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Prospective evaluation of hepatic steatosis in HIV-infected patients with or without hepatitis C virus co-infection

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SUMMARY

Background: Limited data are available on hepatic steatosis (HS) in HIV patients who are not infected with hepatitis C virus (HCV). The aims of this study were to assess the prevalence of HS and its risk factors in HIV patients with and without HCV infection, and to evaluate whether HS correlates with advanced liver fibrosis and/or cardiovascular disease risk.

Methods: Fifty-seven HIV mono-infected and 61 HIV/HCV co-infected patients were enrolled consecutively. All patients underwent liver ultrasound and transient elastography. The main parameters of liver function, HIV and HCV viral loads, CD4+ cell counts, and data on highly active antiretroviral therapy (HAART) were recorded. Cardiovascular disease risk was evaluated using the 10-year Framingham risk score.

Results: HS prevalence in the whole HIV population was 53% (54% in mono-infected patients and 51% in co-infected patients). HS was associated with lipodystrophy and triglyceride values ($p < 0.0001$), metabolic syndrome ($p < 0.0004$), and total cholesterol levels ($p < 0.001$) in both HIV groups. In HIV mono-infected patients, HS was linked with HAART exposure of >1 year ($p < 0.01$). By multivariate analysis, only triglyceride levels ($p < 0.02$) and Framingham risk score ($p < 0.05$) were independently associated with HS in both HIV groups. No correlation was observed between HS and advanced liver fibrosis, measured by transient elastography.

Conclusions: HS was common in HIV patients, occurring in about half of the population. HS was found to be linked with the Framingham risk score, but was not correlated with advanced liver fibrosis. We suggest that in our HIV population with HS, the burden of cardiovascular disease risk is greater than that of liver disease progression.

1. Introduction

Hepatic steatosis (HS) includes a wide spectrum of conditions, characterized by the accumulation of lipid droplets within the cytoplasm of hepatocytes, ranging from fatty infiltration to steatohepatitis. Patients with steatohepatitis are at risk of progressive fibrosis, cirrhosis, and hepatocellular carcinoma.[1]

Before the era of highly-active antiretroviral therapy (HAART), HS prevalence in HIV-infected patients was about 30%,[2] similar to the general population.[3] HIV-infected patients may be at particular risk of HS, as HIV infection per se can result in fat deposition in the liver. In this respect, the chronic inflammatory state induced by the virus itself in patients with active infection, in particular the increase in levels of the pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), may lead to an increased synthesis of triglycerides by different mechanisms and, as a consequence, to their accumulation in fat droplets within the cytoplasm of hepatocytes.[4,5] In addition, new evidence shows that HIV-related microbial translocation may promote hepatic damage due to immune activation.[6] Little is known about the prevalence of HS and its risk factors among patients with HIV infection alone in the post-HAART era. Controversy remains regarding the effects of HAART on HS pathogenesis.[5] Other coexisting morbidities, such as obesity,[7] diabetes mellitus,[8] and alcohol intake, may cause steatogenesis in HIV patients, as is the case in the general population.[6] Also hepatitis C virus (HCV) may induce HS via several molecular mechanisms. Despite this evidence, many divergences persist regarding HS prevalence in HIV/HCV co-infected patients, with values ranging from 23% to 72%.[9]

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In the last few years interest in non-alcoholic fatty liver disease (NAFLD) and its relationship with cardiovascular disease (CVD) risk in the general population has increased. Although Ghouri et al.[10] stated that evidence for this association is weak, there is currently growing clinical evidence supporting the strong association between NAFLD and CVD risk.[11] To date, few data are available on the link between HS and CVD risk in the HIV population.[12]

Liver biopsy is the best diagnostic tool for the diagnosis of HS. However, it is an invasive technique and may result in sampling errors,[13–15] therefore it is not suitable for routine HS assessment. Ultrasonography, because of its low cost, non-invasiveness, and widespread availability, is accepted as an initial screening tool for HS[16,17] and is the most frequently used technique for HS diagnosis.[18] Thus, the aims of this study were to assess the prevalence of HS and its main risk factors in a group of HIV mono-infected and HIV/HCV co-infected patients. In addition, we sought to evaluate whether steatosis is correlated with advanced liver fibrosis, measured as liver stiffness (LS), and CVD risk, evaluated with the 10-year Framingham risk score.

2. Patients and methods

2.1. Study population

The study population consisted of HIV mono-infected and HIV/HCV co-infected patients enrolled consecutively from January 2009 to August 2010, who were followed-up prospectively at our AIDS center. All of these patients underwent ultrasound (US) and transient elastography of the liver.

Patients with acute liver events, hepatocellular carcinoma, chronic hepatitis B, decompensated cirrhosis, a body mass index (BMI) >30 kg/m^2, and/or without LS evaluation were excluded from the study.

Information on age, gender, risk factors for HCV and HIV infections, duration of antiretroviral therapy (ART), and exposure to non-nucleoside reverse-transcriptase inhibitors (NNRTI)/nucleoside reverse-transcriptase inhibitors (NRTI), protease inhibitors (PI), and specific dideoxynucleosides (d-drugs) were all recorded in a database designed for this study. HAART exposure was graded 0 (no HAART) to 2 (1: <1 year; 2: >1 year).

Alcohol intake >20 g/day either at the time of the study or in the past was recorded through patient interviews. BMI was calculated as weight (in kg) divided by height squared (m^2). Diabetes mellitus and impaired fasting glucose (IFG) were defined according to the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus criteria.[19] Diabetes and IFG were analyzed together because of the small number of patients. Smoking status was also evaluated. Metabolic syndrome was diagnosed according to the National Cholesterol Education Program definition.[20] The 10-year CVD risk was assessed in HIV mono-infected and co-infected patients by applying the Framingham risk score.[21] Lipodystrophy was defined as the presence of body fat changes, sub-cutaneous fat loss, and/or increased waist or buffalo hump, recognized by the patients and confirmed by the physicians.[22]

CD4 T-cell counts (most recent value and nadir) and plasma HIV-RNA levels were assessed in all HIV patients. In the HCV-infected patients, HCV genotype and plasma HCV-RNA levels were also recorded. Moreover, baseline complete blood cell count, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and glycemia, were measured in the total study population. The plasma concentration ratio of triglycerides to HDL cholesterol (TG/HDL-C ratio) was also calculated as a surrogate marker of insulin resistance.[23] All the blood tests at baseline were performed on samples from fasting patients.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Ultrasound assessment

Liver US was performed in the morning, after fasting for at least 10 h, by a single operator (MS), using a real-time Philips 5000 HDI apparatus with a 2–5 MHz convex multi-frequency probe.

The hepatic parenchyma echo-pattern was scored as: (1) normal (N), when echoes were homogeneously distributed and liver echogenicity was not increased in relation to the parenchyma of the right kidney; (2) 'bright liver' pattern (BL), characterized by numerous fine-packed, uniformly distributed echoes of high amplitude and increased echogenicity when compared to the parenchyma of the right kidney; (3) coarse pattern (C), represented by the presence of non-homogeneous, coarse, irregular echoes, without posterior beam attenuation;[24] and (4) coarse nodular pattern (CN), when multiple, distinct, weakly hypoechoic nodules (<6 mm in diameter) were widely spread over the liver parenchyma.[25]

BL is the hepatic echo-pattern characteristic of HS. BL severity was scored as: grade 1 (mild steatosis), characterized by increased echogenicity; grade 2 (moderate steatosis), accompanied by increased echogenicity and posterior beam attenuation with slightly impaired visualization of the intrahepatic vessels and diaphragm; grade 3 (severe steatosis), with a marked increase in echogenicity and marked posterior beam attenuation resulting in failure to demonstrate the intrahepatic vessels, diaphragm, and posterior right lobe of the liver.[26]

2.3. Liver fibrosis assessment

Liver fibrosis was assessed by a single certified operator (trained by the manufacturer) using transient elastography (FibroScan®; EchoSens, Paris, France), as previously described.[27] Transient elastography provides an assessment of liver stiffness (LS) expressed in units of kPa.

Advanced liver fibrosis (severe fibrosis and cirrhosis) was defined as a median LS of 9.5 kPa. This cut-off value is strongly correlated with a Metavir score of F3 in HIV/HCV co-infected patients.[28]

2.4. Statistical analysis

A descriptive analysis was carried out for all the clinical and demographic variables. When the data distribution was Gaussian, values were expressed as mean ± standard deviation and their differences were calculated using the Student's *t*-test. Otherwise, data were expressed as median and range and analyzed with the Mann–Whitney *U*-test. Fisher's exact test and the Chi-square test, Mantel–Haenszel Chi-square test, Spearman's rank correlation, and Pearson's correlation were used where appropriate. Multiple logistic regression analysis was performed to estimate the independence of the association between BL and variables significant on univariate analysis. Variables contributing significantly to the fit of the logistic equation were then selected by a step-wise procedure. A p-value of <0.05 was considered significant.

All analyses were performed using the SPSS software package (version 13.0; Chicago, IL, USA).

3. Results

3.1. Study population

One hundred and twenty-two patients were evaluated during the study period. An LS measurement was not obtained for four

Table 1
Baseline clinical and demographic features

Characteristics	HIV ($n=57$)	HIV/HCV ($n=61$)	p-Value
Age (years), mean ± SD	45 ± 9.0	45.2 ± 5.3	NS
Male gender, n (%)	38 (67)	48 (79)	NS
BMI (kg/m^2), mean ± SD	23.6 ± 3.2	23 ± 2.9	NS
Patients under HAART, n (%)	46 (81)	53 (87)	NS
Previous ART (years), median (range)	7 (0.1–17)	10 (0.1–21)	<0.03
Alcohol, n (%)	6 (11)	6 (10)	NS
IFG/diabetes, n (%)	2 (4)	14 (23)	<0.002
Lipodystrophy, n (%)	28 (49)	42 (69)	<0.004
Metabolic syndrome, n (%)	16 (28)	12 (20)	NS
Framingham risk score >10%, n (%)	14 (25)	7 (11)	NS

HCV, hepatitis C virus; SD, standard deviation; BMI, body mass index; HAART, highly active antiretroviral therapy; ART, antiretroviral therapy; IFG, impaired fasting glucose; NS, not significant.

HIV mono-infected patients due to truncular obesity, therefore 118 patients were eligible for inclusion in the study. Fifty-seven of the patients were HIV mono-infected and 61 were HIV/HCV co-infected.

Patient baseline characteristics at the time of US and LS measurement are shown in Table 1. No significant difference was found in mean age or BMI values between the HIV mono-infected and co-infected patients. At the time of US and LS measurement, most of the HIV mono-infected ($n=46$; 81%) and HIV/HCV co-infected ($n=53$; 87%) patients were on HAART. The duration of ART exposure was significantly longer in the co-infected than in the HIV mono-infected patients ($p<0.03$). IFG/diabetes was significantly more frequent in the co-infected than in the HIV mono-infected patients ($p<0.002$), as was lipodystrophy ($p<0.004$). No association was found between the number of HIV mono-infected and co-infected patients and metabolic syndrome.

Table 2 shows the main hematological and virological parameters in the study population. All of the HCV co-infected patients had detectable HCV-RNA and 44 patients had HCV-RNA >700 000 IU/ml. Serum ALT and AST levels were significantly higher in the co-infected than in the HIV mono-infected patients ($p<0.0001$). Total cholesterol, LDL cholesterol, and triglyceride levels were higher in the HIV mono-infected than in the co-infected patients ($p<0.0001$).

Table 2
Main hematological and virological characteristics

Characteristics	HIV ($n=57$)	HIV/HCV ($n=61$)	p-Value
ALT U/l, median (IQR)	23 (7–117)	54 (17–280)	<0.0001
AST U/l, median (IQR)	23 (11–56)	41 (17–281)	<0.0001
Total cholesterol (mg/dl), mean ± SD	207 ± 49	167.3 ± 41.6	<0.0001
HDL cholesterol (mg/dl), mean ± SD	42 ± 10.5	43.8 ± 16.4	NS
LDL cholesterol (mg/dl), mean ± SD	132.7 ± 40.6	95.2 ± 35.3	<0.0001
Triglycerides (mg/dl), median (IQR)	182 (49–615)	117 (50–614)	<0.0001
TG/HDL-C ratio, median (IQR)	3.9 (0.7–22.8)	3 (1–20)	NS
Genotype[a]			
Genotype 3, n (%)	-	19 (31)	-
Genotype 1 (n, %)	-	29 (48)	-
Genotype 2, 4	-	8 (13)	-
HCV-RNA (IU/ml) >700 000, n (%)	-	44 (72)	-
HIV-RNA <47 copies/ml, n (%)	33 (58)	41 (67)	NS
CD4+ count (cells/μl), median (IQR)	447 (35–1093)	422 (35–1208)	NS
CD4+ <200 cells/μl, n (%)	33 (58)	41 (67)	NS

HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, interquartile range; SD, standard deviation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG/HDL-C ratio, triglycerides to high-density lipoprotein cholesterol ratio; NS, not significant.
[a] Genotypes were not known for five patients, because of the non-eligibility of patients for ART.

3.2. Prevalence of BL and association with risk factors

The BL pattern was present in 62 (53%) patients in the total HIV population, of which 31/57 (54%) were HIV mono-infected and 31/61 (51%) were HIV/HCV co-infected (p = not significant). BL of grade 2/3 was present in only in 10 (16%) patients (seven HIV mono-infected and three co-infected).

Table 3 shows the associations between BL and risk factors analyzed in the whole population by univariate analysis. BL was associated with higher BMI ($p<0.05$), lipodystrophy ($p<0.0001$), and metabolic syndrome ($p<0.0004$). Higher values of total cholesterol ($p<0.001$), triglycerides and TG/HDL-C ratio ($p<0.0001$) were also associated with BL.

In addition, undetectable HIV-RNA ($p<0.03$) and CD4+ nadir <200 cells/μl ($p<0.03$) were associated with BL. Although the levels of HIV-RNA were higher in patients with BL than in those without BL (6500 (interquartile range 50–1 110 000) vs. 1750 (interquartile range 60–700 000) copies/ml), the difference was not significant ($z=0.47$; p = not significant). The correlation between the severity of BL and HIV-RNA levels was not significant either (rho = 0.07; p = not significant).

No significant difference was found in cumulative ART exposure between patients with and without BL. Using the HAART exposure score, we found that HAART exposure for more than 1 year was significantly associated with BL in both the total HIV population ($p<0.02$) and the HIV mono-infected patients ($p<0.01$) (Tables 3 and 4). No statistical difference was found when the patients with or without BL in both HIV groups were analyzed for exposure >1 year to the single classes of antiretroviral drugs and specifically d-drugs (Tables 3 and 4). Only the association between BL and NNRTI exposure >1 year was close to statistical significance ($p=0.07$).

Among the HIV mono-infected patients, BL was associated with lipodystrophy, metabolic syndrome ($p<0.02$), and higher values of total cholesterol ($p<0.003$), triglycerides ($p<0.002$), ALT ($p<0.04$), and TG/HDL-C ratio ($p<0.001$) (Table 4). Furthermore, BL was associated with undetectable HIV-RNA ($p<0.001$), CD4+ nadir < 200 cells/μl, and HAART exposure >1 year ($p<0.01$) (Table 4).

In the HIV/HCV co-infected patients, lipodystrophy ($p<0.003$), metabolic syndrome ($p<0.03$), and higher values of total cholesterol ($p<0.04$), triglycerides ($p<0.02$), and TG/HDL-C ratio ($p<0.05$) were associated with BL (Table 4).

3.3. Association between BL and other studied parameters (LS and Framingham risk score)

No association was observed between BL and liver fibrosis or between BL grading and advanced liver fibrosis (measured as LS). Figure 1 shows the correlation between the hepatic parenchyma echo-patterns and LS. Increasing values of LS were correlated with an advanced liver fibrosis ultrasound echo-pattern (coarse and coarse nodular echo-pattern) (rho = 0.24; $p<0.02$).

Framingham risk score values were significantly correlated with BL in both the HIV mono-infected and HIV/HCV co-infected patients (rho = 0.3; $p<0.004$).

3.4. Factors associated with BL on multivariate analysis

Among all the variables significantly associated with BL on univariate analysis, only triglyceride levels (β = 0.15; odds ratio

Table 3
Relationship between bright liver echo-pattern (BL negative/BL positive) and studied variables in the whole HIV population

Variables	Total HIV		Univariate analysis
	BL neg ($n=56$)	BL pos ($n=62$)	p-Value
Age (years), mean ± SD	44.2 ± 8.8	45.8 ± 5.5	NS
BMI (kg/m^2), mean ± SD	22.7 ± 2.9	23.8 ± 3.2	0.05
Alcohol use, n (%)	3 (5)	10 (16)	NS
Lipodystrophy, n (%)	23 (41)	47 (76)	0.0001
Metabolic syndrome, n (%)	5 (9)	23 (37)	0.0004
Total cholesterol (mg/dl), mean ± SD	170 ± 39	201 ± 53	0.001
HDL cholesterol (mg/dl), mean ± SD	44.7 ± 14	41.4 ± 13.7	NS
LDL cholesterol (mg/dl), mean ± SD	106.4 ± 37.2	120.4 ± 45	NS
Triglycerides (mg/dl), median (IQR)	111 (50–380)	180.5 (49–615)	0.0001
TG/HDL-C ratio, median (IQR)	2.6 (1–18)	4.2 (0.7–228)	0.0001
IFG/diabetes, n (%)	6 (11)	10 (16)	NS
ALT (U/l), median (IQR)	34.5 (7–188)	40.5 (7–280)	NS
AST (U/l), median (IQR)	29 (13–281)	31 (11–229)	NS
LS (kPa), median (IQR)	6.8 (3.2–48)	7 (3.3–33.8)	NS
HIV-RNA <47 copies/ml, n (%)	29 (52)	45 (73)	0.03
CD4 count <200 cells/μl, n (%)	30 (54)	46 (74)	0.03
Basal CD4 count cells/μl, median (IQR)	429 (73–1208)	460 (35–1009)	NS
Previous ART (years), median (IQR)	8.5 (0.1–18)	9 (0.33–21)	NS
HAART exposure >1 year, n (%)	39 (70)	57 (92)	0.02
PI use[a] (n)	31[b]	48[c]	NS
NNRTI use[a] (n)	17[d]	38[b]	NS
NRTI non-d-drug use[a] (n)	32	50	NS
NRTI d-drug use[a] (n)	35[b]	51[e]	NS

SD, standard deviation; NS, not significant; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IQR, interquartile range; TG/HDL-C ratio, triglycerides to high-density lipoprotein cholesterol ratio; IFG, impaired fasting glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LS, liver stiffness; ART, antiretroviral therapy; HAART, highly active antiretroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; d-drug, dideoxynucleosides.

[a] Exposure for >1 year.
[b] Data missing for three patients in the univariate analysis.
[c] Data missing for two patients in the univariate analysis.
[d] Data missing for five patients in the univariate analysis.
[e] Data missing for one patient in the univariate analysis.

Table 4
Presence of BL negative/BL positive ultrasound echo-pattern and associated variables in the two study groups

Variables	HIV		p-Value	HIV/HCV		p-Value
	BL neg ($n=26$)	BL pos ($n=31$)		BL neg ($n=30$)	BL pos ($n=31$)	
Age (years), mean ± SD	44.4 ± 11.2	45.6 ± 6.7	NS	44.0 ± 6.1	45.9 ± 4.1	NS
BMI (kg/m^2), mean ± SD	23.0 ± 3.4	24.2 ± 2.9	NS	22.5 ± 2.4	23.5 ± 3.4	NS
Alcohol use, n (%)	1 (4)	5 (16)	NS	1 (3)	5 (16)	NS
Lipodystrophy, n (%)	8 (31)	20 (65)	0.02	15 (50)	27 (87)	0.003
Metabolic syndrome, n (%)	3 (12)	13 (42)	0.02	2 (7)	10 (32)	0.03
Total cholesterol (mg/dl), mean ± SD	187.5 ± 34	224.1 ± 53	0.003	156.2 ± 38	178 ± 42.7	0.04
HDL cholesterol (mg/dl), mean ± SD	44.4 ± 9.8	40.2 ± 10.8	NS	45.1 ± 17	42.65 ± 16	NS
LDL cholesterol (mg/dl), mean ± SD	121.5 ± 36.3	142.1 ± 42.2	NS	93.4 ± 33.4	97.2 ± 37.7	NS
Triglycerides (mg/dl), median (IQR)	120 (62–261)	193 (49–615)	0.002	103 (50–380)	127 (81–614)	0.02
TG/HDL-C ratio, median (IQR)	2.6 (1–6.1)	4.7 (0.7–22.8)	0.001	2 (1–18)	3 (1–20)	0.05
IFG/diabetes, n (%)	1 (4)	1 (3)	NS	5 (17)	9 (29)	NS
ALT (U/l), median (IQR)	20 (7–80)	29 (7–117)	0.04	50.5 (17–188)	51 (18–280)	NS
AST (U/l), median (IQR)	21 (13–38)	25 (11–56)	NS	40.5 (21–281)	45 (22–229)	NS
LS (kPa), median (IQR)	5.6 (3.2–13.7)	6.2 (3.3–26.6)	NS	9.7 (4.3–48.8)	9.8 (4.3–33.8)	NS
HIV-RNA <47 copies/ml, n (%)	9 (35)	24 (77)	0.001	20 (67)	21 (68)	NS
CD4 nadir <200 cells/μl, n (%)	10 (38)	23 (74)	0.01	20 (67)	23 (74)	NS
Basal CD4 count, cells/μl, median (IQR)	438 (169–1093)	486 (35–964)	NS	407 (73–1208)	444 (35–1009)	NS
Previous ART (years), median (IQR)	5.5 (0.1–17)	8 (0.33–17)	NS	10 (0.16–18)	10 (0.42–21)	NS
HAART exposure >1 year, n (%)	15 (58)	28 (90)	0.01	24 (80)	29 (94)	NS
PI use[a] (n)	11	22	NS	20[b]	26[c]	NS
NNRTI use[a] (n)	10[c]	18	NS	7[b]	20[b]	NS
NRTI d-drug use[a] (n)	14	25	NS	21[b]	26[d]	NS
Genotype 3, n (%)	-	-	-	9 (30)	10 (32)	NS

BL, bright liver echo-pattern; HCV, hepatitis C virus; SD, standard deviation; NS, not significant; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IQR, interquartile range; TG/HDL-C ratio, triglycerides to high-density lipoprotein cholesterol ratio; IFG, impaired fasting glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LS, liver stiffness; ART, antiretroviral therapy; HAART, highly active antiretroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; d-drug, dideoxynucleosides.

[a] Exposure for >1 year.
[b] Data missing for three patients in the univariate analysis.
[c] Data missing for two patients in the univariate analysis.
[d] Data missing for one patient in the univariate analysis.

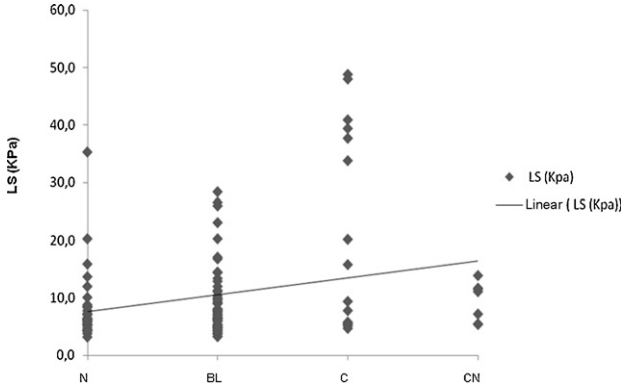

Figure 1. Correlation between hepatic ultrasound echo-pattern and liver stiffness in the study population (N, normal homogeneous echo-pattern; BL, bright liver echo-pattern; C, coarse echo-pattern; CN, coarse nodular echo-pattern). Hepatic ultrasound echo-pattern was significantly correlated with liver stiffness (rho = 0.24; $p < 0.02$).

(OR) 1.1, 95% confidence interval (CI) 1.05–1.3, $p < 0.02$) and Framingham risk score ($\beta = 1.4$; OR 4.3, 95% CI 1.1–17.7, $p < 0.05$) were independently associated with BL on multiple logistic regression analysis.

4. Discussion

The present study shows that the prevalence of HS, assessed by US, is high in both HIV mono-infected and HIV/HCV co-infected patients (54% and 51%, respectively). At present, data on the prevalence and risk factors of HS in HIV mono-infected patients are limited. In some studies, histological non-alcoholic steatohepatitis has been diagnosed only in patients who have undergone liver biopsy due to unexplained transaminase elevation or lipodystrophy.[8,29,30] To our knowledge, only three US-based studies on HS prevalence in HIV patients have been published. In one study, HS prevalence was 31%,[31] while a lower HS prevalence (13%) was found by Lesi et al.[32] in an HIV-positive African cohort and by Ryan et al.[33] in patients with only severe HS. The lower HS prevalence found in these former studies compared to our results may be due to the enrollment of Afro-American patients, who have been reported as having a lower HS prevalence in comparison with Caucasians, as well as the exclusion of patients with significant alcohol consumption.[31]

Several factors were found to be associated with HS in our HIV mono-infected and co-infected patients in the univariate analysis. Biochemical abnormalities, such as high total cholesterol and triglyceride values and metabolic syndrome were found in patients with HS.[9,31,34] As regards other metabolic factors, mean BMI was relatively low, as is frequently observed in HIV patients, and was not associated with HS.[30] Among the HIV-related factors, undetectable HIV-RNA was associated with HS in the mono-infected patients. As previously hypothesized,[35,36] it appears that undetectable HIV-RNA is a surrogate for HAART use. CD4+ nadir below 200 cells/μl was also associated with HS in our HIV mono-infected patients, only on univariate analysis. Discrepant data are reported in the literature on the relationship between immune status assessed by CD4+ cell count and HS;[9] nevertheless a recent study on the natural history of HS in co-infected patients showed that high CD4+ levels were associated with less progression of HS.[37]

Likewise, as regards the associations found between most of the described metabolic abnormalities and HS, there was a significant relationship only on univariate analysis between HS and HAART exposure for more than 1 year in both the total HIV population and the HIV mono-infected patients. As regards the lack of a significant association between BL and exposure for more than 1 year to single classes of antiretroviral drugs, the small number of patients could have played a crucial role.

In a recent meta-analysis,[9] only one out of eight studies evaluating PIs, NRTIs, and NNRTIs in co-infected patients showed a weak association between PI use and HS, and only McGovern et al.[35] found an association between NRTI use and HS. Guaraldi et al.[12] showed that cumulative NRTI exposure was an independent predictor of NAFLD. In this regard, lipodystrophy syndrome, which affects 40–50% of HIV patients under HAART and is often linked to insulin resistance, hypertriglyceridemia, low HDL levels, and metabolic syndrome, was associated with HS in both HIV mono-infected and co-infected patients.[8,38,39] In our HIV population, indirect signs of HAART-related effects such as lipodystrophy, higher triglycerides, total cholesterol levels, and undetectable HIV-RNA appear to corroborate the relationship between HAART exposure and HS. However, further studies are needed to confirm the role of HAART in HS pathogenesis and its natural history.

Ideally HS would be assessed by liver biopsy, but because of its invasiveness and risks this may be considered unethical for asymptomatic HIV mono-infected patients. In addition, ultrasonography has demonstrated a higher sensitivity in the detection of HS in patients without known liver disease and obesity,[26,40] as was the case for our HIV mono-infected patients with low BMI. However, the sensitivity of US in the diagnosis of HS is lower when infection with HCV genotype 1–2 occurs. This may explain in part the higher, even if not significant, HS prevalence found in HIV mono-infected compared with HCV co-infected patients. Nevertheless, the reduced need for liver biopsy in particular among co-infected patients with HCV genotype 2–3, due to the urgency of beginning and the better response to antiviral treatment, has encouraged the use of non-invasive methods in the assessment of both HS and fibrosis. In this regard, the prevalence of HS in co-infection is in agreement with what has been reported in other studies.[9] No association with HCV factors such as HCV genotype or viral load was found, and this may be due to the small percentage of patients infected with genotype 3. Another limitation of ultrasonography is its inability to distinguish simple HS from steatohepatitis.

Transient elastography has already been validated for the measurement of liver fibrosis in HIV and HCV seropositive patients.[28,41] Currently, this has been demonstrated to be a reliable tool in the non-invasive assessment of fibrosis, especially at the advanced stages in histological non-alcoholic steatohepatitis patients,[42] although in co-infected patients fibrosis may be overestimated by the presence of HS.[43] Nevertheless we did not find an association between BL and fibrosis or severity of BL and advanced liver fibrosis (measured as LS), in either HIV group. In this regard, Woreta et al.[37] recently showed that the majority of HIV/HCV co-infected patients (most of whom were black and had HCV genotype 1) had no HS or trivial HS, and compared with patients with significant HS, a significant difference in liver fibrosis was not found. Interestingly they showed there was no progression of HS in most patients.

On multiple logistic regression analysis, the only variables associated with HS without advanced liver fibrosis in the total HIV patients were both triglyceride levels and Framingham risk score. Several studies have reported the association of NAFLD with multiple CVD risk factors, including metabolic syndrome and insulin resistance.[11] The biological mechanisms by which NAFLD may contribute to a higher risk of CVD are not fully understood. Ectopic fat deposition, of which HAART-lipodystrophy is an expression, may play a leading role in the development of insulin resistance, inflammation, and NAFLD, and may be a source of multiple factors implicated in atherogenesis.[44] Calza et al.[45] recently showed that a longer exposure to ART and a diagnosis

of lipodystrophy syndrome were significantly associated with metabolic syndrome and diabetes, which confers an increased risk of CVD. Since in our HIV patients HS and CVD shared several risk factors (hypertriglyceridemia, HAART-lipodystrophy, metabolic syndrome, TG/HDL-C ratio, and total cholesterol), we suppose that HS should also be considered an early marker of CVD. These findings support the use of appropriate measures for maintaining triglycerides within the normal range in order to prevent both fatty liver and CVD in HIV patients. In addition, we suggest that in our HIV population with HS, the burden of CVD risk is greater than that of liver disease progression. Further studies are needed to evaluate the role of HS as an independent CVD risk factor in this population.

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