Enferm Infecc Microbiol Clin. 2021;xxx(xx):xxx–xxx

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Original article

Synergic effect of metabolic syndrome and lipodystrophy on oxidative stress and inflammation process in treated HIV-patients

Carmen María González-Domenech[a,*], Isaac J. Plaza-Andrades[b], Lourdes Garrido-Sanchez[b,*], María Isabel Queipo-Ortuño[c]

[a] Microbiology Department, Sciences College, University of Málaga, Málaga, Spain
[b] Unidad de Gestión Clínica de Endocrinología y Nutrición del Hospital Virgen de la Victoria, Instituto de Investigación Biomédica de Málaga (IBIMA), UMA, Málaga, Spain
[c] Unidad de Gestión Clínica Intercentros de Oncología Médica, Hospitales Universitarios Regional y Virgen de la Victoria, Instituto de Investigación Biomédica de Málaga (IBIMA)-CIMES-UMA, Málaga, Spain

ARTICLE INFO

Article history:
Received 4 September 2020
Accepted 23 November 2020
Available online xxx

Keywords:
Metabolic syndrome
Lipodystrophy
HIV
Protease inhibitors
Inflammation

ABSTRACT

The aim of this study was to assess the effect of lipodystrophy (LD) associated to metabolic syndrome (MS) on oxidative stress and inflammation in a cohort of 243 HIV-infected patients with MS, all of them under three different antiretroviral regimens. We collected immunovirological, biochemical and metabolic data, as well as anthropometric measurements. In addition, cardiovascular risk was also assessed by means of Atherogenic Index of Plasma (API) and Framingham Risk Score. The MS-LD patient set was characterized by a lower initial lymphocyte CD4 count and CD4/CD8 ratio and a higher initial viral load than the group without LD. We also found worse lipidic and glycaemic profiles (with lower HDL-cholesterol and higher triglyceride and glucose levels) in the MS-LD group. BMI, systolic blood pressure and Framingham score were significantly increased compared to MS-Non LD. In addition, patients with MS and LD had significantly higher levels of carbonylated proteins, lipid peroxidation, IL-6 and IL-8, as well as a significant decrease in the levels of leptin, adiponectin and antioxidant activities of catalase, super oxide dismutase and glutathione associated enzymes. In MS-LD HIV-1 patients, a significant negative correlation was found between Framingham Risk Score and the antioxidant biomarkers, however a positive association was found between API and protein-C reactive and carbonylated proteins. Segregating by ART, the above-mentioned conditions were worse within the MS-LD group whose treatment contained protease inhibitors, such as lopinavir. In conclusion, HIV-1 infected patients treated for at least six months, especially with regimens including PIs, showed a worsening of inflammatory process and oxidative stress.

Efecto sinérgico del síndrome metabólico y la lipodistrofia en el estrés oxidativo y el proceso de inflamación en pacientes con VIH que reciben tratamiento

Palabras clave:
Síndrome metabólico
Lipodistrofia
VIH
Inhibidores de la proteasa
Inflamación

RESUMEN

El objetivo de este estudio fue evaluar el efecto de la lipodistrofia (LD) asociada al síndrome metabólico (SM) en el estrés oxidativo y la inflamación en una cohorte de 243 pacientes con VIH y SM, todos en tratamiento con pautas antirretrovirales diferentes. Recopilamos datos inmunovirológicos, bioquímicos y metabólicos, así como medidas antropométricas. Además, el riesgo cardiovascular también se evaluó mediante el índice de plasma aterogénico (API) y la puntuación de riesgo de Framingham. El grupo de pacientes con SM-LD se caracterizó por un recuento inicial de linfocitos CD4 y una relación CD4/CD8 inferiores y una carga vírica inicial más alta que el grupo sin LD. También observamos peores perfiles lipídicos y glucémicos (con menor colesterol HDL y niveles más altos de triglicéridos y glucosa) en el grupo de SM-LD. El IMC, la presión arterial sistólica y la puntuación de Framingham aumentaron significativamente en comparación con el grupo de SM-sin LD. Además, los pacientes con SM y LD tenían niveles

* Corresponding authors.
E-mail addresses: cmgodo@uma.es (C.M. González-Domenech), lourgarrido@gmail.com (L. Garrido-Sanchez).

https://doi.org/10.1016/j.eimc.2020.11.019

significativamente más altos de proteínas carboniladas, peroxidación lipídica, IL-6 e IL-8, así como una disminución significativa de los niveles de leptina, adiponectina y actividades antioxidantes de la catalasa, superóxido dismutasa y enzimas asociadas al glutatión. En los pacientes con SM-LD VIH-1, se observó una correlación negativa significativa entre la puntuación de riesgo de Framingham y los biomarcadores antioxidantes, sin embargo, se observó una asociación positiva entre el API y la proteína C reactiva y las proteínas carboniladas. Al segregarse por ART, las condiciones mencionadas anteriormente fueron peores en el grupo de SM-LD, cuyo tratamiento incluía inhibidores de la proteasa, como el lopinavir. En conclusión, los pacientes con VIH-1 tratados durante al menos seis meses, especialmente con pautas que incluían IP, mostraron un empeoramiento del proceso inflamatorio y el estrés oxidativo.

Introduction

Combined antiretroviral therapy (ART) for those patients infected with HIV has positively impacted on HIV-infected adults' mortality, with a substantial decline since the mid-1990s.[1,2] Moreover, the combination of different antiretroviral agents has represented a significant improvement of life expectancy and quality. However, the rise in longevity subsequently involves a greater likelihood of developing aging-associated and non-infectious diseases similar to the general population, such as cardio-metabolic disorders.[3–6] When these metabolic conditions cluster and manifest together, they are defined as a metabolic syndrome (MS), which includes abdominal obesity, insulin resistance, glucose intolerance, dyslipidaemia and hypertension. The importance of MS lies in the power to predict future cardiovascular diseases, such as heart disease and stroke, as well as type 2 diabetes mellitus (T2DM).[7]

The viral infection itself, through chronic inflammation and immune dysfunction, and, especially, the use of ART is associated with MS and body fat redistribution or lipodystrophy (LD).[1,6,8–13] Moreover, certain ART regimens, particularly those based on protease inhibitors (PIs), were found to increase MS prevalence.[6,14,15] However, establishing a precise figure of prevalence of MS in the HIV-infected population is not possible due to the considerable heterogeneity in the diagnostic criteria for MS.[6]

LD and MS are often associated in HIV-infected patients under antiretroviral treatment, but the former does not seem to be a predisposing factor for the latter.[16,17] However, LD should be defined as a syndrome characterized not only by body shape changes with generalized or partial absence of adipose tissue, but also by other metabolic issues, some of them common to those in MS, such as a significantly higher prevalence of insulin resistance (and hypertriglyceridemia) and hyperlipidaemia with low HDL-C levels.[17–20]

In HIV-1 cohorts, metabolic and morphological changes are proved to be subordinate to hormonal and adipokine levels; hypoadiponectinemia appears to be associated with insulin resistance and lipid disorders.[21,22] In addition, leptin plasma values seem to depend on LD pattern; leptin deficiency is clearly correlated with lipoatrophy while higher leptin levels are linked to mixed forms of lipodystrophy besides central lipoaccumulation.[21,23] Regarding the inflammatory markers, IL-6 and C-reactive protein levels are significantly higher in patients with MS.[22]

In this study, we analyze the role of ART over the immune system activation and influence on oxidative stress when HIV-1 infected patients are suffering MS and LD simultaneously.

Methods

Study population

The study was undertaken at the Regional Hospital Carlos Haya, Malaga, in southern Spain, from January of 2011 to December of 2012. The cohort comprised 243 HIV-infected patients diagnosed with MS, and on stable (uninterrupted) and first-line ART, treated for at least six months (a mean of 28.6 months; IQR: 6–78 months). Patients having MS fulfilled three or more of the following factors, according to the International Diabetes Federation (IDF) criteria: triglycerides (TG) > 150 mg/dL, HDL-cholesterol < 40 mg/dL in men and <50 mg/dL in women, systolic blood pressure (SBP) > 130 mmHg and/or diastolic blood pressure (DBP) > 85 mmHg, fasting glucose ≥ 110 mg/dL, and obesity as defined by a waist circumference > 94 cm in men and >80 cm in women.[24]

Antiretroviral regimen of our patients was composed by one of the following combinations: (a) two nucleoside reverse transcriptase inhibitors (NRTIs) plus a boosted PI: Tenofovir/emtricitabine + lopinavir/ritonavir; and two different ART strategies consisting of two NRTIs plus non-nucleoside reverse transcriptase inhibitors (NNRTIs): (b) Tenofovir/emtricitabina + efavirenz, and (c) Abacavir/lamivudina + efavirenz.

We segregated the patients within the cohort according to the presence or not of lipodystrophy, which was evaluated by clinical examination. We did not make any difference among LD patterns (lipoatrophy, lipohypertrophy or mixed forms), because of the lack of significant differences between them.

As exclusion criteria, we considered different conditions with impact on anthropometric or biochemical measures: Clinical signs and symptoms of acute inflammation or other concomitant infections 6 months prior to inclusion in the study, as well as the presence of AIDS events within the three months prior to inclusion; lactic acidosis induced by antiretroviral drugs; obese patients (BMI > 30 kg/m^2), organic disease (*i.e.*, renal or respiratory failures), which altered the analysis of the parameters of oxidative stress and proinflammatory cytokines; and active drugs addiction. In addition, use of non-steroidal anti-inflammatory drugs (NSAIDs) and/or statins were also considered as exclusion criterium. Patients who stopped ART or switched from PI to NNRTI or *vice versa* during the study period were also excluded.

This study was performed in line with the principles of the Declaration of Helsinki. In addition, approval was obtained by the Ethics and Research Committee of the Regional University Hospital Carlos Haya, Malaga, Spain. All the patients included signed an Informed Consent, containing explicit agreement to use the clinical, anthropometrical, and laboratory data under confidentiality and being anonymized, as performed here.

Laboratory measurements

Blood samples from all subjects were collected after a 12-hour fast. Serum was separated and immediately frozen at –80 °C. Biochemical parameters of duplicate serum samples were measured by standard enzymatic methods (Randox Laboratories Ltd.,

Please cite this article in press as: González-Domenech CM, et al. Synergic effect of metabolic syndrome and lipodystrophy on oxidative stress and inflammation process in treated HIV-patients. Enferm Infecc Microbiol Clin. 2021. https://doi.org/10.1016/j.eimc.2020.11.019

Table 1
Anthropometric, biochemical and metabolic variables, as well as cardiovascular risk factors in the 243 HIV-infected patients with metabolic syndrome and comparison of the groups with and without lipodystrophy.

Variables	Cohort segregation		*p*-Value
	MS-LD	MS-non LD	
Number of patients	119 (49.0)	124 (51.0)	–
Male gender	47 (39.5)	56 (45.16)	NS
Age (years)	43.5 ± 7.1	43.4 ± 10.9	NS
Smokers (n = 80)	42 (35.0)	38 (31.0)	
DBP (mmHg)	87.1 ± 10.7	85.1 ± 8.1	NS
SBP (mmHg)	141.0 ± 8.1	131.1 ± 9.3	<0.001
Treatment duration (months)	31.9 ± 10.8	29.8 ± 8.9	NS
Inmunovirological status			
Nadir lymphocyte CD4 count (cells/μL)	294.2 ± 50.1	291.6 ± 54.4	NS
Initial lymphocyte CD4 count	530 ± 56	589 ± 53	<0.001
Initial viral load (copies/mL)	291.1 ± 39.7	249.6 ± 44.2	<0.001
CD4/CD8 ratio	0.5 ± 0.2	0.6 ± 0.3	<0.001
Anthropometric measurements			
BMI (kg/m^2)	28.5 ± 7.6	24.3 ± 6.1	<0.001
Hip (cm)	99.2 ± 11.6	94.5 ± 6.4	0.016
Waist (cm)	92.3 ± 8.7	84.1 ± 5.8	<0.001
Waist-hip ratio	1.1 ± 0.06	1.1 ± 0.05	NS
Weight (kg)	78.5 ± 9.4	65.1 ± 8.8	<0.001
Biochemical and metabolic variables			
Creatinine (mg/dL)	0.9 ± 0.2	1.1 ± 0.8	NS
Uric acid (mg/dL)	4.7 ± 1.1	4.5 ± 1.5	NS
CPR (mg/dL)	2.9 ± 2.1	2.1 ± 2.8	NS
HDL-cholesterol (mg/dL)	42.1 ± 13.2	47.5 ± 11.2	0.034
LDL-cholesterol (mg/dL)	137.4 ± 41.6	134.1 ± 30.5	NS
Total cholesterol (mg/dL)	207.4 ± 28.9	204.0 ± 27.7	NS
Triglycerides (mg/dL)	180.9 ± 16.7	157.5 ± 12.5	<0.001
Glucose (mg/dL)	126.3 ± 11.8	108.7 ± 12.2	<0.001
Cardiovascular risk assessments			
Atherogenic Index of Plasma	4.4 ± 0.8	4.3 ± 1.1	NS
Framinghan Risk Score (%)	6.1 ± 5.1	3.5 ± 4.8	0.014

The quantitative variables are expressed as mean ± SD, and the qualitative variables in *n* (%).
Abbreviations: BMI: body mass index; CPR: C-reactive protein; DBP: diastolic blood pressure; HIV: human immunodeficiency virus; HOMA-IR: homeostasis model assessment of insulin resistance; LD: lipodystrophy; MS: metabolic syndrome; NS: non-significant; SBP: systolic blood pressure.

Antrim, UK). Low-density lipoprotein cholesterol (LDL) was calculated by the Friedewald formula. Meanwhile, leptin, C-reactive protein (CPR), adiponectin, IL-6 and IL-8 were analyzed by enzyme immunoassay kits (DSL, Webster, TX, and DRG Diagnostics, respectively). We also used commercial kits (Cayman Chemical, Ann Arbor, MI) to determine in plasma total antioxidant capacity (TAC), and the activities of glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), glutathione S-transferase (GSH-Tf), superoxide dismutase (SOD), carbonylated proteins, lipoperoxides (LPO) and catalase (CAT).

Atherogenic index of plasma (AIP)

AIP predicts cardiovascular risk, as a reflection of the balance between the atherogenic and protective lipoproteins.[25] AIP is the result of Log (TG/HDL_C), considering AIP < 0.11 as low risk; AIP between 0.11 and 0.21, intermediate risk; and AIP > 0.21 with increased risk.

Framingham risk score

The Framingham Risk Score is a gender-specific algorithm used to estimate the 10-year cardiovascular risk of an individual, identifying men and women at increased risk for future cardiovascular events.[26] Coronary heart disease (CHD) risk at 10 years can be calculated with the help of the Framingham Risk Score; individuals with low risk have 10% or less CHD risk at 10 years, 10–20% for intermediate risk, and high risk when 20% or more.

Statistical analysis

The statistical analysis was performed with SPSS v.11.5 for Windows (Chicago, IL, USA). The comparison of groups with and without LD within the HIV-infected and MS cohort was performed by the non-parametric Mann–Whitney test. In addition, the Kruskal–Wallis test was employed to compare variables among the three different ART regimen-based groups. The Spearman correlation coefficient was calculated to estimate the correlation among variables. Comparative values were considered to be statistically significant when the test result was ≤0.05. The results are given as the mean ± standard deviation (SD).

Results

Anthropometric, biochemical and immunovirological characteristics

The overall cohort, comprising 243 HIV-infected patients with MS was segregated, according to the presence or not of LD, into two groups of 119 and 124 individuals respectively. Anthropometric, biochemical and metabolic characteristics of both groups are shown in Table 1. Within the MS with LD group (hereinafter MS-LD group), lipoatrophy was the most frequent pattern (45%), followed by a mixed pattern (34%) and finally, by lipohypertrophy (21%). The MS-LD patients set was characterized by an initial lymphocyte CD4 count and CD4/CD8 ratio lower, and higher initial viral load than the other group without LD (530 ± 56 *vs.* 589 ± 53 cells/μL, $p < 0.001$; 0.5 *vs.* 0.6, $p < 0.001$; and 291.1 ± 39.7 *vs.* 249.6 ± 44.2 copies/mL,

Please cite this article in press as: González-Domenech CM, et al. Synergic effect of metabolic syndrome and lipodystrophy on oxidative stress and inflammation process in treated HIV-patients. Enferm Infecc Microbiol Clin. 2021. https://doi.org/10.1016/j.eimc.2020.11.019

Table 2
Inflammation and oxidative stress variables in the cohort, segregating by the presence or not of lipodystrophy.

Variables	Cohort segregation		*p*-Value
	MS-LD	MS-Non LD	
Adiponectin (μg/mL)	11.0 ± 3.4	12.9 ± 3.2	0.022
Carbonylated proteins (nmol/mL)	184.7 ± 90.1	61.1 ± 26.6	<0.001
CAT (nmol/min/mL)	55.2 ± 19.6	59.8 ± 16.2	0.034
GSH-Px (nmol/min/mL)	625.8 ± 86.4	822.0 ± 94.2	<0.001
GSH-Rd (nmol/min/mL)	635.3 ± 116.4	687.7 ± 119.4	0.019
GSH-Tr (nmol/min/mL)	3.7 ± 1.9	4.3 ± 2.4	NS
IL-6 (pg/mL)	4.7 ± 2.1	2.7 ± 1.5	<0.001
IL-8 (ng/mL)	1.9 ± 0.8	1.6 ± 0.5	0.026
Leptin (ng/mL)	2.5 ± 1.8	4.6 ± 1.9	<0.001
LPO (μM)	8.4 ± 2.2	6.6 ± 2.4	<0.001
SOD (U/mL)	2.8 ± 0.7	3.3 ± 0.8	0.008
TAC (mM)	1.7 ± 0.2	1.8 ± 0.2	0.031

The quantitative variables are expressed as mean ± SD.
Abbreviations: CAT: catalase activity; GSH-Px: glutathione peroxidase activity; GSH-Rd: glutathione reductase activity; GSH-Tr: glutathione S-transferase activity; LD: lipodystrophy; LPO: lipid peroxidation; MS: metabolic syndrome; SOD: superoxide dismutase activity; TAC: total antioxidant capacity.

Table 3
Correlation between inflammatory and oxidative stress biomarkers with Atherogenic Index of Plasma and Framingham Risk Score, in HIV-1 patients with MS according to the presence or not of lipodystrophy.

HIV-1 patients with metabolic syndrome (*n* = 243) LD (*n* = 119)							
	PCR	CP	CAT	GSH-Px	GSH-Rd	TAC	
Framingham (%)				−0.886¶		−0.620¶	
AIP	0.333¶	0.900¶	−0.821¶		−0.999¶	−0.825¶	
Non-LD (*n* = 124)							
	Adiponectin	IL-6	PCR	CAT	GSH-Px	GSH-Rd	TAC
Framingham (%)		0.284¶	0.438*	−0.264¶			−0.658¶
AIP	0.475¶		0.333¶	−0.414*	−0.869*	−0.742¶	

Abbreviations: CAT: catalase activity; CP: carbonylated proteins; GSH-Px: glutathione peroxidase activity; GSH-Rd: glutathione reductase activity; GSH-Tr: glutathione S-transferase activity; LD: lipodystrophy; PCR: reactive C protein TAC: total antioxidant capacity.
Pearson correlation coefficient:
* $p < 0.001$.
¶ $p < 0.05$.

$p < 0.001$, respectively). We also found in the MS-LD group lower HDL-cholesterol levels whereas waist, hip, weight, BMI, systolic blood pressure, CRP, triglycerides, glucose values and Framingham score was significantly increased compared to the MS-Non-LD (Table 1).

Inflammatory and oxidative stress biomarkers

With regard to the inflammation and oxidative stress variables in the cohort (Table 2), the MS-LD patients showed significantly higher levels of interleukins (IL-6 and IL-8), carbonylated proteins and lipid peroxidation (LPO). On the contrary, adiponectin and leptin values, CAT, SOD, TAC, GSH-Px, GSH-Tr and GSSG-Rx activities were increased in patients with MS-Non-LD.

Correlation between cardiovascular risk and inflammatory and oxidative stress biomarkers

In MS-LD HIV-1 patients a significant negative correlation was found between the Framingham Risk Score and the antioxidant biomarkers GSH-Px and TAC and between AIP and CAT, GSH-Rd and TAC, as well as, a significant positive correlation being also observed between AIP and PCR and CP (Table 3). On the other hand, in MS-Non-LD patients, the correlation of both cardiovascular risk markers was significantly positive with the reactive C protein and negative with CAT. Other positive and negative correlations were shown in MS-Non-LD between the Framingham Risk Score and IL-6 and TAC and between AIP and adiponectin and GSH-Rd and GSH-Px, respectively (Table 3).

Variability associated to antiretroviral therapy

As depicted in Table 4, we found anthropometric, biochemical and immunovirological differences according to the ART followed for each group of HIV-1 patients with MS, as well as presenting or not LD. Within the MS-LD group, those whose treatment contained PIs (Kaletra®) showed the lowest HDL-cholesterol and the highest triglyceride levels, besides a significantly greater cardiovascular risk than the rest of patients from the cohort. In addition, the MS-LD group who were being treated with Truvada® and Kaletra® also presented a worsening of the lipid and glycaemic profiles in comparison to MS-Non-LD patients.

Discussion

In this study, we have analyzed the synergic effect of LD and MS on oxidative stress and the triggering of inflammation in HIV-infected patients treated for at least six months. Moreover, we have also focused on the role of different ARTs regarding the immune system activation, body composition and other biochemical and metabolic variables.

Lipodystrophy is developed in the course of HIV infection under intensive ARTs. Several grave metabolic complications have been described in association with fat redistribution, such as insulin

Please cite this article in press as: González-Domenech CM, et al. Synergic effect of metabolic syndrome and lipodystrophy on oxidative stress and inflammation process in treated HIV-patients. Enferm Infecc Microbiol Clin. 2021. https://doi.org/10.1016/j.eimc.2020.11.019

Table 4
Metabolic effects and cardiovascular risk in HIV-1 patients, with MS presenting or not LD, under different antiretroviral therapies.

Variables	HIV-1 patients with MS (n = 243)					
	LD (n = 119)			Non-LD (n = 124)		
	2 NRTIs + bPI[a]	2 NRTIs + NNRTIs[b]	2 NRTIs + NNRTIs[c]	2 NRTIs + bPI[a]	2 NRTIs + NNRTIs[b]	2 NRTIs + NNRTIs[c]
Number of patients	**40 (33.6)**	**41 (34.4)**	**38 (32.0)**	**39 (31.5)**	**42 (33.9)**	**43 (34.6)**
Male gender	23 (57.5)	24 (58.5)	21 (55.2)	22 (56.4)	23 (54.7)	22 (51.1)
Age (years)	36.0 ± 4.7	41.5 ± 9.5	37.7 ± 10.5	35.2 ± 5.8	40.3 ± 9.8	38.3 ± 3.5
DBP (mmHg)	89.3 ± 13.6*	85.0 ± 7.1	85.5 ± 12.2	84.9 ± 11.6	83.7 ± 11.7	84.5 ± 9.4
SBP (mmHg)	135.4 ± 14.7*	131.7 ± 7.0	129.5 ± 17.9	131.5 ± 10.1	130.3 ± 5.5	129.9 ± 14.5
Treatment duration (months)	30.8 ± 8.2	29.3 ± 7.6	34.6 ± 10.5	31.7 ± 6.7	26.7 ± 5.6	32.8 ± 7.8
Inmunovirological status						
Initial viral load (log copies/mL)	280.4 ± 8.5*	226.3 ± 9.5	250.8 ± 6.3&	250.2 ± 4.3	223.4 ± 5.6	232.9 ± 7.5
Initial lymphocyte CD4 count (cells/μL)	348.5 ± 61.8*	359.6 ± 43.6	489.9 ± 68.8&	435.2 ± 93.4	368.5 ± 38.3	564.7 ± 87.5
CD4/CD8 ratio	0.5 ± 0.1&	0.4 ± 0.2	0.6 ± 0.1*	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2
Anthropometric measurements						
BMI (kg/m^2)	29.2 ± 4.5	29.5.2 ± 3.9	25.6 ± 4.4	23.05 ± 3.2	29.3 ± 7.9	24.5 ± 3.4
Hip (cm)	101.2 ± 9.1*	97.6 ± 7.0	95.8 ± 6.3	88.3 ± 7.8	94.5 ± 8.5	94.0 ± 8.2
Waist (cm)	93.5 ± 4.2*	92.0 ± 12.6	87.9 ± 8.9	77.2 ± 5.6	89.3 ± 10.9	84.7 ± 12.0
Waist-hip ratio	0.9 ± 0.06	1.0 ± 0.2	1.0 ± 0.04	0.8 ± 0.2	0.9 ± 0.3	1.0 ± 0.08
Weight (kg)	79.6 ± 12.0&	79.7 ± 8.8	75.3 ± 10.7&	69.5 ± 10.6	78.02 ± 5.2	68.6 ± 15.7
Biochemical and metabolic variables						
Creatinine (mg/dL)	1.1 ± 0.1*	0.7 ± 0.1	1.0 ± 0.05	0.9 ± 0.08	0.7 ± 0.05	1.0 ± 0.5
Uric acid (mg/dL)	5.2 ± 0.4&	4.2 ± 1.9	5.4 ± 1.6	4.6 ± 0.7	4.1 ± 1.6	5.0 ± 1.0
CPR (mg/dL)	2.9 ± 0.8	2.9 ± 0.3	1.9 ± 0.1&	2.9 ± 0.5	2.9 ± 0.2	1.8 ± 0.2
HDL-cholesterol (mg/dL)	37.2 ± 4.4*	43.0 ± 10.5	41.5 ± 8.5	43.9 ± 6.8	47.3 ± 12.3	44.9 ± 9.2
LDL-cholesterol (mg/dL)	132.9 ± 27.3&	126.1 ± 12.1	143.2 ± 12.5*	114.7 ± 21.9	124.3 ± 11.3	116.8 ± 11.4
Total cholesterol (mg/dL)	198.2 ± 26.8	203.2 ± 11.4	206.7 ± 14.8*	193 ± 22.3	199.6 ± 9.5	189.0 ± 12.8
Triglycerides (mg/dL)	206.2 ± 19.9*	169.4 ± 15.3	188.8 ± 26.4	193.2 ± 16.3	167.6 ± 12.6	176.7 ± 34.6
Glucose (mg/dL)	106.5 ± 11.3&	137.6 ± 6.3&	115.0 ± 11.9&	90.6 ± 9.8	104.2 ± 9.6	103.7 ± 10.6
Cardiovascular risk assessments						
Atherogenic index	5.2 ± 0.8*	3.4 ± 1.0	4.8 ± 1.1	3.2 ± 0.4	3.3 ± 0.9	4.4 ± 1.2
Framinghan Risk Score (%)	7.3 ± 1.4*	3.2 ± 1.3	4.6 ± 2.5	3.5 ± 1.9	3.0 ± 1.2	4.4 ± 2.2

Abbreviations: BMI: body mass index; bPI: boosted protease inhibitors; CPR: C-reactive protein; DBP: diastolic blood pressure; HIV: human immunodeficiency virus; HOMA-IR: homeostasis model assessment of insulin resistance; LD: lipodystrophy; MS: metabolic syndrome; NRTI: nucleoside reverse transcriptase inhibitors; NNRTIs: non-nucleoside reverse transcriptase inhibitors; SBP: systolic blood pressure; TAD: diastolic blood pressure; TAS: systolic blood pressure.
The quantitative variables are expressed as mean ± SD, and the qualitative variables in n (%).

* Statistically significant difference regarding all remaining subgroups.

& Statistically significant difference with regards to the same treatment set in the other subgroup (MS LD/MS non-LD), or within the corresponding subgroup (MS LD/MS non-LD).

[a] Tenofovir/emtricitabine + lopinavir/ritonavir;

[b] Tenofovir/emtricitabina + efavirenz;

[c] Abacavir/lamivudina + efavirenz.

resistance and dyslipidaemia.[17,27] Therefore, we decided to study the contribution of LD on components of MS when both conditions appear simultaneously.

Although drug toxicity has been strongly related to LD, leading us to assess it further, fat distribution can also be influenced by individual susceptibility as well as by the role of the virus itself.[18,19] HIV infection *per se* is typically associated with dyslipidaemia and consequently with a higher risk of cardiovascular disease.[28,29] The initial conditions of our cohort were characterized by low HDL-cholesterol and high triglyceride values, as well as an increased systolic blood pressure and a Framingham score, with sharper levels in the MS-LD group (Table 1), as previously found in similar cohorts.[17,23] Furthermore, the dysregulated levels of inflammation-modulating cytokines that control lipid metabolism are suggested as mechanisms for HIV-induced dyslipidaemia.[22,30,31] In that sense, the inflammation markers measured in our cohort delivered higher levels of IL-6 and IL-8 in the MS-LD group in comparison to MS Non-LD (Table 2). With regard to adipocytokines, we found serum leptin decreased in the MS-LD group, as also seen in other studies.[27] However, the fat redistribution had no significant effect on adiponectin concentrations, as Freitas and col. observed previously.[23] Moreover, these values were similar to those in non-infected HIV controls with MS,[27] and higher than in the HIV-patients with LD,[23,27] considering the studies mentioned.

Regarding oxidative stress-biomarkers (Table 2), high levels of carbonylated proteins and LPO were found in MS-LD patients. Furthermore, the metabolic protective function of glutathione, through GSH-dependent enzymatic activities (GSH-Px, GSH-Tr and GSSG-Rx), were also decreased in the same group of patients. Therefore, the simultaneous presence of LD and MS seems to worsen oxygen radical damage in the HIV population. On the other hand, and as mentioned before, inflammation markers were also increased in MS-LD patients. The results obtained are backed by up-to-date bibliography, showing an interdependence between oxidative stress and inflammation, and that the simultaneous coexistence of both would contribute to many chronic diseases such as diabetic complications, cardiovascular and neurodegenerative diseases.[32–34]

The impossibility to cure HIV infection currently implies the need of long-term ARTs. According to the ART followed by each group, we found anthropometric, biochemical and immunovirological differences (Table 4). As expected, those patients within the MS-LD group whose treatment contained PIs (Kaletra®) showed the lowest HDL-cholesterol and the highest triglyceride levels, as well as a worsening of the glycaemic profile and a significantly greater cardiovascular risk than the rest of patients from the cohort. PIs possess a high genetic barrier to the development of drug resistance of HIV, with key mutations rarely occurring.[35,36] However,

Please cite this article in press as: González-Domenech CM, et al. Synergic effect of metabolic syndrome and lipodystrophy on oxidative stress and inflammation process in treated HIV-patients. Enferm Infecc Microbiol Clin. 2021. https://doi.org/10.1016/j.eimc.2020.11.019

Table 5
Variability of inflammatory and oxidative stress biomarkers in HIV-1 patients with MS presenting or not LD, and under different antiretroviral therapies.

Variables	HIV-1 patients with MS ($n=243$)					
	LD ($n=119$)			Non-LD ($n=124$)		
	2 NRTIs + bPI[a]	2 NRTIs + NNRTIs[b]	2 NRTIs + NNRTIs[c]	2 NRTIs + bPI[a]	2 NRTIs + NNRTIs[b]	2 NRTIs + NNRTIs[c]
Number of patients	**40 (33.6)**	**41 (34.4)**	**38 (32.0)**	**39 (31.5)**	**42 (33.9)**	**43 (34.6)**
Adiponectin (μg/mL)	10.1 ± 2.4*	11.1 ± 4.1	11.6 ± 3.2&	13.8 ± 2.6	11.8 ± 9.0	14.8 ± 4.2
Carbonylated proteins (nmol/mL)	279.7 ± 68.8*	59.7 ± 10.6	57.4 ± 11.8	210.8 ± 73.6	57.7 ± 10.8	56.2 ± 10.1
CAT (nmol/min/mL)	57.8 ± 8.7&	64.6 ± 13.7	57.6 ± 10.2	67.7 ± 13.4	65.5 ± 14.3	55.3 ± 12.4
GSH-Px (nmol/min/mL)	551.6 ± 51.0*	621.9 ± 116.8	595.9 ± 82.8	610.2 ± 88.3	632.9 ± 114.2	616.8 ± 80.1
GSH-Rd (nmol/min/mL)	513.8 ± 45.3*	527.9 ± 59.3	534.1 ± 20.8&	574.7 ± 68.4	520.9 ± 68.1	588.0 ± 26.5
GSH-Tr (nmol/min/mL)	2.5 ± 1.0*	4.3 ± 2.6	3.6 ± 0.7	5.0 ± 1.1	4.2 ± 2.7	3.4 ± 1.2
IL-6 (pg/mL)	5.4 ± 0.1*	1.3 ± 1.1	1.7 ± 1.2	1.3 ± 0.3	1.4 ± 0.8	1.6 ± 0.6
IL-8 (ng/mL)	2.8 ± 0.5*	1.5 ± 0.3	1.8 ± 0.6	1.9 ± 0.7	1.5 ± 0.5	1.6 ± 0.6
Leptin (ng/mL)	1.7 ± 0.01*	2.6 ± 1.3	2.7 ± 1.2	3.2 ± 1.3	2.7 ± 2.2	2.8 ± 1.4
LPO (μM)	8.9 ± 0.9*	6.9 ± 1.2	6.9 ± 1.9	6.8 ± 1.5	6.7 ± 0.6	6.4 ± 1.3
SOD (U/mL)	2.5 ± 0.7&	2.2 ± 0.2	3.0 ± 0.9&	2.9 ± 0.4	2.1 ± 0.5	3.4 ± 0.7
TAC (mM)	1.4 ± 0.2*	1.7 ± 0.1	1.6 ± 0.3&	1.8 ± 0.2	1.7 ± 0.3	1.9 ± 0.2

Abbreviations: CAT: catalase activity; GSH-Px: glutathione peroxidase activity; GSH-Rd: glutathione reductase activity; GSH-Tr: glutathione S-transferase activity; LD: lipodystrophy; LPO: lipid peroxidation; NRTI: nucleoside reverse transcriptase inhibitors; NNRTIs: non-nucleoside reverse transcriptase inhibitors; SOD: superoxide dismutase activity; TAC: total antioxidant capacity.
The quantitative variables are expressed as mean ± SD, and the qualitative variables in *n* (%).
* Statistically significant difference regarding all remaining subgroups.
& Statistically significant difference with regards to the same treatment set in the other subgroup (LD/non-LD), or within the corresponding subgroup (LD/non-LD).
[a] Tenofovir/emtricitabine + lopinavir/ritonavir;
[b] Tenofovir/emtricitabina + efavirenz;
[c] Abacavir/lamivudina + efavirenz.

long-term treatment based on PIs are typically linked to metabolic syndromes, such as dyslipidaemia or insulin-resistance.[35] So, our results are in line with the literature.

Markers of inflammation and oxidative stress in HIV-1 patients with MS presenting LD and following a regimen based on Truvada® and Kaletra® were worse overall than in those out of this set (Table 5). Once more, the association of ARTs containing lopinavir/ritonavir to oxygen radical damage is well documented.[37] This fact does not seem to be related to the therapeutic group in general, since atazanavir and darunavir are well tolerated, even improving some lipid and renal variables.[37–39] Thus, effects on glucose and lipid levels as well as impact on oxidative stress, when observed with these PIs, may mainly be caused by ritonavir-boosting, rather than by themselves. Moreover, it has been reported that treated patients with darunavir/ritonavir when the boosting agent changed to cobicistat, levels of total cholesterol, LDL and triglycerides decreased, whereas levels of HDL increased.[40,41]

On the other hand, when we reviewed literature about the role of other types of ARTs different from PIs and with a most recent use in HIV treatment, we found a moderate antiinflamatory effect for maraviroc (a CCR5 inhibitor) and for dolutegravir (an integrase inhibitor, INSTI).[41] In addition, both maraviroc and the INSTIs exert a neutral impact on lipid and glucose metabolism, but some INSTI like raltegravir resulted in a weight gain and a global fat accumulation (for a review see Lagathu et al., 2019[37]).

Our study was carried out considering only some kinds of treatments with a limited use at the present time. However, our results can lead the way to similar assessments with most recent ARTs and make possible further comparisons.

In conclusion, we found a clear contribution of fat redistribution and metabolic disturbances to inflammatory process and oxidative stress in HIV-1 infected patients treated for at least six months, being more pronounced this effect when the regimen included PIs.

Funding

This work was supported by the Spanish Ministry of Science and Innovation [SAF 2010-17213]; the Regional Ministry of Health from the Andalusian Government [SAS 111226]; "Miguel Servet Type II" grant [CPI13/00003 to M.I.Q.O. and CPII18/00030 to L.G.S.] from ISCIII, co-funded by the European Regional Development Fund (ERDF); "Nicolas Monardes" research program from the Regional Ministry of Health from the Andalusian Government [C-0030-2018 to M.I.Q.O. and C-0028-2018 to L.G.S.].

Conflicts of interest

None of the authors have any conflict of interest to declare.

Acknowledgements

We thank Dr. Juan de Dios Colmenero Castillo for their assistance with some patients, and also Richard Carlsson for help with the English language.

References

1. Murray CJL, Ortblad KF, Guinovart C, Lim SS, Wolock TM, Roberts DA, et al. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990–2013: a systematic analysis for the Global Burden of Disease Study. Lancet (London, England). 2014;384:1005–70.
2. World Health Organization. HIV/AIDS, Key facts; 2019. https://www.who.int/news-room/fact-sheets/detail/hiv-aids [accessed 3.12.19].
3. Guaraldi G, Orlando G, Zona S, Menozzi M, Carli F, Garlassi E, et al. Premature age-related comorbidities among HIV-infected persons compared with the general population. Clin Infect Dis. 2011;53:1120–6.
4. Lemoine M, Lacombe K, Bastard JP, Sébire M, Fonquernie L, Valin N, et al. Metabolic syndrome and obesity are the cornerstones of liver fibrosis in HIV-monoinfected patients. AIDS. 2017;31:1955–64.
5. Lemoine M, Serfaty L, Capeau J. From nonalcoholic fatty liver to nonalcoholic steatohepatitis and cirrhosis in HIV-infected patients: diagnosis and management. Curr Opin Infect Dis. 2012;25:10–6.
6. Nguyen KA, Peer N, Mills EJ, Kengne AP. A meta-analysis of the metabolic syndrome prevalence in the global HIV-infected population. PLOS ONE. 2016;11:e0150970.
7. Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, et al. Metabolic syndrome and risk of incident cardiovascular events and death. A systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol. 2007;49:403–14.
8. Bonfanti P, Giannattasio C, Ricci E, Facchetti R, Rosella E, Franzetti M, et al. HIV and metabolic syndrome. J Acquir Immune Defic Syndr. 2007;45:426–31.
9. Ledergerber B, Furrer H, Rickenbach M, Lehmann R, Elzi L, Hirschel B, et al. Factors associated with the incidence of type 2 diabetes mellitus in HIV-infected participants in the Swiss HIV cohort study. Clin Infect Dis. 2007;45:111–9.
10. Palacios R, Santos J, González M, Ruiz J, Márquez M. Incidence and prevalence of the metabolic syndrome in a cohort of naive HIV-infected patients:

prospective analysis at 48 weeks of highly active antiretroviral therapy. Int J STD AIDS. 2007;18:184–7.
11. De Wit S, Sabin CA, Weber R, Worm SW, Reiss P, Cazanave C, et al. Incidence and risk factors for new-onset diabetes in HIV-infected patients. Diabetes Care. 2008;31:1224–9.
12. Husain NE, Ahmed MH. Managing dyslipidemia in HIV/AIDS patients: Challenges and solutions. HIV AIDS (Auckl) 2014; 7:1–10. eCollection 2015.
13. Rogalska-Płońska M, Grzeszczuk A, Rogalski P, Łucejko M, Flisiak R. Metabolic syndrome in HIV infected adults in Poland. Kardiol Pol. 2018;76:548–53.
14. Krishnan S, Schouten JT, Atkinson B, Brown T, Wohl D, McComsey GA, et al. Metabolic syndrome before and after initiation of antiretroviral therapy in treatment-naive HIV-infected individuals. J Acquir Immune Defic Syndr. 2012;61:381–9.
15. Echecopar-Sabogal J, D'Angelo-Piaggio L, Chanamé-Baca DM, Ugarte-Gil C. Association between the use of protease inhibitors in highly active antiretroviral therapy and incidence of diabetes mellitus and/or metabolic syndrome in HIV-infected patients: a systematic review and meta-analysis. Int J STD AIDS. 2018;29:443–52.
16. Jericó C, Knobel H, Sorli ML, Montero M, Guelara A, Pedro-Botet J. Síndrome metabólico en pacientes con lipodistrofia infectados por el VIH. Clin Investig Arterioscler. 2006;18:51–6.
17. Freitas P, Carvalho D, Souto S, Santos AC, Xerinda S, Marques R, et al. Impact of Lipodystrophy on the prevalence and components of metabolic syndrome in HIV-infected patients. BMC Infect Dis. 2011;11:246.
18. Behrens GM, Stoll M, Schmidt RE. Lipodystrophy syndrome in HIV infection what is it, what causes it and how can it be managed? Drug Saf. 2000;23:57–76.
19. Norris A, Dreher HM. Lipodystrophy syndrome: the morphologic and metabolic effects of antiretroviral therapy in HIV infection. J Assoc Nurses AIDS Care. 2004;15:46–64.
20. Fardet L, Vigouroux C, Capeau J. Lipodystrophies. Rev Med Intern. 2013;34:614–22.
21. Tsiodras S, Perelas A, Wanke C, Mantzoros CS. The HIV-1/HAART associated metabolic syndrome – novel adipokines, molecular associations and therapeutic implications. J Infect. 2010;61:101–13.
22. Espiau M, Yeste D, Noguera-Julian A, Soler-Palacín P, Fortuny C, Ferrer R, et al. Adiponectin, leptin and inflammatory markers in HIV-associated metabolic syndrome in children and adolescents. Pediatr Infect Dis J. 2017;36:e31–7.
23. Freitas P, Carvalho D, Santos AC, Madureira AJ, Martinez E, Pereira J, et al. Adipokines, hormones related to body composition, and insulin resistance in HIV fat redistribution syndrome. BMC Infect Dis. 2014;14:347.
24. International Diabetes Federation (IDF). The IDF consensus worldwide definition of the Metabolic Syndrome; 2006. https://www.idf.org/e-library/consensus-statements/60-idfconsensus-worldwide-definitionof-the-metabolic-syndrome [accessed 3.12.19].
25. Onat A, Can G, Kaya H, Hergenç G. "Atherogenic index of plasma" (log_{10} triglyceride/high-density lipoprotein-cholesterol) predicts high blood pressure, diabetes, and vascular events. J Clin Lipidol. 2010;4:89–98.
26. Wilson PW. Estimation of cardiovascular risk in an individual patient without known cardiovascular disease. In: Basow DS, editor. UpToDate textbook of medicine. Waltham, MA: Massachusetts Medical Society, and Wolters Kluwer publishers; 2010.
27. Estrada V, Martínez-Larrad MT, González-Sánchez JL, de Villar NG, Zabena C, Fernández C, et al. Lipodystrophy and metabolic syndrome in HIV-infected patients treated with antiretroviral therapy. Metabolism. 2006;55:940–5.
28. Boullart AC, De Graaf J, Stalenhoef AF. Serum triglycerides and risk of cardiovascular disease. Biochim Biophys Acta. 2012;1821:867–75.
29. Willig AL, Overton ET. Metabolic complications and glucose metabolism in HIV infection: a review of the evidence. Current HIV/AIDS Rep. 2016;13:289–96.
30. Gori E, Mduluza T, Nyagura M, Stray-Pedersen B, Gomo ZA. Inflammation-modulating cytokine profile and lipid interaction in HIV-related risk factors for cardiovascular diseases. Clin Risk Manag. 2016;12:1659–66, eCollection 2016.
31. Brites-Alves C, Luz E, Netto EM, Ferreira T, Diaz RS, Pedroso C, et al. Immune activation, proinflammatory cytokines, and conventional risks for cardiovascular disease in HIV patients: a case–control study in Bahia, Brazil. Front Immunol. 2018;9:1469.
32. Cachofeiro V, Goicochea M, de Vinuesa SG, Oubiña P, Lahera V, Luño J. Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. Kidney Int Suppl. 2008;111:S4–9.
33. Biswas SK. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? Oxid Med Cell Longev. 2016;2016:5698931.
34. He JT, Zhao X, Xu L, Mao CY. Vascular risk factors and Alzheimer's disease: blood-brain barrier disruption, metabolic syndromes, and molecular links. J Alzheimers Dis. 2020;73:39–58.
35. Lv Z, Chu Y, Wang Y. HIV protease inhibitors: a review of molecular selectivity and toxicity. HIV AIDS (Auckl). 2015;7:95–104.
36. Aoki M, Das D, Hayashi H, Aoki-Ogata H, Takamatsu Y, Ghosh AK, et al. Mechanism of Darunavir (DRV)'s high genetic barrier to HIV-1 resistance: a key V32I substitution in protease rarely occurs, but once it occurs, it predisposes HIV-1 To develop DRV resistance. MBio. 2018:9, pii: e02425-17.
37. Lagathu C, Béréziat V, Gorwood J, Fellahi S, Bastard JP, Vigouroux C, et al. Metabolic complications affecting adipose tissue, lipid and glucose metabolism associated with HIV antiretroviral treatment. Expert Opin Drug Saf. 2019;18:829–40.
38. Hileman C, Longenecker C, Carman T, Milne G, Labbato DE, Storer Nj, et al. Relationship between total bilirubin and endothelial function, inflammation and oxidative stress in HIV-infected adults on stable antiretroviral therapy. HIV Med. 2012;13:609–16.
39. Vizcarra P, Fontecha M, Monsalvo M, Vivancos MJ, Rojo A, Casado JL. Efficacy and safety of dolutegravir plus boosted-darunavir dual therapy among highly treatment-experienced patients. Antivir Ther. 2019;24:467–71.
40. Echeverría P, Bonjoch A, Puig J, Ornella A, Clotet B, Negredo E. Significant improvement in triglyceride levels after switching from ritonavir to cobicistat in suppressed HIV-1-infected subjects with dyslipidaemia. HIV Med. 2017;18:782–6.
41. Afonso P, Auclair M, Caron-Debarle M, Capeau J. Impact of CCR5, integrase and protease inhibitors on human endothelial cell function, stress, inflammation and senescence. Antivir Ther. 2017;22:645–57.

Please cite this article in press as: González-Domenech CM, et al. Synergic effect of metabolic syndrome and lipodystrophy on oxidative stress and inflammation process in treated HIV-patients. Enferm Infecc Microbiol Clin. 2021. https://doi.org/10.1016/j.eimc.2020.11.019