Metabolic syndrome and obesity are the cornerstones of liver fibrosis in HIV-monoinfected patients

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Background: Metabolic syndrome (MetS) and nonalcoholic fatty liver disease have become a common finding in HIV-infected patients. However, the severity, risk factors and pathogenesis of liver fibrosis in this population have been poorly documented.

Objectives: To assess the impact of MetS on liver fibrosis and analyze the association between MetS, liver fibrosis and markers of adipose tissue and macrophage activation.

Methods: In a matched cohort of HIV-1-monoinfected patients with and without MetS, after exclusion of other causes of liver disease, we assessed liver stiffness measurement and measured levels of serum adipokines, homeostasis model assessment index and soluble CD163 (sCD163) and CD14 as markers of fat, insulin resistance and macrophage/monocyte activation, respectively.

Results: A total of 468 HIV-monoinfected individuals were enrolled; 405 (203 with MetS/202 without MetS) were analyzed. Patients with MetS were older and 49% had insulin resistance. The prevalence of significant liver fibrosis ($\geq$F2) was higher in patients with MetS [25.1%, 95% confidence interval (19.3–31.2)] compared with those without MetS [7.9%, (4.6–12.5), $P < 0.0001$]. In multivariable analysis, obesity [odds ratio: 3.9 (95% CI 2.1–7.1)] and homeostasis model assessment [1.1 (1.06–1.2)] were independent factors of significant fibrosis and remained associated after adjustment on MetS. Serum levels of adipokines and sCD163 were significantly associated with the degree of liver fibrosis. When adjusted on MetS, leptin and sCD163 remained strongly associated with fibrosis/cirrhosis, whereas HIV parameters and antiretroviral therapy were not.

Conclusion: In HIV-monoinfected patients, MetS is an important risk factor of liver fibrosis. Adipose tissue and macrophage activation might be key players in the development of liver fibrosis but the exact mechanisms need to be elucidated.

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Introduction

Over the last two decades, metabolic syndrome (MetS) defined by central obesity, high blood pressure (BP) and impaired glucose and lipid homeostasis [1], has become a growing concern in HIV-infected individuals [2]. In addition, a number of HIV-infected patients present central fat redistribution that aggravates the dysmetabolic

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phenotype [3]. Metabolic disorders in HIV-infected patients result from dysregulation of fat redistribution with insulin resistance induced by aging, life style modifications, antiretroviral therapy (ART) and the virus itself [4].

In HIV-uninfected people, MetS is the main cause of nonalcoholic fatty liver disease (NAFLD) which encompasses a large spectrum of chronic liver diseases from simple steatosis, nonalcoholic steatohepatitis (NASH) to fibrosis and cirrhosis. The severity of liver fibrosis is the strongest predictor of disease-specific mortality in non-HIV patients with NAFLD, cardiovascular disease being one of the major causes of death [5]. As a result, international guidelines on NAFLD/NASH recommend to systematically screen patients with NAFLD for liver fibrosis using noninvasive markers as a first step [6].

Following the significant advances in the management of HIV and viral hepatitis infections and the growing incidence of metabolic disorders globally, NAFLD has become a new concern in HIV-infected patients. NAFLD is estimated to affect about one-third of HIV-monoinfected patients according to the methods of assessment [7–15]. However, only a few studies have documented the prevalence and risk factors of liver fibrosis in HIV patients with NAFLD [15]. Most of these studies [9,11,12,16,17], except a recent one [14], have included a limited number of patients and the pathophysiology of liver fibrogenesis in this population remains poorly understood.

Adipose tissue dysfunction and monocyte/macrophage activation have emerged as new concepts in the development of NAFLD and fibrosis in non-HIV patients, suggesting a Kupffer cell activation in the development of liver fibrosis [18–20], but this has not been documented in HIV patients with NAFLD. HIV-treated patients have increased circulating levels of markers of monocyte and macrophage activation that is soluble CD163 (sCD163) and CD14 (sCD14), suggesting persisting active innate immune dysfunction despite effective ART. In HIV-infected patients, high serum level of sCD163 is an independent predictor of vascular inflammation [21] and all-cause mortality [22]. Obesity is also associated with high circulating levels of sCD14 and sCD163 in HIV-individuals [23]. The relationship between serum levels of sCD14 or sCD163 and liver fibrosis has been poorly assessed in HIV-patients although these makers are associated with liver inflammation and fibrosis in non-HIV patients with chronic hepatitis [24] or NAFLD [18,19]. sCD163 levels have been shown to be associated with biochemical markers of fibrosis (APRI) and liver transaminases in HIV/HCV coinfected patients [25] but there is no indication regarding HIV-monoinfected patients. Very recently, increased sCD163 levels were found to be correlated with incident liver disease in HIV-infected individuals. However, no association with liver fibrosis and cirrhosis was observed, probably due to a lack of statistical power [26]. Adipokines and cytokines for example adiponectin, leptin and interleukin-6 (IL-6), produced by adipose tissue, are associated with the degree of liver damage in non-HIV patients with alcoholic or nonalcoholic liver disease [20,27] but have been poorly analyzed in HIV-infected patients with chronic liver disease.

From a matched cohort of nonalcoholic HIV-monoinfected patients with and without MetS, the following study aimed to assess the impact of MetS on the proportion and severity of liver fibrosis using transient elastography and to analyze the association between MetS, liver fibrosis and markers of adipose tissue and macrophage activation.

Patients and methods

Study population

METAFIB is an exposed–unexposed cohort of HIV-monoinfected patients followed in the department of infectious diseases and tropical medicine of Saint-Antoine University Hospital, Paris, France. Individuals included in the cohort were identified from the local computerized database and evaluated for inclusion at their first visit to the clinic during the study period (January 2011–December 2012). They were adult patients with HIV-1 infection diagnosed at least 5 years earlier. The exposure was defined by the presence of MetS according to the International Diabetes Federation [1] criteria after exclusion of excessive alcohol consumption ($\geq$30 g/day) and other causes of chronic liver diseases: infection with hepatitis B or C viruses, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, biliary obstruction, alpha1-antitrypsin deficiency, hemochromatosis and Wilson's disease. Participants with uncontrolled congestive heart failure or transaminases at least 10 times the upper limit of normal were not enrolled in the study. Patients nonexposed to MetS were matched to exposed ones on age ($\pm$5 years), sex and duration of HIV infection ($\pm$2 years) on a 1 : 1 ratio. Participants were enrolled in the cohort study after providing written consent. The study was approved by the local ethic committee and conducted according to the Helsinki declaration.

Demographic and clinical data

Demographic (age and sex), life style (smoking habit and alcohol consumption), dietary habit (evaluated by a certified dietician using a standardized national questionnaire (InVS/Cnam, France), anthropometric [waist and hip circumference, body mass index (BMI)] and clinical data (blood pressure, type 2 diabetes, medication and medical history) were collected at time of enrollment and recorded in standardized forms by two single trained investigators.

Assessment of liver fibrosis

Liver fibrosis was evaluated by liver stiffness measurement (LSM) obtained in fasting patients using transient elastography (Fibroscan 502, M probe; Echosens, Paris, France) and was performed by trained experienced operators according to the manufacturer's protocol. Results were expressed in kilopascal (kPa) as the median value of 10 successful acquisitions. Failure was defined as no single successful measurement (valid shot = 0) and unreliable measurement was defined as interquartile range (IQR)/LSM of more than 0.30 when LSM is at least 7.1 kPa [28]. Invalid LSM was defined as the total of failure and unreliable LSM values. To estimate fibrosis stages, we used the following cutoffs which were previously validated in HIV-uninfected patients with biopsy proven NAFLD [29]: 7.1, 8.7 and 10.3 kPa for F2, F3 and F4, respectively.

Laboratory investigations

Blood samples were collected after a 12-h overnight fast for determination of liver function tests [aspartate aminotransferase, alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP)], glucose, cholesterol (total, LDL, HDL), triglycerides and insulin. Measurements of circulating insulin were centralized and performed using a high specific immunoassay (Architect; Abbott Laboratories, Rungis, France). Insulin resistance was assessed by using the Homeostasis Model Assessment Method index (HOMA-IR) as follows: fasting insulin (mU/l) × fasting plasma glucose (mmol/l)/22.5. Insulin resistance was defined by a HOMA-IR index at least 2.5. Immunovirological parameters were also collected. $CD4^+$ T-cell count and $CD4^+/CD8^+$ ratio were quantified using standard measurements, whereas nadir $CD4^+$ T-cell count was obtained from patient records prior to inclusion. Ultra sensitive HIV viral load was measured using an adapted Cobas AmpliPrep/Cobas TaqMan HIV-1 assay (Roche Diagnostics, Meylan, France; quantification limit: 1 copy/ml). Patients were defined as having either detectable (≥1 copies/ml) or undetectable (<1 copy/ml levels of HIV-viremia).

Leptin, high sensitivity IL-6 and markers of monocyte/macrophage activation (sCD14 and sCD163) were measured by using an ELISA (Quantikine leptin, IL-6, sCD14, sCD163; R&D Systems, Oxford, UK). Serum adiponectin, which detects total full-length mature adiponectin, and high molecular weight adiponectin level were also measured by ELISA (ALPCO, Salem, New Hampshire, USA). High sensitivity C-reactive protein (CRP) was measured by immunonephelometry (IMMAGE; Beckman-Coulter, Brea, California, USA).

Statistical analysis

The prevalence of significant fibrosis using noninvasive markers of fibrosis in European or American HIV-monoinfected patients varies between 15 and 20% [14,30]. Using the method of Demidenko [31] and assuming a type I error of 0.05 and a probability of having a prevalence of fibrosis up to 15 or 20%, the expected total number of patients needed to attain a power of 0.80 was 227 to 259. Descriptive data are presented as means ± SD, median IQR or number (*n*, %). Patients with or without fibrosis at least F2, at least F3 and at least F4 were compared (groups are mutually exclusive) by chi-squared test for categorical variables and Wilcoxon rank-sum test for continuous variables. To determine the factors associated with fibrosis, we calculated crude odds ratios (ORs) with 95% confidence interval (95% CIs) by univariate modeling in the entire population and adjusted on the presence of MetS. Variables associated with liver fibrosis on univariate analysis with *P* less than 0.2 were entered in a backward stepwise multivariate logistic regression model provided for each level of fibrosis. All analyses were performed with STATA v12.1 (StataCorp, College Station, Texas, USA) and *P* less than 0.05 was considered significant.

Results

Study population

During the inclusion period, 468 HIV-monoinfected individuals [mainly male (89%), mean age 53 (9) years, mean BMI 24.6 (5.3) kg/m^2] were enrolled: 246 with MetS and 222 without MetS. LSM values were invalid in 63 (13.4%) patients. Therefore, 405 patients (203 with MetS and 202 without MetS) were included in the final analysis (Supplementary Fig. 1, http://links.lww.com/QAD/B146). Patients with failure of LSM were mainly women (11.3 versus 3%, $P = 0.007$), with MetS (6.9 versus 0.9%, $P = 0.001$) or high BMI (32 versus 24 kg/m^2, $P = 0.00001$). Patients with unreliable LSM did not differ from the study population on age ($P = 0.08$), obesity ($P = 0.3$), presence of MetS ($P = 0.3$), sex ($P = 0.08$) or BMI ($P = 0.2$).

The characteristics of the study population according to the presence of MetS are summarized in Table 1. Patients with MetS were older and had more metabolic disorders than those without MetS. As expected patients with MetS had a higher number of dysmetabolic features regarding glycemia, triglycerides and HDL cholesterol than patients without MetS (but lower LDL cholesterol) and 99 (49%) of them had insulin resistance as defined by HOMA-IR at least 2.5 (compared with 8.5% in patients without MetS). Evaluation of dietary habits did not find significant difference between the two groups, except that patients without MetS had a higher consumption of wine and reported more exercise than those with MetS (Supplementary Table 1, http://links.lww.com/QAD/B129).

Prevalence of clinically significant fibrosis and cirrhosis

In the study population, the mean value (SD) of LSM was 5.6 kPa (2.2) with a minimum and maximum value of

Table 1. Characteristics of the study population according to the presence of metabolic syndrome.

	Study population, $n=405$	Patients with MetS, $n=203$	Patients without MetS, $n=202$	P value
Mean age, [years (SD)]	53 (9)	54 (9)	52 (8)	0.02
Males, *n* (%)	359 (89)	183 (90)	176 (87)	0.4
Mean BMI [kg/m^2 (SD)]	24.6 (5.3)	26.0 (4.7)	23.2 (5.5)	0.0001
Mean Waist circumference [cm (SD)]	91.9 (10.3)	97.7 (8.9)	86.2 (8.1)	0.0001
Obesity: BMI ≥ 30 kg/m^2, *n* (%)	34 (8.4)	27 (13.3)	7 (3.5)	0.0001
Type 2 diabetes, $n=404$, *n* (%)	33 (8.2)	31 (15.3)	2 (1.0)	0.0001
HIV parameters				
CD4$^+$ cells count (cells/μl)	617 (257)	628 (239)	607 (273)	0.2
CD4$^+$ nadir (cells/μl)	213 (144)	194 (138)	234 (148)	0.005
CD4$^+$/CD8$^+$ ratio	0.85 (0.39)	0.84 (0.40)	0.87 (0.38)	0.3
Duration of HIV infection (years)	17.0 (7.0)	16.9 (6.8)	17.0 (7.3)	0.9
CDC stage C, *n* (%)	99 (24.4)	50 (24.6%)	49 (24.3%)	0.9
US HIV1-VL <copies/ml, *n* (%)	234 (59.9)	88 (44.0)	69 (35.8)	0.1
HIV1-VL < 20 copies/ml, *n* (%)	323 (79.8)	160 (78.8)	163 (80.7)	0.7
Present exposure to PI, *n* (%)	222 (54.8)	118 (58.1)	104 (51.5)	0.2
Duration of exposure to PI (months)	48.9 (31.5)	47.2 (28.9)	52.8 (34.1)	0.3
Present exposure to D4T, DDI or ZDV, *n* (%)	24 (6.0)	14 (6.9)	10 (5.0)	0.6
Duration of exposure to D4T, DDI or ZDV (months)	103.2 (41.1)	88.8 (44.2)	123.5 (26.8)	0.07
Metabolic parameters				
HOMA score	2.58 (3.15)	3.61 (3.48)	1.53 (2.36)	0.0001
HOMA score ≥2.5, *n* (%)	116 (29)	99 (49)	17 (8.5)	0.0001
Total cholesterol (mmol/l)	4.96 (1.04)	4.85 (1.07)	5.08 (1.02)	0.009
HDL-cholesterol (mmol/l)	1.21 (0.37)	1.06 (0.30)	1.36 (0.37)	0.0001
LDL-cholesterol (mmol/l)	2.95 (1.04)	2.80 (1.25)	3.11 (0.74)	0.0001
Triglycerides (mmol/l)	1.89 (1.78)	2.40 (1.65)	1.37 (1.75)	0.0001
Glucose (mmol/l)	5.47 (1.20)	5.85 (1.48)	5.09 (0.64)	0.0001
Hepatic parameters				
AST (IU/l)	29 (20)	31 (19)	27 (20)	0.0001
ALT (IU/l)	34 (27)	41 (34)	28 (15)	0.0001
GGT (IU/l)	52 (52)	62 (64)	42 (33)	0.0001
ALP (IU/l)	70 (21)	71 (22)	69 (20)	0.6
Platelet count (/μl)				
LSM (kPa)	5.6 (2.19)	6.3 (2.6)	4.9 (1.5)	0.0001
LSM ≥ 7.1 kPa	67 (16.5)	51 (25.1)	16 (7.9)	0.0001
Serum inflammatory markers	$n=396$	$n=199$	$n=197$	
Hs CRP (mg/l)	3.50 (4.94)	3.69 (4.88)	3.32 (5.01	0.07
Hs IL-6 (pg/ml)	2.22 (4.33)	2.16 (3.40)	2.28 (5.12)	0.4
Leptin (ng/ml)	7.99 (11.48)	10.52 (13.19)	5.44 (8.76)	0.0001
Total adiponectin (μg/ml)	4.39 (2.83)	3.36 (2.27)	5.44 (2.95)	0.0001
HMW adiponectin (μg/ml)	2.13 (1.95)	1.39 (1.44)	2.87 (2.12)	0.0001
Leptin/adiponectin ratio	2.56 (4.76)	3.92 (6.16)	1.19 (1.87)	0.0001
sCD14 (ng/ml)	2116.14	2204.3 (736.0)	2027.1 (1019.3)	0.0001
sCD163 (ng/ml)	711.03 (293.82)	778.1 (298.6)	643.3 (273.4)	0.0001

Categorical variables are expressed as raw numbers and percentages, continuous variables are reported as means and SD. ALP, alkaline phosphatise; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; DDI, didanosine; D4T, stavudine; GGT, gamma-glutamyl transpeptidase; HMW, high molecular weight; HOMA, homeostasis model assessment; Hs, highly sensitivity; LSM, liver stiffness measurement; MetS, metabolic syndrome; PI, protease inhibitor; sCD14, soluble CD14; sCD163, soluble CD163; US, ultra sensitive; ZDV, zidovudine.

2.4 and 17.1 kPa. Mean LSM was higher in patients with MetS compared with those without MetS [6.3 (2.6) versus 4.9 (1.5) kPa, $P<0.0001$].

Proportions of patients with clinically significant fibrosis (F2), extensive fibrosis (F3) or cirrhosis (F4) are presented in Fig. 1. Based on LSM values, the prevalence of significant fibrosis was higher in patients with MetS [25.1%, 95% CI (19.3−31.2)] as compared with those without MetS [7.9%, 95% CI (4.6−12.5), $P<0.0001$]. Similarly, the prevalence of cirrhosis was much higher in patients with MetS [(8.4%, 95% CI (4.5−13.1)] as compared with patients without MetS [0.9%, 95% CI (0.1−3.5), $P<0.0001$].

Risk factors for clinically significant fibrosis and cirrhosis

On univariate analysis, BMI, obesity, MetS, Centers for Disease Control and Prevention (CDC) stage, HOMA at least 2.5, low HDL cholesterol, high triglycerides, liver enzyme levels, CD4$^+$/CD8$^+$ ratio were significantly associated with fibrosis and cirrhosis (data not shown). Duration of HIV infection and type of ART exposure were not associated with fibrosis/cirrhosis.

Table 2 reports the results of the association between levels of fibrosis and covariates after adjustment on MetS (bivariate analysis). The risk of a higher level of fibrosis and eventually cirrhosis steadily increased with BMI, in

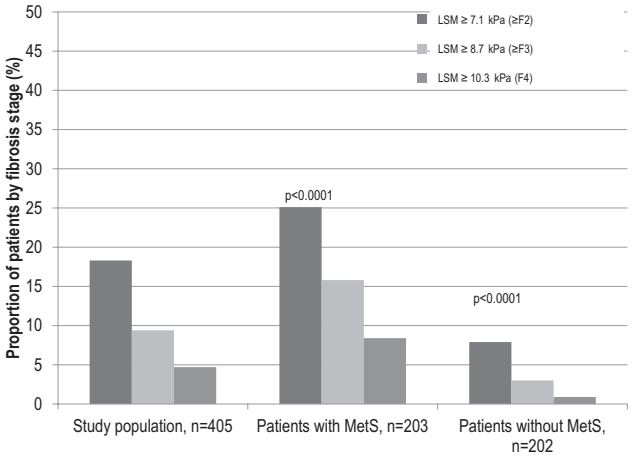

Fig. 1. Proportion of patients by stage of fibrosis and stratified on metabolic syndrome.

particular obesity defined by a BMI at least 30 kg/m^2, waist circumference, GGT and ALP levels, type 2 diabetes, HOMA score, circulating levels of leptin, leptin/adiponectin ratio and sCD163 independently of MetS.

MetS remained consistently associated with an excess risk of fibrosis after adjustment on all former variables. Other variables consistently associated with increased risk of liver fibrosis in the multivariate analysis were obesity, and CDC-stage B. Insulin resistance defined by HOMA-IR at least 2.5 and $CD4^+/CD8^+$ ratio were also strongly associated with extensive fibrosis (F3) and also marginally with cirrhosis for HOMA-IR ($P = 0.09$) (Supplementary Table 2, http://links.lww.com/QAD/B129).

Serum levels of adipokines, soluble CD14 and CD163

Patients with MetS had higher circulating levels of leptin and markers of macrophage activation (sCD14 and

Table 2. Association between levels of liver fibrosis and covariates after adjustment on the presence of metabolic syndrome (bivariate analysis).

	Liver fibrosis ≥F2 OR (95% CI)	Liver fibrosis ≥F3 OR (95% CI)	Cirrhosis OR (95% CI)
Age (years)	0.99 (0.97–1.03)	1.02 (0.98–1.05)	1.009 (0.96–1.06)
Male	0.98 (0.41–2.36)	0.72 (0.21–2.49)	1.04 (0.23–4.79)
BMI (kg/m^2)	**1.13 (1.05–1.21)**	**1.07 (1.02–1.13)**	**1.09 (1.03–1.16)**
Waist circumference (cm)	1.03 (0.99–1.06)	**1.05 (1.009–1.09)**	**1.09 (1.03–1.15)**
Obesity: BMI ≥ 30 kg/m^2	**3.13 (1.45–6.73)**	**3.59 (1.52–8.49)**	**5.29 (1.88–14.94)**
Type 2 diabetes	1.46 (0.64–3.32)	2.09 (0.85–5.17)	**3.44 (1.18–10.06)**
HIV parameters			
$CD4^+$ cells count (cells/μl)	0.99 (0.98–1.001)	1.00 (0.99–1.001)	1.00 (0.99–1.002)
$CD4^+$ nadir (cells/μl)	1.00 (0.99–1.002)	1.00 (0.99–1.002)	1.00 (0.99–1.003)
$CD4^+/CD8^+$ ratio	0.86 (0.43–1.73)	0.46 (0.18–1.23)	0.93 (0.28–3.08)
Duration of HIV infection	1.005 (0.97–1.04)	1.00 (0.95–1.05)	0.98 (0.91–1.05)
CDC stage C	1.33 (0.77–2.27)	0.78 (0.39–1.56)	0.35 (0.13–0.93)
US HIV1-RNA < 1 copies/ml	1.48 (0.85–2.58)	1.30 (0.65–2.61)	1.88 (0.73–4.85)
Present exposure to PI	1.004 (0.99–1.02)	0.92 (0.46–1.83)	0.81 (0.32–2.07)
Present exposure to D4T, DDI or ZDV	1.62 (0.60–4.38)	1.86 (0.58–5.99)	2.98 (0.77–11.47)
Metabolic parameters			
HOMA score (mU/mmol)	**1.09 (1.02–1.17)**	**1.12 (1.04–1.21)**	**1.012 (1.03–1.23)**
Total cholesterol (mmol/l)	1.08 (0.85–1.39)	1.02 (0.74–1.41)	0.76 (0.47–1.24)
HDL-cholesterol (mmol/l)	0.58 (0.23–1.45)	0.93 (0.31–2.80)	1.08 (0.24–4.82)
LDL-cholesterol (mmol/l)	0.91 (0.69–1.20)	1.05 (0.81–1.37)	0.86 (0.51–1.43)
Triglycerides (mmol/l)	**1.14 (1.09–1.30)**	0.99 (0.80–1.22)	0.90 (0.62–1.30)
Glycemia (mmol/l)	0.95 (0.76–1.17)	1.02 (0.80–1.31)	1.19 (0.89–1.60)
Hepatic parameters			
AST (IU/l)	1.008 (0.99–1.02)	1.004 (0.99–1.02)	1.007 (0.99–1.02)
ALT (IU/l)	1.004 (0.99–1.01)	1.001 (0.99–1.01)	1.002 (0.99–1.02)
GGT (IU/l)	**1.005 (1.00–1.009)**	**1.005 (1.0001–1.01)**	**1.005 (1.00008–1.01)**
ALP (IU/l)	**1.02 (1.006–1.03)**	**1.02 (1.0008–1.03)**	**1.03 (1.01–1.05)**
Serum inflammatory markers	$n = 396$	$n = 199$	$n = 197$
Us CRP (mg/l)	1.04 (0.99–1.09)	1.01 (0.95–1.08)	1.06 (0.98–1.13)
Hs IL-6 (pg/ml)	1.00 (0.94–1.07)	0.99 (0.91–1.10)	1.02 (0.92–1.12)
Leptin (ng/ml)	**1.03 (1.005–1.05)**	**1.02 (1.001–1.05)**	**1.03 (1.006–1.06)**
Total adiponectin (μg/ml)	0.97 (0.87–1.1)	1.06 (0.93–1.2)	0.90 (0.70–1.17)
HMW adiponectin (μg/ml)	0.99 (0.83–1.17)	1.11 (0.92–1.34)	0.85 (0.57–1.29)
Leptin/adiponectin ratio	**1.12 (1.04–1.20)**	**1.05 (0.99–1.11)**	**1.08 (1.01–1.15)**
sCD14 (ng/ml)	0.99 (0.99–1.00)	1.00 (0.99–1.00)	0.99 (0.99–1.00)
sCD163 (ng/ml)	**1.01 (1.00–1.00)**	**1.01 (1.00–1.001)**	**1.001 (1.0–1.002)**

ALP, alkaline phosphatise; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; DDI, didanosine; D4T, stavudine; GGT, gamma-glutamyl transpeptidase; HMW, high molecular weight; HOMA, homeostasis model assessment; Hs, highly sensitivity; LSM, liver stiffness measurement; PI, protease inhibitor; sCD14, soluble CD14; sCD163, soluble CD163; US, ultra sensitive; ZDV, zidovudine. Parameters significantly associated with fibrosis are indicated in bold.

Table 3. Serum adipokines and macrophage activation markers stratified according to the severity of liver fibrosis.

	Fibrosis ≥F2, $n=66$	Fibrosis <F2, $n=330$	P value	Fibrosis ≥F3, $n=38$	Fibrosis <F3, $n=358$	P value	Cirrhosis, $n=17$	No cirrhosis, $n=388$	P value
Hs CRP (mg/l)	4.56 (5.17)	3.29 (4.88)	0.0008	3.95 (3.77)	3.46 (5.05)	0.04	5.32 (4.38)	3.41 (4.96)	0.002
Hs IL6 (pg/ml)	2.22 (4.66)	2.42 (2.02)	0.03	2.16 (1.38)	2.23 (4.54)	0.01	2.51 (1.60)	2.21 (4.43)	0.02
Leptin (ng/ml)	**12.86 (16.92)**	**7.02 (9.80)**	**0.0001**	**13.89 (15.24)**	**7.37 (10.84)**	**0.0001**	**18.0 (18.0)**	**7.49 (10.84)**	**0.0001**
Total adiponectin (μg/ml)	**3.72 (2.86)**	**4.53 (2.81)**	**0.004**	**4.00 (3.47)**	**4.43 (2.76)**	**0.04**	**3.18 (2.16)**	**4.45 (2.84)**	**0.01**
HMW adiponectin (μg/ml)	**1.71 (2.09)**	**2.22 (1.92)**	**0.003**	**1.90 (2.60)**	**2.16 (1.88)**	**0.02**	**1.30 (1.59)**	**2.17 (1.96)**	**0.003**
Leptin/adiponectin ratio	**5.36 (9.68)**	**2.00 (2.60)**	**0.0001**	**5.47 (7.98)**	**2.25 (4.18)**	**0.0001**	**8.16 (10.41)**	**2.28 (4.11)**	**0.0001**
sCD14 (ng/ml)	2136.0 (765.2)	2112.2 (915.8)	0.3	2184.5 (720.9)	2108.9 (908.4)	0.1	2008.8 (626.9)	2121.5 (903.2)	0.8
sCD163 (ng/ml)	**797.5 (301.1)**	**693.7 (289.7)**	**0.003**	**829.9 (294.1)**	**698.4 (291.4)**	**0.003**	**893.7 (300.6)**	**701.8 (290.9)**	**0.003**

CRP, C-reactive protein; HMW, high molecular weight; Hs, highly sensitivity; sCD14, soluble CD14; sCD163, soluble sCD163. Parameters significantly associated with fibrosis are indicated in bold.

sCD163) and lower levels of adiponectin than patients without MetS, whereas serum levels of CRP and IL-6 did not differ between the two groups (Table 1). Serum level of leptin was positively correlated with BMI ($r=0.45$, $P<0.0001$). As well, serum levels of leptin and sCD163 were related to HOMA-IR ($r=0.28$ for sCD163, $r=0.24$ for leptin, both $P<0.0001$). An inverse correlation was observed between serum adiponectin levels and BMI ($r=-0.17$) or insulin resistance ($P=-0.23$ both $P<0.0001$).

The inflammatory markers that is leptin, leptin/adiponectin ratio and sCD163 were positively associated with the degree of fibrosis independently of MetS (Tables 2 and 3, Fig. 2), whereas the anti-inflammatory marker adiponectin was negatively associated (Tables 2 and 3). Adipokines and the other markers were also associated with the degree of fibrosis in univariate analysis but the association disappeared after adjustment on MetS except for leptin, leptin/adiponectin ratio and sCD163 (Table 2). sCD163 was also correlated with ALT ($r=0.14$,

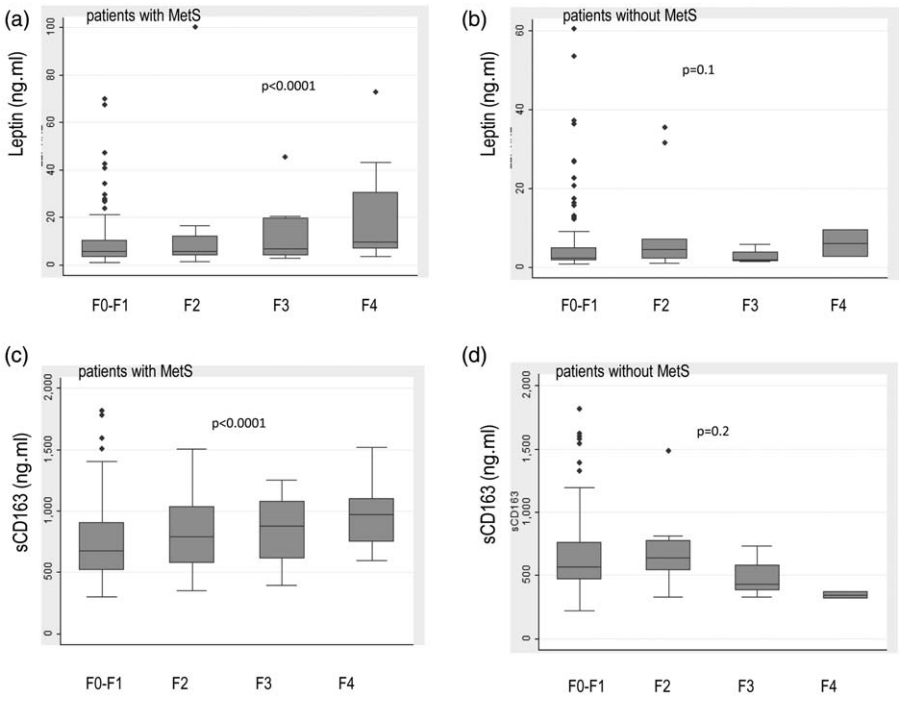

Fig. 2. Circulating levels of leptin (a and b) and soluble CD163 (c and d) according to the degree of fibrosis in patients with metabolic syndrome (a and c) and without metabolic syndrome (b and d). Boxes represent interquartile range (25–75th percentiles) with median, whiskers show upper and lower adjacent values, circles are outside values.

$P = 0.007$), but no association was observed between sCD14 levels and transaminases.

Discussion

Using LSM (Fibroscan) as a noninvasive marker of fibrosis, this large MetS exposed–unexposed cohort study provides strong evidence to consider HIV patients with MetS at high risk of liver fibrosis; obesity and insulin resistance being key players in hepatic fibrogenesis in this population independently of HIV known infection duration and severity and exposure to antiretroviral drugs.

We found that 25.1% of HIV monoinfected patients with MetS had significant fibrosis defined by LSM at least 7.1 kPa and 8.4% had suspected cirrhosis, whilst less than 8% of HIV patients without MetS had suspected fibrosis. After adjustment on the presence of MetS, we identified obesity and its biomarker (leptin), type 2 diabetes and insulin resistance (and its associated factor leptin/adiponectin ratio) as associated factors of fibrosis and cirrhosis. Obesity remained the strongest predictor of fibrosis in multivariable analysis independently of HIV-related parameters. These findings support the 'adipocentric concept' [32] of chronic liver disease and fibrosis in HIV monoinfection.

Our study has been focused on liver fibrosis rather than steatosis for three main reasons: first, a large number of studies have assessed the prevalence of liver steatosis in HIV-monoinfected patients with an estimate at 35% (95% CI 29–42) [15], and the deleterious impact of simple steatosis is considered as mild; second, fibrosis is the hallmark of the severity of chronic liver disease and the strongest predictor of liver-related mortality in non-HIV cohorts of NAFLD [5,33] and third, the proportion of significant liver fibrosis and cirrhosis and their risk factors have been poorly documented in HIV-monoinfected patients [15]. Although only one study suggested good performance of transient elastography for the detection of fibrosis in HIV patients with NAFLD [34], its excellent performances have been reported in non-HIV patients with better results than standard biochemical markers (i.e. APRI or Fibrosis-4) [35].

To date, only a few studies assessed the proportion of liver fibrosis using Fibroscan in nonalcoholic HIV-monoinfected patients and none of these studies compared the prevalence and severity of fibrosis between at-risk and unselected populations. In 300 consecutive HIV-monoinfected patients, Vuille-Lessard *et al.* [14] found 15% of patients with suspected significant fibrosis defined by LSM at least 7.1 kPa and 2.3% were classified as cirrhotics. In this study, BMI, diabetes mellitus and hypertension were independent factors of fibrosis and cirrhosis. Another study reported a 17.6% rate of significant fibrosis (LSM > 7.4 kPa) among 125 unselected HIV-monoinfected patients and interestingly MetS was an independent factor of fibrosis and cirrhosis [OR 3.99, 95% CI (1.001–16.09)] [30]. Liver biopsy-based studies have included a limited number of selected patients [9,11,12,16,36,37] and reported similar rates of significant fibrosis (15 to 30%) [12,15,16,36–38].

As previously reported [14,30], the level of liver transaminases was not identified as a marker of fibrosis in our study, suggesting that normal transaminases levels in HIV patients cannot exclude the presence of liver fibrosis.

In our study, HIV-related parameters were not clearly associated with the presence and severity of liver fibrosis. Despite a statistical association between $CD4^+/CD8^+$ ratio and extensive fibrosis (F3) and CDC stage B and cirrhosis, the relationship between the degree of fibrosis and the severity of HIV infection ($CD4^+$, $CD4^+/CD8^+$, nadir $CD4^+$, viral load) or ART regimen was not confirmed. Only a few studies analyzed factors associated with fibrosis in HIV-monoinfected patients; our findings are in line with previously published data. Lombardi *et al.* [30] and Morse *et al.* [36] did not find any association between fibrosis and either the duration and severity of HIV infection or the past or present use of ART, arguing against an impact of ART and HIV infection on liver fibrosis. This has been confirmed by a recent systematic review which analyzed risk factors of liver fibrosis in HIV-monoinfected patients with NAFLD [15].

In HIV-monoinfected patients, the mechanisms of hepatic fibrogenesis remain to be determined. As observed in non-HIV patients, our study suggests that in HIV-monoinfected patients, insulin resistance which is closely associated with obesity and the MetS, is central in the development of liver fibrogenesis. Although insulin resistance and MetS have been previously reported to play a role in liver steatogenesis in HIV infection [15,39], its role on liver fibrogenesis has been poorly analyzed in HIV patients. By contrast the influence of MetS and insulin resistance on liver fibrogenesis has been well documented in experimental studies and non-HIV patients [40]. In-vitro studies have shown that insulin promotes hepatic stellate cell differentiation into myofibroblast-like cells and the production of connective tissue growth factor expression; both leading to an excess amount of extracellular matrix components. Insulin resistance also induces hepatocyte apoptosis through lipid peroxidation, reticulum endoplasmic stress and oxidative stress and is associated with the release of profibrogenic cytokines (i.e. transforming growth factor-beta) and adipokines or cytokines (i.e. leptin, IL-6) [40].

Our study confirms that HIV-monoinfected patients with MetS have altered circulating concentrations of adipokines and increased levels of monocyte and macrophage activation markers (sCD14 and sCD163). This is in line with previous data, which reported high levels of leptin

and low level of adiponectin in HIV patients with MetS [41] and identified obesity in HIV patients as an independent factor of monocyte and macrophage activation [23]. However, one study, including a small number of HIV patients, did not find any significant correlation between sCD163 and sCD14 and some features of the MetS [42].

Significantly, only circulating levels of leptin, a reflect of fat mass, leptin/adiponectin ratio, a marker of insulin resistance and sCD163, a stronger marker of hepatic Kupffer cell activation than sCD14, were significantly associated with the degree of liver fibrosis in our study, independently of MetS. In these aging patients, treated for HIV infection for a median duration of 17 years, a phenotype of lipodystrophy was also commonly observed. To be able to analyze central fat accumulation we measured waist circumference in addition to BMI. High BMI and leptin, as a marker of total fat mass, were more strongly associated with fibrosis than waist circumference, stressing for the role of increased fat mass rather than of the lipodystrophic phenotype. In non-HIV patients with NAFLD, the role of adipose tissue and its adipokines in particular leptin and adiponectin have been extensively studied [20,43,44] and even identified as predictors of liver injuries [45]. In contrast, there is a paucity of data on sCD14 and sCD163 in non-HIV patients with NAFLD and fibrosis. Three studies conducted in NAFLD patients have found an association between sCD163 and the severity of fibrosis [18,19,22], but conflicting results have been reported on the correlation between serum sCD14 and liver fibrosis.

To best of our knowledge, no data on serum levels of adipokines or sCD14 and sCD163 and the degree of fibrosis in HIV monoinfected patients have been published so far. We found that levels of sCD163 were significantly associated with the degree of liver fibrosis and were correlated with ALT levels, suggesting a key role of Kupffer cells and hepatic macrophages in hepatic inflammation and fibrosis in HIV-monoinfected patients. In contrast, no association was found among sCD14, liver fibrosis and transaminases. It is likely that macrophage activation as measured by high level of sCD163 occurs in response to inflammatory state related to obesity and insulin resistance. However, the exact mechanisms of macrophage activation in hepatic fibrogenesis in HIV patients need to be confirmed.

Here we found that an increase in fat mass, associated with increased leptin levels, decreased adiponectin levels and with insulin resistance, was playing a major role in liver fibrosis, as observed in non-HIV infected individuals, supporting the adipocentric concept of liver fibrogenesis. Moreover, the independent association of sCD163 levels and fibrosis is in favor with a hepatic immune activation phenotype. Therefore, both mechanisms are probably involved.

The implication of increased fat mass and altered adipokine profile together with insulin resistance on the occurrence of cardiovascular disease has been previously demonstrated. Increased sCD163 levels are associated with vascular inflammation in HIV-infected patients [21]. Therefore, altered patterns associated with liver fibrosis in HIV-infected patients might be also responsible for an increased risk of cardiovascular disease in these patients, but this remains to be shown.

Our study has some limitations: first, we used a noninvasive marker of fibrosis which performances and cutoff have not been specifically validated in our study population. In addition Fibroscan did not allow for the assessment of the entire study population due to invalid results in 63 (13%) patients. Second, we did not histologically confirm the diagnosis of significant fibrosis and cirrhosis. We were also unable to look at the proportion of patients with NASH which diagnosis still relies on histology. Third, our study is a cross-sectional study and longitudinal follow-up is highly needed to assess the impact of liver fibrosis on morbidity and mortality in the HIV population. Finally, our study included mainly male patients. A recent study suggested that the degree of liver steatosis is significantly lower in HIV-infected women as compared with non-HIV women; therefore, our findings might differ in HIV-monoinfected women [46].

In summary, in HIV-monoinfected patients, MetS is an important risk factor of liver fibrosis. Obesity and insulin resistance are key factors associated with liver fibrosis independently of the duration of HIV infection and ART exposure. Adipose tissue and macrophage activation probably play an important role in the development of fibrosis in HIV-monoinfected patients, but the exact mechanisms need to be elucidated. Systematic screening for liver fibrosis should be performed in HIV-monoinfected patients with obesity or MetS independently of the normality of transaminases levels.

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Author's contribution: J.-L.M., M.L. and K.L. designed the study. M.L., L.F., M.S., K.L., P.-M.G., N.V. and J.-L.M. recruited the patients and were responsible for the clinical assessment of the patients. M.S. was in charge of the study management. K.L. was in charge of the statistical analysis. J.P.B., S.F. and J.C. have performed the

measurement of the metabolic and inflammatory biomarkers. M.L., K.L. and J.C. wrote the article which has been fully reviewed by J.-L.M. All the authors read and approved the article.

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Conflicts of interest

There are no conflicts of interest.

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