Title: Impact of Lipodystrophy on the Prevalence and Components of Metabolic Syndrome in HIV-infected patients

Running title: HIV-Lipodystrophy and Metabolic Syndrome

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Abstract

In HIV-infected patients combination antiretroviral therapy (cART) is associated with clinical lipodystrophy (CL) and metabolic abnormalities (MA). Lipodystrophy per se is associated with MA similar to metabolic syndrome (MS). The impact of HIV-CL on the MS is not clear. **Aims:** to evaluate the prevalence of MS and its 5 components, and to determine if the presence of CL was associated with an increased prevalence of MA in HIV-infected patients on cART. **Methods:** We evaluated 345 HIV-patients using two different MS definitions (NCEP-ATPIII-2005 and IDF-2005). **Results:** CL was present in 58.7% of the patients. The prevalence of MS was 52.2% (ATPIII) and 43.2% (IDF), and not significantly different between patients with (W) or without (WT) CL, regardless of the used definition (ATPIII WCL 52.9% vs WT CL 51.1%; p=0.738; IDF WCL 41.3% vs WTCL 46.0%; p=0.379). Moderate concordance was observed between the 2 definitions (kappa = 0.484; p<0.001) and after gender stratification a good one in women (kappa= 0.759; p< 0.001). According to the presence of CL, no differences were found in systolic and diastolic BP, glucose and total cholesterol levels. However, patients WCL had lower waist circumference, HDL-C and higher triglycerides levels. A significantly higher prevalence of hypertriglyceridemia, low HDL-C levels, and lower prevalence of abdominal obesity were observed in patients WCL. **Conclusions:** The prevalence of MS was high in these HIV-infected patients. Although lipodystrophy had no impact on the prevalence of MS, it was associated with an increased prevalence of hypertriglyceridemia and low HDL-cholesterol levels, both important cardiometabolic risk factors.

**Key Words:** Metabolic syndrome, Lipodystrophy, HIV infection
Introduction

Combination antiretroviral therapy (cART) has changed the course of HIV infection, leading to a significant reduction in AIDS related morbidity and mortality [1]. However, cART is known to be associated with changes in fat distribution (lipodystrophy) and metabolic abnormalities, including insulin resistance, dyslipidemia, and increased blood pressure (BP) [2, 3].

The metabolic syndrome (MS), a common condition in the general population, refers to a constellation of cardiovascular disease (CVD) risk factors, including increased waist circumference (WC), disorders in glucose and lipid metabolism, and hypertension [4, 5]. Each of these risk factors individually increases cardiovascular risk, but the MS itself has been shown to be a powerful independent risk factor for cardiovascular morbidity and diabetes [6]. Still, it remains controversial if MS adds further in the identification of high risk individuals [7-9]. HIV-infected lipodystrophic patients share several components of the MS. The prevalence of the MS in HIV-infected patients has been reported to range from 4.4 to 45.4% [9-23].

HIV-lipodystrophy is considered to be an adverse effect of cART, not limited to a specific drug or class of drugs [24]. It seems to affects up to half or even more HIV-infected patients receiving cART [25]. However, few studies address the prevalence of MS in patients with lipodystrophy [12, 14, 17].

The aim of this study was to evaluate the prevalence of MS and each of its 5 components in HIV-infected adult patients, with or without lipodystrophy under cART, and to determine if the presence of lipodystrophy was associated with an increased prevalence of metabolic alterations.
Methods

Subjects

As part of a cross-sectional study, 345 non-institutionalized, HIV-infected Caucasian adults (239 men), on cART referred from the Infectious Diseases to the Endocrinology Out-patient Clinic of Hospital São João due to lipodystrophy or any metabolic disorders were included. Only patients on cART were included, since lipodystrophy is mainly related to ART drugs. The study protocol was approved by the Hospital Ethical Committee and all patients provided informed consent.

Clinical assessment:

For each patient the following information was collected, using a standardized protocol: demographic data (age, gender), length of HIV infection, history of diabetes, hypertension, use of antidiabetic, antihypertensive or lipid-lowering drugs and length of cART. Weight, height, WC and resting BP were measured as previously described [26]. Body weight was measured using TANITA (Tanita®, model TBF 300) scale and height was measured to the nearest centimetre in the standing position using a wall stadiometer (Holtain Limited Crymych, Dyfed®).

Clinical lipodystrophy (CL) and central fat accumulation or abdominal prominence, were defined as previously described [27]. All clinical assessments were performed by the same practitioner (PF). A venous blood sample was drawn after a 12-hour overnight fast. All the samples were analyzed at the central laboratory of our hospital. Plasma glucose, total cholesterol, HDL cholesterol (HDL-C) and triglycerides were determined using automatic standard routine enzymatic methods.

The CD4+ cell count was determined by flow cytometry and plasma RNA-VIH was measured by a quantitative reverse transcriptase polymerase chain reaction, which had a lower limit of detection of 50 copies/mL.
Metabolic Syndrome definitions

Two different MS definitions were used: the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATPIII) modified by American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement (AHA/NHLBI) in 2005 [28] and the International Diabetes Federation (IDF) definition [29].

Statistical analysis

Data were presented as mean and standard deviation (SD) for quantitative variables and compared using the Student-t test or the Mann-Whitney test as appropriate. Categorical variables were described as counts and proportions, and compared using the chi-square or Fisher’s exact test. Kappa coefficients were estimated to analyze the statistical agreement between ATP III as modified by the AHA/NHLBI and IDF definitions of MS.

Statistical analysis was performed using the SPSS version 17.0 software (SPSS Inc., Chicago, Illinois, USA). All probabilities were two tailed and p values <0.05 were regarded as significant.

Results

Of the 345 HIV-1 infected patients on cART included in this study, 58.7 % presented CL. Table 1 shows the characteristics of the study sample according to the presence of CL. Patients with CL were more frequently male, older, had longer length of infection, longer length of cART and lower weight, BMI and WC. Also, this group of patients had significantly higher CD4+ cell counts and a significantly higher prevalence of virological suppression. No significant differences in HIV risk factors or in cART
regimen composition were found between patients with or without CL. In the total of the patients the prevalence of hepatitis C co-infection was 28.8%, without significantly differences between patients with or without CL.

Regarding BMI distribution, when compared to those without CL, men and women with CL, were more frequently classified as normal or underweighted (Table 2). According to the presence of CL, no differences were found in systolic and diastolic BP, glucose and total cholesterol levels. However, patients with CL had lower WC, lower HDL-C and higher triglycerides levels (Table 1).

**Metabolic syndrome**

In our sample, the prevalence of MS was 52.2% and 43.2%, according to ATPIII and IDF criteria, respectively. No significant differences in the prevalence of MS were observed in patients with or without CL, regardless of the used definition (table 3). Moderate concordance was observed between the 2 definitions (kappa = 0.484; p<0.001). After gender stratification, a moderate agreement was observed in men (kappa= 0.374; p< 0.001), and a good one in women (kappa= 0.759; p< 0.001). Using both SM criteria, no significant differences in the prevalence of CL were found between patients with and without MS [{with MS 60.6% (109 patients) vs without MS 58.8 % (97 patients); p= 0.822 for ATPIII} and {with MS 57.0% (85 patients) vs without MS 61.7 % (121 patients); p= 0.442 for IDF}].

No significant differences in MS prevalence were found between patients with and without CL (Table 1). Also, no significant difference according to gender and presence of CL was observed regarding the prevalence of MS (Table 3). However, men and women with lipodystrophy had significantly different prevalences of MS when stratified by BMI classes, regardless the criteria used to define MS (Table 4). Men and women
with CL presented a significantly higher prevalence of MS in the normal or underweight class (Table 2). A significantly higher prevalence of hypertriglyceridemia, low HDL-C levels and lower prevalence of abdominal obesity were observed in patients with CL. No differences were found in the number of MS features according to ATPIII and IDF criteria in patients with and without CL (Table 1).

Regarding the aggregate of MS criteria using the ATP definition, the most frequent aggregate number was 2, except for men without CL. On the other hand, by the IDF definition, the most frequent aggregate number was 3, except for women without CL (table 5 and 6), no significant increase of MS prevalence with age was observed, in patients with or without CL (data not shown).

Concerning the frequency of the individual ATPIII criteria for MS, we found a significant lower prevalence of central obesity in men with CL, no other significant differences being observed. No significant differences were found in the frequency of the five IDF criteria for MS in men (table 5).

In women with CL, using either ATPIII or IDF criteria, we found a statistically higher frequency of hypertriglyceridemia and low HDL-C levels. According to ATPIII criteria, women with CL had a lower frequency of central obesity (Table 6).

No significant differences were found between the cumulative number MS features according to the presence of CL in both genders (Table 5 and 6).

**Discussion**

Some aspects of the present study need to be highlighted. To our knowledge, this is the first study conducted in HIV-infected patients focusing specifically on the role of CL on the prevalence of MS. Another particularity is that all patients were on cART. Other studies analysed the presence of MS in naïve or on cART HIV-infected patients, with sub-analyses concerning the presence of CL in MS patients [12, 14, 17].
cART can induce a large spectrum of metabolic disturbances. On the other hand, lipodystrophy *per se*, including genetic lipodystrophy syndromes, is associated with lipid and metabolic disturbances, having many similarities with the MS [2, 30-34]. The effect of cART on the molecular processes of metabolic disturbances has been extensively studied [35] and its contribution to an increased risk of premature and accelerated atherosclerosis in HIV infection is well recognized [36, 37]. Our aim was to determine if lipodystrophy is associated with a different metabolic pattern, namely MS. HIV-lipodystrophy patients may constitute a subset of patients in whom diabetes and cardiovascular risk may not be accurately predicted by the WC cut-offs, because these may not mirror the extent of lipid disturbances and insulin resistance [17]. On the other hand, it is unknown if MS is more frequent in the lipodystrophy patients [9, 17]. We found a high prevalence of MS compared with other studies [9-23]. This finding may be related to a selection bias in the referral to the Endocrinology clinic or reflect the fact that all patients were on cART. Since our main goal was to evaluate the influence of lipodystrophy in MS features, only patients on cART were included. And, it is not our aim to determine the prevalence of MS in the Portuguese HIV population. The low MS prevalence observed in Jericó study probably results of the high proportion of intravenous drug users, which concurred also to the low prevalence of abdominal obesity founded. In our study, the prevalence of intravenous drug users was 24.6 % in patients without CL and 30.6 % in patients with CL (not significantly different). Studies in HIV-infected individuals have revealed different prevalences of MS: similar, higher or lower than the control group [9-23]. It also seems to be higher than the 22 to 24% prevalence rate reported for the general US population [38]. Other data suggest that the increased prevalence of MS among HIV-infected persons may be more
reflective of the burgeoning epidemic of obesity than a predominant effect of cART [39].

This prevalence of MS in HIV-infected patients under cART seems to be higher than the prevalence observed in the general Portuguese population. In a study of Santos et al, the crude prevalence of MS in a sample of 1436 adults living in our Hospital reference area was lower than in our study: 23.9% (27% in female and 19.1% in male) and 14.5% after age-adjustment using the European standard population. In the general population, the prevalence of MS is highly age-dependent in both genders, regardless of the definition used, and its frequency peaks in the sixth decade declining thereafter [26].

The vast majority of the studies published used NCEP III criteria for estimating the prevalence of MS, and only two considered both criteria. In our study the concordance between the two definitions was similar (kappa=0.484) to that observed in the multicentric study of Samaras [17] (kappa=0.46). The stratification by gender increased the concordance in our sample for women (kappa=0.759) but not for men, which may reflect the mean of WC [90.9 (13.3) cm] in women, which is higher than the cut-off of both MS definitions, being this not true for men [(92.5(11.4) cm)].

In our sample, the prevalence of MS was lower using the IDF definition compared to the AHA/NHLB criteria either in the total of the sample or in men with or without CL. In women (total of the sample and with or without CL) was higher when we used the IDF definition. This probably mirrors the IDF definition: central obesity is compulsory criteria, women had higher mean of WC and the cut-off for this feature was lower. Patients with CL had lower WC, and as proposed by Capeau J, the cut-off used in HIV-infected patients, who despite a lower WC mean, had increased visceral fat [33], cannot be equal to that considered for the general population. The decrease in WC resulting of less subcutaneous fat is “protective” of MS tag maintaining the cardiometabolic risk.
No differences in the prevalence of ATPIII or IDF defined MS between patients with and without CL, were observed. So, we were not able to demonstrate that lipodystrophy is associated with a different metabolic pattern. Estrada showed a non-significantly higher prevalence of MS in patients with lipodystrophy compared to those without lipodystrophy (18.2% vs 10.6%) [14].

Analyzing the data the other way round, Samaras showed that the prevalence of CL was 57.2% in the total of patients, and 73% and 79% in those with MS defined by respectively IDF and ATPIII criteria [17]. In patients without MS, CL was less frequent. In subjects with MS, according to IDF criteria, the presence of CL was not associated with worse metabolic features. In contrast, those with lipodystrophy but without MS had a significantly worse metabolic profile (higher total cholesterol, triglycerides and glucose levels, insulin resistance, and lower HDL-C levels), similar for IDF and ATP-III criteria, suggesting that CL has metabolic implications equivalent to MS. In our patients, we didn’t find significantly differences in the prevalence of lipodystrophy in patients with and without MS, according to both criteria. In the Jericó study, as in Samaras, lipodystrophy was more common among MS participants (50.4 vs 33.8; p=0.0001) [12]. However in our study, we didn’t observe that MS was more frequent in patients with CL (Table 1), although Squillace, found that CL was strongly associated with MS [22].

In Jericó study, the prevalence of MS in HIV-infected patients significantly increased with age, 5.1% in patients younger than 30 years of age and 27% in those aged 50-59 years [12]. A similar pattern was reported by Mangili [10]. In our sample, we didn’t observe any difference in the distribution of MS prevalence according to age. This may reflect the homogeneity of age strata in our sample, when compared to other studies (only 12% of our sample had 60 years or more of age).
Regarding gender differences, similarly to our study, Mangili reported that significantly more women than men had MS [10].

Concerning the number of MS components, in our study and according to IDF definition we had 25% of patients with two features of MS, lower when compared to other studies (table 1). In Samara study, 49% of patients had at least two features of MS, namely elevated lipid levels, but were not classified as having MS as their WC were below the cut-off definitions [17]. In Jericó study, the MS prevalence was 17%, with 69.3% of patients showing one or more features of MS, 35.8 % two or more, 4.5 % three or more and 0.1% five features [12].

In what concerns the prevalence of the individual features of MS, we found that the two least prevalent features were high fasting glucose level and high WC, the latter being the least prevalent in the patients with CL. The most frequent features were high triglyceride and low HDL-C levels, followed by high BP. According to the presence of CL, no differences were found in systolic and diastolic BP, glucose and total cholesterol mean levels. However, patients with CL had lower WC and HDL-C and higher triglyceride levels. Bonfatti and Sobieszczyk, also observed that glucose and BP abnormalities were the least common components [18, 21]. Lipid abnormalities were also the most prevalent components in other studies [9, 10, 12, 13, 16, 17].

In contrast to our results, Sattler found that hypertension is more frequent in HIV lipodystrophic in HIV-infected patients on cART [40].

Obesity is an important risk factor for MS in the general population and in HIV-infected persons [39]. Many HIV-infected patients maintain or gain weight as they survive longer with improved treatment regimens. In Jacobson’s cohort, more than 35% of the patients were overweight, 18% being obese, and less than 2% had a BMI lower than 18.5 kg/m² [13]. We found that 32.6% of men and 33.3% of women were overweight.
and that 10.4% of men and 20% of women were obese. In addition, we observed a higher prevalence of overweight and obesity among the patients without CL (Table 4, 5). Although not proving causation, because of the cross-sectional nature of the study design, we can hypothesize that the high frequency of overweight and obesity (that are related to MS) in patients without CL may contribute to the observed absence of difference in the MS prevalence between patients with or without CL.

Worm described that MS patients at the enrolment were more likely to have received the NRTI and protease inhibitor classes of drugs and had higher CD4 counts and lower HIV-RNA levels than those without the MS [9]. We observed that patients with CL had higher CD4 cell count and higher viral suppression rate.

Nowadays, HIV-infected patients have a growing lifespan as a result of successful treatment. The cumulative effects of infection, its treatment and ageing may play a role in the occurrence of metabolic abnormalities. Certainly, life-style risk factors for CVD and diabetes, such as poor dietary habits, low level of physical activity, and smoking are also common in HIV-infected adults [41].

There is currently a debate regarding the identity of MS, its pathophysiology, pedagogic utility and prognostic capacity. Regarding the identity there is substantial confusion between the clinical definition and respective cut-off values proposed by various organizations (NCEP-ATPIII, IDF, WHO, AACE, etc). Some studies reported outcomes for patients without type 2 diabetes. Even in the absence of type 2 diabetes, MS is associated with an increased risk of CV mortality, myocardial infarction and stroke. Also, the pro-inflammatory state seems to be an important underlying feature of MS. There is a lot of controversy if the prognostic significance of the metabolic syndrome exceeds the risk associated with the sum of its individual components. MS differentiates itself from short-term risk calculators in that it does not include age, and
can therefore indicate high risk at any age. Whatever the uncertainties of definition and aetiology, metabolic syndrome is associated with a 2-fold increase in cardiovascular outcomes and a 1.5-fold increase in all-cause mortality. The concept of MS was introduced mainly to emphasize the need for these patients to reshape their lifestyle, to have better nutritional habits and to be physically more active in order to lose weight (especially visceral fat)[42].

Our study had some limitations. Although we included all the patients referred from the Infectious Disease Department to our Department we can’t exclude any bias in the referral, since some patients could have been referred because they had some degree of glucose intolerance or dyslipidemia. We didn’t exclude patients with previously diagnosed diabetes or hypertension to HIV infection. We neither can discard the influence of the pre-HIV body composition, the cumulative exposure time of each drug and the nadir value of CD4 factors that could contribute to the risk lipoatrophy or abdominal prominence that were not evaluated. It is difficult to compare results on the prevalence of MS because of the heterogeneity in populations regarding past and actual cART regimens and treatment adherence. In what concerns CD4 count we only presented the values in the moment of cross sectional evaluation, because we don’t have the nadir value.

Our data showed that among HIV patients on cART, the prevalence of MS can be remarkably high, particularly considering the mean age of our sample. This finding could explain why HIV patients may have an increased risk for CVD [1]. It is also very important to consider other factors that dynamically influence the prevalence of MS, including national trends in obesity, nutrition, and physical activity, and genetic/ethnic susceptibility to the syndrome. Our results were not representative of the general HIV population in Portugal.
Conclusions

The prevalence of metabolic syndrome in this HIV patient population was high. No significant differences were found in the prevalence of MS between patients with or without clinical lipodystrophy. In women there was a high concordance between IDF and ATPIII metabolic syndrome definitions. The fact that the prevalence of increased waist circumference was found to be low in men with clinical lipodystrophy could explain the lower concordance between the two metabolic syndrome definitions. In this patient population, lipodystrophy was associated with an increased prevalence of hypertriglyceridemia and low HDL-cholesterol levels, both important cardiometabolic risk factors.

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Transparency declarations

None to declare
References


Additional files provided with this submission:

Additional file 1: Tabelas SM 1-4.doc, 93K
http://www.biomedcentral.com/imedia/2334666875226733/supp1.doc
Additional file 2: Tabelas SM 5-6.doc, 53K
http://www.biomedcentral.com/imedia/7830736745226733/supp2.doc