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ORIGINAL ARTICLE

Characterization of gut microbiota composition in HIV-infected patients with metabolic syndrome

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Abstract

The presence of metabolic syndrome (MS) per se or its separated components in HIV-infected patients contributes to an accelerated aging and increased cardiovascular risk. Gut microbiota (GM) dysbiosis has been linked with chronic inflammation associated with MS in a general non-infected population. However, no studies concerning GM have been performed in HIV-infected patients with MS. The aim of this study was to analyze bacterial translocation, inflammation, and GM composition in HIV-infected patients with and without MS. A total of 51 HIV-infected patients were recruited and classified according to the presence of MS (40 patients without MS and 11 with MS). Markers of bacterial translocation, inflammation, and cardiovascular risk were measured and GM was analyzed using 16S rRNA gene deep sequencing. No differences were observed among both HIV-infected groups in the bacterial translocation markers LBP and sCD14. A tendency to increase the inflammatory markers IL-6 ($p = 0.069$) and MCP-1 ($p = 0.067$) was observed in those patients suffering from MS. An increase in the cardiovascular risk markers PAI-1 ($p = 0.007$) and triglycerides/HDL cholesterol ratio ($p < 0.0001$) was also found in the MS group. No significant changes were observed at phylum level although a decrease in the abundance of seven genera and seven bacterial species, including some anti-inflammatory bacteria, was observed in HIV-infected patients with MS. To summarize, the presence of MS was not accompanied by major changes in GM, although the reduction observed in some anti-inflammatory bacteria may be clinically useful to develop strategies to minimize inflammation and its future deleterious consequences in these HIV-infected patients.

Keywords HIV infection · Metabolic syndrome · Gut microbiota composition · Bacterial translocation · Inflammation · Cardiovascular risk

Abbreviations

ALT	Alanine aminotransferase
ART	Antiretroviral treatment
AST	Aspartate aminotransferase
BBH	Best blast hit
BLAST	Basic local alignment search tool
BT	Bacterial translocation
CEImLAR	Committee for Ethics in Drug Research in La Rioja
CVD	Cardiovascular diseases
ELISA	Enzyme-linked immunosorbent assay
GALT	Gut-associated lymphoid tissue
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
HIV+	HIV-infected patients
HOMA-IR	Homeostasis model assessment insulin resistance index
IL6	Interleukin-6
LBP	Lipopolysaccharide-binding protein,
LCA	Lowest common ancestor
LDL	Low-density lipoprotein
MCP-1	Monocyte chemotactic protein-1
MS	Metabolic syndrome

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NCEP-ATP III	National Cholesterol Education Program Adult Treatment Program III
PAD	Diastolic blood pressure
PAI-1	Plasminogen activator inhibitor-1
PAS	Systolic blood pressure
PIs	Protease inhibitors
sCD14	Soluble CD14

Introduction

Human immunodeficiency virus (HIV) infection is accompanied by depletion of $CD4^+$ T cells from mucosal sites, particularly from gut-associated lymphoid tissue (GALT) [20]. The depletion of gastrointestinal $CD4^+$ T cells is followed by alterations in the architecture of the lymphoid tissue and by the loss of intestinal mucosal barrier function, which favors the passage of bacteria and bacterial components (such as lipopolysaccharides, DNA, and flagellin) into the *lamina propria* of the gastrointestinal tract and, eventually, into the systemic circulation. This is known as *bacterial translocation* (BT). BT has been shown to persist throughout the course of HIV infection and contributes to the immune activation that is observed in the chronic phases of the infection [4, 26, 30, 36, 37, 44]. This chronic inflammation and activation of the immune system is associated with an accelerated aging in HIV-infected people, contributing to the development of several disorders such as cardiovascular diseases (CVD), non-AIDS cancers, frailty (loss of muscle mass, osteoporosis, and muscle weakness), kidney or liver diseases, neurologic complications, and metabolic syndrome (MS) [14, 15, 37, 46]. In addition and despite the fact that antiretroviral therapy has significantly improved the prognosis of HIV-infected patients, several metabolic abnormalities have also been described in those patients under antiretroviral treatment, especially under proteinase inhibitors (PIs). Among these metabolic side effects associated with the use of PIs is worth noting the presence of MS.

MS is a collection of cardiometabolic risk factors including obesity, hypertension, dyslipidemia, and insulin resistance, increasing the risk for CVD and type 2 diabetes mellitus. The high economic and social burden of MS is still growing; thus, clinical research is urgent to deeply understand the complex pathogenesis of this syndrome.

The prevalence of MS in HIV-infected people is quite similar than that reported for the general population with no HIV infection [38]. In fact, the prevalence of MS in HIV-infected patients ranges from 7 to 47% depending on the MS definition and the study design used, but, in all causes, the presence of this syndrome should be taken into account when designing health strategies, since the presence of MS *per se* or its separate components could be responsible for an increased cardiovascular risk in HIV-infected patients and could be associated with a significant reduction of their quality of life [35, 51].

Dysbiosis (an imbalance in microbiota composition) has been linked with chronic inflammation associated with several pathologies such as MS. In this context, either MS *per se* or its separated components have been associated with higher levels of BT [19, 47] and also with specific changes in the relative abundance of gut microorganisms in non-infected patients with MS [29] or in those subjects/animals suffering from any of the components of this syndrome (reviewed by Festi et al. and de Groot et al.) [13, 18]. More specifically, the abundances of *Sutterella*, *Methanobrevibacter*, and *Lactobacillus* were enriched in those patients with MS, whereas *Akkermansia*, *Odoribacter*, and *Bifidobacterium* were enriched in the healthy group [13, 29]. However, some incongruent results have arisen from these studies due, among other factors, to the differences in the studied populations (animal models and humans are not always comparable), the definition criteria applied etc. Anyway, what seems clear is that the presence of MS in a non-infected population is accompanied by increased BT, increased inflammation, and dysbiosis. However, the effects of MS on GM composition in HIV-infected patients have not been investigated yet. Thus, our objective was to analyze BT, inflammation, and GM composition in HIV-infected patients with MS in comparison with those HIV-infected patients without MS.

Materials and methods

Patient recruitment

A total of 51 Caucasian HIV-infected patients (HIV+) on antiretroviral treatment (ART) for at least 1 year and with viral load < 20 copies/mL for at least 6 months were recruited over 8 months from the Infectious Diseases Department at Hospital Universitario San Pedro (Logroño, Spain) (September 2013 to April 2014). All patients were immune responders. The recruited HIV+ patients were classified according to the presence of MS in accordance with the criteria established by the National Cholesterol Education Program Adult Treatment Program III (NCEP-ATP III). Thus, patients with any three of the following five criteria were diagnosed with MS: elevated waist circumference (≥ 102 cm in men or ≥ 88 cm in women), elevated triglycerides (≥ 150 mg/dL), reduced HDL-cholesterol (< 40 mg/dL in men or < 50 mg/dL in women), elevated blood pressure (≥ 130 mmHg systolic blood pressure or ≥ 85 mmHg diastolic blood pressure), and elevated fasting glucose (≥ 100 mg/dL) [22]. Thus, 40 HIV-infected patients without MS (HIV+MS−) and 11 patients with MS (HIV+MS+) were finally recruited for this study. The following exclusion criteria were applied: < 18 years old; pregnant women; patients treated with antibiotics, anti-inflammatory drugs corticosteroids, immunosuppressive drugs, or probiotics in the last 3 months; individuals with

kidney, coeliac, or inflammatory disease, thyroid disorders, neoplasms, history of intestinal surgery (except appendectomy or cholecystectomy), inflammatory bowel diseases (even if inactive), chronic pancreatitis, or any syndrome related to intestinal malabsorption. Patients receiving statins were also excluded because it was demonstrated that statin therapy causes gut dysbiosis [7, 39].

As a clinical procedure, $CD4^+$ and $CD8^+$ T cell counts and HIV viral load were measured using flow cytometry and COBAS TaqMan 48 Analyzer respectively (Roche Molecular Systems Inc., Branchburg, NJ, USA). Viral load of HBV and HCV were quantified for possible coinfection, and, in case of coinfection, degree of liver fibrosis was measured by FibroScan (Echosens, Paris, France). Patients were classified depending on liver fibrosis degree according to the METAVIR scoring system (F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; F4, cirrhosis or advanced fibrosis) [10].

This study was performed following the Helsinki Declaration and was approved by the Committee for Ethics in Drug Research in La Rioja (CEImLAR) (23 April 2013, reference number 121). All participants provided their written informed consent.

Biochemical parameters and enzyme-linked immunosorbent assay

Plasma and serum samples were collected from peripheral blood after a 12-h fast. Plasma levels of glucose, triglycerides, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using an AutoAnalyzer (Cobas C711, Roche, Madrid, Spain). The remaining samples were centrifuged and stored at − 80 °C in order to carry out the analysis of insulin, lipopolysaccharide-binding protein (LBP), soluble CD14 (sCD14), interleukin-6 (IL6), plasminogen activator inhibitor-1 (PAI-1), and monocyte chemotactic protein-1 (MCP-1) markers. Serum levels of insulin and MCP-1 were quantified by an enzyme-linked immunosorbent assay (ELISA) from EMD Millipore (MA, USA) and Luminex (Minneapolis, USA) respectively. Plasma levels of LBP and serum levels of sCD14 were measured using an ELISA from Hycult Biotech (The Netherlands). IL6 serum levels and PAI-1 plasma levels were measured using an ELISA from R&D (Minnesota, USA). All the analyses were performed in duplicate with commercially available kits and according to the manufacturers' instructions.

The homeostasis model assessment insulin resistance index (HOMA-IR) was calculated as follows: HOMA-IR index = fasting insulin (mU/L) × fasting glucose (mg/dL)/405 according to the report by Matthews et al. [32]. The triglycerides-to-HDL ratio was also calculated as a marker of cardiovascular risk [17, 31].

DNA extraction from stool samples and 16S rRNA gene sequencing

DNA extraction from fresh stool samples was carried out using the DNeasy Blood & Tissue Kit (Qiagen, Venlo, Netherlands). The concentration and the purity of the extracted samples were quantified by a NanoDrop spectrophotometer 1000 (ND-1000; Thermo Scientific, USA).

Samples were amplified for the 16S rDNA hypervariable sequence V4 with the Illumina MiSeq Instrument (reads, 2 × 250 bp) [8]. Bioinformatics analysis of the data was carried out by Era7 Bioinformatics (Granada, Spain). In short, the computational tool FLASh was used to assemble the two reads of each pair to obtain a larger sequence for a more specific taxonomic assignment of the reads. Subsequently, reads were assigned to a taxon based on direct similarity of each read, one-by-one, compared with any sequence 16S included in the Ribosomal Database Project (RDP) through the BLAST (Basic Local Alignment Search Tool) program [11]. Massive BLAST tasks were performed using MG7 system [1, 5]. Two different taxonomic assignment approaches were used: BBH (Best Blast Hit: each read was assigned to the taxon corresponding to the BBH over a threshold of similarity) and LCA (Lowest Common Ancestor: adopted by advanced tools of metagenomic analysis such as the last version of MEGAN 24). The median number of sequences assigned per patient was 99,702.33 with the LCA method, whereas 100,201.53 sequences were obtained with the BBH method. Alpha diversity measures, including number of observed species, alpha index, the Margalef and Chao-1 indexes were determined. To analyze beta diversity, web server METAGENassist was used [2].

Statistical analysis

Results are expressed as mean ± standard error of the mean. Qualitative variables were analyzed using the χ^2 test or Fisher's exact test. Normal distribution of continuous variables was tested with Shapiro-Wilk test. Comparisons were performed with unpaired t test or Mann-Whitney U test depending on the normality of the data. Spearman rank-sum test was used to identify the association between variables. Statistical analysis was carried out using SPSS 19·0 (SPSS® Inc. Chicago, IL, USA) and GraphPad Prism 6 (GraphPad Prism®, La Jolla, CA, USA). α-diversity metrics were performed using R (version 3.2.2; The R foundation for Statistical Computing, Vienna, Austria). p values < 0.05 were considered statistically significant. Data obtained from β-diversity were statistically analyzed using the Wilcoxon

rank-sum non-parametric test and false discovery rate (FDR) < 0.05 was considered significant.

Results

Characteristics of the participants

The prevalence of MS in the HIV-infected patients recruited in our Department in Logroño (Spain) along 8 months and according to the NCEP-ATP III criteria was 21.57%.

Table 1 shows the main characteristics of the studied population. Both groups were very homogeneous in all parameters except for those that are characteristics of the MS. Thus, HIV patients with MS were significantly older than those without MS ($p = 0.035$) and, as expected, patients with MS showed a significant increase in body weight ($p = 0.018$), body mass index ($p = 0.007$), systolic and diastolic blood pressure ($p = 0.027$; $p = 0.002$, respectively), triglycerides ($p < 0.0001$), and total cholesterol ($p = 0.008$) compared to the group without MS. Insulin levels and HOMA-IR index were also significantly increased in patients with MS ($p = 0.021$; $p = 0.003$, respectively). No statistical differences were observed when comparing the different ART combinations among both HIV groups ($P = 0.920$).

Markers of bacterial translocation, inflammation, and cardiovascular risk

No differences were observed when the BT markers LBP and sCD14 were analyzed in both HIV groups (Fig. 1a, b). Higher levels of IL-6 and MCP-1 that approached the threshold of statistical significance were observed in HIV+MS+ subjects when compared to HIV+MS− patients ($p = 0.069$; $p = 0.067$, respectively) (Fig. 1c, d). HIV-infected patients with MS showed higher levels of the cardiovascular risk marker PAI-1 in comparison with those HIV-infected patients without MS ($p = 0.007$) (Fig. 1e). Likewise, HIV patients with MS showed significantly higher values of the triglycerides-to-HDL ratio, which is a marker of cardiovascular risk, compared to the HIV+MS− group ($p < 0.0001$) (Fig. 1f).

Bacterial diversity and gut microbiota composition

α-diversity was assessed using the number of observed species, alpha index, the Margalef, and Chao-1 indexes. No differences were found in these parameters among HIV+MS− and HIV+MS+ patients (Fig. 2).

Regarding GM composition, phyla were dominated by Firmicutes and Bacteroidetes. Thus, HIV+MS− and HIV+MS+ groups showed a relative abundance of 74.32 and 71.22% respectively of these two phyla together. When comparing the relative abundance of the main phyla present in the gut, no significant differences were found. Only in the analysis of Actinobacteria, a $p < 0.05$ was found ($p = 0.032$), although no statistically significance was obtained with FDR (FDR = 0.128).

At lower taxonomic levels, HIV-infected patients with MS showed a decrease in the relative abundance of seven bacterial genera and seven species, whereas no increase was observed in the abundance of any genera or bacterial species. Within the Firmicutes phylum, four genera and six species were reduced in the HIV+MS+ group, including the genera *Eubacterium*, *Roseburia*, *Ruminococcus*, and *Subdoligranulum*, and the bacterial species *Eubacterium eligens*, *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, *Roseburia inulinivorans*, *Ruminococcus flavefaciens*, and *Subdoligranulum* sp. Among the Proteobacteria phylum, *Desulfovibrio* and *Sutterella* genera and the bacterial specie *Sutterella wadsworthensis* were also reduced. Finally, the *Bifidobacterium* genus of the Actinobacteria phylum was also decreased (Table 2). A PCA was performed and clusters representing both groups were overlapped; thus, very small differences in GM profile were observed among HIV-infected patients independently of suffering from MS (Fig. 3). The clustering of samples was represented by their respective 95% confidence interval ellipse and results were plotted according to the first two principle components accounting for 54.6% of the total variation (Component 1 = 30.4% and component 2 = 24.2%).

Associations between bacteria and physiological and biochemical parameters

The relative abundance of Coriobacteriales bacterium was positively associated with serum glucose levels ($r = 0.362$, $p = 0.009$), whereas it was negatively associated with systolic and diastolic blood pressures ($r = -0.365$, $p = 0.013$; $r = -0.336$, $p = 0.023$, respectively). Negative correlations between *F. prausnitzii* and the BT marker sCD14 ($r = -0.398$, $p = 0.007$) and triglyceride levels ($r = -0.466$, $p = 0.001$) were detected. A significant negative correlation was also observed between *R. intestinalis* and LDL levels ($r = -0.332$, $p = 0.032$). The abundance of *R. flavefaciens* was negatively correlated with the cardiovascular risk marker PAI-1 ($r = -0.345$, $p = 0.020$) and also with LDL levels ($r = -0.374$, $p = 0.015$) (Table 3).

Discussion

This study describes what happens in the gut in HIV-infected patients that suffer from MS in comparison with those HIV-infected people not suffering from such syndrome. We have reported that the presence of MS in HIV-infected patients was not accompanied by major changes in GM composition. Only

Table 1 Characteristics of HIV-infected patients

		HIV+MS−	HIV+MS+	*p* value
No. of patients		40	11	
Gender (male)		27/40 (67.5%)	7/11 (63.64%)	1
Age (years)		48.38 ± 0.89	52.30 ± 1.10	0.035
Body weight (kg)		66.95 ± 1.63	76.05 ± 3.98	0.018
Body mass index (kg/m^2)		22.78 ± 0.74	27.81 ± 2.04	0.007
Systolic blood pressure (mmHg)		127 ± 2.31	137.8 ± 4.29	0.027
Diastolic blood pressure (mmHg)		80.43 ± 1.80	92.78 ± 1.93	0.002
CD4 nadir count (cells/mm^3)		246.5 ± 27.33	260 ± 69.10	0.831
CD4 count (cells/mm^3)		715.5 ± 45.15	619.7 ± 154.1	0.314
T4/T8 index		0.93 ± 0.05	0.77 ± 0.09	0.106
Time since diagnosis of HIV infection (years)		15.82 ± 1.12	19 ± 2.47	0.206
AIDS		18/40 (45%)	4/11 (36.36%)	0.737
Coinfection with hepatitis B Virus		1/40 (2.5%)	0/11 (0%)	1
Coinfection with hepatitis C Virus		22/40 (55%)	5/11 (45.45%)	0.574
Mode of transmission		IVDU, 16/40 (40%) HS, 17/40 (42.5%) MSM, 2/40 (5%) Vertical, 1/40 (2.5%) IVDU/HS, 0/40 (0%) Unknown, 4/40 (10%)	IVDU, 4/11 (36.36%) HS, 2/11 (18.18%) MSM, 0/11 (0%) Vertical, 0/11 (0%) IVDU/HS, 1/11 (9.09%) Unknown, 4/11 (36.36%)	0.090
Degree of hepatic fibrosis		NO, 18/40 (45%) F1, 7/40 (17.5%) F2, 5/40 (12.5%) F3, 7/40 (17.5%) F4, 3/40 (7.5%)	NO, 7/11 (63.63%) F1, 1/11 (9.09%) F2, 1/11 (9.09%) F3, 2/11 (18.18%) F4, 0/11 (0%)	0.939
Antiretroviral treatment		NRTIs+PIs, 11/40 (27.5%) NRTIs+NNRTIs, 18/40 (45%) NRTIs+INSTIs, 6/40 (15%) Others, 5/40 (12.5%)	NRTIs+PIs, 4/11 (36.4%) NRTIs+NNRTIs, 4/11 (36.4%) NRTIs+INSTIs, 2/11 (18.2%) Others, 1/11 (9.1%)	0.920
Advanced degree of hepatic fibrosis		10/40 (25%)	2/11 (18.18%)	1
Time on ART (years)		12.28 ± 0.97	14.64 ± 2.29	0.287
Time on the last ART (years)		6.03 ± 2.90	3.36 ± 2.25	0.006
Biochemical tests	Plasma triglycerides (mg/dL)	103.6 ± 5.78	210.7 ± 24.74	<0.0001
	Plasma total cholesterol (mg/dL)	181.2 ± 5.71	214.7 ± 7.91	0.008
	Plasma LDL (mg/dL)	113.6 ± 5.58	132.9 ± 13.68	0.154
	Plasma HDL (mg/dL)	52.77 ± 2.53	42.90 ± 4.41	0.069
	Plasma triglycerides/HDL	3.01 ± 0.17	6.06 ± 0.75	< 0.0001
	Plasma AST (U/L)	20.84 ± 1.07	19.89 ± 2.03	0.678
	Plasma ALT (U/L)	24.94 ± 1.89	23.67 ± 3.15	0.946
	Plasma glucose (mg/dL)	89.83 ± 1.63	93.27 ± 3.23	0.335
	Serum insulin (μU/mL)	12.38 ± 0.54	16.69 ± 1.83	0.021
	HOMA-IR index	2.74 ± 0.13	3.81 ± 0.43	0.003

Quantitative data are presented as mean values ± SEM, whereas qualitative data are indicated as percentage. A p value of < 0.05 was considered significant

AIDS, acquired immunodeficiency syndrome; *ALT*, alanine aminotransferase; *ART*, antiretroviral therapy; *AST*, aspartate aminotransferase; *F0*, no fibrosis; *F1*, portal fibrosis without septa; *F2*, portal fibrosis and few septa; *F3*, numerous septa without cirrhosis; *F4*, cirrhosis or advanced fibrosis; *HDL*, high-density lipoprotein; *HIV+MS−*, HIV-infected patients without metabolic syndrome; *HIV+MS+*, HIV-infected patients with metabolic syndrome; *HOMA-IR*, homeostasis model assessment for insulin resistance; *HS*, heterosexual; *IVDU*, intravenous drug user; *IVDU/HS*, intravenous drug user and multiple heterosexual contacts; *LDL*, low-density lipoprotein; *MSM*, men who have sex with men; *NRTIs+INSTIs*, nucleoside reverse transcriptase inhibitors and integrase strand transfer inhibitors; *NRTIs+NNRTIs*, nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors; *NRTIs+PIs*, nucleoside reverse transcriptase inhibitors and protease inhibitors

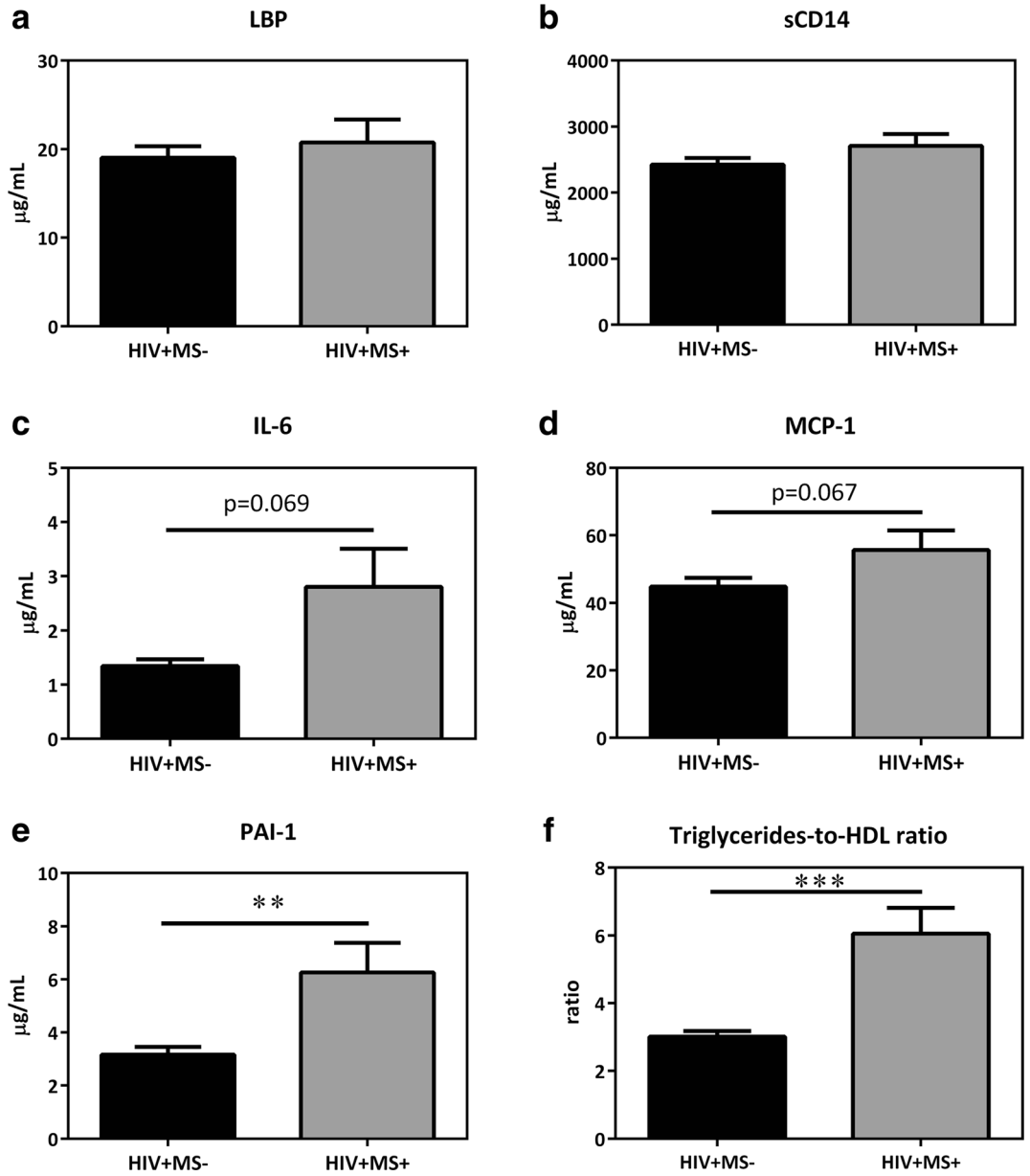

Fig. 1 **a**, **b** Bacterial translocation, **c**, **d** inflammation, and **e**, **f** cardiovascular risk markers in HIV-infected patients with and without metabolic syndrome. Each bar represents the mean ± SEM. $p < 0.05$ was considered significant. HIV+MS+, HIV-infected patients without metabolic syndrome; HIV+MS+, HIV-infected patients with metabolic syndrome. LBP, lipopolysaccharide-binding protein; IL-6, interleukin 6; MCP-1, monocyte chemotactic protein 1; PAI-1, plasminogen activator inhibitor-1; sCD14, soluble CD14; triglycerides/HDL ratio, triglyceride-to-HDL-cholesterol ratio

minimal deviations with potential clinical impact have been observed when comparing HIV patients with and without MS. Thus, the presence of MS was associated with a reduction in the butyrate-producing bacteria *E. eligens*, *R. intestinalis*, *R. inulinivorans*, and *Subdoligranulum* sp., which could constitute, along with the lower abundance of *F. prausnitzii*, reliable markers of future cardiovascular events in HIV-infected patients.

We have also calculated the prevalence of MS in our HIV cohort recruited along 8 months. Thus, the prevalence of MS in HIV people in our Hospital, according to the NCEP-ATP III criteria, was 21.57%, which was similar to the overall prevalence observed in HIV-infected population worldwide and in Europe using the same criteria (24.6 and 24.1%, respectively) [38]. When comparing this prevalence with previous studies carried out in Spain, similar rates were also observed (10–25%) [12, 24, 41]. In addition, the prevalence observed was also within the range of the 10–84% observed in the uninfected population [25], which indicates that the prevalence of this syndrome in HIV-infected people is not higher than in the general population. However, and even though the prevalence is similar, the metabolic changes begin at earlier ages in HIV-

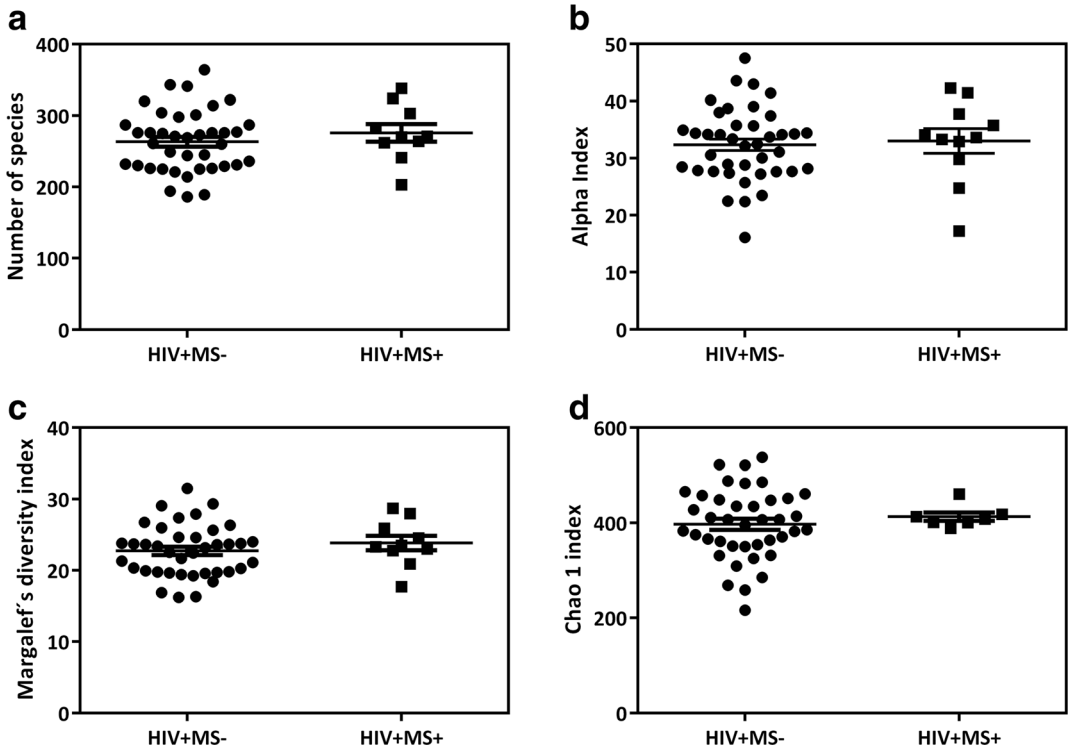

Fig. 2 α-diversity measurements in HIV-infected patients with and without metabolic syndrome. Four indices were used to represent the α-diversity: **a** Number of species. **b** Alpha index. **c** Margalef's diversity index. **d** Chao 1 index. Each bar represents the mean ± SEM. $P < 0.05$ was considered significant. HIV + MS+, HIV-infected patients without metabolic syndrome; HIV + MS+, HIV-infected patients with metabolic syndrome

infected people due to the chronic inflammatory state caused by HIV infection and the prolonged use of antiretroviral drugs that leads to the development of different metabolic alterations [38, 46]. Thus, the presence of MS in a HIV-infected population should be taken into account when designing health strategies.

Table 2 The presence of metabolic syndrome in HIV-infected patients was associated with a decrease in the relative abundance of seven genera and seven species in comparison with HIV patients without metabolic syndrome

Phylum	Taxonomic group	Category	FDR
Firmicutes	*Eubacterium*	Genus	0.012
Firmicutes	*Eubacterium eligens*	Species	0.002
Firmicutes	*Faecalibacterium prausnitzii*	Species	0.037
Firmicutes	*Roseburia*	Genus	7.47×10^{-4}
Firmicutes	*Roseburia intestinalis*	Species	0.002
Firmicutes	*Roseburia inulinivorans*	Species	8.85×10^{-4}
Firmicutes	*Ruminococcus*	Genus	3.59×10^{-4}
Firmicutes	*Ruminococcus flavefaciens*	Species	0.002
Firmicutes	*Subdoligranulum*	Genus	0.012
Firmicutes	*Subdoligranulum sp.*	Species	0.002
Proteobacteria	*Desulfovibrio*	Genus	0.019
Proteobacteria	*Sutterella*	Genus	0.002
Proteobacteria	*Sutterella wadsworthensis*	Species	0.002
Actinobacteria	*Coriobacteriales bacterium*	–	0.002
Actinobacteria	*Bifidobacterium*	Genus	0.009

A false discovery rate (FDR) < 0.05 was considered significant

Our previous studies reported that HIV-infected people presented higher plasma levels of sCD14, a marker of BT, when compared to a control/healthy population [48]. However, when these HIV-patients were split out depending on the presence of MS in this study, no differences were observed among them despite the fact that an increase of BT has been reported in non-HIV people with MS or suffering from any of its separated components when compared with a healthy population [27, 45]. These results could suggest that HIV-infection is responsible for such increase in BT and the presence of MS in these patients does not potentiate such actions.

Analysis of GM composition using PCA revealed that HIV + MS− and HIV + MS+ groups clustered together, indicating very little differences in GM profile. In fact, no differences were observed in α-diversity among groups. Conversely, the study carried out by Lim et al. found that the presence of MS in uninfected subjects was accompanied by a reduction in bacterial diversity [29]. The differences could be due to the fact that HIV by itself induces a strong decrease in α-diversity and MS does not translate into a higher reduction.

No significant differences were observed among both HIV groups at the phylum level, however, at lower taxonomic

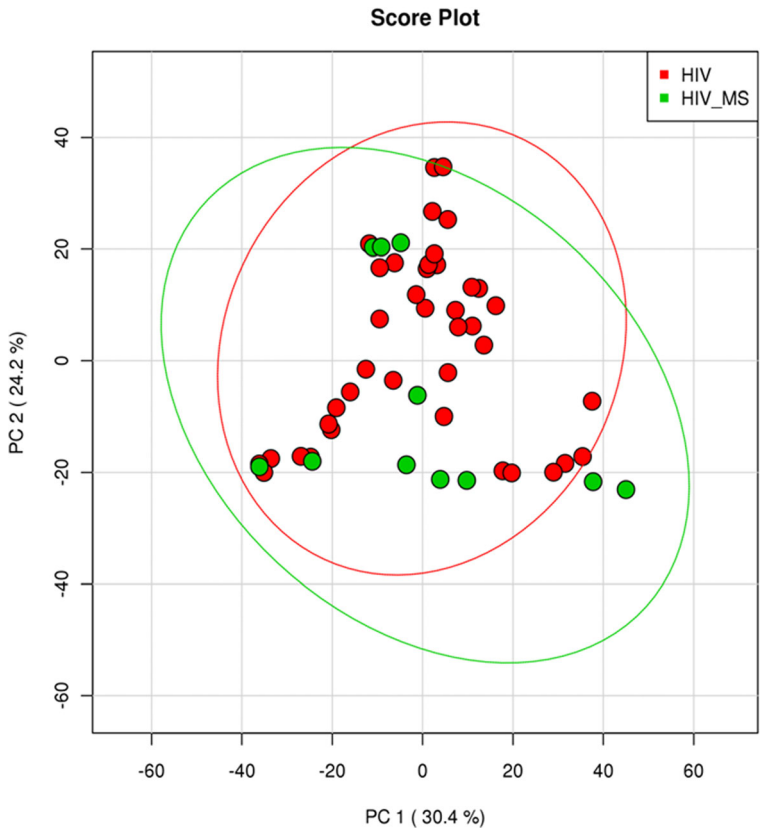

Fig. 3 Principal component analysis (PCA) of HIV-infected patients according to the presence of metabolic syndrome. Each circle represents a sample: red circles represent the HIV-infected patients without metabolic syndrome (HIV) and green circles represent the HIV-infected patients with metabolic syndrome (HIV_MS)

levels, the presence of MS was accompanied by a lower relative abundance of *Desulfovibrio* genus, which is known as a hydrogen sulfide producer and associated with damage in the gut barrier [3]. This is the first study where a reduction in the abundance of this genus has been reported in HIV-infected patients with MS in comparison with those not suffering from such syndrome. Only the study from Zhang et al. found a higher number of bacteria belonging to the Desulfovibrionaceae family in fresh fecal samples from animal models of diet-induced MS, specifically in Apoa-I knockout mouse and in wild-type C57BL/6J mice [52]. However, these discrepancies could be due to the fact that animal models not always mimic what happen in humans. In addition and more importantly, the HIV-infection impact on GM composition cannot be compared among both studies. Thus, the decline observed in this endotoxin producer in HIV-infected patients with MS and its clinical implications needs to be further investigated.

In general, the vast majority of the depleted bacteria observed in HIV-infected patients with MS were commensal and belong to Firmicutes phylum, including *R. intestinalis*, *R. inulinivorans*, *F. prausnitzii*, and some members of the

Table 3 Associations (Spearman's rank correlation coefficients) among the abundance of bacterial genera and species and biochemical and physiological parameters defining the presence of metabolic syndrome in HIV-infected patients

Bacteria	Marker	Spearman Rho	Significance (two tail)
Coriobacteriales bacterium	Glucose	0.362	0.009
	PAS	− 0.365	0.013
	PAD	− 0.336	0.023
Faecalibacterium prausnitzii	Triglycerides	− 0.466	0.001
	sCD14	− 0.398	0.007
Roseburia intestinalis	LDL	− 0.332	0.032
Ruminococcus	Triglycerides	− 0.358	0.011
Ruminococcus flavefaciens	LDL	− 0.374	0.015
	PAI-1	− 0.345	0.020

A p value of < 0.05 was considered significant. p value not adjusted by FDR

LDL, low-density lipoprotein; *PAD*, diastolic blood pressure (mmHg); *PAI-1*, plasminogen activator inhibitor-1; *PAS*, systolic blood pressure (mmHg); *sCD14*, soluble CD14

Subdoligranulum genus, all of them butyrate producers [49]. Butyrate plays an important role at the intestinal level by contributing to intestinal mobility, epithelial defense barrier, and reduction of inflammation [6]. In fact, butyrate has been considered in the last years to be among the top targets since depletion of this microbe-derived metabolite is linked to several diseases and seems to facilitate establishment of enteric pathogens by disrupting colonization resistance [42, 50]. A reduction in colonic butyrate-producing bacteria was found by Dillon et al. in HIV-infected patients, which was associated with increased BT and immune activation [16]. Although, in our study, butyrate has not been specifically measured, the reduction in butyrate-producing bacteria in HIV patients with MS is clear and could suggest an unhealthier gut and increased gut inflammation compared to HIV patients without MS. In fact, a trend towards increased systemic inflammation was observed in those patients with MS.

In the same line, the reduction observed in the abundance of *F. prausnitzii*, similarly to what has been observed in non-HIV-infected patients with MS, is of great interest [23]. This bacterium presents anti-inflammatory properties and it was demonstrated that it can improve intestinal barrier function in animal models with low grade or acute inflammation, being recognized as a biomarker of intestinal health [9, 28, 34]. Therefore, a lower abundance of *F. prausnitzii* could suggest that HIV patients with MS have a greater inflammatory state, which, in turn, could be associated with a greater cardiovascular risk. Overall, these results underline the need to monitor these patients even after immunological control with antiretrovirals in order to avoid, if possible, the deleterious effects derived from loss of protection at the gut level.

The genus *Bifidobacterium* (Actinobacteria phylum) includes bacteria with known beneficial effects. In fact, several probiotics are based on different bacterial strains belonging to this genus [43]. Interestingly, the presence of MS was associated with a decrease in *Bifidobacterium* when intestinal microbiota of both uninfected subjects [29] and HIV patients of our study were analyzed. Thus, these patients could benefit from these probiotics in order to reduce the incidence of future events associated with inflammation.

To sum up, the presence of MS was associated with a decrease in the relative abundance of several bacteria with known anti-inflammatory roles and, therefore, could suggest loss of protection against future cardiovascular events. Thus, the increased cardiovascular risk observed in HIV-infected patients with MS (as observed with PAI-1 and the triglycerides-to-HDL ratio) could be associated with the reduction of these bacteria. These bacteria could constitute reliable markers of future cardiovascular events in HIV-infected people with MS. Thus, it would be of great interest to carry out a follow-up study that could demonstrate if the administration of such bacteria as probiotics could lead to a reduction in cardiovascular risk and in the development of cardiovascular events.

One of the limitations of the present study was the difference in age among both HIV groups, despite the similarities observed in all other parameters (time under stable antiretroviral treatment, families of ART used, etc.). In fact, GM composition can be modulated by age [40]. For this reason, stratification by age was carried out in the HIV subjects (median age 49 years) in order to evaluate whether the changes observed in GM composition in patients with MS were due to age or by the presence of MS itself. As no changes in GM composition were observed at all the levels analyzed (genera and bacterial species), the reduction observed in the abundance of several bacteria seems to be associated with the presence of MS itself and not secondary to age. Similarly, no significant differences were observed in gut microbiota profile among the HIV-infected patients with MS and a group of non-infected people with MS ($n = 6$) (FDR > 0.3 for all ranks analyzed, data not showed) which suggest that the changes described herein are due to the presence of MS and not secondary to infection. Larger cohorts will be needed to confirm such studies. Finally, other potential limitation of the present study could be the small number of HIV-infected patients with MS included, although this sample size was similar to other metagenomic studies [21, 33].

In summary, the presence of MS in HIV-infected patients was not accompanied by major changes in GM composition and only minimal deviations with potential clinical impact have been observed when compared with HIV patients without MS. The reduced relative abundance observed in some relevant bacterial species, such as *F. prausnitzii*, suggests a greater inflammatory state at the intestinal level that could underline the higher CVD risk observed in these patients. Our study also highlights the potential usage of some of these bacteria as targets to develop strategies to modulate gut microbiota in order to minimize gut inflammation and its future deleterious consequences. More studies are needed to confirm such results.

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Authors' contributions Study design: P.P.M. and J.A.O. Data collection: P.P.M., J.A.O., and M.J.V.M. Performed the experiments: E.R.F. and M.J.V.M. Analyzed the data: J.M.L.R. and M.J.V.M. Interpretation of the data and wrote the paper: P.P.M., J.A.O., and M.J.V.M. Reviewed and/or edited the manuscript: all authors.

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Compliance with ethical standards

Conflict of interest J.A. Oteo has received travel grants from Gilead Sciences, MSD, and Janssen.

Other authors have nothing to declare.

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