Nonalcoholic Steatohepatitis and Hepatic Fibrosis in HIV-1–Monoinfected Adults With Elevated Aminotransferase Levels on Antiretroviral Therapy

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(See the Editorial Commentary by Crum-Cianflone on pages 1579–81.)

Background. Persistent aminotransferase elevations are common in human immunodeficiency virus (HIV)–infected patients on antiretroviral therapy (ART), including those without hepatitis B or C coinfection, but their clinical significance is unknown.

Methods. HIV-infected adults with aminotransferase levels elevated above the upper limit of normal for ≥6 months while receiving ART, and without chronic viral hepatitis or other known causes of chronic liver disease, underwent a detailed metabolic assessment and liver biopsy.

Results. Sixty-two HIV-infected subjects completed the study. Forty (65%) had clinically significant liver pathology, including 34 (55%) with nonalcoholic steatohepatitis (NASH) and 11 (18%) with bridging fibrosis, 10 of whom also had NASH. Nonspecific abnormalities alone were seen in 22 (35%) subjects, including mild steatosis, mild to moderate inflammation, and evidence of drug adaptation. Insulin resistance, obesity, and the presence of either of 2 minor alleles in the *PNPLA3* gene were significantly associated with increased risk of NASH and fibrosis. NASH and/or fibrosis were not associated with duration of HIV infection or ART, specific antiretroviral drugs, history of opportunistic infection, immune status, or duration of aminotransferase elevation.

Conclusions. HIV-infected adults with chronic aminotransferase elevations while receiving ART have a high rate of liver disease. Noninvasive testing can help identify liver disease in such patients, but liver biopsy is necessary to definitively identify those at risk for liver disease progression and complications. Longitudinal follow-up of this cohort will better characterize the natural history of aminotransferase elevations in this population and identify noninvasive biomarkers of liver disease progression.

Keywords. hepatotoxicity; liver biopsy; insulin resistance; PNPLA3.

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Aminotransferase elevations are common in human immunodeficiency virus (HIV)–infected patients, occurring in 20%–60% of patients on antiretroviral therapy (ART), even in the absence of viral hepatitis coinfection [1–3]. Causes of hepatotoxicity in HIV-infected adults include coinfection with hepatitis B or C, alcohol abuse, nonalcoholic fatty liver disease (NAFLD), and medication, including antiretroviral, toxicity. A possible

contribution to chronic liver injury by HIV infection itself or the associated immunodeficiency is controversial [4, 5]. Prior studies in HIV-monoinfected patients with aminotransferase elevations suggest a high prevalence of clinically significant liver disease, including nonalcoholic steatohepatitis (NASH) and hepatic fibrosis [6, 7].

Patients with aminotransferase elevations require additional visits for laboratory studies and clinical assessments, and often undergo interruptions and changes in ART [8]. However, the impact of chronic aminotransferase elevations, and any related liver damage, on morbidity and mortality has not been well defined [9, 10]. As a consequence, at present there are no established guidelines as to when ART should be changed or discontinued in the setting of mild to moderate aminotransferase elevations. After evaluation for viral hepatitis and other reversible causes of liver enzyme elevations, most clinicians elect to monitor these elevations, given their uncertain clinical significance.

To better understand the clinical and pathologic correlates of chronic aminotransferase elevations in HIV-infected adults receiving ART and without evidence of chronic viral hepatitis, such patients were prospectively enrolled in a cross-sectional study to examine the prevalence and clinical correlates of antiretroviral-associated liver disease, including steatosis, steatohepatitis, and fibrosis.

METHODS

Study Population

Participants were enrolled from 2007 to 2013, after referral by local community care providers or from within the National Institutes of Health Intramural HIV/AIDS Research Program.

HIV-infected adults receiving combination ART for ≥1 year, with aspartate aminotransferase (AST) or alanine aminotransferase (ALT) elevations greater than the upper limit of normal (ULN; ie, AST >34 U/L or ALT >41 U/L) on ≥3 occasions over at least 6 months were eligible if they had no evidence of active viral hepatitis, hereditary or autoimmune liver disease, or hemochromatosis; no ongoing alcohol abuse; and no contraindication to liver biopsy. The institutional review board of the National Institute of Allergy and Infectious Diseases approved the protocol, and all participants provided written informed consent.

After enrollment, participants completed a questionnaire and interview regarding demographic variables, medical history, current and previous medication use including ART, alcohol consumption, and illicit drug use. A physical examination and routine anthropometric measurements were performed. History of opportunistic infection, cumulative ART exposure, $CD4^+$ T-cell counts, HIV viral load, and prior liver-related testing results were obtained through review of available medical records.

Laboratory Testing

Laboratory assessment prior to liver biopsy included complete blood counts, AST, ALT, alkaline phosphatase, gamma-glutamyl transpeptidase, bilirubin, and albumin. HIV RNA was measured by polymerase chain reaction (PCR; RealTime HIV-1 Assay, Abbott), and $CD4^+$ T-cell counts were measured using flow cytometry.

Additional laboratory evaluation for liver disease included assessment of serum iron, total iron binding capacity, ferritin, ceruloplasmin, antinuclear antibody, antimitochondrial antibody, anti–smooth muscle antibody, and alpha-1 antitrypsin phenotype. Hepatitis A, B, and C serologies and quantification of hepatitis B virus (HBV) DNA (COBAS AmpliPrep/COBAS TaqMan HBVv2.0, Roche) and hepatitis C virus (HCV) RNA (COBAS AmpliPrep/COBAS TaqMan HCV Test, Roche) were also performed.

In the first 49 participants, serum samples were tested for immunoglobulin M (IgM) and immunoglobulin G (IgG) against hepatitis E virus (HEV) using enzyme immunoassays [11]. Samples positive for either underwent further testing for HEV RNA using reverse transcription PCR [12].

The AST-to-platelet ratio index (APRI) [13], FIB-4 index [14], and the NAFLD Fibrosis Score (NAFLD-FS) [15] were calculated as noninvasive markers of fibrosis, using baseline parameters.

Metabolic laboratory assessment included fasting insulin, glucose, homeostatic model assessment of insulin resistance (HOMA-IR) [16], cholesterol and triglycerides, and oral glucose tolerance testing (OGTT). The diagnosis of metabolic syndrome was made according to American Heart Association/National Heart, Lung and Blood Institute criteria [17].

Imaging

Computed tomography scanning of the abdomen was performed to evaluate for hepatosplenomegaly and other anatomic abnormalities. Axial images were obtained from the dome of the diaphragm to the iliac crests and then, following intravenous contrast administration, during arterial and venous phases. Precontrast images were used for measurement of liver and spleen attenuation and liver-to-spleen attenuation ratio. Mean attenuation of 3 measurements was reported. The liver-to-spleen attenuation ratio was considered normal if ≥1 [18]. The segmentation method was used for liver and spleen volume measurements. A single scan at level L4/5, performed prior to administration of intravenous contrast, was used for measurement of abdominal subcutaneous and visceral fat volume [19].

Liver Histology

Percutaneous liver biopsy was performed under ultrasound guidance using an 18-gauge needle. One liver biopsy fragment was fixed in formalin. Paraffin-embedded sections were stained with hematoxylin and eosin, Masson trichrome, with special

stains for iron and copper, and for reticulin. Liver biopsies were read by a single liver pathologist (D. E. K.).

Fibrosis and inflammatory activity were scored according to the modified histology activity index (Ishak) scoring system [20]. Steatosis was graded on a scale of 0–4 based on the percentage of cells with fat. Histological features of NAFLD, such as steatosis, inflammation, and hepatocyte ballooning, were further scored according to the NASH Clinical Research Network (CRN) scoring system [21].

Single-Nucleotide Polymorphism Analysis

Participants were genotyped for 2 single-nucleotide polymorphisms (SNPs) in the gene encoding patatin-like phospholipase domain-containing protein 3 (*PNPLA3* or adiponutrin), an enzyme involved in triglyceride metabolism, previously reported to be associated with elevated ALT levels, and histologic parameters of NASH and fibrosis severity [22, 23]. The minor allele of rs738409 C/G is a nonsynonymous SNP, encoding an Ile148-Met change, whereas SNP rs2281135 is located in an intron. DNA was obtained from stored peripheral blood mononuclear cells. The TaqMan SNP genotyping assays (C_7241_10 and C_15875080_10), were performed on a QuantStudio 12 K Flex PCR System (Applied Biosystems).

Case-control allelic association Fisher exact test and quantitative association analyses were performed using the program PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) [24]. Log-transformed ALT and AST values were used. A P value of <.05 was considered significant.

Statistical Analyses

Group comparisons were performed using the nonparametric Mann–Whitney rank-sum test for continuous variables and exact χ^2 methods for categorical variables. Fisher exact test was used for binary variables. Stepwise logistic regression analysis was used to identify independent predictors of NASH. Because of the limited number of events, logistic regression was not used to identify predictors of fibrosis. Because of the large number of comparisons, 2-sided P values <.05 were considered suggestive, whereas P values <.01 were considered more definitive. Analyses were performed using StatXact and S-Plus.

RESULTS

Study Population

Sixty-two subjects underwent liver biopsy. Table 1 summarizes the demographic and clinical parameters of these patients. Of note, most of the cohort was male (94%). Median time from HIV diagnosis was 17.5 years (range, 2.3–22.8 years). The cohort was highly treatment experienced, with a median time on ART of 12.9 years (range, 1.7–22.8 years). Median $CD4^+$ T-cell nadir was 180 cells/μL (range, 6–599 cells/μL), and 26 participants (42%) had a history of opportunistic infection.

The median duration of AST and/or ALT elevation at enrollment was 3.7 years (range, 1.1–11.4 years). At screening, ALT was greater than twice the ULN (>82 IU/L) in 24 (39%) participants.

Median body mass index (BMI) was 27.6 kg/m^2 (range, 15.3–47.1 kg/m^2) with 47% of participants overweight and 31% obese. Using waist circumference, 35% of men and 75% of women met the metabolic syndrome criteria for abdominal obesity [17]. OGTT, performed in 57 participants, identified diabetes mellitus in 4 (7%) previously undiagnosed participants and impaired glucose tolerance in 13 (21%) additional participants. HOMA-IR was calculated using fasting insulin and glucose values obtained at the time of study entry from participants without known diabetes mellitus (n = 60). Forty-nine (82%) participants had a HOMA-IR score >2.6, consistent with a clinical diagnosis of insulin resistance, and 35 (58%) met the more specific score of >3.8 [25, 26]. Twenty-six of 56 participants without diabetes (46%) met criteria for metabolic syndrome.

Anti-HEV IgG was positive in 5 of 49 tested samples (10%); all were anti-IgM and HEV RNA PCR negative. Twenty-nine participants (47%) reported complete alcohol abstinence, and the remaining 33 (53%), all male, reported occasional alcohol use of <100 g/week. Five participants (8%) reported a distant (>5 years prior to enrollment) history of alcohol abuse.

Liver Biopsy

Liver biopsy results (N = 62) are summarized in Table 2, and selected histology images are presented in the Supplementary Figure. Steatosis involving >5% of the hepatocytes (NAFLD) was present in the biopsies of 45 (73%) participants, 20 (32%) of whom had moderate to severe steatosis. Three-quarters of these biopsies (34 participants or 55% of the total cohort) had inflammation and hepatocellular ballooning consistent with the diagnosis of NASH [21]. Of those with NASH, 8 had no fibrosis, 16 had evidence of mild fibrosis (NASH CRN fibrosis score >0 or Ishak fibrosis score = 1), and 10 had bridging fibrosis. Three participants had fibrosis (Ishak stage ≥1) without NASH. Additional findings on biopsy included portal venopathy (1 participant) and nodular regenerative hyperplasia (NRH) (2 participants).

Twenty-two participants (35%) had nonspecific abnormalities including mild steatosis, mild inflammation, and hepatocellular ground-glass cytoplasmic changes consistent with drug adaptation.

Correlates of Liver Pathology

One of the goals of this study was to identify noninvasive markers of liver pathology that could potentially be used in the clinical management of patients. Compared with patients without

Table 1. Selected Demographic and Clinical Characteristics of HIV-Infected Adults With Chronic, Unexplained Elevations in Aminotransferases While on Antiretroviral Therapy (N = 62)

Characteristic	No. (%) or Median (Range)
Age, y	50 (17–67)
Male sex	58 (94%)
Race (self-identified)	
White	40 (65%)
Black	5 (8%)
Asian	2 (3%)
Not selected/mixed race	15 (24%)
Ethnicity (self-identified)	
Hispanic/Latino	18 (29%)
Not Hispanic/Latino	44 (71%)
Time since HIV diagnosis, y	17.5 (2.3–27.8)
$CD4^+$ nadir, cells/μL	180 (6–599)
$CD4^+$ count at enrollment, cells/μL	548 (105–1631)
HIV RNA load, copies/mL	<40 (<40–726)
Total duration of antiretroviral therapy, y	12.9 (1.7–22.8)
Antiretroviral therapy at enrollment	
NRTI	60 (97%)
Zidovudine	11 (18%)
Stavudine	3 (5%)
Didanosine	0 (0%)
Lamivudine or emtricitabine	57 (92%)
Tenofovir	44 (71%)
Abacavir	14 (23%)
NNRTI	33 (53%)
Protease inhibitor	29 (47%)
Integrase inhibitor	14 (23%)
Efuvirtide	1 (2%)
Maraviroc	1 (2%)
Previous antiretroviral exposure per patient	
No. of agents by class	
NRTI	2 (0–7)
NNRTI	1 (0–2)
Protease inhibitor	1 (0–5)
Lipodystrophy, clinical history	26 (42%)
Duration of AST and/or ALT elevation at biopsy, y	3.7 (1.1–11.4)
AST, U/L (normal range, 9–34 U/L)	46 (19–411)
ALT, U/L (normal range 6–41 U/L)	72 (27–1244)
ALT ≤82 U/L (≤2× ULN)	38 (61%)
ALT > 82–123 U/L (2–3× ULN)	12 (19%)
ALT >123 U/L (3× ULN)	12 (19%)
GGT, U/L (normal range, 11–52 U/L)	97 (20–977)
Platelet count, × 1000/μL (normal range, 150–400 × 1000/μL)	208 (79–380)
BMI, kg/m^2	27.6 (15.3–47.1)
Overweight (25–29.9)	29 (48%)
Obese (≥30)	19 (31%)
Waist circumference, cm (n = 53)	96 (63–147)
Waist-to-hip circumference ratio	1.0 (0.8–1.1)
Known diabetes mellitus	2 (3%)
2-hour glucose (oral 75-g glucose tolerance test) (n = 57)[a]	
Normal (<140 mg/dL)	39 (68%)
Impaired glucose tolerance (140–199 mg/dL)	14 (25%)
New diagnosis diabetes mellitus (≥200 mg/dL)	4 (7%)
HOMA-IR (n = 60)[a]	4.5 (0.2–154.3)
HOMA-IR > 2.6	49 (82%)
HOMA-IR > 3.8	35 (58%)
Use of antilipid therapy, any	29 (47%)
Statin use	22 (35%)
Total cholesterol, mmol/L	191 (95–422)
HDL cholesterol, mmol/L	39 (16–114)
Triglycerides, mmol/L	193 (51–553)
Abdominal CT imaging (n = 60)	
Decreased liver density, consistent with steatosis (clinical read)	17 (28%)
Hepatomegaly	6 (8%)
Splenomegaly	8 (13%)
Abdominal CT image analysis (n = 60)	
Adipose tissue volume at L4/5, cm^3	
Subcutaneous	142 (8–592)
Visceral	171 (16–451)
Subcutaneous/visceral ratio	1.1 (0.1–16.3)
Liver and spleen attenuation, HU	
Liver attenuation	54 (19–74)
Spleen attenuation	52 (12–126)
Liver/spleen attenuation ratio	1.1 (0.3–2.0)
Liver/spleen attenuation ratio <1.0	19 (32%)
Liver volume, cm^3	2211 (1447–4930)
Spleen volume, cm^3	371 (119–1011)

Table 1 continued.

Median and range presented unless noted otherwise.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CT, computed tomography; GGT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; HOMA-IR, homeostatic model assessment of insulin resistance; HU, Hounsfield units; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleos(t)ide reverse transcriptase inhibitor; ULN, upper limit of normal.

[a] Two participants with known diabetes mellitus did not undergo oral glucose tolerance testing and were not included in the HOMA-IR calculations.

significant fibrosis, patients with liver biopsy evidence of significant fibrosis (Iskak stage ≥2) had higher levels of inflammation and steatosis on liver biopsy, reflecting the association of NASH with fibrosis in this cohort (Supplementary Data). Given that fibrosis and NASH are more likely to progress to clinically significant liver disease, we looked for markers that could distinguish patients with fibrosis or NASH from patients with nonspecific findings on biopsy. Three participants with portal venopathy/NRH were excluded from these comparisons.

Table 2. Liver Biopsy Findings (n = 62)

Histologic Feature or Diagnosis	No. (%)
Steatosis	
None to trace (0) (<5%)	17 (27)
Mild (1) (5%–25%)	25 (40)
Moderate (2) (25%–50%)	13 (21)
Severe (3–4) (50%–75%)	7 (11)
Fibrosis score[a]	
None (0)	43 (69)
Mild (1)	7 (11)
Moderate (2)	1 (2)
Bridging (3–4)	11 (18)
Cirrhosis (5–6)	0 (0)
Diagnosis	
Nonspecific changes	22 (35)
Steatohepatitis	34 (55)
Steatohepatitis with any fibrosis	26 (42)
Steatohepatitis with bridging fibrosis	10 (16)
Fibrosis (Ishak stage ≥1) without evidence of steatohepatitis	3 (5)
Bridging fibrosis without steatohepatitis	1 (2)
Portal venopathy/nodular regenerative hyperplasia	3 (5)

All biopsies had at least 10 portal tracts for assessment. Median biopsy length was 15 mm (range, 7–24 mm); 59 of 62 (95%) biopsies were ≥10 mm in length.

[a] Fibrosis classified according to the Ishak modified histology activity index scoring system [20].

As summarized in Table 3, AST was higher in patients with Ishak stage ≥2 compared with those with little or no fibrosis (61 U/L vs 38 U/L; $P = .03$); however, ALT levels did not differ significantly (81 U/L vs 68 U/L; $P = .27$). AST/ALT ratio and FIB-4 were higher and NAFLD-FS was lower in patients with Ishak stage ≥2; APRI was similar between groups. Liver and spleen volumes did not differ significantly between those with and without fibrosis.

AST, ALT, and APRI were higher in the NASH cohort compared with the nonspecific changes group (Table 3); however, AST/ALT ratio, FIB-4, and NAFLD-FS were similar between groups.

Liver attenuation and liver-to-spleen attenuation ratios were significantly reduced in NASH patients compared with those with nonspecific changes on biopsy. No significant difference was seen in the volume of visceral adipose tissue (VAT) or VAT-to-subcutaneous adipose tissue ratio between the groups.

BMI, waist circumference, waist-to-hip ratio, and 2-hour glucose during OGTT were higher in patients with NASH compared with those without NASH. Fasting insulin levels and HOMA-IR were also higher in the NASH cohort, although fasting glucose did not differ between groups. High-density lipoprotein cholesterol was lower in patients with NASH; however, no significant differences were seen between the groups in other lipid parameters or in use of anti-lipid therapies including statins. The prevalence of metabolic syndrome did not differ between the groups.

No significant difference was seen between those with and those without significant fibrosis or in those with and without NASH in HIV-specific parameters, including $CD4^+$ T-cell count, $CD4^+$ nadir, history of opportunistic infection, time since HIV diagnosis, duration of antiretroviral use, or exposure to antiretroviral agents by class (Supplementary Data). No association was seen between NASH and a clinical diagnosis of lipodystrophy (data not shown).

In multivariate analysis, liver attenuation and liver-to-spleen ratio were the strongest predictors of NASH.

SNP Analysis

The call rates for both SNPs examined were 100%. A significantly higher frequency of both minor alleles, known to be in strong linkage disequilibrium with each other, was seen in patients with NASH, each with odds ratios of 3.9 compared with participants with nonspecific changes on liver biopsy ($P < .004$; Table 4). Although differences in racial distribution between the pathologic subgroups may account for some of the differences in observed frequency, frequency remained significantly higher in those with NASH, even when the analysis was limited to white, not Hispanic/Latino, patients (Table 4). Furthermore, on univariate analysis, each SNP highly correlated with increased ALT levels, steatosis grade, and fibrosis score (Table 5).

DISCUSSION

This prospective study, which represents the largest biopsy series to date, identified a high prevalence of clinically significant liver disease in HIV-monoinfected adults with elevated aminotransferases while receiving ART. NAFLD was found in 73%, NASH in 54% and bridging fibrosis in 17% of the cohort. Fibrosis occurred with NASH in the majority of patients: 26 of 31 (84%) with any fibrosis and 10 of 11 (91%) patients with bridging fibrosis.

Our findings are consistent with prior observations in smaller cohorts. In a French study of 30 HIV-monoinfected adults with elevated aminotransferases for ≥6 months, liver biopsy identified NASH in 53% and bridging fibrosis in 13% [6]. Similarly, in a US cohort of 14 patients, steatosis was seen in 65%, NASH in 26%, and bridging fibrosis in 14% [7].

Rates of NASH and fibrosis in our cohort are higher than those reported in HIV-negative and viral hepatitis–negative populations undergoing liver biopsy for evaluation of elevated aminotransferases, although differences exist in study populations, biopsy indications, and staging systems. In a US cohort of 81 patients with AST or ALT >1.5 × ULN on at least 2 occasions over 6 months, NASH was seen in 32% and bridging

Table 3. Comparison of Selected Clinical and Research Parameters by Fibrosis Score and Diagnosis

Characteristic	Ishak Fibrosis Score[a] on Liver Biopsy			Biopsy Diagnosis[a,b]		
	<2 (n = 47)	≥2 (n = 12)	*P* Value	Nonspecific Changes (n = 22)	NASH (n = 34)	*P* Value
Demographics						
Age, y	49 (21–67)	53 (45–63)	.12	50 (21–67)	50 (31–63)	.37
Male sex, No. (%)	44 (94%)	11 (92%)	.81	20 (91%)	32 (94%)	.65
Race (self-identified), No. (%)			.57			.14
White	32 (68%)	7 (58%)		17 (77%)	18 (53%)	
Black	4 (9%)	0 (0%)	.05	1 (5%)	3 (9%)	.39
Asian	0 (0%)	2 (17%)		0 (0%)	1 (3%)	
Not selected/mixed race/other	11 (23%)	3 (25%)		4 (18%)	11 (32%)	
Ethnicity			1			.16
Hispanic/Latino	15 (32%)	4 (33%)		4 (18%)	14 (41%)	
Not Hispanic/Latino	32 (68%)	8 (67%)		18 (82%)	20 (59%)	
Abdominal CT imaging						
Adipose tissue volume at L4/5						
Subcutaneous adipose tissue, mm^3	142 (8–592)	223 (36–568)	.07	142 (8–357)	148 (18–592)	.13
Visceral adipose tissue, mm^3	162 (16–451)	201 (127–268)	.08	165 (18–342)	188 (34–451)	.26
Visceral to subcutaneous ratio	1.2 (0.1–16.3)	0.8 (0.5–5.2)	.45	1.2 (0.1–16.3)	1 (0.3–5.2)	.24
Liver attenuation, HU	58 (19–74)	45 (23–62)	.007	63 (51–74)	46 (19–72)	<.001
Spleen attenuation, HU	52 (12–126)	51 (33–59)	.43	52 (33–60)	52 (12–126)	.38
Liver/spleen attenuation ratio	1.1 (0.3–2.0)	0.9 (0.4–1.3)	.04	1.2 (1.0–1.7)	0.9 (0.3–2.0)	<.001
Liver volume, cm^3	2222 (1447–4930)	2258 (1277–3293)	1	2140 (1447–3029)	2371 (1806–4930)	.18
Spleen volume, cm^3	355 (119–768)	532 (120–1011)	.06	331 (173–768)	381 (119–1011)	.47
Laboratory studies						
Platelets $\times 10^9$/μL	212 (106–376)	192 (79–380)	.39	217 (149–376)	205 (79–380)	.45
Albumin, g/dL	4.1 (3.6–4.6)	4.1 (3.2–4.6)	.42	4.0 (3.7–4.4)	4.1 (3.2–4.6)	.04
AST, U/L	38 (19–411)	61 (27–187)	.04	34 (19–89)	55 (26–411)	.02
ALT, U/L	68 (27–1244)	81 (45–182)	.27	60 (27–109)	84 (35–1244)	.005
ALT > 82 U/L (2× ULN), No. (%)	18 (38%)	5 (42%)	.61	5 (23%)	17 (50%)	.04
Duration of AST and/or ALT elevation at biopsy, y	3.5 (1.1–10.8)	5.0 (1.5–11.4)	.2	3.7 (1.1–10.8)	3.5 (1.1–10.8)	.4
GGT, U/L	93 (20–805)	99 (37–158)	.88	73 (20–574)	102 (29–805)	.18
AST/ALT	0.55 (0.33–1.49)	0.71 (0.51–1.03)	.01	0.58 (0.40–1.37)	0.62 (0.33–1.49)	.45
APRI [13]	0.6 (0.2–6.6)	0.7 (0.4–7.0)	.11	0.53 (0.22–1.16)	0.72 (0.25–6.96)	.02
FIB-4 [14]	1.1 (0.3–4.3)	1.5 (0.9–8.1)	.03	1.1 (0.3–2.0)	1.2 (0.6–8.1)	.28
<1.45 (no to mild fibrosis)	32 (68%)	5 (42%)		14 (64%)	22 (65%)	
>3.25 (advanced fibrosis)	2 (4%)	3 (25%)		0 (0%)	4 (12%)	
NAFLD fibrosis score [15]	−1.82 (−4.67–0.22)	−1.23 (−2.56–1.69)	.02	−1.85 (−4.67–0.22)	−1.61 (−1.61–1.69)	.14
≤ −1.455 (low probability of fibrosis)	31 (66%)	6 (50%)		16 (73%)	19 (56%)	
>0.676 (high probability of fibrosis)	0 (0%)	2 (17%)		0 (0%)	2 (6%)	
Metabolic indicators						
Body mass index, kg/m^2	26.8 (15.3–47.1)	30.0 (24.1–33.3)	.14	25.4 (21.4–33.7)	29.1 (15.3–47.1)	<.001
Waist circumference, cm	95 (63–147)	101 (87–119)	.22	91 (82–118)	101 (63–147)	.006
Hip circumference, cm	97 (78–130)	98 (87–114)	.62	95 (89–113)	100 (78–130)	.03
Waist-to-hip ratio	0.98 (0.81–1.13)	1.03 (0.95–1.08)	.1	0.96 (0.85–1.10)	1.01 (0.81–1.13)	.03
2-hour glucose (OGTT), mg/dL	116 (64–180)	154 (97–275)	.01	109 (64–162)	128 (81–275)	.03
Hemoglobin A1C, %	5.6 (4.4–6.2)	6.3 (4.4–6.7)	.04	5.5 (4.7–6.1)	5.7 (4.4–6.7)	.03
Glucose, fasting, mg/dL	102 (71–157)	108 (92–141)	.01	102 (83–157)	103 (71–141)	.61

Table 3 continued.

Characteristic	Ishak Fibrosis Score[a] on Liver Biopsy			Biopsy Diagnosis[a,b]		
	<2 (n = 47)	≥2 (n = 12)	*P* Value	Nonspecific Changes (n = 22)	NASH (n = 34)	*P* Value
Insulin, fasting, mg/dL	15.2 (1.2–75.3)	25.4 (12.3–504)	.04	12.7 (1.9–40.0)	21.9 (1.2–930.0)	.002
HOMA-IR	3.9 (0.2–18.2)	7.1 (2.9–154.1)	.03	3.4 (0.4–8.5)	6 (0.2–284)	.003
Triglycerides, mmol/L	185 (51–521)	291 (71–553)	.17	168 (51–520)	220 (55–553)	.32
Cholesterol, total, mmol/L	191 (95–422)	198 (122–276)	.96	176 (109–422)	197 (95–299)	.36
HDL cholesterol, mmol/L	42 (23–114)	33 (16–54)	.007	44 (27–76)	36 (16–114)	.009
Metabolic syndrome, presence, No. (%)	15 (32%)	10 (83%)	.001	8 (36%)	15 (45%)	.43
HIV-related factors						
Time from HIV diagnosis, y	17.5 (2.3–27.8)	17.1 (3.8–24.8)	.72	18.2 (2.7–24.7)	16.3 (2.3–27.8)	.37
Total $CD4^+$ T-cell count, cells/μL	539 (105–1631)	592 (138–1525)	.62	498 (105–1115)	580 (138–1631)	.4
$CD4^+$ T-cell percentage	30 (7–49)	31 (8–47)	.67	31 (8–49)	28 (7–48)	.19
$CD4^+$ T-cell nadir, historical, cells/μL	195 (<10–599)	160 (<10–423)	.75	189 (12–561)	178 (6–599)	.41
History of opportunistic infection, No. (%)	21 (45%)	4 (33%)	.48	8 (36%)	17 (50%)	.59
ART duration at biopsy, y	12.4 (1.7–22.8)	13.0 (2.7–21.6)	.96	12.9 (3.2–20.6)	11.1 (1.7–22.8)	.33

Data are presented as median (range) unless noted otherwise.

Abbreviations: ALT, alanine aminotransferase; APRI, aspartate aminotransferase–to–platelet ratio index; ART, antiretroviral therapy; AST, aspartate aminotransferase; CT, computed tomography; GGT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; HOMA-IR, homeostatic model assessment of insulin resistance; HU, Hounsfield units; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OGTT, oral glucose tolerance testing; ULN, upper limit of normal.

[a] Three participants with portal venopathy/nodular regenerative hyperplasia were excluded from this analysis.

[b] Three participants with fibrosis without steatohepatitis were excluded from this analysis.

fibrosis and cirrhosis in 7% [28]. In a French cohort of 272 adults with ALT greater than the ULN on at least 3 occasions over 6 months, liver biopsy identified NASH in 33% and advanced fibrosis in 10% [29].

Taken together, these results suggest that NASH and fibrosis are frequently overlooked and potentially serious complications in HIV-infected patients on ART. Our study demonstrates that there is considerable overlap in historical, laboratory, and imaging findings in those with nonspecific changes and simple steatosis on liver biopsy and those with NASH and/or fibrosis, making liver biopsy necessary for identification of NASH and advanced fibrosis in HIV-infected patients with unexplained elevations in aminotransferase levels. Transient elastography (Fibroscan) has been shown to have good sensitivity and specificity for detection of advanced fibrosis in HIV/viral hepatitis–coinfected patients as well as in HIV-negative NASH populations and may be useful in the current study population (unpublished observations). Liver biopsy can best identify patients at risk for progressive liver disease, largely at a stage where close monitoring and, in some cases, intervention can alter the natural history and potentially improve long-term outcomes.

The high prevalence of NAFLD and NASH seen in our study raises concerns about long-term prognosis in this population. Studies have shown increased morbidity and mortality in non-HIV-infected patients with NAFLD, including increased risk of cardiovascular disease and malignancy; outcomes are worse in those with NASH compared to those with simple steatosis [30]. Compared with patients with advanced liver disease and cirrhosis due to hepatitis C, patients with NAFLD and advanced fibrosis or cirrhosis have lower rates of liver-related complications but equivalent mortality [31]. Furthermore, NAFLD is an independent risk factor for hepatocellular carcinoma, accounting for 16% of cases, with more than a third of cases occurring in the absence of cirrhosis in some reports [32]. Early diagnosis and effective management of fatty liver disease in HIV-infected adults may result in the prevention or delay of these complications. Diet modification and exercise to reduce weight, vitamin E supplementation, and treatment with antidiabetic medication have shown benefit in HIV-negative patients with NAFLD, although the benefits of these interventions in HIV-associated NAFLD have not been studied [33].

Fatty liver disease in HIV has been associated with a number of factors, including factors associated with NAFLD in the general population such as sex, obesity, insulin resistance, and hypertriglyceridemia, and with HIV-related factors including ART and lipodystrophy. ART is associated with metabolic abnormalities, including hypertriglyceridemia, insulin resistance, peripheral fat loss and central fat accumulation, and

Table 4. Allele Frequency of 2 Single-Nucleotide Polymorphisms in PNPLA3 in HIV-Monoinfected Adults With Elevated Aminotransferases and in Reference Populations

		HIV-Monoinfected Adults With Elevated Aminotransferases (Current Report)								
SNP	Allele (Minor/ Major)	All (N = 62)	NS Liver Biopsy Changes (n = 22)	NASH (n = 34)	*P* Value, NS vs NASH[a]	Odds Ratio, NS vs NASH (95% CI)	NS, White, Not Hispanic/ Latino (n = 19)	NASH, White, Not Hispanic/ Latino (n = 19)	*P* Value, NS vs NASH, White, Not Hispanic/ Latino Only[a]	Odds Ratio, NS vs NASH, White, Not Hispanic/ Latino Only (95% CI)
rs738409	G/C	0.371/0.629	0.204/0.796	0.500/0.500	.003	3.9 (1.6–9.3)	0.147/0.853	0.421/0.579	.018	4.2 (1.3–13.3)
rs2281135	A/G	0.323/0.677	0.159/0.841	0.426/0.574	.004	3.9 (1.5–10.1)	0.088/0.912	0.342/0.658	.011	5.4 (1.4–21.0)

		Reference Populations			
		1000 Genomes Project [27]			NAFLD, HIV-Negative, White [22]
SNP	Allele (Minor/ Major)	All-Population Frequency	White Frequency	Mexican Frequency	
rs738409	G/C	0.284/0.716	0.213/0.787	0.598/0.402	0.505/0.495
rs2281135	A/G	0.272/0.728	0.178/0.822	0.545/0.455	0.368/0.632

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; NAFLD, nonalcoholic fatty liver disease; NASH,nonalcoholic steatohepatitis; NS, nonspecific; SNP, single-nucleotide polymorphism.

[a] Two-sided χ^2 test for comparison of allelic frequency between participants with nonspecific changes on liver biopsy and those with NASH.

Table 5. Univariate Association of Single-Nucleotide Polymorphism Genotype With Selected Laboratory Parameters and Histologic Parameters of Disease Severity

SNP	AST	ALT	Steatosis	HAI Inflammation Score	Ishak Fibrosis Score
rs738409	.012	<.001	.003	<.001	.001
rs2281135	.016	<.001	.009	.013	.006

Data are presented as *P* values.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HAI, histologic activity index; SNP, single-nucleotide polymorphism.

mitochondrial toxicity, which are associated with increased risk of NAFLD [34]. However, the contribution of ART to risk of hepatic steatosis remains uncertain [35, 36]. We were unable to demonstrate any association between NAFLD and current or previous thymidine analogue or protease inhibitor use, with exposure to specific antiretroviral medications classes, or with cumulative ART exposure. Because our study enrolled only patients receiving ART and did not include a comparison cohort of HIV-infected untreated patients, we cannot draw any conclusions about the contribution of HIV or ART per se to the high rates of NASH and fibrosis in our cohort.

Markers of insulin resistance were strongly associated with NASH. OGTT identified 4 previously undiagnosed cases of diabetes and 13 participants with impaired glucose tolerance. The high prevalence of insulin resistance suggests that patients with unexplained elevations in aminotransferases should be evaluated for insulin resistance with OGTT. The early detection of impaired glucose tolerance could allow intervention with lifestyle modifications or medication to potentially delay or avoid development of diabetes mellitus [37].

Our study is the first to demonstrate an association between polymorphisms in the *PNPLA3* gene and biopsy-documented steatosis and fibrosis, as well as aminotransferase levels, in HIV-monoinfected patients. In other populations, these polymorphisms have been strongly associated with severity of steatosis and fibrosis in NAFLD [22], alcoholic liver disease, chronic hepatitis C, and, more recently, with risk of hepatocellular carcinoma [38]. *PNPLA3* is a protein with triacylglycerol lipase and acylglycerol O-acyltransferase activities, but may have additional roles in liver metabolism [39]. A recent cross-sectional study of the Multicenter AIDS Cohort Study (MACS) found that the non-CC variant of rs738049 in the *PNPLA3* gene was associated with computed tomography–defined hepatic steatosis in HIV-positive patients, 12% of whom were HCV coinfected, but not in HIV-negative patients [40]. The strong association with pathology in our cohort together with the MACS data suggests that these variants play a significant, potentially additive role in risk of liver disease in HIV-infected populations.

Three participants, all of whom had previously received didanosine, were found to have liver pathology associated with noncirrhotic portal hypertension, NRH and portal venopathy. Noncirrhotic portal hypertension has previously been associated with ART, especially didanosine [41].

Limitations of our study include selection bias of patients referred and willing to undergo liver biopsy, underreporting of alcohol use, and sampling error inherent in biopsy-based studies.

In conclusion, chronic elevations in serum aminotransferases in HIV-infected adults receiving ART are associated with significant pathology in a high proportion of patients. Long-term follow-up of this cohort will provide important information about the clinical consequences of these findings.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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