART), medical attention has been distracted from the

management of HIV infection to the care of osteopenia or osteoporosis (Morse and Kovacs, 2006). The high prevalence of HIV-related osteoporosis is likely the result of heterogeneous causes and the interplay of host, viral and specific antiretroviral factors (Morse and Kovacs, 2006; Amorosa and Tebas, 2006; Cazanave *et al.*, 2008; Gibellini *et al.*, 2010). Bone is constantly undergoing a remodelling process, in a synchronized balance between resorption and formation, which can be altered during HIV-infection. A direct effect of HIV on bone cells, the persistent activation of pro-inflammatory cytokines and alterations in the metabolism of vitamin D have most often been addressed as possible causes of HIV-related osteoporosis even though we are still

Corresponding author

Antonio Di Biagio
Clinica Malattie Infettive
IRCCS A.O.U. San Martino-IST
Largo Rosanna Benzi, 10
16132 Genova, Italy
E-mail: antonio.dibiagio@hsanmartino.it

far from a complete understanding of its pathologic pathway (Kuhne *et al.*, 2001; Fessel and Hurley, 2003; Fausto *et al.*, 2006). Moreover, HIV-infected women also present the same risk factors for bone pathologies as the general population, such as genetic pattern, physical inactivity, decreased intake of calcium and vitamin D, cigarette smoking, alcohol use, steroid exposure and hormonal factors (Walker Harris and Brown, 2012; Amorosa and Tebas, 2006a; Dolan *et al.*, 2004). In this context, the evaluation of bone quality is of major clinical importance. Dual x-ray energy absorptiometry (DXA) is the currently used technique for assessing bone status, even if some limitations of this technique have been widely acknowledged well beyond studies of HIV-infected populations, including being a bone size-dependent measure (Amorosa and Tebas, 2006a; Amorosa and Tebas, 2006b; Szulc *et al.*, 2005). In addition, in assessing bone status and fracture risk, factors other than bone density should be taken into account, like elasticity and biomechanical characteristics of bone. As an alternative and/or integrative technique to DXA, quantitative ultrasound (QUS) has recently been proposed as a screening method for osteopenia/osteoporosis, since it proved to be accurate and precise in evaluating bone mass (Wuster *et al.*, 2000). Furthermore, QUS can predict pathologic fractures to an even greater extent than DXA (Langton *et al.*, 1984; Wasnich *et al.*, 1987; Black *et al.*, 1992; Di Biagio *et al.*, 2007). QUS of phalanx (f-QUS) is a non invasive and low-cost technique that can be used extensively for assessing bone mineralization and structure (Wuster *et al.*, 2000; Barkmann *et al.*, 2000; Gimeno Ballester *et al.*, 2001; Halaba and Pluskiewicz, 2004). Allowing easily repeated safe measurements, QUS may prove valuable to monitor how changes in bone mineralization and structure evolve over time in the HIV-infected population and to evaluate the short-term effects of different antiretroviral drug regimens on bone (Rosso *et al.*, 2010). We sought to assess bone quality using f-QUS technology in HIV-infected women, comparing them with a group of sex and age-matched healthy controls. We also looked for correlations between HIV-related risk factors and f-QUS parameters with a cross-sectional analysis of data obtained from a cohort of HIV-infected women.

METHODS

Study participants

We performed QUS evaluations of bone status within a cohort HIV-infected women followed regularly at San Martino Hospital in Genoa. Patients were consecutively enrolled between May and September 2006. There were no exclusion criteria based on lymphocyte T CD4+ counts or HIV-RNA load. All patients provided written informed consent. HIV-negative women were drawn from a historical control group of healthy subjects.

Interview data

We interviewed our patients about their medical history and lifestyle. Interview data included age, race (Caucasian, Caribbean, Latin American or African), menopausal status, personal history of fracture, parental history of hip fracture, smoking history (never, former or current regular smoking of more than 20 cigarettes/day), alcohol use (number of alcohol units per day, classified as 3 units/day or more), substance abuse [ever or recent (past 5 years) drug use], history of rheumatoid arthritis, estimated glomerular filtration rate, statins, hormonal contraception, or hormone replacement therapy and glucocorticoid exposure (≥ 6 months). Calcium intake was empirically considered adequate in patients consuming 2 or more portions per day of dairy products. Patients were stratified for exercise habits into sedentary and current or previous sport activities (at least twice/week). Based on medical records, we evaluated years, stage, and risk factors of HIV-infection (according to CDC classification), presence of chronic active hepatitis B (HBV), defined as positivity for hepatitis B surface antigen (HBsAg) for more than 6 months, with detectable serum HBV-DNA, and/or hepatitis C (HCV) co-infection (HCV-RNA positive). Patients' physical examination included weight, height, and BMI measurements and clinical lipodystrophy evaluation. Lipodystrophy was classified as the presence of at least one of these features:

- fat loss in the face (sunken cheeks), arms, legs or buttocks;
- enlargement of the breasts, increased abdominal girth or dorso-cervical fat accumulation;
- mixed pattern (both peripheral atrophy and central accumulation of fat).

We calculated the number of therapeutic lines received, duration and type of (cART): 3 nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), or 2 NRTIs + protease inhibitors (PIs), or non nucleoside reverse transcriptase inhibitors, (NNRTI) and NRTIs (particularly, months of exposure to didanosine, stavudine, tenofovir, zidovudine), respectively. We measured alkaline phosphatase, creatinine, cholesterol, triglycerides, viral load and lymphocyte T CD4+ cells count.

We also evaluated serum markers of bone formation, such as osteocalcin and bone specific alkaline phosphatase, and urine concentration of N-TX telopeptide levels, as a bone resorption marker.

Osteocalcin is the principal non-collagen protein of bone, but measuring it may present some serious limitations deriving from its short half-life, in the order of a few minutes, its rapid renal clearance, and the fact that it is quite fragile, being subject to fragmentation.

For this reason, we decided to evaluate also bone-specific alkaline phosphatase, for its relatively long half-life (1-2 days).

Bone mass assessment

Scans of the distal diaphysis of the proximal phalanx of II-V fingers of the dominant hand were taken with a DBM Sonic 1200 ultrasound unit (IGEA, Carpi, Italy). The instrument is equipped with two probes mounted on an electronic caliper. The emitter is positioned on the medial surface of the phalange, and the receiver is positioned on the opposite side. The emitter probe generates a US signal with a frequency of 1.25 MHz. A good coupling between the probes and skin is achieved with interfacing gel.

The graphic tracing obtained reflects the characteristics of the electrical signal generated by US after crossing the phalanx soft tissues and bone. The following f-QUS variables were examined:

1. Amplitude-dependent speed of sound (AD-SoS, expressed in m/s), i.e., the velocity at which the US crosses the phalanx, calculated by dividing the distance between the probes by the time taken by the first signal received with a predetermined minimum amplitude value (2 mV). This variable is influenced by structural characteristics of bone and gives information on bone mineralization and bone mass.

2. Ultrasound bone profile index (UBPI), a combination of graphic trace-associated parameters, has shown a high correlation with Ad-SoS after adult age, while correlation is poor before 30. Wuster *et al.* hypothesized that UBPI may be related mostly to bone tissue characteristics like elasticity and structure, rather than density. UBPI has shown good sensitivity and specificity in discriminating hip-fractured from non-fractured subject of the same age (Wuster *et al.*, 2000).

Measurements were performed by the same single observer (repeatability as root mean square coefficient of variation was 0.81 for AD-SoS). Calibration and time stability of the device were checked daily with an internal calibration procedure.

According to the World Health Organization criteria, osteoporosis was defined as -3.2 T score for AD-SoS, corresponding to 1900 m/s, or -3.14 T-score for UBPI, corresponding to ≤ 0.39 ; while osteopenia was defined as AD-SoS or UBPI under -1 T-score. Risk of fracture, expressed with UBPI, was considered for value ≤ 0.39 (Wuster *et al.*, 2000).

AD-SoS z-score were calculated as the difference between the individual observed value and the mean value for age and gender determined in the reference population, divided by the corresponding standard deviation (SD).

Statistical analysis

The statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 11.0 for Windows. Comparisons between groups and multivariate analyses were carried out using an ANOVA model. Correlations were evaluated using the Pearson correlation coefficient.

RESULTS

Our cohort included 87 HIV-infected women. The patients' characteristics are shown in Table 1. Ten (11%) patients had developed AIDS. Median plasma viral load was <50 copies/ml (range <50-60000); median plasma CD4 cell count was 495 cells/ μ l (range 10-1229). HCV coinfection (HCV-RNA positive) occurred in 30 patients (34%). No patients had HBV coinfection. At the time of the survey, 76 patients (84%) were taking cART: 19%

were treated with 3 NRTIs, 37% with PIs- and 44% with an NNRTI-based regimen; the remaining 11 (16%) did not receive any treatment (93%) or monotherapy with lamivudine (7%); 81% were in premenopausal status; 41% were former intravenous drug users (IVDU).

According to WHO Study Group criteria, two pa-

TABLE 1 - Patients characteristics.

Age mean [range]	43 years [21;73]
Weight mean [range]	59,06 kg [37;85]
BMI mean [range]	22,7 [15;31,2]
Height mean [range]	1,61 cm [1,42; 1,76]
Age of menarche mean [range]	13 years [9;17]
Age of hiv infection diagnosis median [range]	14 years [0;28]
AIDS diagnosis	10/87 (11%)
HCV coinfection	30/87 (34%)
Pregnancies mean [range]	1,66 [0;6]
Spontaneous abortion mean [range]	0,84 [0;6]
Post-menopausal women	13/89
Years of menopause mean [range]	6,94 [1;23]
Creatinine mean [range]	0,8 mg/dl [0,5-2,3]
Cholesterol mean [range]	187 mg/dl [91;305]
Triglycerides mean [range]	127 mg/dl [48;400]
CD4 count mean [range]	495 cells/ μ l [10; 1229]
Viral load mean [range]	<50 copies/ml [<50;60000]
HAART at the time of survey	76 (84%)
Bone alkaline phosphatase mean [range]	12,9 U/l [2,5;40,8]
Osteocalcin mean [range]	10,5 ng/ml [2,0;30,7]
N-terminal telopeptide (NTX) mean [range]	650,914 μ g/mmol [73,000; 3001,000]
Lipodistrophy	35/87

tients (2%) were osteoporotic, while 30 subjects (34.4%) were osteopenic at QUS. UBPI was pathologic in 5.7% of patients. As reported by other authors (Soballa *et al.*, 1998), we found a high correlation between AD-SoS and UBPI, $r=0.730$. AD-SoS values, plotted as a function of age in relation to normative curves obtained in the reference population (± 1 SD), are shown in Figure 1a. UBPI values plotted as a function of age in relation to normative curves obtained in the reference population (± 1 SD) are shown in Figure 1b. UBPI was significantly reduced in our population ($p<0.05$).

In univariate analyses, factors associated with lower AD-SoS were duration of HIV infection ($r: -0.20$, $p<0.04$), number of combined antiretroviral regimen, AZT ($r: -0.21$, $p<0.05$), and PI-based regimens ($r: 0.30$, $p<0.001$), lipodistrophy ($r: 0.24$, $p<0.01$), urine concentration of N-TX ($r: 0.24$, $p<0.05$), greater BMI ($r: -0.33$, $p<0.001$). UBPI was related to duration of cART ($r: -0.21$, $p<0.05$), use of AZT ($r: -0.21$, $p<0.05$), use of PI ($r: -0.20$, $p<0.05$), lipodistrophy ($r: 0.25$, $p<0.01$) and HIV-RNA load ($r: -0.21$, $p<0.05$). Both AD-SoS and UBPI values were not significantly related to osteocalcin and bone specific alkaline phosphatase levels.

Association analysis (ANOVA test) among QUS variables and lifestyle factors (smoking history, alcohol use, dairy product consumption, exercise habits, substance abuse, personal history of fracture, parental history of hip fracture, history of rheumatoid arthritis, estimated glomerular filtration rate, statins, hormonal contraception, or hormone replacement therapy and glucocorticoids exposure) did not reveal any significant association.

The factors significantly related to AD-SoS values in HIV woman (meeting the criterion of on univariate analysis $P<0.05$) were included in a multivariate linear regression model with AD-SoS as dependent variable: results of the regression analysis are summarized in Table 2. These data confirmed that BMI and duration of HIV infection had a strong correlation with bone disease, using phalangeal QUS. For comparison we used a control group of 499 HIV-negative women originally from the same geographical area (northern of Italy). HIV-infected women and control subject were matched for age (42.8 versus 42.9 years, $p=0.9657$), weight (60.8 versus 59.4 kg,

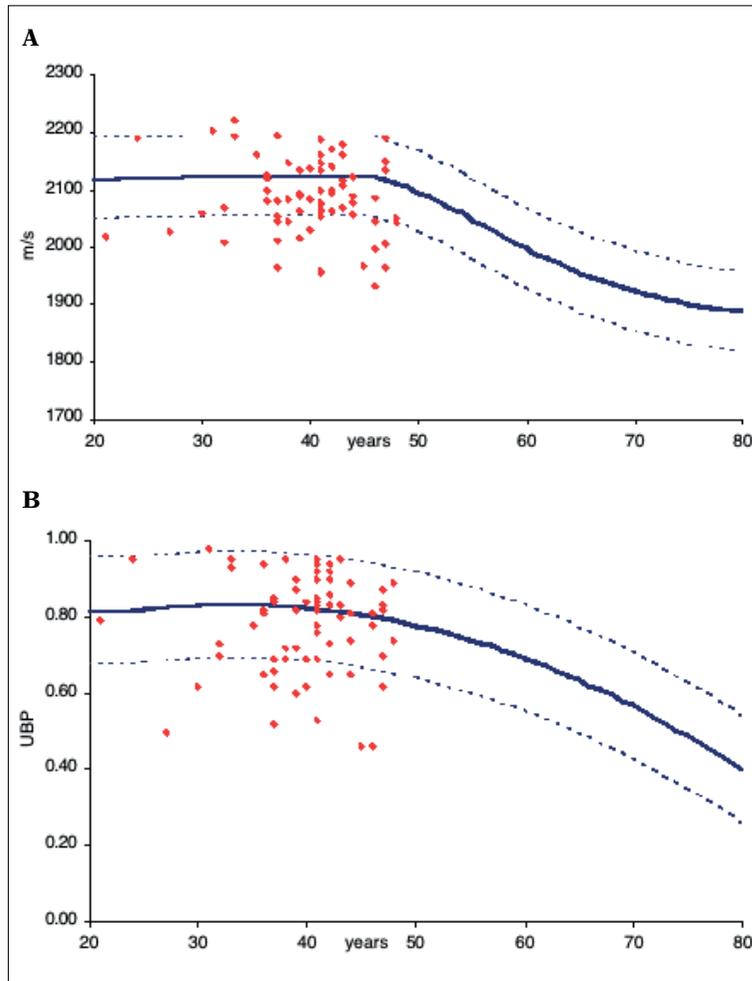


FIGURE 1 - AD-SoS (a) AND UBPI (b) of HIV-infected patients, as a function of chronological age, in relation to normative curves obtained in the reference population.

TABLE 2

	Coefficients ^a									
	Unstandardized Coefficients		Standardized Coefficients			95% Confidence Interval for B		Correlations		
	B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound	Zero-order	Partial	Part
(Constant)	2236.798	64.797		34.520	.000	2107.428	2366.169			
HIV time diagnosis	-2.398	1.244	-.226	-1.928	.048	-4.882	.085	-.206	-.231	-.205
PI treatment	17.972	17.729	.118	1.014	.314	-17.425	53.368	.261	.124	.108
Lipodistrophy	19.686	15.246	.146	1.291	.201	-10.753	50.124	.200	.157	.137
BMI	-7.562	2.032	-.412	-3.721	.000	-11.620	-3.505	-.357	-.416	-.396

^aDependent Variable: AD-SoS.

p=0.2539), height (161 versus 161 cm, p=0.8310), age of menarche (12.8 versus 12.9 years, p=0.5742) and number of pregnancy (1.8 versus 1.8, p=0.8880).

AdSoS (2100 versus 2070 m/s, p=0.0013) and UBPI (0.89 versus 0.84, p<0.0001) values were lower in HIV group. Differences were also found in T-score values (-0.34 versus -0.77, p=0.0013).

DISCUSSION

Dual x-ray energy absorptiometry (DXA) is the gold standard method for diagnosis of osteopenia (WHO study group, 1994), but it has also some limitations that are widely acknowledged well beyond studies of HIV-infected populations, including being a bone size-dependent measure (Amorosa and Tebas, 2006a; Amorosa and Tebas, 2006b; Szulc *et al.*, 2005). Moreover it seems that other factors (other than bone mineral density) like elasticity and biomechanical characteristics of bone are also important in bone fragility. QUS is increasingly used for osteopenia/osteoporosis risk assessment, because of its low cost, portability and lack of exposure to ionizing radiation (Baroncelli *et al.*, 2006). The most widely validated technique is QUS at the heel. f-QUS is a most recent technique, and its performance characteristics have been widely examined in other studies (Wuster *et al.*, 2000; Baroncelli *et al.*, 2006).

Our cross-sectional study confirmed the data of increased incidence of bone disease in HIV-infected women. Previous studies have well documented the association between HIV infection and decreased bone mass density measured with DEXA (Walker Harris and Brown, 2012).

Some factors have been involved in this condition, such as: severity and duration of HIV infection, antiretroviral agents and duration of cART, viral and immunologic factors, which can both interfere with the cross-talk between osteoblast and osteoclast cells into the bone remodeling unit, body mass index, lipodystrophy, metabolic abnormalities, methadone maintenance programs, opiate use, as well as genetic influences and lifestyle factors (Kuhne *et al.*, 2001; Fessel and Hurley, 2003; Fausto *et al.*, 2006).

Our observation that low BMD, measured with f-QUS, is associated with HIV infection, even in the presence of significant excess body mass, sup-

ports findings by other researchers that body composition factors may play a paradoxical role in affecting BMD. One possible explanation for this finding is the lower bioavailability of 25-hydroxyvitamin D observed in patients with increased body mass index, because of its seizure in abdominal adipose tissue.

As is already known, the duration of HIV infection plays an important role in the bone remodeling process as a result of direct viral effects and chronic immune activation, promoting osteoclastogenesis and bone resorption.

In conclusion, phalangeal QUS technology seems able to detect bone abnormalities in HIV-infected women, and to monitor bone response to factors able to interfere with bone turnover in these subjects. Therefore, early recognition and management of reduced bone quality by this easy-to-perform examination may be useful for a better management of HIV-infected patients in routine clinical care, in particular as a screening procedure, to become an important part of their long-term health maintenance.

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