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Increased Body Mass Index and Type 2 Diabetes Are the Main Predictors of Nonalcoholic Fatty Liver Disease and Advanced Fibrosis in Liver Biopsies of Patients With Human Immunodeficiency Virus Monoinfection

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Background. Liver disease is an important cause of morbidity and mortality in people living with human immunodeficiency virus (PLWH), of which nonalcoholic fatty liver disease (NAFLD) is an increasingly recognized cause. There are limited data investigating NAFLD in HIV monoinfection and histologically defined disease. We aimed to identify who is at risk of fibrosis, NAFLD, and nonalcoholic steatohepatitis (NASH) among PLWH and explore the diagnostic accuracy of noninvasive markers of fibrosis.

Methods. This was a retrospective, cross-sectional, international, multicenter study including patients with HIV monoinfection, without chronic viral hepatitis or other known causes of chronic liver disease, who underwent liver biopsy for abnormal liver biochemistry and/or clinical suspicion of liver fibrosis.

Results. A total of 116 patients from 5 centers were included. Sixty-three (54%) had NAFLD, of whom 57 (92%) had NASH. Overall, 36 (31%) had advanced fibrosis (≥F3) and 3 (3%) had cirrhosis. Of the 53 cases without NAFLD, 15 (28%) had advanced fibrosis. Collagen proportionate area was similar between cases with and without NAFLD (3% vs 2%). Body mass index was independently associated with NAFLD (aOR, 1.2; 95% CI, 1.08–1.34), and type 2 diabetes was independently associated with advanced fibrosis (aOR, 3.42; 95% CI, 1.00–11.71). The area under the curve for advanced fibrosis was 0.65 and 0.66 for both NAFLD Fibrosis Score (NFS) and FIB-4. Cutoff values of −1.455 (NFS) and 1.3 (FIB-4) have negative-predictive values of 0.80 and 0.82, respectively.

Conclusions. Advanced fibrosis is strongly associated with type 2 diabetes in PLWH. Serological markers require further optimization.

Keywords. NAFLD; NASH; fibrosis; HIV; histopathology.

Nonalcoholic fatty liver disease (NAFLD) is defined by the presence of hepatic steatosis in the absence of secondary causes such as excessive alcohol consumption. It encompasses a large spectrum of disease from nonalcoholic fatty liver (NAFL; "simple steatosis") to hepatocyte inflammation/ballooning defined as nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis.

A major limitation in the literature to date on NAFLD in people living with human immunodeficiency virus (HIV; PLWH) has been little available data based on disease stage defined by liver histology. Despite the advances of noninvasive markers, NASH remains a histological diagnosis and the gold standard for fibrosis staging is liver biopsy. Therefore, accurate phenotyping of patients with NAFLD requires a histological diagnosis, and this is particularly needed in special populations such as PLWH in whom noninvasive markers have not been well validated [1] and the pathogenesis of liver disease is potentially more complex.

To facilitate research collaborations within this field, an international consortium of clinical academics with expertise on HIV-NAFLD was established: the Steatohepatitis in HIV Emerging Research (SHIVER) Group. This study represents the

inaugural project for the group. The primary objective was to assess the histopathological features of liver biopsies performed in HIV monoinfection and identify risk factors associated with fibrosis, NAFLD, and NASH. The secondary objective was to assess the performance of noninvasive tests for the diagnosis of liver fibrosis in patients with HIV monoinfection using liver histology as a reference standard.

METHODS

Study Population
This study was a retrospective international, multicenter, cross-sectional study. Five centers from the United Kingdom (Imperial College and Royal Free Hospital), Italy (University of Modena), the United States (University of California San Francisco), and Canada (McGill University) collected liver biopsy samples from adult (age ≥18 years) cases with HIV monoinfection. Exclusion criteria were positive hepatitis C antibody or hepatitis B surface antigen; current or recent (within 6 months) alcohol excess defined as 21 units or more per week for men and 14 or more units per week for women; concurrent life-threatening illness, active malignancy, AIDS-defining illness, or evidence of other chronic liver disease at the time of liver biopsy, including biliary disease, autoimmune hepatitis, Wilson's disease, and hereditary hemochromatosis; and long-term exposure to steroids or amiodarone. Clinical data nearest to the time of liver biopsy and within 6 months were collected, including basic demographics and anthropometrics, liver biochemistry, HIV history including drug exposure and HIV-specific complications, and medical comorbidities. The Triglyceride Glucose Index (TGI) was used as a surrogate marker of insulin resistance: TGI = ln(triglycerides [mg/dL] × fasting glucose [mg/dL]) [2].

Liver Biopsy and Histological Analysis
Liver biopsies were performed as part of the clinical evaluation of patients with unexplained elevations in liver transaminases and/or clinical suspicion of liver fibrosis, either by percutaneous or transjugular approach, with a minimum core of 10 mm.

Liver samples were paraffin fixed and formalin embedded, and stained with hemotoxylin and eosin and Sirius red. Liver biopsy slides were centrally read by an expert liver histopathologist (R. G.) blinded to clinical and biological data, scored according to the NASH Clinical Research Network (CRN) system (Nonalcoholic Steatohepatitis [NAS] score) [3] and classified as NAFLD or NASH according to the Fatty Liver Inhibition of Progression (FLIP) algorithm [4]. Fibrosis stage was defined by the Brunt classification [5].

Collagen proportionate area (CPA) was quantified on picro-Sirius red–stained formalin-fixed, paraffin-embedded sections using previously published methods [6]. Image capture was carried out at 4× objective magnification on a Zeiss Azioskop 50 using a Zeiss Axiocam ICc5 camera. Image analysis was carried out by A. H. in a single center (Royal Free Hospital, London, UK) on the captured images using a custom script for Zeiss Axiovison software: segmentation of tissue and collagen was achieved in an RGB color-space, followed by a manual editing step to remove areas not related to pathological collagen deposition, such as structural collagen in portal tracts and image artefacts. The CPA was calculated as the amount of collagen expressed as a percentage proportion of overall biopsy tissue area, as previously published [6].

Noninvasive Markers of Liver Fibrosis
The following noninvasive markers of fibrosis were calculated: FIB-4 = age (years) × aspartate aminotransferase (AST; IU/L)/platelets $(10^9/L)$ × √ alanine aminotransferase (ALT; IU/mL) [7]; NAFLD Fibrosis Score (NFS) = −1.675 + 0.037 − age (years) + 0.094 − body mass index (BMI; kg/m^2) + 1.13 × impaired fasting glucose/diabetes (yes = 1, no = 0) + 0.99 × AST:ALT ratio − 0.013 × platelet count $(×10^9/L)$ − 0.66 × albumin (g/dL) [8].

Statistical Analysis
Continuous variables are expressed as means (SD) for parametric data or medians (interquartile range [IQR]) for nonparametric data, and ordinal variables as frequencies (%). Groups with and without advanced (≥F3) fibrosis were compared using unpaired t test or Mann-Whitney U and chi-square tests, as appropriate. Multivariate logistic regression models for NAFLD and advanced fibrosis were built using biologically relevant variables, presented as odds ratios (ORs) with 95% confidence intervals (CIs). Noninvasive markers of fibrosis were compared with CPA and the gold standard of NAS fibrosis stage, and the diagnostic accuracy was assessed with area under the receiver operator curve (AUROC), sensitivity, specificity, positive-predictive values and negative-predictive values (NPVs), and positive likelihood ratios and negative likelihood ratios (LR−). Analyses were performed using IBM SPSS Statistics version 20.

RESULTS

Population Characteristics
A total of 116 patients were included in the study between August 2001 and February 2019 (Imperial College, including St Mary's Hospital and Chelsea and Westminster Hospital: n = 39; Royal Free Hospital: n = 39; University of Modena and Reggio Emilia, Modena, Italy: n = 12; University of California San Francisco, USA: n = 14; McGill University Health Centre, Montreal, Canada: n = 12). The demographic and clinical characteristics of patients are included in Table 1. The mean (±SD) age was 48.4 ± 10.4 years and patients were mainly non-Hispanic white (72.9%) males (93.2%) with suppressed HIV viral load (94.1%). The mean (±SD) BMI was 29.2 ± 5.5 kg/m^2, with rates of diabetes, hypertension, and dyslipidemia of 21.2%, 44.9%, and 39.8%, respectively.

Table 1. Study Population Demographic Data

Characteristics	Values (N = 116)
Age, years	48.4 (10.9)
Male, n (%)	110 (93.2)
Ethnicity, n (%)	
White, European	86 (72.9)
White, Hispanic	5 (4.2)
Black	13 (11.0)
Other	12 (10.3)
Body mass index, kg/m^2	29.2 (5.5)
Diabetes, n (%)	25 (21.2)
Hypertension, n (%)	53 (44.9)
Dyslipidemia, n (%)	47 (39.8)
Time since HIV, years	13.0 (7.0–21.0)
CD4 nadir, cells/mm^3	162.5 (36.8–277.5)
Time from diagnosis to ART, months	11 (1–45)
Duration of ART, years	9.0 (5.0–17.0)
NRTIs, months	158 (63–216)
NNRTIs, months	41 (6–95)
PIs, months	22 (0–116)
IIs, months	0 (0–1)
D-drugs, months	0 (0–54)
Platelets, ×10^9/L	206 (66)
ALT, IU/L	68 (45–107)
AST, IU/L	46 (32–63)
ALP, U/L	91 (74–111)
Bilirubin, μmol/L	10 (7–17)
Albumin, g/L	43.1 (5.2)
Cholesterol, mmol/L	4.8 (1.1)
Triglycerides, mmol/L	1.9 (1.2–3.1)
LDL cholesterol, mmol/L	2.7 (2.1–3.4)
HDL cholesterol, mmol/L	1.2 (0.5)
Fasting glucose, mmol/L	5.9 (2.1)
TGI	505.6 (445.8–608.2)
Detectable HIV viral load, %	7 (5.9)
CD4, cells/μL	638.1 (297.3)
CD8, cells/μL	875 (586–1209)
CD4:CD8	1.0 (0.5–1.0)

Data are presented as n (%), means (SD), or medians (IQR) according to distribution.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; D-drug, dideoxynucleoside analog; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; II, integrase inhibitor; IQR, interquartile range; LDL, low-density lipoprotein; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TGI, Triglyceride Glucose Index.

Histopathological Characteristics

Sixty-three of 116 patients (54.3%) had at least 5% macrovesicular steatosis consistent with the diagnosis of NAFLD, of whom 57 (90.5% cases with NAFLD, 49.1% of entire cohort) had NASH as defined by the FLIP algorithm (steatosis, ballooning, and lobular inflammation) [4]. Most cases (48/116, 41.4%) without NAFLD had nonspecific mild lobular inflammation, and 5 cases had features most in keeping with a drug reaction. No vascular liver disease (eg, nodular regenerative hyperplasia) was reported.

There was liver fibrosis in 102 of 116 (87.9%) cases, including 35 (30.2%) stage 1, 28 (24.1%) stage 2, 36 (31.0%) stage 3, and 3 (2.6%) with cirrhosis (Table 2). The subset of cases (53/116)

without steatosis were also reported by the Ishak staging system, and included 21 of 53 (39.6%) stage 0, 9 (17.0%) stage 1, 8 (15.1%) stage 2, 11 (20.8%) stage 3, 1 (1.9%) stage 4, 2 (3.8%) stage 5, and 1 (1.9%) stage 6. The median CPA of all cases was 3.0% (2.0–5.0). The CPA was identical between stages F0 and F2 (2%) but then increased significantly above F3 (F3, 5.0% [IQR, 3.0–7.8]; F4, 20.0% [IQR, 4.0–20.0]) (Figure 1).

Body Mass Index Is the Key Predictor of Nonalcoholic Fatty Liver Disease in HIV Monoinfection

The characteristics of subjects with NAFLD were compared with those with no steatosis on liver biopsy. On univariate analysis, factors significantly associated with NAFLD were increased BMI (OR, 1.15; 95% CI, 1.06–1.25; $P = .001$), type 2 diabetes (OR, 2.63; 95% CI, 1.00–6.90; $P = .050$), hypertension (OR, 2.43; 95% CI, 1.14–5.17; $P = .021$), dyslipidemia (OR, 2.18; 95% CI, 1.01–4.70; $P = .047$), increased CD4 count (OR, 1.02; 95% CI, 1.01–1.04; $P = .002$), and increased CD8 count (OR, 1.01; 95% CI, 1.00–1.02; $P = .009$). Increased AST:ALT ratio (OR, .008; 95% CI, .02–.39; $P = .002$) and previous use of dideoxynucleoside analogs (D-drugs) (OR, .40; 95% CI, .19–.88; $P = .022$) were associated with no steatosis (Table 3). Two multivariate models were built to investigate for variables

Table 2. Summary of Histological Characteristics of Study Population Liver Biopsies

Histological Feature or Diagnosis	Values
Steatosis	
None (<5%)	53 (45.7)
Mild (5–33%)	34 (29.3)
Moderate (34–66%)	28 (24.1)
Severe (>66%)	1 (0.9)
NASH[a]	57 (49)
Ballooning, 0/1/2	39 (33.6)/49 (42.2)/28 (24.1)
Inflammation, 0/1/2/3	14 (12.1)/72 (62.1)/22 (19.0)/8 (6.9)
NAS score[b,c]	4.1 (1.2)
NAS <3	2 (3.2)
NAS 3–4	41 (65.1)
NAS >4	20 (31.7)
Drug reaction	5 (4.3)
Fibrosis[c]	
None	14 (12.1)
F1	35 (30.2)
F1a	3 (2.6)
F1b	21 (18.1)
F1c	11 (9.5)
F2	28 (24.1)
F3	36 (31.0)
F4	3 (2.6)
Nonspecific changes	48 (41.4)

Data are presented as n (%).
Abbreviations: CRN, Clinical Research Network; NAFLD, nonalcoholic fatty liver disease; NAS, Nonalcoholic Steatohepatitis (score); NASH, nonalcoholic steatohepatitis.
[a]NASH is defined as the presence of steatosis, ballooning, and lobular inflammation.
[b]NAS score is only reported on the subset of cases with NAFLD (n = 63).
[c]NAS score and fibrosis stage reported according to the NASH CRN system.
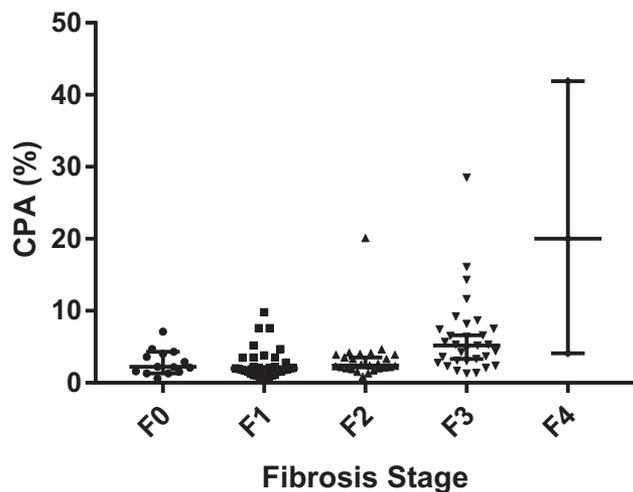
Figure 1. CPA per fibrosis stage (Brunt). Data are presented as medians ± IQRs: F0, 2.0% (1.8–4.0%); F1, 2.0% (1.0–3.3%); F2, 2.0% (2.0–4.0%); F3, 5.0% (3.0–7.8%); F4, 20.0% (4.0–20.0%). Abbreviations: CPA, collagen proportionate area; IQR, interquartile range.

independently associated with NAFLD, both adjusted for age, BMI, and CD4 nadir—model 1 (metabolic): age (years), BMI (kg/m^2), type 2 diabetes, hypertension, dyslipidemia, CD4 nadir (cells/μL); model 2 (HIV): age (years), BMI (kg/m^2), lifetime D-drug exposure, CD4 (cells/μL), CD8 (cells/μL), CD4 nadir (cells/μL). Body mass index was the only variable independently associated with NAFLD (model 1: adjusted OR [aOR], 1.20; 95% CI, 1.08–1.34; P = .001; model 2: aOR, 1.20; 95% CI, 1.08–1.33; P = .001). Body mass index remained the only independent predictor of NAFLD when TGI replaced type 2 diabetes in the model (Table 4, Supplementary Table 1). More subjects with NAFLD had advanced (≥F3) fibrosis (22.6% vs 42.9%; P = .022), but the CPA was similar between those with and without NAFLD (3.0% [2.0–5.0] vs 2.0% [2.0–5.0]; P = .445) (Figure 2).

Type 2 Diabetes Is an Independent Predictor of Advanced Fibrosis
A total of 39 of 116 (34%) subjects had advanced (≥F3) fibrosis on liver biopsy (Table 5). On univariate analysis, a diagnosis of NAFLD (OR, 2.56; 95% CI, 1.14–5.78; P = .023), type 2 diabetes (OR, 5.26; 95% CI, 2.05–13.50; P = .001), hypertension (OR, 2.65; 95% CI, 1.20–5.85; P = .016), dyslipidemia (OR, 3.12; 95% CI, 1.40–6.94; P = .005), duration of antiretroviral therapy (ART) (OR, 1.06; 95% CI, 1.00–1.11; P = .036), and time since HIV diagnosis (OR, 1.08; 95% CI, 1.03–1.13; P = .002) were associated with advanced fibrosis (Table 6).

A multivariate model was built using the significant variables from multivariate analysis. Duration of ART and time since HIV infection could not be included together due to collinearity; therefore, duration of ART was selected due to

uncertainty between time of diagnosis and duration of infection. In the model, type 2 diabetes had the strongest association with advanced fibrosis (aOR, 3.42; 95% CI, 1.00–11.71; P = .050). When the TGI was used in place of type 2 diabetes in the model, it was the only variable significantly associated with advanced fibrosis (aOR per 10-unit increase, 1.04; 95% CI, 1.00–1.07; P = .033) (Supplementary Table 2).

In a subanalysis on subjects without NAFLD comparing those with (15/53) and without (38/53) advanced fibrosis (≥ Ishak stage 3), the only significant difference between the groups on univariate analysis was longer time since HIV diagnosis (11.5 [6.8–18.3] vs 21.0 [13.0–26.0] years; P = .005) but age was similar (49.0 ± 12.0 vs 50.0 ± 8.0 years; P = .761).

Performance of Noninvasive Markers for Advanced Fibrosis
The diagnostic accuracy of noninvasive markers of fibrosis was evaluated using liver histology as a reference. FIB-4 and NFS had poor diagnostic accuracy for advanced liver fibrosis, with AUROCs for FIB-4 and NFS to detect advanced fibrosis of .65 (95% CI, .53–.76) and .66 (95% CI, .56–.80) (all subjects), .64 (95% CI, .49–.79) and .64 (.49–.79) (subjects with NAFLD only), and .72 (95% CI, .55–.88) and .73 (95% CI, .57–.89) (subjects without NAFLD only), respectively (Figure 3).

A CPA value of 7.6% or greater is predictive of long-term adverse outcomes [6]. Both FIB-4 and NFS performed more robustly at identifying cases with a CPA of 7.6% or greater than advanced fibrosis as defined by NAS CRN staging, where the AUROCs of FIB-4 and NFS for CPA of 7.6% or greater were .84 (95% CI, .74–.93) and .81 (95% CI, .70–.91) (all subjects), .82 (95% CI, .69–.95) and .78 (95% CI, .64–.92) (subjects with NAFLD only), and .88 (95% CI, .75–1.00) and .83 (95% CI, .68–.98) (subjects without NAFLD only), respectively (Figure 4).

Validated cutoff values for ruling out advanced fibrosis are less than 1.3 for FIB-4 [9] and less than −1.455 for NFS [8]. Using these values, the sensitivity, NPV, and LR− were 0.72, 0.80, and 0.66 for FIB-4 and 0.84, 0.82, and 0.43 for NFS (Table 7A). These tests were better at ruling out CPA of 7.6% or greater, where the sensitivity, NPV, and LR− were 0.93, 0.98, and 0.16 for FIB-4 and 0.93, 0.97, and 0.20 for NFS (Table 7B).

DISCUSSION

This study reports the largest known sample of liver biopsies in HIV monoinfection, read by a central expert liver pathologist, in which the primary risk factor identified for NAFLD was BMI and for advanced fibrosis was type 2 diabetes.

NAFLD is common in PLWH, but understanding more clearly which patients progress to NASH and advanced fibrosis will help clinicians to appropriately risk-stratify patients for

Table 3. Demographic and Clinical Characteristics of Subjects According to the Presence of Nonalcoholic Fatty Liver Disease on Liver Biopsy (Defined as Macrovesicular Steatosis ≥5%)

Characteristics	Steatosis <5% (n = 53)	Steatosis ≥5% (n = 63)	P
Age, years	49.3 (10.9)	47.7 (11.0)	.458
Male, n (%)	49 (92.5)	61 (96.8)	.289
Black ethnicity, n (%)	9 (17.0)	4 (6.3)	.071
Body mass index, kg/m^2	27.3 (5.5)	30.9 (5.0)	<.001
Diabetes, n (%)	7 (13.2)	18 (28.6)	.045
Hypertension, n (%)	18 (34.0)	35 (55.6)	.020
Dyslipidemia, n (%)	16 (30.2)	31 (49.2)	.045
Time since HIV, years	14.0 (7.0–21.5)	11.5 (6.8–21.3)	.565
CD4 nadir, cells/mm^3	143.0 (39.0–238.5)	189.5 (32.3–294.3)	.419
Time from diagnosis to treatment, months	11.5 (2.0–37.8)	11.0 (1.0–50.5)	1.000
Duration of ART, years	10.0 (6.0–17.5)	8.0 (4.8–16.3)	.415
NRTIs, months	180 (80–253)	122 (62–204)	.076
NNRTIs, months	33 (8–94)	50 (4–107)	.448
PIs, months	57 (0–130)	3 (0–106)	.061
IIs, months	0 (0–0)	0 (0–12)	.027
Previous D-drugs (yes/no), n (%)	31 (58.5)	23 (36.5)	.021
Platelets, ×10^9/L	193 (71)	217 (58)	.047
ALT, U/L	52 (32–76)	78 (59–137)	<.001
AST, U/L	39 (27–62)	50 (41–63)	.019
AST:ALT	0.87 (0.41)	0.65 (0.23)	<.001
ALP, U/L	96 (75–112)	89 (72–108)	.174
Bilirubin, μmol/L	12.0 (7.0–18.0)	10.0 (7.0–16.3)	.507
Albumin, g/L	42.9 (6.8)	43.3 (3.4)	.709
Cholesterol, mmol/L	4.7 (1.0)	4.9 (1.1)	.288
Triglycerides, mmol/L	1.8 (1.1–2.5)	2.1 (1.4–3.3)	.126
LDL, mmol/L	2.6 (0.9)	2.8 (1.0)	.186
HDL, mmol/L	1.2 (0.4)	1.1 (0.5)	.265
Fasting glucose, mmol/L	5.7 (2.5)	6.1 (1.8)	.367
TGI	480.5 (405.4–587.0)	514.5 (460.7–656.8)	.040
Detectable VL, %	1 (1.9)	6 (9.5)	.082
CD4, cells/μL	541 (261)	720 (303)	.001
CD8, cells/μL	739 (494–1025)	900 (673–1339)	.019
CD4:CD8	1.0 (0.4–1.0)	0.9 (0.5–1.0)	.816
CPA, %	2.0 (2.0–5.0)	3.0 (2.0–5.0)	.445
Fibrosis			
≥F2	27 (50.9)	40 (63.5)	.173
≥F3	12 (22.6)	27 (42.9)	.022

Data are presented as n (%), means (SD), or medians (IQR) according to distribution.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; D-drug; dideoxynucleoside analog; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; II, integrase inhibitor; IQR, interquartile range; LDL, low-density lipoprotein; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TGI, Triglyceride Glucose Index; VL, HIV viral load.

further investigation, such as a liver biopsy, and initiate appropriate management.

In this study, subjects were selected from 5 centers in Europe and North America who had had a liver biopsy without another cause of chronic liver disease. Approximately half (54%) had macrovesicular steatosis consistent with NAFLD, only 4% had a drug reaction, and interestingly, 41% had nonspecific features. Of the 63 subjects with NAFLD, 57 (92%) had NASH, and 58% of the whole cohort had ≥F2 fibrosis, reflecting the selection criteria in centers to biopsy patients with a high pretest probability of more advanced disease.

Collagen proportionate area was used as a quantitative measure of fibrosis, which showed that the quantity of collagen deposition is similar between stages F0 and F2 but then increases steeply from F3 to F4. This helps explain the decline in prognosis with F3 fibrosis, whereas it matches population controls for at least 20 years for F2 and 30 years for F0–F1 fibrosis [10]. The subjects with cirrhosis (n = 3) had a wide range of CPA scores (4.1%, 20.0%, and 41.9%), illustrating an important limitation of current staging systems in which patients with cirrhosis are crudely grouped together despite large differences in collagen content.

Table 4. Univariate and Multivariate Analysis of Risk Factors for Nonalcoholic Fatty Liver Disease

| | Univariate | | Multivariate | | | |
| | | | Model 1 | | Model 2 | |
	OR (95% CI)	*P*	aOR (95% CI)	*P*	aOR (95% CI)	*P*
Age	.99 (.95–1.02)	.455	.96 (.91–1.01)	.128	1.01 (.96–1.06)	.681
BMI	1.15 (1.06–1.25)	.001	1.20 (1.08–1.34)	.001	1.20 (1.08–1.33)	.001
Black ethnicity	.33 (.10–1.15)	.081	…		…	
Diabetes	2.63 (1.00–6.90)	.050	1.07 (.27–4.21)	.928	…	
Hypertension	2.43 (1.14–5.17)	.021	3.14 (.98–10.11)	.055	…	
Dyslipidemia	2.18 (1.01–4.70)	.047	1.28 (.41–3.99)	.668	…	
Duration of ART	.98 (.94–1.03)	.470	…		…	
D-drugs[a]	.40 (.19–.88)	.022	…		.36 (.13–1.02)	.053
CD4	1.02 (1.01–1.04)	.002	…		1.00 (1.00–1.00)	.177
CD8	1.01 (1.00–1.02)	.009	…		1.00 (1.00–1.00)	.094
CD4 nadir	1.02 (.99–1.04)	.226	1.00 (1.00–1.00)	.940	1.00 (1.00–1.00)	.744

Model 1 (Metabolic): age (per year), BMI (per kg/m^2), diabetes, hypertension, dyslipidemia, CD4 nadir (per 10 cells/µL). Model 2 (HIV): BMI (per kg/m^2), diabetes, lifetime D-drugs, CD4 (per 10 cells/µL), CD8 (per 10 cells/µL), CD4 nadir (per 10 cells/µL).

Abbreviations: aOR, adjusted odds ratio; ART, antiretroviral therapy; BMI, body mass index; CI, confidence interval; D-drug; dideoxynucleoside analog; HIV, human immunodeficiency virus.

[a]Past exposure in lifetime.

Fibrosis quantitation with CPA could have prognostic utility in identifying subjects at high risk of decompensation, which may aid in the identification of patients at risk of hepatic complications [6]. This should also be considered in clinical trial designs for antifibrotic drugs in NASH, both in subjects with and without HIV, where a continuous variable of fibrosis content may be a more sensitive measure of antifibrotic effect compared with semi-quantitative staging, which suffers from significant interobserver variability [11]. A limitation to implementing this technology has been a lack

Figure 2. CPA in subjects with and without NAFLD. Abbreviations: CPA, collagen proportionate area; NAFLD, nonalcoholic fatty liver disease.

of outcome data for CPA levels, but this has recently been addressed by a study showing that CPA independently predicts clinical outcomes [6].

The main feature differentiating patients with and without NAFLD was BMI, which was significantly increased in patients with NAFLD (30.9 vs 27.3 kg/m^2), and this remained independently associated in the multivariate analysis. Clearly, as in the general population, obesity is a hallmark characteristic of NAFLD in PLWH [9, 12, 13]. Some studies have shown an association with nucleoside reverse transcriptase inhibitor (NRTI) exposure and NAFLD [14], possibly through an indirect effect on fat metabolism and redistribution, but this has not consistently been the case [15] and is not supported in this study, where ART exposure was not predictive of NAFLD. It is surprising that D-drug exposure tended towards a protective effect in the univariate and multivariate analysis, but in a cohort with a median ART duration of approximately 10 years and median D-drug exposure of 0 months, the individual exposure of subjects was likely to have been very low towards the end of the era when they were prescribed, so the reported effect may be subject to confounding. There are emerging data on excess weight gain following use of integrase inhibitors, and further evaluation is required to understand if this may result in an increased risk of developing NAFLD [16].

Body mass index was not associated with an increased risk of advanced fibrosis, whereas type 2 diabetes remained independently associated in the multivariate analysis. This was supported by the significant association with increased TGI, a surrogate marker of insulin resistance [2]. A paired biopsy study of subjects with NASH (n = 83) without HIV
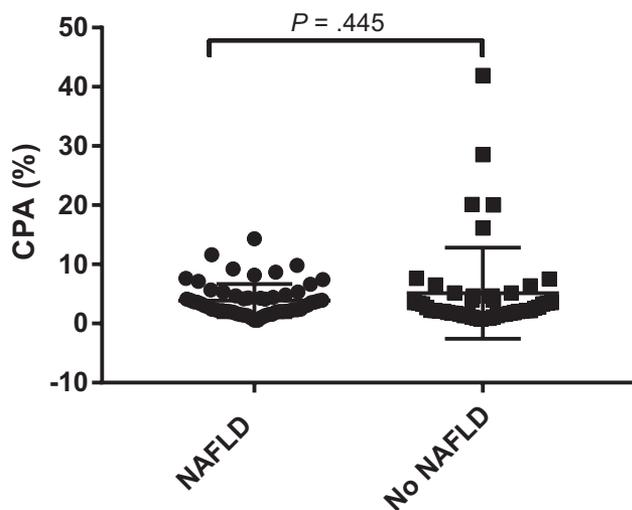
Table 5. Demographic and Clinical Characteristics of Subjects According to the Presence of Advanced Fibrosis (Defined as ≥F3 Fibrosis by Brunt Classification)

Characteristics	<F3 (n = 77)	≥F3 (n = 39)	P
Age, years	47.2 (10.5)	50.9 (11.5)	.082
Male, n (%)	73 (94.8)	37 (94.8)	.988
Black ethnicity, n (%)	12 (15.6)	1 (2.6)	
Body mass index, kg/m^2	28.8 (5.5)	30.1 (5.5)	.242
Diabetes, n (%)	9 (11.7)	16 (41.0)	<.001
Hypertension, n (%)	29 (37.7)	24 (61.5)	.015
Dyslipidemia, n (%)	24 (31.2)	23 (59.0)	.005
Time since HIV, years	10.5 (5.0–18.8)	20.0 (11.0–24.0)	.001
CD4 nadir, cells/mm^3	171.0 (48.0–299.3)	137.0 (19.5–216.0)	.175
Time from diagnosis to treatment, months	6.5 (1.0–37.8)	14.0 (2.0–78.0)	.157
Duration of ART, years	8.0 (4.0–15.0)	13.0 (6.0–20.0)	.038
NRTIs, months	132 (52–226)	172 (72–211)	.377
NNRTIs months	26 (2–88)	64 (28–112)	.054
PIs, months	23 (0–110)	22 (0–120)	.594
Previous D-drugs (yes/no), n (%)	33 (45.2)	21 (53.8)	.150
Platelets, ×10^9/L	213 (57)	193 (144–252)	.122
ALT, U/L	67 (41–101)	73 (52–137)	.176
AST, U/L	44 (30–62)	54 (42–72)	.017
AST:ALT	0.74 (0.36)	0.75 (0.31)	.852
ALP, U/L	93 (74–111)	90 (75–102)	.377
Bilirubin, µmol/L	10.0 (6.9–17.1)	11.0 (7.5–18.6)	.484
Albumin, g/L	43.2 (5.7)	42.8 (4.2)	.699
Cholesterol, mmol/L	4.9 (1.0)	4.6 (1.2)	.172
Triglycerides, mmol/L	1.8 (1.2–3.1)	2.0 (1.2–3.3)	.711
LDL, mmol/L	2.8 (0.8)	2.4 (1.2)	.044
HDL, mmol/L	1.2 (0.4)	1.1 (0.7)	.626
Fasting glucose, mmol/L	5.4 (1.1)	7.1 (3.1)	<.001
TGI	475.4 (431.4–574.3)	534.0 (505.0–764.6)	.001
Detectable VL, %	3 (3.9)	4 (10.3)	.174
CD4, cells/µL	626 (262)	661 (357)	.555
CD8, cells/µL	902 (683–1264)	764 (514–1044)	.050
CD4:CD8	1.0 (0.4–1.0)	1.0 (0.7–1.0)	.539
NAFLD	36 (46.8)	27 (69.2)	.022
NFS	−1.9 (−3.0 to 1.0)	−0.7 (−2.2 to 0.2)	.002
FIB-4	1.1 (0.8–1.8)	1.7 (1.0–2.1)	.008

Data are presented as n (%), means (SD), or medians (IQR) according to distribution. Groups were compared by Mann-Whitney test (discrete data) and chi-square (categorical). $P < .05$ was considered statistically significant.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; D-drug; dideoxynucleoside analog; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; II, integrase inhibitor; IQR, interquartile range; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD Fibrosis Score; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TGI, Triglyceride Glucose Index; VL, HIV viral load.

demonstrated similar results, in which fibrosis progression was independently linked to type 2 diabetes [17], and a study using transient elastography in PLWH has also shown that liver fibrosis is significantly more common in patients with the metabolic syndrome, of which type 2 diabetes is a key feature [18]. Therefore, in PLWH, development of metabolic complications of obesity, particularly diabetes, represents an increased risk for liver fibrosis and the potential for higher liver-related morbidity.

An interesting observation in our study was the high rate of nonspecific findings in cases with no evidence of significant steatosis, including 60% with some evidence of fibrosis and 28% with Ishak stage 3 or higher fibrosis, representing a group with significant underlying liver damage but no known cause of chronic liver disease. This included the patients with cirrhosis, possibly through "burnt-out" NASH, but this observation does raise further questions about the natural history of liver fibrosis in patients with HIV, particularly since the time since HIV diagnosis was the main variable associated with fibrosis in subjects without steatosis. There were insufficient cases to conduct a multivariate analysis in this subgroup, which warrants further investigation, particularly with longitudinal follow-up.

Table 6. Univariate and Multivariate Analysis of Risk Factors for Advanced Fibrosis

	Univariate		Multivariate Model 1	
	OR (95% CI)	*P*	aOR (95% CI)	*P*
Age	1.03 (1.00–1.07)	.085	…	
BMI	1.04 (.97–1.12)	.242	…	
Black ethnicity	.14 (.02–1.14)	.066	…	
NAFLD	2.56 (1.14–5.78)	.023	2.47 (.96–6.39)	.062
Type 2 diabetes	5.26 (2.05–13.50)	.001	3.42 (1.00–11.71)	.050
Hypertension	2.65 (1.20–5.85)	.016	.99 (.34–2.89)	.983
Dyslipidemia	3.12 (1.40–6.94)	.005	1.88 (.69–5.13)	.221
Duration of ART	1.06 (1.00–1.11)	.036	1.03 (.97–1.09)	.394
D-drugs[a]	1.82 (.80–4.12)	.152	…	
CD4	1.71 (.76–3.86)	.195	…	
CD8	1.00 (1.00–1.00)	.124	…	
CD4 nadir	1.00 (1.00–1.00)	.205	…	
Time since HIV infection	1.08 (1.03–1.13)	.002	…	

Multivariate model: BMI (per kg/m^2), NAFLD (≥5% steatosis on liver biopsy), type 2 diabetes, hypertension, dyslipidemia, duration of ART (per year).

Abbreviations: aOR, adjusted odds ratio; ART, antiretroviral therapy; BMI, body mass index; CI, confidence interval; D-drug, dideoxynucleoside analog; HIV, human immunodeficiency virus; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio.

[a]Past exposure in lifetime.

How, therefore, should we select patients to send for further evaluation including liver biopsy? Targeted screening for NAFLD should certainly be considered in PLWH who are obese, a practice that becomes even more important in those with accompanying metabolic complications, especially type 2 diabetes. Current guidelines for NAFLD recommend risk stratification of patients at risk of NAFLD and liver fibrosis with noninvasive markers, including FIB-4 and NFS [9]. The accuracy of these serological markers of fibrosis were evaluated in this population of PLWH and the AUROC values were poor. A recent large (n = 452) cross-sectional study by Boursier et al [19] in subjects with NAFLD in the general population validating these markers against a liver biopsy gold standard also showed these tests only have a modest AUROC for diagnosing advanced fibrosis: 0.732 for NAFLD and 0.780 for FIB-4. However, these scores are primarily applied using cutoff values designed to optimize the NPV of the test, a practice that has been successfully applied to stratify patients for referral to secondary care from the community [20]. Using previously validated cutoffs for FIB-4 [21] and NFS [8] in this study, there was an NPV of 0.80 and 0.82 for FIB-4 and NFS. The performance of these markers was similar to the Boursier et al study where the optimized cutoffs of −1.036 (NFS) and 1.515 (FIB-4) had NPVs of 0.81 and 0.82, respectively [19]. The performance improved in the prediction of CPA of 7.6% or greater (NPVs of 0.98 for FIB-4 and 0.97 for NFS), but overall, about one-quarter of cases with advanced fibrosis could be mis-classified as low risk, supporting concerns about the accuracy of these markers in HIV-associated NAFLD [1].

The main limitations of the study are its retrospective design with data from a selected population and heterogeneous indications for liver biopsy, and a lack of elastography data. However, the strength of the study is the large, multicenter international collection of centrally reviewed liver biopsy data in a field where, to date, only a few, small studies with liver biopsy in patients with HIV monoinfection have been published. Although significant alcohol excess was an exclusion criterion, we did not have data on moderate alcohol use. However, our cutoff approximates that used in NAFLD trials.

Conclusions

In HIV monoinfection, advanced liver fibrosis is strongly associated with type 2 diabetes and NAFLD with elevated BMI. Liver fibrosis may be evident in PLWH and with no known established cause of chronic liver disease. The biochemical markers of fibrosis (FIB-4 and NFS) require further validation in this population.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.
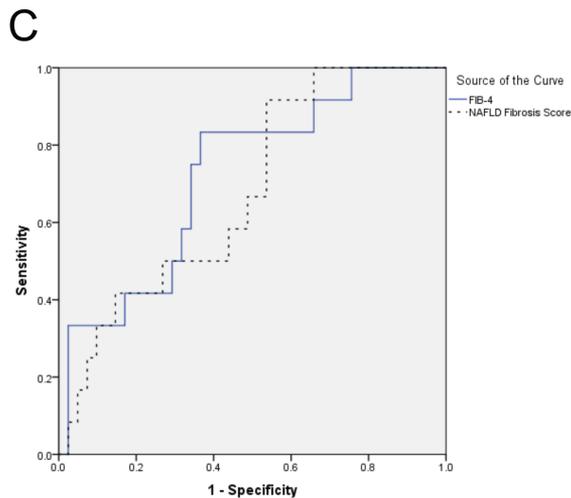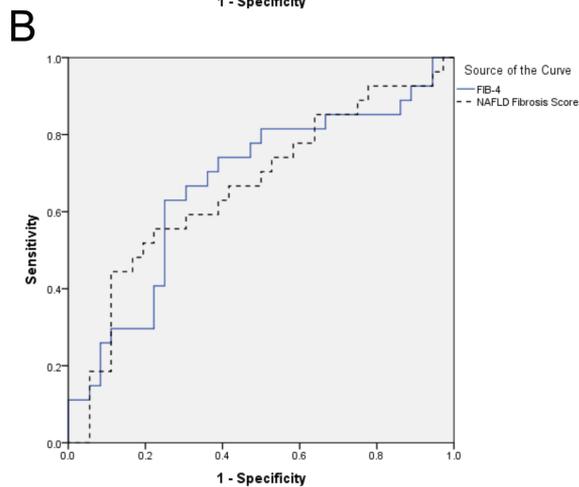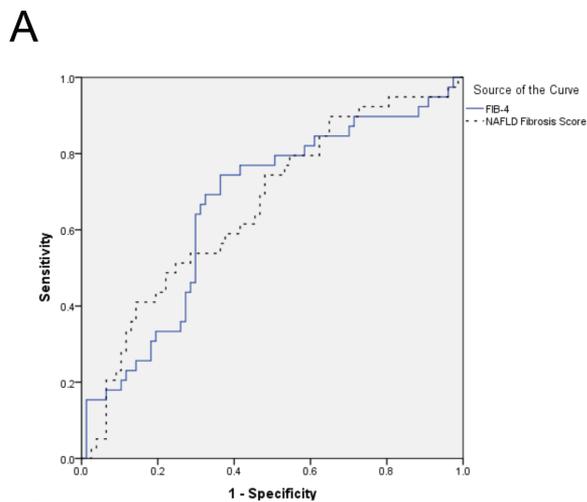
Notes

Figure 3. ROC curves assessing the performance of the noninvasive markers for detecting advanced (≥F3) fibrosis. *A*, All cases. AUROC FIB-4, 0.659; NFS, 0.688; combined, 0.716. *B*, NAFLD cases only. AUROC FIB-4, 0.655; NFS, 0.684; combined, 0.662. *C*, Non-NAFLD cases only. AUROC FIB-4, 0.722; NFS, 0.679. Abbreviations: AUROC, area under the receiver operator curve; NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD Fibrosis Score; ROC, Receiver Operating Characteristic.
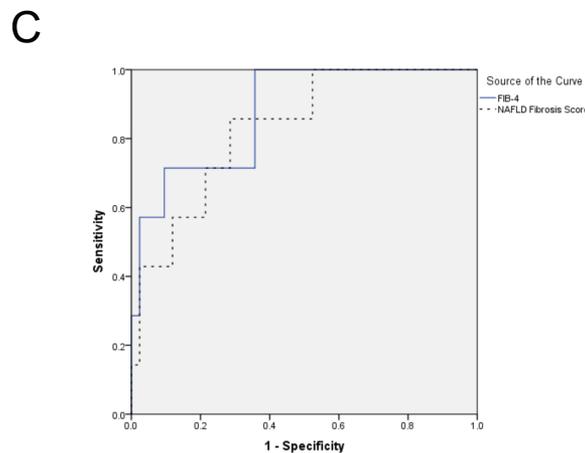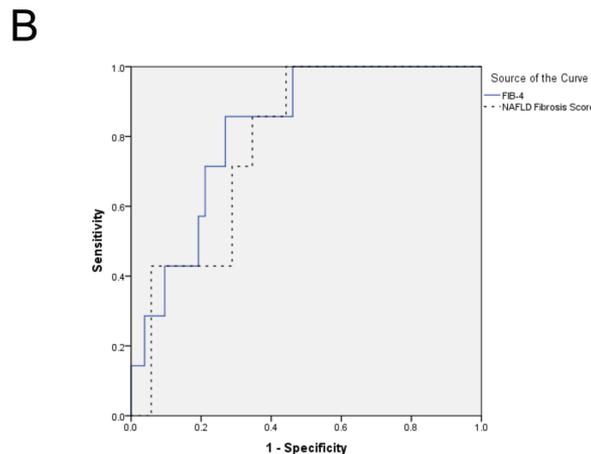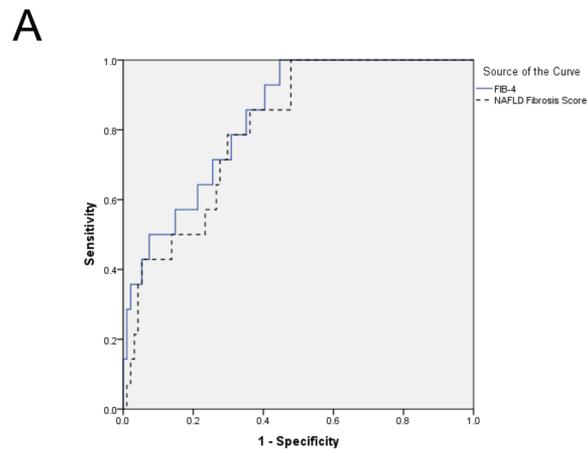
Figure 4. ROC curves assessing the performance of the noninvasive markers for detecting CPA ≥7.6% (n = 14/116). *A*, All cases. AUROC FIB-4, 0.836; NFS, 0.805; combined, 0.784. *B*, NAFLD cases only. AUROC FIB-4, 0.819; NFS, 0.780. *C*, Non-NAFLD cases only. AUROC FIB-4, 0.878; NFS, 0.830. Abbreviations: AUROC, area under the receiver operator curve; NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD Fibrosis Score; ROC, Receiver Operating Characteristic.

Table 7. Diagnostic Accuracy of FIB-4 and NFS for Advanced Fibrosis (A) and Collagen Proportionate Area (B) >7.6%

	Cutoff	Sensitivity	Specificity	PPV	NPV	LR+	LR−
A							
FIB-4	1.3	0.72	0.43	0.46	0.80	1.26	0.66
NFS	−1.455	0.84	0.36	0.40	0.82	1.32	0.43
B							
FIB-4	1.3	0.93	0.44	0.24	0.98	1.65	0.16
NFS	−1.455	0.93	0.35	0.18	0.97	1.44	0.20

Abbreviations: LR+, positive likelihood ratio; LR−, negative likelihood ratio; NFS, NAFLD Fibrosis Score; NPV, negative-predictive value; PPV, positive-predictive value.

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