Etiology and Severity of Liver Disease in HIV-Positive Patients With Suspected NAFLD: Lessons From a Cohort With Available Liver Biopsies

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Background: Spectrum of liver injury among HIV-positive people is wide; in particular, prevalence of nonalcoholic fatty liver disease (NAFLD) seems to be higher compared with HIV-negative people.

Methods: We retrospectively evaluated all liver biopsies performed at Royal Free Hospital from 2000 to 2017 in HIV monoinfected patients with abnormal transaminases, to assess the underlying cause of liver disease and to characterize the extent of fibrosis. We furthermore evaluated the diagnostic accuracy of FIB4 and FibroScan as noninvasive tools for fibrosis assessment.

Results: Ninety-seven patients were included. Most common histological findings were NAFLD (28%), nonspecific changes (26%), and normal histology (13%). Twenty percent of the patients had significant fibrosis and 11% had advanced fibrosis. FIB4, at a cutoff of 1.3, had a specificity of 82% and negative predictive value (NPV) of 95% for exclusion of advanced fibrosis. FibroScan was available in 28% patients and 33% had a liver stiffness $\geq$7.5 kPa. FibroScan showed a specificity of 77% and NPV of 94% for exclusion of significant fibrosis. Among patients with NAFLD (n = 27), 18% had advanced fibrosis, whereas the majority (56%) did not have any fibrosis. The NPV of FIB4 for advanced fibrosis in these patients was 93%.

Conclusions: Among HIV-positive patients with elevated transaminases, a surprisingly high number of patients had nonsignificant changes or even normal histological findings. The prevalence of NAFLD was lower than reported in other series. Use of noninvasive tools with a high NPV for significant fibrosis can help reduce the number of required biopsies.

Key Words: HIV, liver histology, liver fibrosis, FIB4, FibroScan

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INTRODUCTION

HIV infection is a major global health issue with an estimated 36.7 million people living with HIV (PLWH) worldwide by the end of 2016.[1] The widespread availability of combination antiretroviral therapy (cART), in particular in countries with resource-rich settings, has led to near-normalization of life expectancy in HIV-positive people with access to testing and early ART. The "Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D)" study demonstrated the reduction in HIV-related mortality among PLWH but at the same time highlighted the increase in the proportion of patients dying from other causes and importantly, chronic liver disease (CLD) accounted for 10% of deaths during the years 2009–2011.[2]

HIV-positive people with hepatitis B virus/hepatitis C virus (HCV) coinfection are at higher risk of developing hepatic complications particularly at the commencement of ART therapy.[3,4] Nevertheless, irrespective of the presence of coinfection, liver disease and abnormal liver blood tests (LFTs) are more prevalent in people who are HIV-positive compared with the general population.[5] Despite the growing attention to this issue, clinicians still struggle to characterize etiology of abnormal LFTs in HIV monoinfected people[6,7] because the extent of liver abnormalities that can affect PLWH is vast.[8] Nonalcoholic fatty liver disease (NAFLD) is the most frequent underlying cause of abnormal LFTs among HIV monoinfected people[9–11] with a prevalence that seems to be higher than in the general population.[12] Moreover, there are efforts to validate tools of noninvasive fibrosis assessment among HIV-positive people, particularly in those with NAFLD.[13]

We therefore performed a retrospective review of all liver biopsies performed in our center from 2000 to 2017 in HIV monoinfected patients with abnormal LFTs; the aims were first, to assess the underlying cause of abnormal LFTs and to characterize the extent of liver fibrosis and, second, to

test the performance of noninvasive tests, particularly FIB4, in assessing fibrosis severity.

PATIENTS AND METHODS

This is a retrospective study in HIV monoinfected patients with available liver histology and any grade of abnormal LFTs for ≥3 months at the time of biopsy. Abnormal LFTs were defined as an alanine transaminase (ALT) value >35 U/L and/or aspartate transaminase (AST) >31 U/L according to the normal range values of the laboratory. We considered all the liver biopsies performed at Royal Free Hospital, an acute trust in North London with tertiary HIV and liver centers, from January 2000 to June 2017 in patients with diagnosed HIV who were aged 18 years or older. Liver biopsies were identified using the trust pathology departmental clinical database with "HIV" and "liver" as searching terms. Clinical and patient data were obtained from the HIV clinical database. Exclusion criteria were: HBsAg positivity; previous or current HCV infection (anti-HCV positivity); concurrent infection (opportunistic or nonopportunistic) or treatment for suspected infection at time of biopsy; liver malignancy; liver biopsies performed on transplanted or explanted livers or on focal liver lesions; samples deemed suboptimal according to the pathologist's assessment on the biopsy report; biopsies performed in patients with hepatic decompensation or lack of sufficient clinical data.

For each patient, we collected demographic, anthropometric, and clinical data [presence of diabetes, dyslipidemia, hypertension, cardiovascular disease, and lipodystrophy; duration since HIV diagnosis; exposure to cART; time between HIV diagnosis and cART initiation; duration of cART; exposure to nucleoside/tide reverse transcriptase inhibitors (NRTI), nonnucleoside/tide reverse transcriptase inhibitors (NNRTI), protease inhibitors, integrase inhibitors, and d-drugs (stavudine, didanosine, and zalcitabine) at the time of biopsy]. Diabetes, hypertension, and dyslipidemia were identified from medical records as listed diagnoses or when antidiabetic, antihypertensive, or lipid-lowering medications were prescribed, respectively.

Use of tobacco and illicit drugs and the degree of alcohol consumption were retrieved from the clinical records; alcohol abuse was defined as a daily consumption >30 g/d for men and >20 g/d for women.

Blood test results at the time of biopsy (or within ±6 months if not available) were collected (full blood count, AST, ALT, alkaline phosphatase, gamma-glutamyl transpeptidase, bilirubin, albumin, international normalized ratio, creatinine, urea, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein, triglycerides, HbA_1C, fasting glycemia, thyroid-stimulating hormone, fT4, CD4 cell count, CD8 cell count, CD4/CD8 ratio, and HIV viral load).

Histological findings were classified in 11 categories: normal findings, nonspecific changes, NAFLD, drug-induced liver injury, alcohol-related liver disease, mixed conditions (alcohol-related damage associated to other causes of liver damage), autoimmune liver diseases, rare metabolic liver diseases (hemochromatosis, alpha-1 antitrypsin deficiency), nodular regenerative hyperplasia, biliary diseases, and cryptogenic cirrhosis.

Fibrosis stage was assessed using a 0–4 point scale based on the report from the pathologist (0 = absent, 1 = mild, 2 = significant, 3 = advanced, 4 = cirrhosis), and disease-specific scoring systems used by the pathologist were converted into this scale. Biopsies included in the group "nonspecific changes" were assessed for grade of inflammation on a 0–3 point scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe). Biopsies in keeping with NAFLD were subclassified in simple steatosis (nonalcoholic fatty liver disease (NAFL): steatosis in ≥5% of hepatocytes) and nonalcoholic steatohepatitis (NASH: concurrent presence of steatosis, lobular inflammation, and ballooning degeneration).

To assess the accuracy of noninvasive measures of liver fibrosis, FIB4[14] was calculated for each patient [age (y)*AST (U/L)/platelet count (10^9/L)*√ALT (U/L)].[15] A cutoff of <1.3 was used for exclusion of advanced fibrosis.

Liver stiffness assessed by FibroScan (Echosens, Paris, France) was also considered for a subset of patients who had FibroScan performed within 1 year of the biopsy. FibroScan has been widely demonstrated as a reliable tool to detect liver fibrosis but no established cutoffs exist for differentiating fibrosis stages. We considered a cutoff of 7.5 kPa for detection/exclusion of significant fibrosis.[16]

Statistical Analysis

Distribution of continuous variables was assessed and presented as mean ± SD (parametric data) or median (interquartile range; nonparametric data). Tests of normality were used to assess the distribution of variables. Categorical variables were presented as frequencies and percentages (n, %). Univariate analysis was performed to find determinants of significant and advanced fibrosis. Comparison between categorical (fibrosis severity) and continuous variables was performed using the Student T-test for normal variables and the Mann–Whitney test for not normal ones. Comparison between categorical variables was performed with the χ^2 test. A 2-tailed p-value ≤0.05 was considered statistically significant. Only variables with p-value ≤0.05 were entered in the multivariable analysis. Multivariable logistic regression was used to identify independent predictors of significant and advanced fibrosis. All data were analyzed using the statistical package SPSS (version 22; IBM, New York, NY).

RESULTS

Selection of Biopsies and Baseline Characteristics

We collected a total of 177 biopsies. From this initial pool, we excluded biopsies performed in patients with opportunistic and nonopportunistic infections (n = 26), missing clinical data (n = 17), coinfection with HCV/hepatitis B virus (n = 16), explanted/transplanted livers (n = 7), multiple biopsies performed in the same patient, in which cases the most recent biopsy was used (n = 7), hepatic decompensation (n = 4), malignancy (n = 2), and inadequate sample quality (n = 1). Finally, 97 biopsies fulfilled our selection criteria. All included biopsies were performed to

TABLE 1. Characteristics of the Study Population

Variable	Total Study Population (n = 97)	Patients With NAFLD (n = 27)	Patients Without NAFLD (n = 70)	*P*
Age, y ± SD	47 ± 10	48 ± 10	47 ± 10	0.49
Male, n (%)	79 (81)	27 (100)	52 (74)	**0.002**
BMI, kg/m^2 ± SD	27 ± 6	29 ± 5	26 ± 7	**0.003**
Ethnicity, n (%)				
White	64 (66)	21 (78)	43 (61)	0.16
Black	29 (30)	4 (15)	25 (36)	0.05
Others (Hispanic, Asian, mixed)	4 (4)	2 (7)	2 (3)	0.31
ETOH abuse, n (%)	17 (20)	—	17 (29)	—
Smoking status, n (%)				
Never	29 (39)	9 (39)	20 (38)	1
Past	22 (29)	7 (30)	15 (29)	1
Current	24 (32)	7 (30)	17 (33)	1
Diabetes mellitus, n (%)	11 (11)	5 (19)	6 (9)	0.17
Arterial hypertension, n (%)	20 (21)	8 (30)	12 (17)	0.26
Dyslipidemia, n (%)	46 (47)	22 (81)	24 (34)	**<0.001**
History of coronary disease, n (%)	4 (4)	3 (11)	1 (1)	0.064
History of lipodystrophy, n (%)	29 (30)	8 (30)	21 (30)	1
Laboratory results				
Platelet count, 10^9/L ± SD	217 ± 76	216 ± 52	219 ± 84	0.83
ALT, UI/L (IQR)	71 (69)	76 (57)	61 (71)	0.25
Ferritin, ng/mL (IQR)	214 (477)	181 (289)	224 (596)	0.45
Tot cholesterol, mmol/L ± SD	4.9 ± 1.2	5.1 ± 1.2	4.9 ± 1.2	0.33
HDL cholesterol, mmol/L ± SD	1.3 ± 0.5	1.1 ± 0.3	1.4 ± 0.5	**<0.001**
Triglyceride, mmol/L (IQR)	1.7 (1.6)	2.5 (1.7)	1.4 (1.3)	**<0.001**
Glycemia, mmol/L (IQR)	5.1 (1.7)	5.4 (1.7)	5 (1.6)	0.21
CD4 count, cell/UL (IQR)	507 (361)	636 (273)	448 (337)	0.09
HIV RNA undetectable (<50 copies/mL), n (%)	72 (74)	20 (74)	52 (74)	0.72
HIV RNA when detectable, copies/mL (IQR)	18,624 (50,692)	11,541 (41,563)	22,215 (91,179)	0.52
Duration of HIV infection, months (IQR)	126 (111)	129 (130)	129 (113)	0.48
HAART, n (%)				
Naive	8 (8)	1 (4)	7 (10)	0.44
Experienced	89 (92)	26 (96)	63 (90)	0.44
On treatment	86 (89)	25 (93)	61 (87)	0.72
Duration of HAART, months (IQR)	100 (86)	100 (90)	101 (89)	0.7
NRTI exposure, n (%)	87 (92)	25 (96)	62 (90)	0.44
NNRTI exposure, n (%)	66 (72)	16 (62)	50 (76)	0.2
Protease inhibitors exposure, n (%)	65 (68)	19 (73)	46 (67)	0.63
Integrase inhibitor exposure, n (%)	5 (6)	2 (8)	3 (5)	0.62
Entry inhibitor exposure, n (%)	2 (2)	0 (0)	2 (3)	1
D-drug exposure, n (%)	51 (55)	16 (62)	35 (53)	0.49

D-drugs are zalcitabine, stavudine, and didanosine.
Bold entries are statistically significant *P*-value (<0.05).
ETOH, alcohol; HAART, highly active antiretroviral therapy; IQR, interquartile range.

investigate abnormal LFTs and/or to determinate fibrosis severity.

Table 1 summarizes the baseline characteristics of the population. Mean age of the population was 47 ± 10 years, 81% were male, and 66% of the population were of white ethnicity. Mean body mass index (BMI) was 27 ± 6 kg/m^2. Documentation on alcohol consumption was available for 85 (88%) patients, 20% of which reported hazardous alcohol use. Forty-seven percent of patients had dyslipidemia, 11% had diabetes, and 4% had a history of cardiovascular disease (3 acute myocardial infarctions and 1 chronic myocardial ischemia). Most of the patients had undetectable HIV viral load (74%) and were on ART at the time of biopsy (89%). Three patients stopped cART before the biopsy (2 patients 5 months before and 1 patient 3 years before), whereas 8 patients had never been on cART at the time of the biopsy; thus, 92% of patients were cART experienced (past or current exposure to cART) with a median duration of cART of 100 months. Median duration since HIV diagnosis was 126 months. The majority of the patients were NRTI (92%),

NNRTI (72%), protease inhibitors (68%), and d-drugs (55%) experienced.

Histological Findings

The most common histological findings were NAFLD (n = 27, 28%) and nonspecific changes (n = 25, 26%). Twelve biopsies (13%) showed a completely normal picture. Less common findings were consistent with drug-induced liver injury (n = 8, 8%), alcoholic liver disease (n = 5, 5%), nodular regenerative hyperplasia (n = 5, 5%), rare metabolic liver disease (n = 4, 3 with hemochromatosis and one with alpha-1 antitrypsin deficiency), mixed conditions associated with some degree of alcohol damage (n = 4, of which 3 in keeping with alcohol and NASH based on clinical history and metabolic risk factors and one showing alcohol damage plus signs of porphyria cutanea tarda), autoimmune disease (n = 3, 3% of which 2 in keeping with treated autoimmune hepatitis and 1 consistent with autoimmune cholangitis), biliary disease (n = 2, one secondary biliary cirrhosis due to gallstones and one HIV cholangiopathy), and cryptogenic cirrhosis (n = 2, of which one probably associated with heart failure).

Significant fibrosis, defined as ≥F2 stage, was present in 19 (20%) patients, whereas advanced fibrosis, defined as ≥F3 stage, was present in 11 (11%) patients. The majority of patients had no fibrosis (n = 61, 63%). Among biopsies that showed nonspecific changes, more than half had mild inflammation (n = 19, 76%) and absence of fibrosis (n = 18, 72%) (see Table 1, Supplemental Digital Content 1, http://links.lww.com/QAI/B266, summarizes the histological findings).

Factors associated with the presence of significant fibrosis in the univariate analysis were history of coronary artery disease, lower HDL cholesterol levels, detectable HIV viral load, and diagnosis of NAFLD. These variables (excluding history of coronary artery disease due to the small number of cases) were included in the multivariable analysis, and only HDL cholesterol level remained significantly associated with the presence of significant fibrosis at histology. No significant association was found with any other variable.

NAFLD Subgroup

Compared to the non-NAFLD population, the NAFLD cohort was composed only of men (100%) with a higher BMI (29 kg/m^2), higher prevalence of dyslipidemia (81%), lower level of HDL, and higher level of triglycerides. No difference was found in any other variable traditionally linked to NAFLD (diabetes, hypertension). Baseline characteristics of patients with NAFLD are shown in Table 1. Among the 27 biopsies in keeping with NAFLD, 15 (55%) had simple steatosis, 8 (30%) met the diagnostic criteria for NASH, and 4 (15%) had NASH-cirrhosis. Significant and advanced fibrosis were present in 9 (33%) and 5 (18%) biopsies, respectively. More than a half of the biopsies (n = 15, 56%) showed no fibrosis. Table 2 summarizes the histological findings in the NAFLD subgroup.

TABLE 2. Histological Findings of Patients With NAFLD (n = 27)

	n (%)
Severity of NAFLD	
Steatosis	15 (55)
NASH	8 (30)
Cirrhosis	4 (15)
Tot	27 (100)
Breakdown for single histological finding	
Steatosis	
Mild	9 (33)
Moderate	15 (56)
Severe	3 (11)
Inflammation	
Absent	7 (26)
Mild	16 (59)
Moderate	4 (15)
Cellular ballooning	
Absent	16 (59)
Few	10 (37)
Many	1 (4)
Fibrosis	
Stage 0	15 (56)
Stage 1	3 (11)
Stage 2	4 (15)
Stage 3	2 (7)
Stage 4	3 (11)

The presence of NAFLD of any severity was associated with the presence of significant fibrosis only in the univariate analysis.

Accuracy of FIB4 in Excluding Advanced Fibrosis

The prevalence of FIB4 scores <1.3, 1.3–2.67, and >2.67 was 38%, 46%, and 16%, respectively. Table 3 illustrates the matching between histology and FIB4 values in our cohort: FIB4 had 82% specificity and 95% negative predictive value (NPV) for ruling out advanced fibrosis. In the NAFLD subgroup, the prevalence of FIB4 scores <1.3, 1.3–2.67, and >2.67 was 52%, 41%, and 7%, respectively. Specificity and NPV for the exclusion of advanced fibrosis were similar to the general cohort (80% and 93%, respectively).

Accuracy of Liver Stiffness Assessed by FibroScan in Detecting Significant Fibrosis

FibroScan values within 1 year of the biopsy were available for 27 (28%) patients and 9 (33%) of them had a liver stiffness >7.5 kPa. FibroScan had a sensitivity of 80% (4/5), specificity of 77% (17/22), and NPV of 94% for significant fibrosis. In the NAFLD subgroup, 9 patients had valid FibroScan values of which 6 (67%) were >7.5 kPa. Sensitivity, specificity, and NPV were 100%, 60%, and 100%, respectively. Among patients who had FIB4 <1.3

TABLE 3. Matching Between FIB4 Values and Fibrosis Stage in all Patients (n = 97) and in the NAFLD Subgroup (n = 27)

	Total Study Population			Patients With NAFLD		
	FIB4 <1.3	FIB4 ≥1.3	Tot	FIB4 <1.3	FIB4 ≥1.3	Tot
Fibrosis stage <3	35	51	86	13	9	22
Fibrosis stage ≥3	2	9	11	1	4	5
Tot	37	60	97	14	13	27
Specificity	82% (9/11)			80% (4/5)		
Sensitivity	41% (35/86)			59% (13/22)		

and FibroScan (11 patients), 6 had a liver stiffness <7.5 kPa, and liver histology was in keeping with absence of fibrosis in all of them; among the 5 patients with a liver stiffness ≥7.5 kPa, only 1 patient had advanced fibrosis on histology. The correlation between FIB4 and FibroScan values is shown in Table 2, Supplemental Digital Content 1, http://links.lww.com/QAI/B266.

DISCUSSION

In this study, we evaluated the underlying cause of abnormal LFTs in HIV monoinfected patients referred for liver biopsy and assessed the severity of liver injury. Our study highlighted that NAFLD and nonspecific changes were the most common findings in this population. We also found that a significant proportion of HIV people with raised transaminases had a completely normal histological picture, and the majority undergoing a liver biopsy had mild or no fibrosis. Based on this, we examined our cohort for the potential utility of a simple serological test (FIB4) in excluding severe liver disease and we demonstrated its excellent performance in ruling out advanced fibrosis (NPV 95%). In addition, in a small subgroup of patients with available FibroScan results, we showed that FibroScan had an equally excellent performance in excluding significant fibrosis (NPV 94%).

Although this study was not designed to establish the overall prevalence of NAFLD in HIV monoinfected patients, it did confirm the significant prevalence of NAFLD among people with HIV (28% in those biopsied in our study). Interestingly, a recent meta-analysis shows that the global prevalence of NAFLD among the general population detected by different imaging techniques is 25.2%[17]: taken together, these data suggest that the prevalence of NAFLD among HIV patients might not significantly differ compared to the general population.

In line with our findings, several other studies have demonstrated that the burden of NAFLD in HIV monoinfected patients may be similar to that in the HIV-negative population.[12] Of note, studies that included HIV patients with elevated LFTs and available liver histology documented a high prevalence of NAFLD, in some cases almost 3 times higher than that observed in our study.[9–11,18] The discrepancy between these data and ours is partially due to different diagnostic methods, patient inclusion criteria and, for older studies, different histological criteria used to define NASH. In our study, we did not exclude a priori patients with alcohol abuse (20% of our cohort) or patients with known history of CLD, although only a small percentage (20%) had a diagnosis or high suspicion of CLD before undergoing liver biopsy. Exact definition of NASH has to be carefully evaluated when considering older studies because several changes has been made over the years in the diagnosis of NASH, before the current histological criteria.[19]

Taking all this into account, we believe that the prevalence of NAFLD in HIV cohorts identified from published studies, especially those with histological endpoints, may be an overestimation due to selection bias. Interestingly, NAFLD patients in our cohort differed significantly compared with non-NAFLD patients only for gender, BMI, and some characteristics of lipid profile (a more frequent diagnose of dyslipidemia, more elevated triglyceride levels, and lower HDL levels), whereas no difference in diabetes and hypertension prevalence was found; this could be due to a type II error related to the small sample size, although we cannot exclude differences in the mechanisms of development of NAFLD among HIV patients and uninfected population.

The second most common histological finding in our study was consistent with nonspecific changes. This includes biopsies with only minimal steatosis, inflammation, or fibrosis and nondiagnostic features, which did not allow the pathologist to formulate a specific diagnosis. Moreover, more than a half of our biopsies (72%) showed no evidence of fibrosis. These findings are not uncommon; in other studies on HIV monoinfected patients with available liver histology, nonspecific abnormalities or biopsies without significant steatosis/inflammation were found in 13%–35% of cases.[9–11] Moreover, in our study, a substantial number of biopsies reassuringly showed a completely normal picture (13%). Minimal lesions or normal histological pictures are also a common finding in studies involving HIV-negative populations. For example, de Ledinghen et al[20] reported liver biopsy results in 272 HIV-negative patients with unexplained chronically elevated ALT, which showed normal/almost normal liver in 20% of cases.

These results raise 2 main issues. First, as already noticed by Morse,[11] there is a considerable overlap in the clinical, biochemical, and imaging background among patients with nonspecific/nondiagnostic abnormalities and frequent pathological conditions such as NAFLD and NASH, suggesting the need to develop further reliable criteria to discriminate between these 2 liver conditions. Second, it is important to re-evaluate the criteria clinicians rely on to perform a liver biopsy and to incorporate a more widespread use of noninvasive tests for diagnosis and fibrosis assessment before performing a liver biopsy in order to prevent unnecessary risks.

Therefore, we evaluated the extent and the severity of liver fibrosis among HIV monoinfected people because fibrosis is the strongest predictor of liver-related mortality and is independently associated with long-term overall mortality in NAFLD.[21,22] More than half of the included patients had no evidence of fibrosis (63%), whereas significant and advanced fibrosis was detected in 19% and 11%,

respectively, in line with the prevalence reported in similar studies.[12] In our study, the only factor independently associated with significant fibrosis was a lower level of HDL, whereas we could not identify predictive factors for advanced fibrosis. This is likely due to a type II error due to the relatively small number of patients and the heterogeneity in the diagnoses. Data from literature showed that abnormal lipid profile, especially low HDL and high triglycerides levels, has a higher prevalence in metabolic syndrome (MS) in HIV-positive compared to HIV-negative people with MS. In addition, not only is MS more prevalent in HIV-positive people, but also the composition of it differs compared with the general population.[23,24] However, there is some evidence that HIV-positive people with NAFLD are more likely to have features of severe liver injury than HIV-negative people with NAFLD[18,25] despite a lower BMI and more intense physical activity.[26] This suggests that factors other than those traditionally linking NAFLD and MS components can drive liver injury in these patients, such as the HIV infection per se or the prior use of ART.

Neither diabetes nor BMI was found to predict liver fibrosis in our cohort, likely due to the small sample size or the relatively low BMI and prevalence of diabetes in our population. Our findings contrast with the data from other studies,[27–30] a meta-analysis,[12] and from data on HIV-negative patients where insulin resistance is associated with severe fibrosis.[31,32] No factor associated with HIV infection or cART correlated with the presence of significant or advanced fibrosis in our population. Although we had a small sample size to be able to detect such differences, most of the studies in HIV-positive people have so far failed to convincingly associate HIV infection and its related therapy to NAFLD and fibrosis. Some exceptions are the studies by Guaraldi, who correlated NRTI exposure with the presence of NAFLD detected by CT[33] and studies by Blanco, Matthews, and Pembroke, who found an association, respectively, between DDI/D4T exposure, HIV viremia, longer duration of HIV, and detectable HIV viral load with higher fibrosis stages assessed with FibroScan.[28,34,35] These discrepancies could be due to patient exposure to earlier generations of ART with greater liver toxicity.

Considering the importance of detecting fibrosis in patients with suspected liver injury, we tested 2 different noninvasive tools, FIB4 and FibroScan, looking at their potential utility in avoiding unnecessary biopsies.

FIB4 was initially validated in a cohort of HIV-HCV coinfected patients[14] showing a good performance in ruling out advanced fibrosis. In our cohort, a cutoff of 1.3 had a sensitivity of 41% and a specificity of 82%, respectively, for advanced fibrosis, and NPV was 95% in the general cohort and 93% in the NAFLD subgroup. Using FIB4 to triage our patients, we could have spared almost 40% (37/97) biopsies at the expense of a minor percentage of patients (5%, 2/37) who would have been incorrectly classified. This obviously translates to a double benefit because it saves costs and reduces the risk related to invasive procedure for the patient.

The drawback of such a kind of test is the relatively high percentage (46% in our cohort) of results included in the gray zone, which need to be further investigated. FibroScan, as already demonstrated in HIV monoinfected patients,[36] had a sensitivity of 80%, specificity of 77%, and NPV of 94% in ruling out significant fibrosis. Importantly, noninvasive fibrosis tests perform better in excluding rather than detecting fibrosis.[37]

Overall, these results support the validity of using noninvasive tests to triage HIV patients with suspected liver injury according to their risk of significant or advanced fibrosis[38] in order to select the appropriate patients for a liver biopsy. Because there were a significant number of HIV monoinfected patients with normal histology or nonspecific minimal changes, the use of noninvasive fibrosis tests would ensure a more careful selection before proceeding to a biopsy.

Limitations of the study are first, its retrospective nature and second, the lack of central reading for liver biopsies as we relied only on the final report made by the local pathologist. The small sample size limited the statistical power to detect associations with NAFLD and liver fibrosis. Because the referral for liver biopsy was based on an individual clinical decision, our population was also characterized by heterogeneity.

In conclusion, we showed that the most common causes of abnormal LFTs among HIV monoinfected patients referred for liver biopsy are NAFLD and nonspecific changes and that these two conditions could not be distinguished based on LFT's elevation only. Indeed, raised LFTs should not trigger automatically a decision for a liver biopsy because the probability of finding normal/near-normal histology is high. Patients should be triaged using noninvasive tools of fibrosis assessment, such as FIB4 and FibroScan; those with low values on these tests should only undergo biopsy if there are specific clinical concerns because the probability of advanced fibrosis is very low.

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