Calcaneal quantitative ultrasound (QUS) and dual X-ray absorptiometry (DXA) bone analysis in adult HIV-positive patients

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SUMMARY

Human immunodeficiency virus (HIV)-infected patients have an increased risk of developing osteopenia or osteoporosis compared with healthy individuals. Our aim was to compare dual X-ray absorptiometry (DXA), the gold standard for measuring bone mineral density (BMD), with bone quantitative ultrasound (QUS), an alternative technique for predicting fractures and screening low BMD, at least in postmenopausal populations. We analyzed DXA and QUS parameters to investigate their accuracy in the diagnosis and prediction of bone alterations in a cohort of 224 HIV-1-positive patients. The speed of sound (SOS), broadband ultrasound attenuation (BUA) and stiffness index (SI) parameters showed a moderate correlation with DXA, especially with total-body BMD (r coefficient of 0.38, 0.4 and 0.42 respectively), particularly in the female subgroup. In addition, multivariate analysis of HIV-positive patients assessed for vertebral fractures indicated that QUS was more effective than DXA at predicting the risk of fracture. QUS can be used as an additional tool for analyzing bone density in HIV-positive patients and its case of use and low cost make it especially suitable for resource-limited settings where DXA is not employed.

KEY WORDS: HIV, QUS, DXA, Bone.

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INTRODUCTION

Several studies have demonstrated that the human immunodeficiency virus (HIV), together with certain combinations of antiretroviral drugs, induces a significant bone loss in HIV-positive patients in comparison with uninfected individuals (Borderi *et al.*, 2009; Brown & Qaqish, 2006; Brown & McComsey, 2006). These observations suggest that HIV infection is an independent risk factor for reduced bone mineral density (BMD) (Womack *et al.*, 2011). Osteoporosis in HIV-infected patients is emerging as a problem worldwide, and its management has been improving over the past decade through the use of biochemical and instrumental approaches and, most recently, anti-osteoporotic drugs (McComsey *et al.*, 2010). Osteoporosis is significantly associated with an increase in bone fractures, which, in turn, can negatively affect life expectancy (Womack *et al.*, 2011). The diagnosis of osteopenia and osteoporosis is currently determined by DXA (Kanis *et al.*, 2008). DXA measures the BMD at specific anatomical sites (i.e. the femur, lumbar spine and wrist), and it can be expressed as both a

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T-score and a Z-score (NIH Consensus 2001). DXA is currently considered the gold standard for the diagnosis of osteoporosis. Although DXA effectively describes mineralization status, this technique is less consistent in assessing certain important characteristics of bone structure, such as the trabecular dimensions and bone elasticity, which play important roles in the maintenance of global bone strength evaluation (Chappard *et al.*, 1997; Glüer *et al.*, 1993; Wu *et al.*, 1998). Moreover, because DXA is expensive, it is not an ideal choice for screening for bone mass alterations, particularly in resource-limited countries (Cournil *et al.*, 2012). Hence, a different approach, based on ultrasound, has been proposed to study bone structure. This method, the QUS technique, is able to detect various bone mechanical properties and the trabecular structural orientation through the measurement of parameters represented by the speed of sound (SOS), broadband ultrasound attenuation (BUA) and stiffness (Frost *et al.*, 1999; Frost *et al.*, 2000; Laugier *et al.*, 1997). These three parameters can be used to describe the strength of bone. Additional studies have validated calcaneal QUS for clinical use in osteoporosis and demonstrated that QUS can predict fragility fractures in postmenopausal women and in men over the age of 65, independently of central BMD as determined by DXA (Bauer *et al.*, 1995; Bauer *et al.*, 1997; Bauer *et al.*, 2007; Hans *et al.*, 1996; Khaw *et al.*, 2004; Steward *et al.*, 2006). In this study, we performed DXA and QUS measurements in HIV-positive patients to evaluate the ability of these techniques to accurately screen for bone disease in this population.

MATERIALS AND METHODS

Study population

A total of 224 HIV-positive patients were consecutively enrolled during their periodic clinical evaluations at the Infectious Disease Unit, St. Orsola-Malpighi Hospital, Bologna. The group consisted of 152 men and 72 women with a global mean age of 47.02±9.26 years (range 24-72 years). Most of these patients were Caucasian (213 of 224; 95.1%) and their mean BMI (kg/m^2) was 23.73±3.45. As indicated in the ISCD guidelines, people at risk of osteoporosis were postemenopausal women and men aged 50 or more. Among the 224 HIV-positive patients enrolled in our study, 30 women (41.7%) were in post-menopausal age, whereas 62 men (40.8%) were aged 50 or more.

This cross-sectional study performed QUS (Sahara® Hologic, Bedford, MA) of the calcaneus in all the patients and total-body DXA in 183 patients, L1-L4 vertebral DXA in 187 patients, femur DXA in 186 patients and femoral neck DXA in 185 patients to measure the relative BMD (Z-score and T-score) with DXA Lunar Prodigy Primo TM device (GE Healthcare). In addition, the patients' fracture history was recorded. For a subgroup of patients (53 of 224), a recent lateral spine X-ray was available. The study was approved by the local Independent St. Orsola-Malpighi Hospital Bologna Ethics Committee (protocol number: 3278/2012) and complied with the Code of Ethics (Declaration of Helsinki). Informed consent was obtained from each patient enrolled.

BMD, T-score and Z-score by DXA

In accordance with World Health Organization (WHO) classification criteria, BMD is reported in terms of T-score, which represents the number of standard deviations below the mean for a young, sex- and ethnicity-matched control population. It is calculated using the following formula T-score = (patient's BMD - reference BMD)/SD, where the reference BMD and standard deviation (SD) refer to the control population. The WHO classification criteria identify a T-score values >-1 SD as being normal, between -1 SD and -2.5 SD as indicating osteopenia, and ≤-2.5 SD as indicating osteoporosis (NIH Consensus 2001).

Guidelines issued by the International Society for Clinical Densitometry (ISCD) recommend using the T-score with the diagnostic cut-off values specified by the WHO only for women in menopause. Although definitive data are lacking, it is generally accepted that the same method can be applied to men over the age of fifty if they have at least one major risk factor for osteoporosis. For individuals aged under 50 years, diagnosis is recommended using the Z-score, which compares a patient's BMD with that of a healthy age-, sex- and ethnicity-matched

population. It is calculated using the following formula Z-score = (patient's BMD - reference BMD)/SD, where the reference BMD and SD refer to the mean of the matched population. A Z-score of - 2.0 or lower is defined as "below the expected range for age" and a Z-score above - 2.0 is "within the expected range for age". (Baim *et al.,* 2008).

SOS, BUA, stiffness, T-score and Z-score by QUS
All patients were analyzed by calcaneal QUS. This device consists of two unfocused transducers mounted coaxially on a monitor caliper. One transducer acts as the transmitter, the other as a receiver. The transducers are acoustically coupled to the heel using soft rubber pads and an oil-based coupling gel. The Sahara® Hologic device measures both broadband ultrasound attenuation (BUA, dB/MHz) and speed of sound (SOS, m/s) at a fixed region of interest in the mid-calcaneus, and the BUA and SOS results are combined to provide an estimate of the quantitative ultrasound index (QUI or stiffness) using the formula QUI = 0.41 x (BUA + SOS) - 571. QUS results were also expressed as the corresponding T-scores, and Z-scores using the same cut-offs as those for the DXA measurements. The QUS T- and Z-scores were calculated following the manufacturers' indications.

Statistical analysis
Descriptive statistics included the numbers and percentages of patients, as well as the mean and standard deviation (SD) values. The association between the QUS parameters and the DXA measurements was determined using the Pearson's correlation r coefficient. Multiple linear regression analysis was performed, with the DXA parameters as dependent variables and stiffness, age, sex and BMI as independent variables. Simple regression analysis was used to test for trends among the different variables. A receiver operator characteristic (ROC) curve analysis was performed to assess the performance of the different QUS parameters in the identification of patients with or without a T-score ≤-2.5, for all four sites measured by DXA. Areas under the curve (AUCs), 95% CIs and p-values were recorded. To test the strength of association between T-scores (dichotomized into osteoporotic and non-osteoporotic) from QUS and DXA, a Phi (φ) test of association was performed. Multivariate test analyses were conducted to consider the effects of group variables on QUS and DXA parameters, and Wilks' Lambda (Λ) F statistics were obtained. All tests were two-tailed and only $p<0.05$ was considered statistically significant. All statistical calculations were carried out using SPSS for Windows 20.0 version (IBM Corp., Armonk, NY, USA).

RESULTS

In this cross-sectional study we selected a group of 224 HIV-positive patients (Table 1). All patients were analyzed using calcaneal QUS to measure their SOS (m/sec), BUA (dB/MHz), stiffness, T-score and Z-score. In parallel, total-body DXA was performed in 183 patients, L1-L4 vertebral DXA in 187 patients, femur DXA in 186 patients and femoral neck DXA in 185 patients in order to measure the relative BMD (Z-score and T-score).
The correlation between calcaneal QUS and DXA parameters was determined for all HIV-positive patients using Pearson's test (Table 2). Total-body BMD measurements in HIV-positive patients were significantly associated with calcaneal SOS ($r=0.38$; $p<0.001$), BUA ($r=0.426$; $p<0.001$) and stiffness ($r=0.415$; $p<0.001$). Similar r coefficient values were obtained when lumbar spine, femur and femoral neck BMD were compared with QUS parameters (Table 2). When these data were stratified by sex and correlated with age, the QUS parameters including SOS ($r=-0.377$; $p<0.01$), BUA ($r=-0.414$; $p<0.01$), stiffness ($r=-0.403$; $p<0.01$) and T-score ($r=-0.393$; $p<0.01$) showed an increased Pearson's coefficient in women (Table 3), whereas only the SOS parameter ($r=-0.162$; $p<0.05$) was significant in men, with a very low r coefficient. The same analysis performed with DXA parameters showed a more complex situation. Age was significantly correlated with several DXA parameters in women (Table 3) but in men a correlation was found only for the femoral neck ($r=0.207$; $p<0.05$) and total-hip ($r=0.234$; $p<0.01$) Z-scores.
A multiple regression analysis was carried out to predict DXA parameters from stiffness, age,

TABLE 1 - *General parameters of patients with QUS and DXA data.*

Parameters	*Number of patients (%)*	*Mean (±SD)*
Number of patients (%)	224	
Mean (+SD)	152 (67.9%)	
Women	72 (32.1%)	
Age (years)	224	47.02 (±9.26)
BMI (kg/m^2)	224	23.73 (±3.45)
Ethnicity	224	
Caucasian	213 (95,1%)	
Other	11 (4.9%)	
QUS		
SOS (m/sec)	224	1538.78 (±26.03)
BUA (dB/MHz)	224	63.22 (±14.29)
Stiffness	224	85.82 (±15.79)
T-Score	224	-1.208 (±0.946)
Z-Score	224	-0.596 (±0.919)
DXA		
BMD total body	183	1.124 (±0.106)
T-Score total body	183	-0.645 (±1.238)
Z-Score total body	183	-0.284 (±1.13)
BMD L1-L4	187	1.050 (±0.180)
T-Score L1-L4	187	-1.041 (±1.464)
Z-Score L1-L4	187	-0.721 (±1.427)
BMD femur	186	0.912 (±0.144)
T-Score femur	186	-1.092 (±1.076)
Z-Score femur	186	-0.666 (±0.982)
BMD femoral neck	185	0.858 (±0.148)
T-Score femoral neck	185	-1.275 (±1.093)
Z-Score femoral neck	185	-0.657 (±0.964)
Fracture (spine Rx)	53	
Yes	12 (22.6 %)	
Men	8	
Women	4	
No	41 (77.4 %)	
Men	26	
Women	15	

TABLE 2 - *DXA and QUS parameters with their correlation coefficients.*

	SOS	*BUA*	*Stiffness*	*T-score*	*Z-score*
Total-body BMD	0.380	0.426	0.415	0.361	0.336
Total-body T-score	0.386	0.403	0.411	0.415	0.402
Total-body Z-score	0.322	0.300	0.329	0.352	0.371
L1-L4 BMD	0.282	0.330	0.313	0.293	0.281
L1-L4 T-score	0.309	0.349	0.338	0.337	0.331
L1-L4 Z-score	0.219	0.222	0.231	0.254	0.295
Femur BMD	0.350	0.402	0.386	0.346	0.318
Femur T-score	0.375	0.395	0.400	0.400	0.378
Femur Z-score	0.309	0.335	0.334	0.327	0.360
Femoral-neck BMD	0.303	0.376	0.345	0.304	0.254
Femoral-neck T-score	0.347	0.389	0.379	0.374	0.328
Femoral-neck Z-score	0.265	0.324	0.300	0.289	0.322

Pearson's coefficients calculated between DXA and QUS parameters were indicated. All parameters showed a significant correlation (p-values <0.001). QUS, quantitative ultrasound; SOS, speed of sound; BUA, broadband ultrasound attenuation; DXA, dual energy X-ray absorbptiometry; BMD, bone mineral density.

TABLE 3 - *Correlation coefficients calculated between age and QUS/DXA data*

	Total	*Men*	*Women*
SOS (m/sec)	-0.228 **	-0.162 *	-0.377 **
BUA (dB/MHz)	-0.142 *	-0.016	-0.414 **
Stiffness	-0.207 **	-0.117	-0.403 **
T-score (QUS)	-0.207 **	-0.116	-0.393 **
Z-score (QUS)	0.006	0.075	-0.140
Total-body BMD (DXA)	-0.143	-0.045	-0.332 **
Total-body T-score (DXA)	-0.123	-0.026	-0.328 **
Total-body Z-score (DXA)	0.011	0.058	-0.108
L1-L4 BMD (DXA)	-0.054	0.055	-0.281 *
L1-L4 T-score (DXA)	-0.034	0.092	-0.312 *
L1-L4 Z-score (DXA)	0.146 *	0.187 *	0.032
Femur BMD (DXA)	-0.134	-0.031	-0.337 **
Femur T-score (DXA)	-0.137	-0.029	-0.353 **
Femur Z-score (DXA)	0.117	0.234 **	-0.122
Femoral neck BMD (DXA)	-0.244 **	-0.184 *	-0.369 **
Femoral neck T-score (DXA)	-0.242 **	-0.163	-0.409 **
Femoral neck Z-score (DXA)	0.116	0.207 *	-0.078

Pearson's coefficients (r) were displayed. *p-value<0.05. **p-value<0.01.

sex and BMD. Interestingly, this statistical approach demonstrated that these independent variables significantly predicted all DXA parameters (p<0.0005; Table 4).

In the subgroup of patients (53 subjects) with an available spinal X-ray we observed 12 spinal fractures (22.6% of the entire study population; Tables 1 and 5).

To investigate the specificity and sensitivity of QUS in the discrimination of the presence/absence of osteoporosis, we applied an ROC and the AUCs were calculated to assess the correspondence between QUS and DXA, the latter considered as the gold standard for the diagnosis of osteoporosis (Figure 1). The AUC reflects the potential of QUS to discriminate between normal and osteoporotic patients as diagnosed by DXA. We used the DXA T-scores as a reference, with a cut-off value of -2.5, to discriminate between normal/osteopenic and osteoporotic patients. All QUS parameters significantly predict the osteoporosis risk, with consistently high AUC values. Notably, when we analyze the total-body T-score data subset, the AUCs were consistently high for all QUS parameters, especially for the QUS T-score (AUC=0.820; 95% CI 0.706-0.935; p<0.0005). Figure 1 shows the ROC curve for the total-body DXA T-scores. In parallel, we generated the contingency table to indicate the values of sensitivity and specificity (Tables 6 and 7).

To assess the association between two variables, namely the QUS T-scores and the DXA T-scores, in predicting osteoporotic or normal/osteopenic bone, these variables were dichotomized, with a cut-off of -2.5 SD (i.e. T-scores ≤-2.5 SD for osteoporotic bone, T-scores >-2.5 SD for normal/osteopenic bone). Subsequently, a Phi (φ) test was performed to quantify the strength of association between these variables (Table 8). All the variables showed a strong association when the entire study population was considered (QUS x total-body DXA: φ=0.495, QUS x L1-L4 DXA: φ=0.418, QUS x femur DXA: φ=0.542, QUS x femoral neck DXA: φ=0.429, all values were significant at p<0.0005). When stratified by sex, all four combinations showed a minor but significant correlation in men: it is noteworthy that the QUS T-score association with the DXA T-scores resulted in a substantial increase in the Phi coefficient (QUS x total-body DXA: φ=0.887, QUS x L1-L4 DXA: φ=0.638, QUS x femur (TOTAL HIP) DXA: φ=0.802, QUS x femoral neck DXA: φ=0.598, all values were significant at p<0.0005).

DISCUSSION

This study compared the calcaneal QUS with total-body, femur, femoral neck and L1-L4 vertebral DXA analyses for a group of adult HIV-positive patients. Because DXA is widely considered the gold standard for the measurement of BMD and in the diagnosis of osteoporosis, our first aim was to statistically evaluate QUS parameters (SOS, BUA, stiffness, T-score and Z-score) with respect to DXA parameters (BMD, T-score and Z-score) in the entire HIV-positive study population. Interestingly, the QUS parameters (SOS, BUA, stiffness and T-score) showed a moderate correlation with the DXA parameters. In particular, this correlation (calculated by the r coefficient) was more

TABLE 4 - *Multiple regression analysis.*

	F	*r*	*p*		β	*p*
Total-body BMD	27.60	0.618	<0.0005	*Age*	-0.125	0.045
				QUS Stiffness	0.332	<0.0005
				Sex	-0.186	0.003
				BMI	0.380	<0.0005
Total-body T-score	17.11	0.527	<0.0005	*Age*	-0.088	0.188
				QUS Stiffness	0.372	<0.0005
				Sex	0.157	0.017
				BMI	0.329	<0.0005
Total-body Z-score	9.77	0.424	<0.0005	*Age*	0.063	0.381
				QUS Stiffness	0.351	<0.0005
				Sex	0.230	0.001
				BMI	0.160	0.025
L1-L4 BMD	7.19	0.369	<0.0005	*Age*	-0.017	0.819
				QUS Stiffness	0.286	<0.0005
				Sex	-0.039	0.581
				BMI	0.188	0.010
L1-L4 T-score	8.40	0.395	<0.0005	*Age*	0.007	0.919
				QUS Stiffness	0.327	<0.0005
				Sex	0.081	0.250
				BMI	0.202	0.005
L1-L4 Z-score	7.22	0.370	<0.0005	*Age*	0.194	0.008
				QUS Stiffness	0.288	<0.0005
				Sex	0.208	0.004
				BMI	0.072	0.317
Femur BMD	22.06	0.572	<0.0005	*Age*	-0.120	0.062
				QUS Stiffness	0.306	<0.0005
				Sex	-0.124	0.050
				BMI	0.387	<0.0005
Femur T-score	20.02	0.554	<0.0005	*Age*	-0.127	0.052
				QUS Stiffness	0.345	<0.0005
				Sex	0.143	0.026
				BMI	0.391	<0.0005
Femur Z-score	13.19	0.475	<0.0005	*Age*	0.147	0.033
				QUS Stiffness	0.344	<0.0005
				Sex	0.060	0.374
				BMI	0.286	<0.0005
Femoral neck BMD	21.93	0.572	<0.0005	*Age*	-0.258	<0.0005
				QUS Stiffness	0.241	<0.0005
				Sex	-0.081	0.204
				BMI	0.406	<0.0005
Femoral neck T-score	22.68	0.579	<0.0005	*Age*	-0.258	<0.0005
				QUS Stiffness	0.300	<0.0005
				Sex	0.165	0.010
				BMI	0.429	<0.0005
Femoral neck Z-score	12.79	0.470	<0.0005	*Age*	0.124	0.076
				QUS Stiffness	0.308	<0.0005
				Sex	0.081	0.241
				BMI	0.328	<0.0005

Multiple regression analysis on relationship between DXA parameters and four predictive factors: QUS stiffness, BMI, age and sex. *r*, Pearson's coefficient; F, F statistic; β, standardized beta-coefficient; p, p-value.

TABLE 5

Fracture (spine Rx)							
No (41)				*Yes (12)*			
Sex		*Age (years)*	*BMI (kg/m²)*	*Sex*		*Age (years)*	*BMI (kg/m²)*
Men	*Women*	*Mean (SD)*	*Mean (SD)*	*Men*	*Women*	*Mean (SD)*	*Mean (SD)*
26	15	48 (9.9)	24.3 (2.7)	8	4	54 (7.3)	24.3 (3.3)

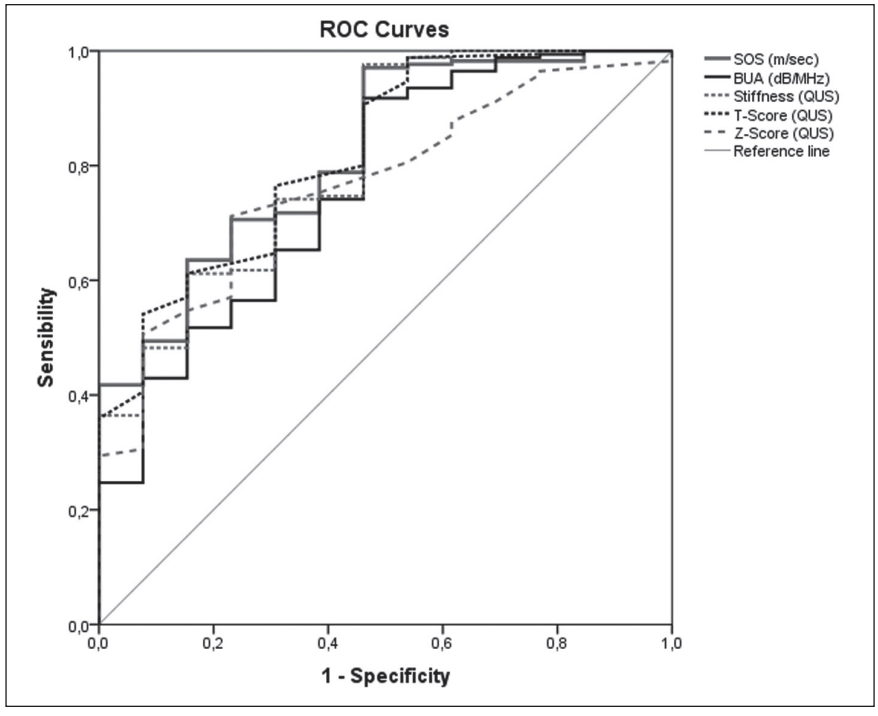

FIGURE 1 - *ROC curves for QUS parameters from HIV-positive patients with or without a diagnosis of osteoporosis, based on total-body DXA T-scores. The threshold was set at -2.5, with osteoporotic patients having T-scores ≤-2.5 and non- osteoporotic patients having T-scores >-2.5.*

TABLE 6 - *Contingency tables including all patients.*

		T-Score (QUS)* T-Score total body (DXA)			
		T-Score total body (DXA)			
		Osteoporosis	*Normal/osteopenic*	*Total*	
T-Score (QUS)	<=-2.5	6	2	8	Sensitivity 46,15%
	>-2.5	7	168	175	Specificity 98,82%
Total		13	170	183	
		T-Score (QUS)* T-Score L1-L4 (DXA)			
		T-Score L1-L4 (DXA)			
		Osteoporosis	*Normal/osteopenic*	*Total*	
T-Score (QUS)	<=-2.5	7	1	8	Sensitivity 30,43%
	>-2.5	16	163	179	Specificity 99,39%
Total		23	164	187	
		T-Score (QUS)* T-Score femur (DXA)			
		T-Score femur (DXA)			
		Osteoporosis	*Normal/osteopenic*	*Total*	
T-Score (QUS)	<=-2.5	6	2	8	Sensitivity 40%
	>-2.5	9	169	178	Specificity 98,83%
Total		15	171	186	
		T-Score (QUS)* T-Score femoral neck (DXA)			
		T-Score femoral neck (DXA)			
		Osteoporosis	*Normal/osteopenic*	*Total*	
T-Score (QUS)	<=-2.5	6	2	8	Sensitivity 27,27%
	>-2.5	16	161	177	Specificity 98,77%
Total		22	163	185	

Gold Standard: DXA T-Scores parameters categorized as osteoporosis (T-score <= -2.5) or normal/osteopenic (T-score > -2.5). Tested variable: QUS T-Score parameter categorized as osteoporosis (T-score <= -2.5) or normal/osteopenic (T-score > -2.5).

TABLE 7 - *Contingency tables including only patients at risk, namely all women in post-menopausal age and men aged 50 or over.*

		T-Score (QUS)* T-Score total body (DXA)			
		T-Score total body (DXA)			
		Osteoporosis	*Normal/osteopenic*	*Total*	
T-Score (QUS)	<=-2.5	6	1	7	Sensitivity 60%
	>-2.5	4	61	65	Specificity 98,39%
Total		10	62	72	
		T-Score (QUS)* T-Score L1-L4 (DXA)			
		T-Score L1-L4 (DXA)			
		Osteoporosis	*Normal/osteopenic*	*Total*	
T-Score (QUS)	<=-2.5	6	1	7	Sensitivity 42,86%
	>-2.5	8	61	69	Specificity 98,39%
Total		14	62	76	
		T-Score (QUS)* T-Score femur (DXA)			
		T-Score femur (DXA)			
		Osteoporosis	*Normal/osteopenic*	*Total*	
T-Score (QUS)	<=-2.5	5	2	7	Sensitivity 62,50%
	>-2.5	3	66	69	Specificity 97,06%
Total		8	68	76	
		T-Score (QUS)* T-Score femoral neck (DXA)			
		T-Score femoral neck (DXA)			
		Osteoporosis	*Normal/osteopenic*	*Total*	
T-Score (QUS)	<=-2.5	5	2	7	Sensitivity 33,33%
	>-2.5	10	59	69	Specificity 96,72%
Total		15	61	76	

Gold Standard: DXA T-Scores parameters categorized as osteoporosis (T-score <= -2.5) or normal/osteopenic (T-score > -2.5). Tested variable: QUS T-Score parameter categorized as osteoporosis (T-score <= -2.5) or normal/osteopenic (T-score > -2.5).

TABLE 8 - *Association between QUS and DXA T-scores in predicting osteoporotic or non-osteoporotic bone.*

T-scores	*Total*		*Men*		*Women*	
	φ	*p*	φ	*p*	φ	*p*
QUS x total-body	0.495	<0.0005	0.246	0.007	0.887	<0.0005
QUS x L1-L4	0.418	<0.0005	0.276	0.002	0.638	<0.0005
QUS x femur	0.542	<0.0005	0.371	<0.0005	0.802	<0.0005
QUS x femoral neck	0.429	<0.0005	0.308	0.001	0.598	<0.0005

Phi (φ) coefficients were displayed. φ, phi test; *p*, p-value.

consistent when the QUS parameters were compared with the DXA, BMD and DXA T-score. Following stratification by sex and correlation with age, women showed a higher r coefficient than the entire study population. The analysis of the QUS data collected with Sahara Hologic device from HIV-positive women showed that this subgroup has lower QUS parameter values than those of uninfected women, suggesting a more rapid decrease in bone strength correlated with HIV-infection and/or cART treatment. Interestingly, higher r coefficients were detected when total-body DXA BMD was compared with the calcaneal QUS parameters (SOS, BUA and stiffness), whereas the lowest r coefficients were obtained when those QUS parameters were compared with L1-L4 DXA BMD.

Bone properties (cortical or trabecular structure), patient characteristics (gender) and, most likely, HIV/cART features (i.e., infection length and different antiretroviral regimens) elicit different correlations between DXA and QUS specific parameters. These data are in accordance with the r coefficient observed in other studies, in which comparative analyses between QUS and DXA data were performed on

healthy individuals, even when different QUS devices were used (Ikeda *et al.,* 2004; Steward *et al.,* 2000). However, the correlations between QUS parameters (SOS, BUA and stiffness) and DXA Z-scores and T-scores in the different body regions are not sufficient to support the hypothesis that calcaneal QUS can be considered an alternative technique to DXA for diagnosing osteopenia/osteoporosis in HIV-positive patients. This confirms the official guidelines of the Osteoporosis Society that still indicate that DXA is the gold standard for osteoporosis diagnosis in healthy donors (National Osteoporosis Foundation, 2010). In fact, discordant results between calcaneal QUS and central DXA have been obtained and are not necessarily an indication of methodological error (Pisani *et al.,* 2013). QUS and DXA scans simply measure different bone characteristics, (bone quality and bone quantity, respectively), and therefore both can return useful integrative information. The DXA technique is able to identify the variations in BMD by analyzing both cortical and trabecular bone. By contrast, DXA is not able to determine the strength of bone architecture, and a normal BMD could be associated with impaired bone structure that has a higher risk of fractures (Stagi *et al.,* 2014). The QUS approach has been introduced as a new tool for bone analysis because it can effectively investigate the bone micro-architecture as well as BMD (Flöter *et al.,* 2011). For example, BUA is dependent on bone trabecular orientation (at least during in vitro testing) and BUA and BMD are also related, suggesting that ultrasound attenuation could represent a parametric synthesis of the bone micro-architecture and bone mass density (Grimal *et al*., 2013). Every QUS parameter provides specific and different information (for example, 99% of SOS variabilty depends on density+elasticity+anisotrophy at 99% whereas BUA variability depends on density+trabecular dimensions+connectivity at 68%).

ìIt is noteworthy that QUS shows good performance even on the metabolically active trabecular metabolic bone compared with cortical structural bone, suggesting a more rapid detection of bone impairment (Navarro *et al.,* 2012). Furthermore, QUS can be used as a test for screening patients who undergo DXA scans, especially when DXA is not available. Because QUS devices are portable and easy to use and carry no radiation-exposure risk, QUS is a useful technique for assessing bone characteristics in resource-limited settings where the more expensive DXA cannot be employed. A recent study of a cohort of African HIV-positive patients performed using only with QUS confirmed the BMD reduction and bone impairment in these patients in comparison with sex- and age-matched healthy individuals. In addition, QUS can be a valuable test in any setting for screening patients who undergo the DXA scan, and for providing specific information that can be usefully integrated with DXA results.

QUS measurements of the calcaneus discriminate effectively between cases and controls with vertebral crush fractures and non-spinal fractures (Liu *et al.,* 2012). Several studies performed in large groups of healthy subjects have shown that QUS is as effective as DXA in the prediction of fracture risk (Pines, 2013). The ability of QUS to identify the risk of fractures was compared with that of DXA in our group of HIV-positive patients. We compared the QUS and DXA results for the 53 patients who were studied with spinal X-rays. The multivariate analysis data for QUS, L1-L4 DXA, sex and age showed that the QUS variables showed a higher and strongly significant F when compared with L1-L4 DXA, especially when we analyzed the age x spinal fracture group. This observation indicates that QUS is preferable to DXA as a method for predicting fractures in adult HIV-positive individuals, although this analysis was performed in a small number of cases. Monitoring changes in bone structure and mineral density is important in the management of HIV-infected patients because HIV infection and certain combinations of antiretroviral treatments elicit progressive bone loss in these patients (Gibellini *et al.,* 2012; Ofotokun & Weitzmann, 2010; Rothman *et al.,* 2012). HIV proteins and some antiretrovirals interfere with bone homeostasis, osteoblast/osteoclasts crosstalk and differentiation (Cotter *et al.,* 2007; Cotter *et al.,* 2008; Gibellini *et al.,* 2008; Gibellini *et al.,* 2010). The decrease in osteoblast activity, the opposing increase in osteoclast-driven bone resorption and the derangement of cytokines related to bone homeostasis such as RANKL

and OPG (Fakruddin & Lawrence, 2005; Konishi *et al.,* 2005; Gibellini *et al.,* 2007; Mora *et al.,* 2007), lead to a reduction in BMD and an increase in fractures in HIV-positive patients (Peters *et al.,* 2013; Womack *et al.,* 2013). To the best of our knowledge, this paper is the first report on the comparative application of QUS and DXA to adult HIV-positive patients. A previous study by Mora and coworkers analyzed the relationship between QUS and DXA in a pediatric cohort achieving a similar correlation between DXA and QUS parameters (Mora *et al.,* 2009).

In conclusion, the correlation observed especially between by DXA and QUS parameters in determining total-body BMD suggests that QUS can be used as an additional diagnostic technique in HIV-positive patients for fracture prediction, a principal clinical endpoint correlated with osteoporosis.

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