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Sonographic assessment of severity in the body fat changes related to the lipoatrophic findings of HIV associated Adipose Redistribution Syndrome (HARS)

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ABSTRACT

Objective: To investigate the diagnostic accuracy of ultrasound (US) to identify the severity of body fat changes due to HIV-related lipoatrophy (LA) by US grading scale (US GS. Methods: US diagnoses based on measurements of the thickness of subcutaneous fat at representative reference points (RPs) for LA were compared to clinical diagnoses based on the HIV Outpatient Study Grading Scale (HOPS-GS). A sample size of 115 patients was required for statistical power of 80%. Results: 73 males [HOPS-GS-0: 31.5%, 41.2%, and 50.5%; HOPS-GS-1 42.5%, 32.5%, and 29%, and HOPS-GS-2/3 26%, 26.2% and 20.5%, for facial, brachial, and crural RPs, respectively] and 42 females [HOPS-GS-0 26%, 43%, and 28.6%; HOPS-GS-1 38%, 38% and 33.3%; and HOPS-GS-2/3 36, 19% and 38.1%, for facial, brachial, and crural RPs, respectively] were recruited. Significant differences were found in US assessments for each corresponding HOPS-GS (p<.003–.0001). Diagnostic thresholds were identified for each degree of LA severity (US-GS 0 vs. US-GS 1 and US-GS2-3) for facial, brachial, and crural LA, with related sensitivity (range: 83–99%), specificity (range: 85–99%), positive predictive values (range: 73–98%) and negative predictive values (range: 82–98%). Compared with clinical LA diagnoses, US-GS correctly diagnosed 80.9% of cases (OR: 8.1; 95%CI: 2.8–23.5, p<.0001). US identified 33% of cases with initial LA (fat loss <1.5mm from diagnostic thresholds) not recognizable by the clinical assessments. Conclusions: US shows good diagnostic accuracy in the assessment of early HIV related LA. US-GS may be valuable in routine management of HIV outpatients to objectively assess lipoatrophic findings.

Introduction

Highly active antiretroviral therapies (HAART) have radically changed the prognosis and life expectancy of patients infected with human immunodeficiency virus (HIV)(1), but their accelerated introduction into clinical practice is associated with unexpected side-effects, including changes in visceral and subcutaneous adipose tissue, lipid and glucidic metabolism, and an increased risk of cardiovascular diseases due to accelerated endothelial dysfunction (2-6).
HIV-associated adipose redistribution syndrome (HARS) is a socially disabling disorder that compromises patients' quality of life, and is a serious challenge in the management of HIV-infected patients (5). There are a number of gaps in the tools available for the diagnosis and treatment of HARS. The score proposed in the HIV Outpatients Study (HOPS-GS)(7) can expose these patients to the risk of misdiagnosis, particularly in the early, less severe stages due to the absence of objective methods to assess body fat changes (BFCs). The lipodystrophy score by the Case Definition Group Study (LCDS)(8) or the Fat Redistribution and Metabolic Changes Study (FRAM)(9) increases the degree of diagnostic accuracy, but are probably too difficult to perform in an outpatient setting because imaging tools, such as Dual Energy X-ray Absorbimetry (DEXA) (10,11) and computed tomography (CT) (12,13), are required. These tools are often not feasible because of the high costs, long waiting lists, and exposure to ionizing radiation.
Using ultrasound (US) to assess BFCs avoids the limitations of other validated imaging tools as it is widely available and cost effective, does not expose patients to ionizing radiation, and it is readily accepted by patients.
Several investigators have already described a high degree of accuracy of US, compared with both anthropometry and tomography, in the assessment of the intra-abdominal and subcutaneous fat thickness (SFT) changes documented in non HIV-related central obesity (14-24).
Moreover, recent studies on HIV patients showed the ability of US to identify BFCs related to HARS (25-29).
Although US is more operator-dependent than other techniques, our US-based measurement technique showed low intra-observer variability and high inter-observer agreement (30).

Moreover, as observed in non HIV subjects (14-24), we recently confirmd a good comparability of CT and US measurements of subcutaneous adipose tissue (31).
Based on these results, the current study addressed two key questions: (1) the accuracy of US in identifying SFT loss related to lipoatrophy (LA) and compared with the HOPS-GS-based clinical diagnoses, and (2) to identify a US-based Grading Score (US-GS) of LA at reference points (RPs) that are clinically representative of BFCs described in HARS.

Patients and Methods

Study population

This cross-sectional study enrolled adult HIV patients (73 males and 42 females) receiving HAART but with no active AIDS-defining illnesses, who had been clinically assessed for HARS. Patients were examined at the Unit of Ultrasound of the Infectious Disease Division of IRCSS S. Matteo Foundation, Pavia, from January to July 2005. Informed consent was obtained from all patients, and the local Ethics Committee approved the study.
Non-obese HIV-infected patients, identified by body-mass index (BMI) <27 kg/m^2, were selected. As reported by Muurahainen et al. at the *1st International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV, San Diego 2007*, patients were chosen to avoid non-lipodystrophic obesity and to select for patients with a higher likelihood of LA.
An age, sex (males: 28.9 years [95% CI: 24.9-32.9]; females: 34.3 years [95% CI: 31.6-36.9]), and BMI (males: 22.1 kg/m^2 [95% CI: 21.2-23.1]; females: 20.5 kg/m^2 [95% CI: 19.8-21.1]) matched healthy control (HC) group (25 males and 24 females) was compared with the HIV patients.

Clinical body fat assessments

At enrolment, the patient completed a detailed interview and underwent an extensive physical examination by experienced clinicians. Additional data for each patient, including virological and immunological status, duration of HIV infection, treatment with a particular antiretroviral drug or drug class, and their duration were considered. HARS was defined by the correspondence between the self-reports of patients and the medical examinations and based on HOPS- GS (7). Fat redistribution was scored separately for the face, arms and lower limbs on a scale of 0 and 3, where 0 is the absence of BFCs, 1 is minimal-mid, 2 is fair, and 3 is severe changes in BFCs.
All patients underwent quantitative US scans of regional body fat.

Echographic body fat assessments

The RPs were based on the correspondence with BFCs described in the clinical examinations. The same experienced sonologist, blinded to the patient's status, performed the US SFT measurements using high-frequency transducers (7.5–13 MHz) from EUB 6500 (HITACHI Medical System).
SFT was assessed at three RPs, avoiding any pressure on the underlying skin, any stand-off pad, and using a minimal quantity of gel to obtain the best resolution of the epidermis (excluded from the SFT measurement) and derma. Patients were examined in a supine position.
The US assessments were performed only when a well-defined horizontal posterior hyperechoic line of the epidermis was obtained. Facial LA was assessed at the deepest point of Bichat's pad using a left nasogenian transversal scan SFT from the outer line of the hyperechoic rim of the malar bone to the inner hyperechoic line of the epidermal layer **(Figure 1a).** For the brachial LA, an assessment of upper limb SFT was performed with dorsal scans of the arm and an anterior flexion of the right elbow joint at a 90° angle, using a longitudinal scan 10 centimeters above the elbow, from the outer edge of superficial fascia of triceps to the inner hyperechoic line of the epidermal layer **(Figure 1b)**. For the crural LA, a long scan was performed 20 cm above the rotula, from the outer edge of superficial fascia of the quadriceps to the inner hyperechoic line of the epidermal layer **(Figure 1c).** Three measurements were obtained for each RP; their mean value was used for statistical analysis.

Statistical analysis

The sample size was fixed to allow at least 80% statistical power using a priori sample size calculator for multiple regression by identifying the minimum required sample size, given an alpha level less than 0.05, numbers of predictors and medium anticipated effect size (*Cohen's d =0.1).* Under these conditions, a sample size of 115 patients evaluated by the 2 compared tests was required.
The mean ± 95% confidence interval (CI) was mea-

Figure 1a.

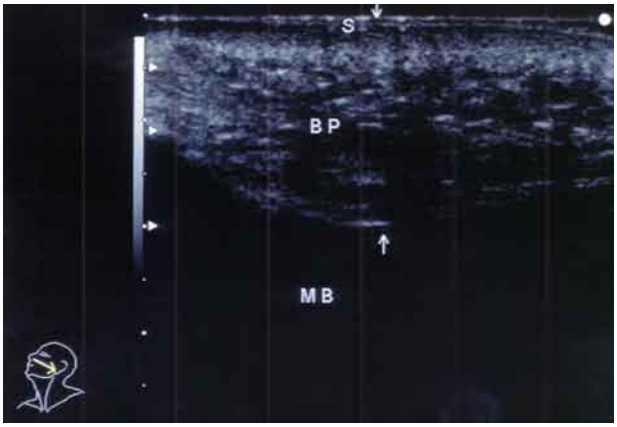
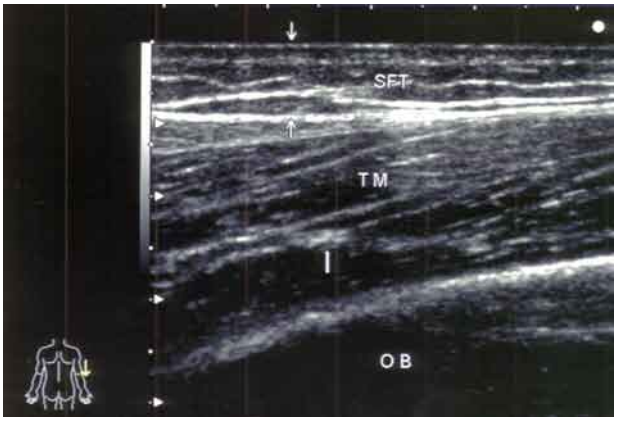


Figure 1b.



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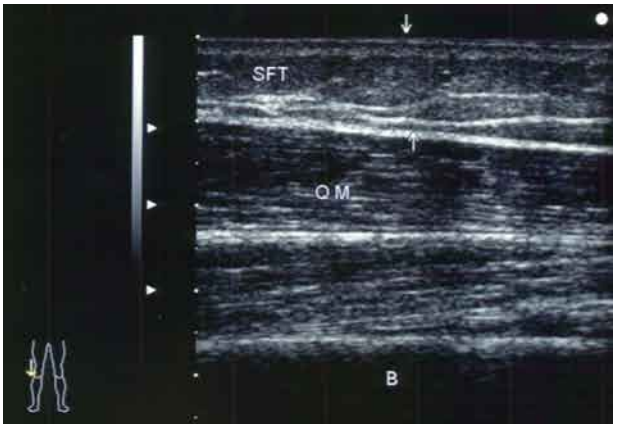


sured for all clinical and demographic data and all the measurements. A *t*-test was used to analyze the clinical and demographic data and to assess statistical differences between mean values of US-based SFT measurements in HCs, and in patients with HOPS-GS0 vs. those with HOPS-GS1 and HOPS-GS2-3, respectively.

Receiver-operating characteristic (ROC) curves were computed, and the area under the curve ± 95% CI was calculated. The US measurements at three separate RPs were compared in HIV patients classified as HOPS-GS0 vs. HOPS-GS1 and HOPS-GS2-3 vs. patients with HOPS-GS ≤1.

Diagnostic thresholds (DTs) were identified for each RP and maximized for both sensitivity and specificity to obtain a US grading scale able to identify patients with mild LA vs. not lipoatrophic, and patients with fair/ severe LA.

ROC curve analysis was performed based on sex, due to differences in body fat distribution between males and females. Using our US-based DTs, we performed a logistic regression to compare all clinical and US diagnoses of LA. The odds ratio with 95% CI was assessed. Significance was identified as $P \leq 0.05$.

Statistical analyses were performed with MEDCALC statistical software (Broekstraat 52 B-9030 Mariakerke, Belgium).

Results

Patients' body fat distribution based on the HOPS-GS for males and females is presented in **Table 1.** **Table 2** summarizes the demographic, clinical, and biohumoral characteristics of male and female patients based on HOPS-GS.

Relationship between US-based SFT measurements and HOPS-GS

Figures **2a** and **2c,** and **3a** and **3c** show the mean values (95% CI) of SFT measurements performed at each RP compared with the clinical assessments in the 3 HOPS-GS classification groups in males and females.

Facial assessments

In male patients, the mean values were 13 (95% CI, 12.4–13.6) mm, 9.9 (95% CI, 9.4–10.4) mm and 5.2 (95% CI, 4.3–6.1) mm for HOPS-GS0, GS1, and GS2-3, respectively (P<.0001: GS0 vs. GS1 and GS 0–1 vs. GS2). In female patients, the mean values were 13.3 (95% CI, 12.5–14.1) mm, 9.96 (95% CI, 9.4–10.5) mm, and 7.1 (95% CI, 6.2–8.1) mm for HOPS-GS0, GS1, and GS2-3, respectively (P <.0001: GS0 vs. GS1 and GS 0–1 vs. GS2). In healthy male controls, the mean value was 12.5 (95% CI, 11.8–13.2) mm (NS HC vs. GS0, P <.0001: HC vs. GS1–2). In healthy female controls, the mean value was 13.95 (95% CI, 12.9– 14.9) mm (NS HC vs. GS0, P <.0001: HC vs. GS1–2).

Brachial assessments

In male patients, the mean values were 5.1 (95% CI, 4.5–5.6) mm, 3.2 (95% CI, 3–3.4) mm, and

Figure 2a.

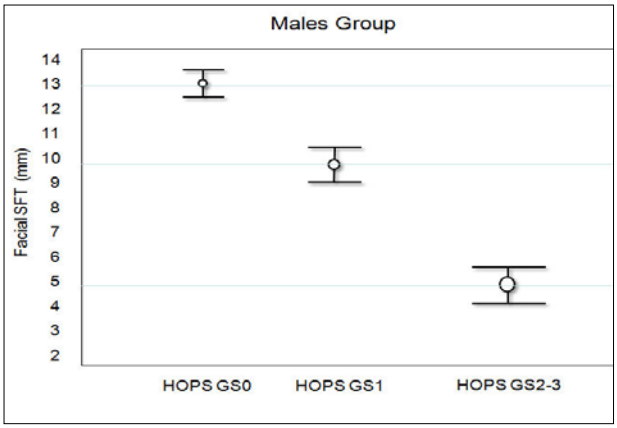


Figure 2b.

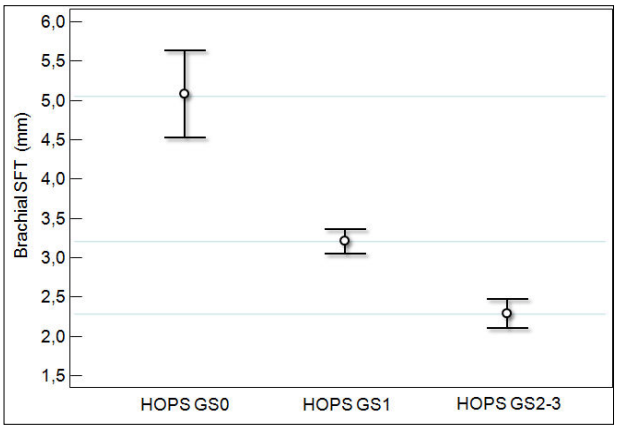
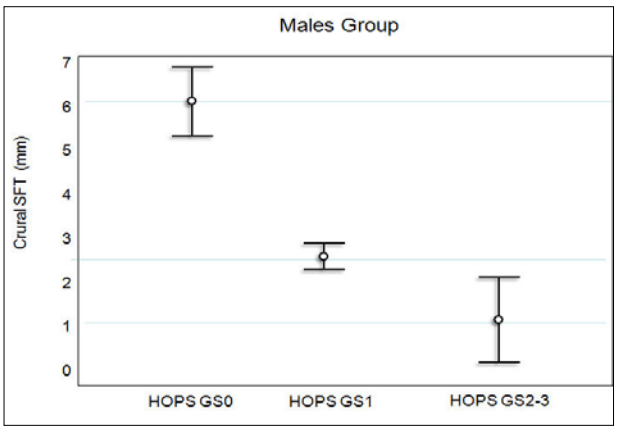


Figure 3b.



2.3 (95% CI, 2–2.5) mm, for HOPS-GS0, GS1, and GS2-3, respectively (*P*<.003: GS0 vs. GS1 and *P* <.0001: GS 0–1 vs. GS2). In female patients, the mean values were 6.2 (95% CI, 5.4–6.9) mm, 4.2 (95% CI, 3.6–4.9) mm, and 3.1 (95% CI, 2.6–3.5) mm, for HOPS-GS 0, GS1, and GS2-3, respectively (*P* <.0001: GS0 vs. GS1 and GS 0–1 vs. GS2). In healthy male controls, the mean value was 4.1 mm (95% CI, 3.7–4.4) (NS HC vs. GS0, *P* <.0001: HC vs. GS1–2). In healthy female controls, the mean value was 6.8 (95% CI, 5.6–7.9) mm (NS HC vs. GS0, *P* <.0001: HC vs. GS1–2).

Crural assessments

In male patients, the mean values were 6.1 (95% CI, 5.3–7) mm, 2.6 (95% CI, 2.2–2.8) mm, and 2 (95% CI, 1.5–2.5) mm for HOPS-GS0, GS1, and GS2-3, respectively (*P* <.0001: GS0 vs. GS1 and GS 0–1 vs. GS2). In female patients, the mean values were 12.3 (95% CI, 10.2–14.3) mm, 7 (95% CI, 6.5–7.5) mm, and 4.6 (95% CI, 3.9–5.3) mm for HOPS-GS 0, GS1, and GS2-3, respectively (*P* <.0001: GS0 vs. GS1 and GS 0–1 vs. GS2). In healthy male controls, the mean value was 5.3 (95% CI, 4.3–6.2) mm (NS HC vs. GS0, *P* <.0001: HC vs. GS1–2). In healthy female controls, the mean value was 12.2 (95% CI, 11–13.4) mm (NS HC vs. GS0, *P* <.0001: HC vs. GS1–2).

***Receiving operator oharacteristics* (ROC) analysis**

ROC curves were determined for the female and male populations according to the 3 different grades of severity of BFCs classified by the clinicians with HOPS-GS0 vs. HOPS-GS1 and HOPS-GS ≤1 vs. HOPS-GS2-3.

Determination of LA diagnostic thresholds

Table 3 shows the optimal DTs obtained for the assessment of facial, brachial, and crural LA with the corresponding sensitivity, specificity and positive (PPV) and negative (NPV) predictive values at each RP.

An echographic grading scale (US-GS) was derived for each RP based on sex.

Males

Facial assessments: DTs <11 mm (sensitivity 92%, specificity 94%, PPV 95%, NPV 91%) and <7 mm (sensitivity 99%, specificity 98%, PPV 95%, NPV 97%), respectively, showed good diagnostic accuracy for distinguishing not lipoatrophic (US-GS0) from mild atrophic patients (US-GS1) and for distinguishing mild LA from fair/severe LA (US-GS2).

Brachial assessments: DTs <4 mm (sensitivity 83%, specificity 95%, PPV 96%, NPV 86%) and <2.5 mm (sensitivity 90%, specificity 97%, PPV 82%, NPV 98), respectively, showed good accuracy for distinguishing lipoatrophic (US-GS0) from mild atrophic patients (US-GS1) and for distinguishing mild LA from fair/severe LA (US- GS2).

Crural assessments: DTs <4 mm (sensitivity 96%, specificity 85%, PPV 81%, NPV 97%) and <2.5 mm (sensitivity 94%, specificity 89%, PPV 73%, NPV 98%), respectively, showed good accuracy for distinguishing non lipoatrophic (US-GS0) from mild atrophic patients (US-GS1) and for distinguishing mild LA from fair/severe LA (US- GS2).

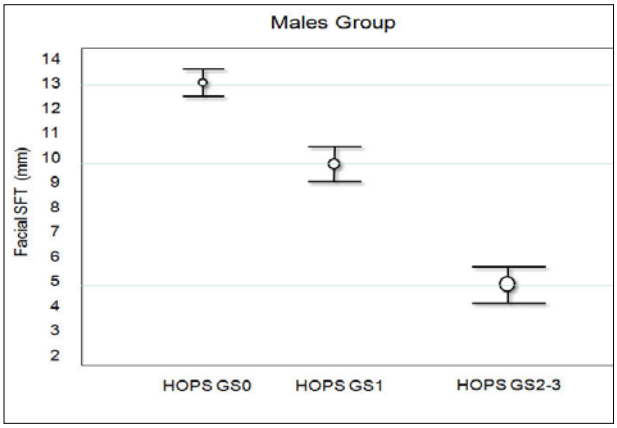
Females

Facial assessments: DTs <11.5 mm (sensitivity 92%, specificity 96%, PPV 92%, NPV 96%) and <8.3 mm (sensitivity 91%, specificity 99%, PPV 98%, NPV 96%), respectively, showed good diagnostic accuracy for distinguishing lipoatrophic (US-GS0) from mild atrophic patients (US- GS1) and for distinguishing mild LA from fair/severe LA (US- GS2).

Brachial assessments: DTs <4.5 mm (sensitivity 83%, specificity 95%, PPV 95%, NPV 82%) and <3.5 mm (sensitivity 86%, specificity 94%, PPV 75%, NPV 97%), respectively, showed good accuracy for distinguishing non lipoatrophic (US-GS0) from mild atrophic patients (US- GS1) and for distinguishing mild LA from fair/severe LA (US- GS2).

Crural assessments: DTs <8 mm (sensitivity 94%,

Figure 3a.



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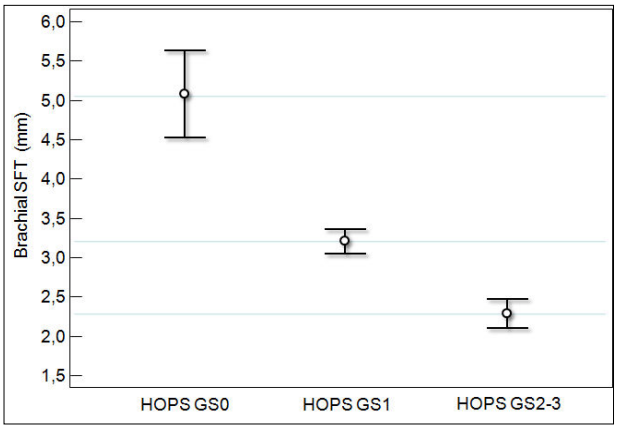
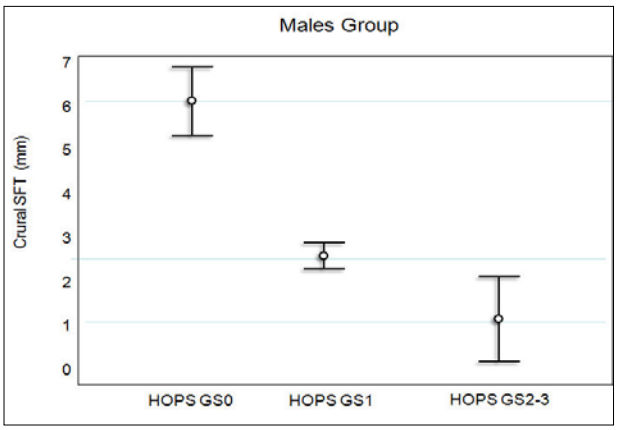


Figure 3c.



specificity 96%, PPV 94%, NPV 96%) and <5.5 mm (sensitivity 93%, specificity 96%, PPV 93%, NPV 95%), respectively, showed good accuracy for distinguishing non lipoatrophic (US-GS0) from mild atrophic patients (US-GS1) and for distinguishing mild LA from fair/severe LA (US- GS2).

A logistic regression was performed to assess the ability of the US-GS to correctly classify cases of LA compared with the HOPS-GS. The results confirmed that the sonologist correctly diagnosed 80.9% of LA cases (regression coefficient: 2.1 [SE: 0.54], *P* <.0001) with an odds ratio of 8.1 (95% CI: 2. 8 to 23.5).
US showed 38 out of 115 (33%) patients misclassified compared with the clinical assessments, including four patients with HOPS-GS0 and seven with HOPS-GS1 in the subgroup with facial LA; seven patients with HOPS-GS0, and 11 with HOPS-GS1 in the subgroup with brachial LA; three patients with HOPS-GS0, five with HOPS-GS1, and one with HOPS-GS2 in the subgroup with crural LA. Clinicians identified LA not recognized by the sonologist in only five cases.
Thirty-three (28.6% of our series) of these cases of US-based LA diagnoses not recognized by clinicians corresponded to patients with minimal SFT losses (measurements ≤1.5 mm from DTs).

Discussion

US assessment of HARS in patients receiving HAART is a controversial topic (32-35), but the accuracy of US has already been demonstrated (26-29), and US diagnoses were shown to be in agreement with clinical methods of classification such as the lipodystrophy score by case definition study, although the sample size in this study was small (25).
The low cost, availability, simplicity and lack of ionizing radiation of US could make it an ideal and practical tool in the diagnosis of HARS compared with CT and DEXA. Moreover, given the complexity and variety of clinical presentations of HARS, US may allow assessments of each district of the body, such as focalized facial LA or focalized lipohypertrophy (cervical lipomatosis), whereas tools such as DEXA cannot (36,37).
We started our investigation on the perspectives of US with preliminary tests on its comparability with other objective tools, particularly the CT scan which is considered to be the gold standard. As described in a recent report US-based SFT measurements were highly comparable to CT scans (31). These data, although obtained from a sample of patients in the current cohort, confirmed the high degree of accuracy of US to assess the subcutaneous and visceral adipose tissue as compared to CT, observed in previous not HIV related studies (14-24).
Because US is more operator-dependent than other techniques, we tested the intra- and inter-observer reliability. As described in a previous report, there was little intra-observer variability with US and good inter-observer agreement (30).
Based on the reliability of our measurement technique at standardized RPs, we decided to assess its accuracy in the diagnosis of HARS.
Given the potential bias that might be caused by the BMI of enrolled patients being too low in the current study, we only evaluated the US assessments of lipoatrophic findings. Moreover, unpublished data showed that SFT could be directly related to the patient's BMI when overweight (BMI >24 kg/m^2) or obese (BMI > 27 kg/m^2) patients were compared with normal weight patients.
Although this does not seem like a significant variable in the current study because so few patients had a BMI greater than 24 kg/m^2 (16 males and 11 females) future studies in a larger population including the three different BMI subgroups (normal, overweight, and obese) are needed.
However, because lipoatrophic findings are predominant in patients with a BMI <24 kg/m^2, the strong correlation with traditional clinical assessments shown in our series of patients with low or normal BMI is an important result.
Significant areas under the ROC curves for the subpopulations compared, distinct DTs with high overall sensitivity and specificity and high predictive values suggest that US is a reliable method for diagnosis of LA.
Additionally, compared with the HOPS-GS, the US-GS shows a high percentage of correctly classified cases of LA with significant odds ratios.

Looking at the ROC curves, the results of US-GS appear to be very high.
Because the ROC analysis is a binary categorization of the patients and does not consider the misclassifications between the individual US-GS and the HOPS-GS, when individual stages of LA severity were taken into account, US-GS misclassified 33% of the patients.
As expected, most of these misclassifications (33 of the 38 misclassified subjects, the 28.6% of 115 enrolled patients) occurred in patients with SFT assessments that were too low (<1.5 mm from the DTs) to be detectable by the human eye, confirming US-based assessments are more accurate than clinical assessments in the early diagnosis of LA.

Differences in SFT diagnostic thresholds for LA diagnosis between men and women, particularly in the mid thigh, agree with previous reports describing differences in the presentation of HARS (38) between male and female HIV patients, and describing evidence of sex-related differences in body fat distribution in the general population (39). However, large differences between DTs for males and females in the mid thigh suggest that a more distal measurement (less than 15 cm from the rotula) may be a more useful RP which would allow more similar DTs in females and males. This has also been observed in previous studies (26,27).
One limitation of this study is the absence of longer follow-up, necessary to confirm US-based LA diagnosis by observing clear progression of the disease. Although a high statistical power was obtained in the selection of our population, a larger number of patients must be evaluated because well defined and validated US-GS could be widely used in practise.
In summary, we feel that the introduction of US-

based SFT measurements within the established three months-based outpatient management of HIV patients when HAART is started is a valid guide to identify early BFCs and in future to use a validated US GS for its severity.
In effect, serial assessments of SFT at our and other RPs of lipodystrophic findings (28), more than other less objective methods could be useful not only to prevent LA in naïve HIV patients, sparing the unfavorable costs of their treatments (40), but could also monitor the progression to advanced stages of LA in patients receiving HAART and/or provide assessments of SFT recovery in response to rescue therapies.

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