AP&T Alimentary Pharmacology and Therapeutics

Clinical, biochemical and histological differences between HIV-associated NAFLD and primary NAFLD: a case–control study

I. Vodkin*, M. A. Valasek†, R. Bettencourt‡, E. Cachay§ & R. Loomba*,‡,¶

*Division of Gastroenterology, Department of Medicine, University of California, San Diego, La Jolla, CA, USA.
†Department of Pathology, University of California, San Diego, La Jolla, CA, USA.
‡Division of Epidemiology, Department of Family and Preventative Medicine, University of California, San Diego, La Jolla, CA, USA.
§Department of Medicine, University of California, San Diego, San Diego, CA, USA.
¶NAFLD Translational Research Unit, Department of Medicine, University of California, San Diego, La Jolla, CA, USA.

Correspondence to:
Dr R. Loomba, Division of Gastroenterology and Epidemiology, School of Medicine, University of California, San Diego, UC 303, MC-0063, 9500 Gilman Drive, La Jolla, CA 92093, USA.
E-mail: roloomba@ucsd.edu

Publication data
Submitted 20 October 2014
First decision 18 November 2014
Resubmitted 23 November 2014
Accepted 24 November 2014
EV Pub Online 11 December 2014

This article was accepted for publication after full peer-review.

SUMMARY

Background
There are limited data regarding the clinical, biochemical and liver histological characteristics of patients with HIV-associated nonalcoholic fatty liver disease (NAFLD), and whether this entity differs in presentation and severity from primary NAFLD

Aim
To examine the clinical and histological differences between HIV-associated NAFLD and primary NAFLD.

Methods
This is a cross-sectional, case–control study comparing patients with HIV-associated NAFLD vs. patients with primary NAFLD. HIV-infected patients were identified from a database of consecutive liver biopsies performed at the University of California at San Diego, over a 13-year period. HIV-infected patients with biopsy-proven NAFLD were selected as cases, after exclusion of other causes of liver disease and hepatic steatosis. Age–sex-matched controls with biopsy-proven primary NAFLD were randomly identified from the same pathology database. All biopsies underwent a standardised, detailed, histological research evaluation by a liver pathologist who was blinded to clinical and case–control status.

Results
Compared to age–sex-matched patients with primary NAFLD ($n = 33$), patients with HIV-associated NAFLD ($n = 33$) had significantly higher mean aspartate aminotransferase ($P < 0.001$), alanine aminotransferase ($P < 0.001$), alkaline phosphatase ($P = 0.003$) and serum triglycerides ($P = 0.024$). Similarly, compared to age–sex-matched primary NAFLD, patients with HIV-associated NAFLD had significantly higher rates of definite steatohepatitis (37% vs. 63%, $P = 0.04$), and more features of liver injury, including lobular inflammation (<0.001) and acidophil bodies (<0.001).

Conclusion
Compared to age–sex-matched primary NAFLD, HIV-associated NAFLD has increased severity of liver disease and a higher prevalence of NASH.

Aliment Pharmacol Ther 2015; **41**: 368–378

doi:10.1111/apt.13052

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has become one of the most common causes of chronic liver disease in Europe and the United States (US).[1–4] NAFLD is defined as the presence of hepatic steatosis in the absence of other causes of liver disease in individuals who consume little or no alcohol. The American Association for the Study of Liver Diseases practice guidelines divide NAFLD into primary and secondary NAFLD.[5] The primary form of NAFLD is associated with metabolic risk factors such as obesity, insulin resistance and the metabolic syndrome.[6–8] Other aetiological factors, such as hepatitis C virus, acquired or genetic metabolic diseases and certain medications (including steroids, methotrexate, amiodarone and some antiretroviral agents), can result in a similar pattern of steatosis and liver injury, which is termed secondary NAFLD.[5, 9] HIV-associated NAFLD is an important cause of secondary NAFLD, which is likely multifactorial, and is linked to HIV-associated lipodystrophy,[10] direct medication effects and metabolic risk factors.

Non-alcoholic fatty liver disease is a spectrum of liver disease that ranges from non-alcoholic fatty liver, which is considered to be nonprogressive form of NAFLD, to non-alcoholic steatohepatitis (NASH), which is considered to be the progressive form of NAFLD. NASH can lead to adverse liver-related outcomes such as cirrhosis, hepatocellular carcinoma and increased risk of death from liver disease in a subset of patients.[11–15]

HIV is a major global health issue, with 35.3 million people living with the disease worldwide, with 2–3 million in the United States and Western and Central Europe.[16] In the era of effective combination antiretroviral therapy (cART), major reductions in acquired immunodeficiency syndrome (AIDS)-related mortality have resulted in a shift towards HIV becoming a chronic illness. Accordingly, liver disease has become the second leading cause of non-AIDS-related deaths, accounting for nearly 7–14% of all deaths in patients with HIV.[17–19] Although viral hepatitis is still the leading cause of liver-related morbidity and mortality in this population, nearly half of the HIV-infected patients that undergo evaluation for unexplained liver test abnormalities are found to have NAFLD.[20] The prevalence of NAFLD is higher in individuals with HIV infection (30–40%) than in the general population.[21, 22] As HIV-infected patients are ageing, there has been a rise in the prevalence of obesity (8–14%)[23, 24] and the metabolic syndrome (14–18%),[25, 26] which will likely further increase the risk of NAFLD in this population.

Despite the high prevalence of NAFLD in the HIV-infected population, limited data exist regarding the clinical, biochemical and histopathological characteristics of HIV-associated NAFLD, and whether this entity differs in clinical presentation and histological severity from primary NAFLD. Therefore, we aimed to examine the differences between HIV-associated NAFLD and primary NAFLD. Our hypothesis is that HIV-associated NAFLD leads to more severe liver disease and higher rates of NASH than primary NAFLD.

METHODS

Study design, setting, inclusion and exclusion criteria

We performed a cross-sectional, case–control study comparing patients with HIV-associated NAFLD vs. patients with primary NAFLD. All HIV-infected patients were identified from a database of consecutive liver biopsies performed at the University of California, San Diego, between 1 December, 1999 and 9 May 2012. Cases included patients with biopsy-proven HIV-associated NAFLD after exclusion of other causes of liver disease and hepatic steatosis. Controls included patients with primary NAFLD who were identified from the same pathology database, and age and sex matched using a computer generated randomisation algorithm. Patients were included if they had the diagnosis of NAFLD or cryptogenic cirrhosis and had liver biopsy tissue available for pathologist review. Exclusion criteria included viral hepatitis or other forms of chronic liver disease as determined by serology and liver histology. Additional exclusion criteria were average alcohol consumption of >20 g daily for men or >10 g daily for women (obtained from the medical record by extensive chart review), the presence of hepatocellular carcinoma or hepatic metastasis, and use of non-antiretroviral medications associated with secondary NAFLD (methotrexate, tamoxifen, amiodarone, glucocorticoids). This study was conducted with the approval of the Institutional Review Board at the University of California, San Diego. Detailed, systematic protocol for derivation of cases and controls is described below.

Derivation of the cohort

During the study period, 4525 liver biopsies were performed. Derivation of the cohort is presented in Figure S1. Of the liver biopsies performed during our study period, 505 were performed in HIV-infected subjects. Of these, 419 were excluded due to the presence of viral hepatitis. An additional 47 were excluded due to other

causes of chronic liver disease or inadequate tissue sample. Of possible HIV-associated NAFLD, 39 cases were reviewed by the pathologist and a further six were excluded due to either a lack of steatosis or a high suspicion for alcoholic liver disease. The remaining 33 cases with NAFLD or cryptogenic cirrhosis were included in the study. Controls were derived from the same pathology database. Of the 4020 biopsies without HIV, 356 cases of possible NAFLD or cryptogenic cirrhosis were identified. Fifty were excluded due to lack of clinical data, alcohol use or presence of secondary NAFLD. The 306 remaining primary NAFLD controls were randomly identified by an independent statistician, and 33 were age and sex matched to our HIV cohort.

Materials and methods

Clinical and biochemical data were collected through a systematic chart review that was uniformly ascertained by a single trained investigator (IV). Demographic and lifestyle data collected included age, sex, ethnicity (Hispanic vs. not) and history of regular tobacco use. Anthropometric data included body mass index (BMI, kg/m^2). Clinical characteristics included the presence of type 2 diabetes (identified by ICD-9 code, fasting blood sugar ≥126, haemoglobin A1c ≥6.5 or use of a glucose lowering agent), hypertension (identified by ICD-9 code or use of a blood pressure lowering agent) and hyperlipidaemia (identified by ICD-9 code or use of a lipid lower agent). HIV-specific clinical data included date of HIV diagnosis, disease duration and history of opportunistic infections. Detailed history on current and past antiretroviral use was also ascertained, including use of nucleoside/tide reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors, protease inhibitors (PIs) and newer classes of antiretroviral medications. Laboratory data collected for all patients included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), albumin, internal normalised ratio, platelet count, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol (HDL), triglycerides, fasting glucose and haemoglobin A1c (HbA1c). HIV-specific laboratory data included CD4 T-cell count, CD4 nadir and HIV viral load (VL). For all demographic, laboratory and clinical parameters, we obtained the closest available data to the date of biopsy. We also calculated AST/ALT ratio, APRI score (defined as [(AST/ULN AST)/platelets] × 100),[27] and FIB-4 score (defined as age (years) × AST [U/L]/(platelets[10^9/L] × (ALT [U/L])$^{1/2}$)).[28] These markers were selected because they rely on easy to obtain laboratory data and have been used in HIV co-infected patients.[15, 29]

Liver histology

Before final inclusion, all selected cases and controls underwent a standardised, detailed, histological research evaluation by a liver pathologist (MAV) who was blinded to clinical status and case/control status. Liver biopsies were scored using the well-validated and previously used NASH CRN Histologic Scoring System.[30] Histology was assessed for the presence and grade of steatosis, pattern of steatosis (zone 1, 3 or panacinar) and presence of microvesicular steatosis. Assessment of inflammation included number of foci of lobular inflammation, severity of portal inflammation and the presence or absence of microgranulomas or lipogranulomas. Other histomorphological parameters evaluated using haematoxylin and eosin (H&E) stain included hepatocyte ballooning, acidophil bodies, Mallory–Denk bodies, glycogenated nuclei and megamitochondria. Periodic Acid–Schiff Diastase stain was used to evaluate for intracytoplasmic globules (as can be seen in alpha 1-anti-trypsin deficiency). Iron stain was also examined. Patients with significant hepatocyte iron were clinically ruled out for genetic haemochromatosis. The degree of fibrosis, assessed using the H&E and Trichrome stains, was divided into four stages, with '0' indicating no fibrosis and '4' indicating the presence of cirrhosis. Based on the global histological evaluation, liver biopsies were classified as no steatosis, simple steatosis, or either possible/borderline or definite steatohepatitis. Steatohepatitis was defined by the presence of steatosis, inflammation and ballooning degeneration with or without the presence of peri-cellular fibrosis. Patients who had no steatosis and were not classified as cryptogenic cirrhosis were excluded from the study (histology evaluation form is available as Appendix S1).

Statistical analysis

Baseline characteristics between the HIV-associated NAFLD and primary NAFLD were compared using either χ^2 or Fisher's exact tests for comparisons between categorical variables, and the two independent samples *t*-test for comparison of mean differences between continuous variables. Univariate analysis was used to identify clinical and histological differences between cases and controls. HIV-specific factors were also assessed for their association with the presence of advanced fibrosis (defined as grade 3 or 4) or steatohepatitis. Age, sex, ethnicity and BMI were then entered into a multivariable-adjusted model to examine the prevalence NASH and advanced

liver disease between the two groups. A two-tailed $P \leq 0.05$ was considered statistically significant. Statistical analyses were performed using SAS version 9.3 (Cary, NC, USA).

RESULTS

Demographic, clinical and biochemical characteristics in HIV-associated compared to primary NAFLD

Table 1 shows the demographic, clinical and biochemical characteristics of the cohort categorised into patients with HIV-associated NAFLD vs. primary NAFLD. The two groups were matched for age and sex, with 79% males with a mean age [±standard deviation (s.d.)] of 44 (±9) years. Both groups had similar rates of tobacco use and similar rates of metabolic risk factors including hypertension, type 2 diabetes mellitus and hyperlipidaemia. Patients with primary NAFLD and HIV-associated NAFLD also had similar BMI, with mean (±s.d.) of 30.6 (±5.4) vs. 29.8 (±6.0) kg/m^2, $P = 0.57$.

Compared to primary NAFLD, patients with HIV-associated NAFLD had significantly higher mean AST (41 U/L vs. 88 IU/L, $P < 0.001$), ALT (62 U/L vs. 146 U/L, $P < 0.001$), ALP (75 U/L vs. 141 U/L, $P = 0.003$), as well as significantly higher triglyceride levels (182 mg/dL vs. 242 mg/dL, $P = 0.02$).

Histological characteristics in HIV-associated compared to primary NAFLD

A standardised, detailed, histological research evaluation of the cohort, categorised into patients with HIV-associated NAFLD vs. primary NAFLD is pre-

Table 1 | Clinical, anthropometric and biochemical differences between HIV-associated and primary NAFLD

Characteristics	HIV-associated NAFLD ($n = 33$)	Primary NAFLD ($n = 33$)	P*
Demographics and lifestyle			
Male, *n* (%)	26 (78.8)	26 (78.8)	1.000
Age (mean years ± s.d.)	44.8 ± 9.4	44.3 ± 9.1	0.822
Hispanic, *n* (%)	17 (51.5)	8 (24.2)	**0.022**
Ever smoked regularly†, *n* (%)	12 (36.4)	11 (34.4)	0.867
Clinical			
Hypertension, *n* (%)	16 (48.5)	13 (40.6)‡	0.524
Type 2 diabetes, *n* (%)	6 (18.2)	7 (21.2)	0.757
Hyperlipidaemia, *n* (%)	21 (63.4)	17 (53.1)‡	0.390
Anthropometric (mean ± s.d.)			
Body mass index (kg/m^2)	29.8 ± 6.0	30.6 ± 5.4 ($n = 31$)	0.575
Hepatology panel (mean ± s.d.)			
AST (U/L)	88.4 ± 54.2	40.8 ± 15.6	**<0.001**
ALT (U/L)	146.2 ± 120.8	62.0 ± 23.6	**<0.001**
AST/ALT ratio	0.7 ± 0.2	0.7 ± 0.2	0.905
Alkaline phosphatase (ALP) (U/L)	140.6 ± 114.3	74.6 ± 23.1	0.003
Albumin (g/dL)	4.2 ± 0.5	4.3 ± 0.5	0.165
Bilirubin, total (mg/dL)	1.0 ± 0.9	0.9 ± 1.0	0.747
International normalised ratio	1.1 ± 0.4‡	1.0 ± 0.1	0.584
Other laboratory studies (mean ± s.d.)			
Platelet count (1000/mm^3)	218.2 ± 67.3	248.0 ± 91.2	0.136
Total cholesterol (mg/dL)	207.5 ± 55.4	191.6 ± 37.3	0.176
HDL cholesterol (mg/dL)	39.4 ± 14.4	44.9 ± 10.5	0.142
LDL cholesterol (mg/dL)	119.6 ± 40.9	115.2 ± 34.9	0.645
Triglycerides (mg/dL)	242.1 ± 108.6	181.7 ± 103.0	**0.024**
HbA1c (%)	5.9 ± 1.3 ($n = 28$)	5.7 ± 0.7 ($n = 27$)	0.495
Fasting serum glucose (mg/dL)	100.7 ± 18.5 ($n = 31$)	101.8 ± 18.9	0.803

s.d., standard deviation; NAFLD, non-alcoholic fatty liver disease; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, haemoglobin A1c. *P*-values in bold are significant with a two-tailed less than or equal to 0.05.

* *P*-values (two-sided) determined from either a Fisher's exact or χ^2 test for categorical variables or *t*-test for continuous variables.

† Ever smoked regularly by self-report.

‡ $n = 32$.

Table 2 | Histological differences between HIV-associated and primary NAFLD

Histological feature*	HIV-associated NAFLD (n = 33), n (%)	Primary NAFLD (n = 33), n (%)	P†
Steatosis			
Grade			
0–1 (0–33%)	19 (57.5)	18 (54.5)	0.961
2 (>33–66%)	9 (27.3)	10 (30.3)	
3 (>66%)	5 (15.2)	5 (15.2)	
Location (predominant)			
Zone 3 (central)	12 (36.4)	13 (39.4)	1.000
Zone 1 (periportal)	1 (3.0)	1 (3.0)	
Azonal/Panacinar	19 (57.6)	18 (54.6)	
None (cryptogenic cirrhosis)	1 (3.0)	1 (3.0)	
Microvesicular steatosis: present	0 (0.0)	0 (0.0)	
Fibrosis			
Stage:			
None (0)	13 (39.4)	14 (42.4)	0.466
Mild/moderate (zone 3), portal/periportal (1A, 1B, 1C)	9 (27.3)	14 (42.4)	
Zone 3 and periportal (2)	5 (15.2)	3 (9.2)	
Bridging (3)	4 (12.1)	1 (3.0)	
Cirrhosis (4)	2 (6.0)	1 (3.0)	
Advanced fibrosis:	6 (18.2)	2 (6.1)	0.258
Inflammation			
Lobular inflammation (score) (no. foci per 200× field)			
0 to <2 foci (0–1)	11 (33.3)	26 (78.8)	**<0.001**
2–4 foci (2)	18 (54.6)	6 (18.2)	
>4 foci (3)	4 (12.1)	1 (3.0)	
Microranulomas: present	5 (15.2)	0 (0.0)	0.053
Lipogranulomas: present	15 (45.5)	18 (54.6)	0.460
Portal inflammation (score)			
None (0)	0 (0.0)	1 (3.0)	0.427
Mild (1)	28 (84.8)	30 (90.9)	
More than mild (2)	5 (15.2)	2 (6.1)	
Liver cell injury			
Ballooning (score):			
None (0)	11 (33.3)	16 (48.5)	0.102
Few (1)	18 (54.6)	17 (51.5)	
Many (2)	4 (12.1)	0 (0.0)	
Acidophil bodies:			
None (0)	12 (36.3)	28 (84.8)	**<0.001**
Rare (1)	15 (45.5)	5 (15.2)	
More than rare (2)	6 (18.2)	0 (0.0)	
Megamitochondria: present	1 (3.0)	0 (0.0)	
Other findings			
Mallory–Denk bodies:			
None (0)	26 (78.8)	22 (66.7)	0.260
Rare (1)	6 (18.2)	11 (33.3)	
More than rare (2)	1 (3.0)	0 (0.0)	
Glycogenated nuclei:			
None (0)	7 (21.2)	6 (18.2)	0.223
Few (1)	21 (63.6)	16 (48.5)	
Many (2)	5 (15.2)	11 (33.3)	
NAFLD Activity Score (NAS)			
0–4	21 (63.6)	27 (81.8)	0.097
5–8	12 (36.4)	6 (18.2)	
Mean ± s.d.	4.24 ± 1.5	3.33 ± 1.1	**0.006**

Table 2 | (Continued)

Histological feature*	HIV-associated NAFLD (n = 33), n (%)	Primary NAFLD (n = 33), n (%)	P†
Diagnostic classification			
Steatohepatitis (diagnosis):			
Cryptogenic cirrhosis	1 (3.0)	1 (3.0)	0.130
Not steatohepatitis (steatosis only)	7 (21.2)	12 (36.4)	
Possible/Borderline	4 (12.1)	8 (24.2)	
Definite steatohepatitis	21 (63.6)	12 (36.4)	
Prevalence of NASH	21 (63.6)	12 (36.4)	**0.027**

* Determination of histological features by expert pathologist using the NASH CRN scoring system.[30] *P*-values in bold are significant with a two-tailed less than or equal to 0.05.

† *P*-values (two-sided) determined from either a Fisher's exact or χ^2 test for categorical variables or *t*-test for continuous variables.

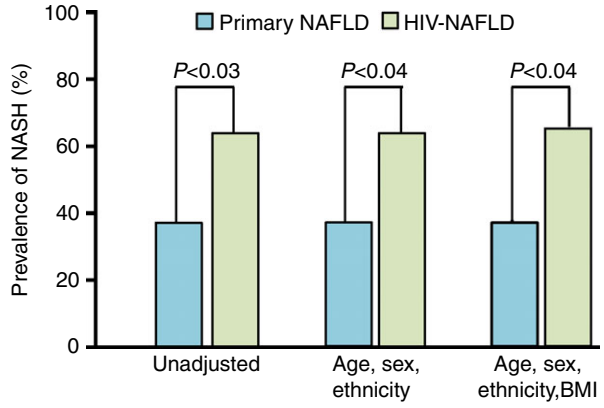

Figure 1 | Prevalence of NASH. HIV-associated NAFLD cases had significantly higher prevalence of NASH compared to primary NAFLD controls. This finding remained significant when adjusted for age, sex, ethnicity and body mass index (BMI). One patient with cryptogenic cirrhosis in each group was excluded from the analysis.

sented in Table 2. Compared to primary NAFLD, patients with HIV-associated NAFLD were more likely to have definite steatohepatitis (36.4% vs. 62.7%, $P = 0.027$, see Figure 1) and higher mean (±s.d.) NAFLD activity score (NAS) (3.33 ± 1.1 vs. 4.24 ± 1.5, $P = 0.006$). They were also more likely to have features of liver injury (see Figure 2), including lobular inflammation (21.2% vs. 66.7%, $P < 0.001$) and acidophil bodies (15.2% vs. 63.7%, $P < 0.004$). Fibrosis stage was similar between the two groups, with most patients having none or mild to moderate fibrosis. Grade and characteristics of steatosis, markers of inflammation and liver cell injury such as portal inflammation and ballooning, and many other features enumerated in Table 2 were not statistically significant.

Disease severity in HIV-associated NAFLD compared to primary NAFLD

Compared to primary NAFLD, patients with HIV-associated NAFLD were more likely to have definite steatohepatitis (36.4% vs. 62.7%, $P = 0.027$) in the univariate analysis. These results remained statistically significant even after multivariable-adjustment for age, sex, ethnicity and BMI (Figure 1).

In addition, compared to primary NAFLD, patients with HIV-associated NAFLD had significantly higher scores for non-invasive markers of advanced disease: APRI and FIB-4. In the univariate model, mean (95% CI) APRI between the two groups was 0.5 (0.2–0.7) vs. 1.2 (0.9–1.5), $P < 0.001$ and FIB-4 was 1.1 (0.7–1.5) vs. 1.8 (1.4–2.2), $P = 0.02$ (Table 3). Significantly fewer patients with HIV-associated NAFLD were in the mild disease group (≤0.5 for APRI[27] and ≤1.45 for FIB-4[28]) with 21.2% vs. 75%, <0.001 for APRI and 51.5% vs. 78.8%, $P = 0.02$ for FIB-4. These data remained significant when adjusted for age, sex, ethnicity and BMI, with mean (95% CI) APRI and FIB-4 of 0.5 (0.2–0.8) vs. 1.1 (0.9–1.4), $P = 0.004$ and 1.1 (0.7–1.6) vs. 1.8 (1.4–2.2), $P = 0.027$ respectively.

HIV-specific clinical and biochemical characteristics

During the initial cohort derivation, 86 biopsies in HIV-infected patients without viral hepatitis were identified and 33 (38%) were found to have NAFLD. Table S1 provides details of HIV-specific factors which were assessed in HIV-associated NAFLD. The mean (±s.d.) CD4 count in our cohort was 612.5 (±310.1) cells/μL. Most of the patients (90.9%) were on cART at the time of liver biopsy and 78.8% had achieved an undetectable VL.

Table 4 shows features of HIV infection categorised into not NASH vs. NASH and not advanced vs. advanced

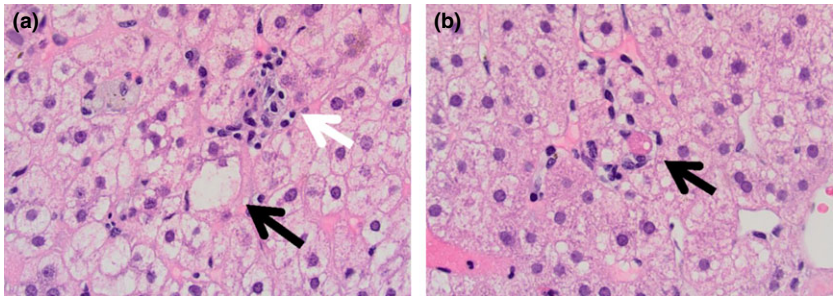

Figure 2 | Pathological lesions in HIV-associated NAFLD. (a) 600× haematoxylin and eosin (H&E) stain showing hepatocyte ballooning (black arrow) and lobular inflammation (white arrow). (b) 600× H&E stain showing acidophil body with associated inflammation.

Table 3 | Multivariable-adjusted models of demographic and laboratory features in HIV-associated compared to primary NAFLD: features of advanced disease

Non-invasive markers of fibrosis	HIV-associated NAFLD (n = 33), n (%)	Primary NAFLD (n = 33), n (%)	P*
APRI†, mean (95% CI)			
Unadjusted	1.2 (0.9–1.5)	0.5 (0.2–0.7)	<0.001
≤0.5, n (%)	7 (21.2)	25 (75.8)	<0.001
≥1, n (%)	11 (33.3)	2 (6.1)	0.005
Age, sex, ethnicity adjusted	1.1 (0.9–1.4)	0.5 (0.2–0.8)	0.003
Age, sex, ethnicity, BMI adjusted	1.1 (0.9–1.4)	0.5 (0.2–0.8)	0.004
FIB4‡, mean (95% CI)			
Unadjusted	1.8 (1.4–2.2)	1.1 (0.7–1.5)	0.020
≤1.45, n (%)	17 (51.5)	26 (78.8)	0.020
≥3.25, n (%)	3 (9.1)	1 (3.0)	0.613
Age, sex, ethnicity adjusted	1.8 (1.4–2.2)	1.1 (0.7–1.5)	0.027
Age, sex, ethnicity, BMI adjusted	1.8 (1.4–2.2)	1.1 (0.7–1.6)	0.027

* P-values (two-sided) determined from Fisher's exact or χ^2 test for categorical variables, or t-test for continuous variables.

† APRI: [(AST/ULN AST)/platelets] × 100. Cutoffs of ≤0.5 and ≥1 with best performance in the literature.[27]

‡ FIB-4: (Age × AST)/(Platelets × (√(ALT)). Cutoffs of ≤1.45 and ≥3.25 show best performance in the literature.[28]

liver disease (defined as bridging fibrosis or cirrhosis). Longer HIV duration was the only variable significantly associated with the presence of NASH, even when adjusted for age, with mean (95% CI) of 63.3 (23.5–103.0) vs. 139.6 (110.6–167.7) months, P = 0.004. Otherwise, there were no differences in the presence or absence of NASH or advanced fibrosis based on HIV-related factors.

DISCUSSION

Main findings

The aim of this study was to provide an in-depth clinical, biochemical and histopathological characterisation of HIV-associated NAFLD, and systematically examine differences between HIV-associated NAFLD and primary NAFLD. Our study demonstrated a significantly higher proportion of NASH as well as other laboratory and histological features of more severe liver injury in HIV-associated NAFLD, despite similar metabolic characteristics in the two groups. This highlights the fact that HIV-infected subjects are a particularly high-risk group for advanced liver disease, even without the presence of viral hepatitis.

In context with the published literature

Previous studies have shown that the prevalence of NAFLD (based upon imaging-based assessment) may be higher in HIV-infected subjects than the general

Table 4 | Features of HIV infection associated with advanced disease

Characteristics	Not NASH* $N = 11$, n (%)	NASH $N = 21$, n (%)	P†	Not advanced $N = 27$, n (%)	Advanced‡ $N = 6$, n (%)	P†
Clinical						
History of AIDS (OI or CD4 < 200), $n = 20$	6 (54.6)	13 (61.9)	0.721	17 (63.0)	3 (50.0)	0.659
History of OI, $n = 11$	5 (45.5)	6 (28.6)	0.442	10 (37.0)	1 (16.7)	0.637
HIV duration (months) mean (95% CI)	62.5 (24.0–100.9)	139.6 (111.8–167.4)	**0.002**	116.6 (85.9–147.3)	126.6 (61.6–191.7)	0.777
Age adjusted HIV duration (months) mean (95% CI)	63.3 (23.5–103.0)	139.2 (110.6–167.7)	**0.004**	118.4 (87.8–148.9)	118.6 (52.8–184.4)	0.995
Medications§						
NRTI only	0 (0)	0 (0)	0.385	0 (0)	0 (0)	0.076
NNRTI + PI	4 (36.4)	9 (42.9)		13 (48.2)	0 (0)	
NNRTI + NRTI	4 (36.4)	5 (23.8)		6 (22.2)	3 (50.0)	
Other	1 (9.1)	6 (28.6)		6 (22.2)	2 (33.3)	
None	2 (18.2)	1 (4.8)		2 (7.4)	1 (16.7)	
HIV panel						
CD4 cells/μL mean (95% CI)	649.8 (458.2–841.4)	611.8 (473.1–750.5)	0.745	623.3 (500.0–746.6)	564.0 (302.4–825.6)	0.679
VL >400 copy/mL, $n = 7$	3 (27.3)	4 (19.1)	0.668	7 (25.9)	0 (0)	0.301

AIDS, acquired immune deficiency syndrome; OI, opportunistic infection; ART, antiretroviral therapy; HIV, human immunodeficiency virus; NRTI, nucleoside/tide reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; VL, viral load. *P*-values in bold are significant with a two-tailed less than or equal to 0.05.

* One patient with cryptogenic cirrhosis excluded from analysis.

† All *P*-values from Fisher's exact test (HIV and CD4 tested with *t*-test).

‡ Defined as bridging fibrosis or cirrhosis.

§ HIV regimen at the time of liver biopsy. History of any prior exposure to stavudine was also evaluated and there was no difference in presence of NASH or advanced disease.

population. Crum-Cianflone *et al.* conducted a cross-sectional study of 216 HIV-infected patients and showed that the prevalence of hepatic steatosis was 31% using an ultrasound-based evaluation. Among the patients who underwent a liver biopsy evaluation in this study, 36% were found to have evidence of NAFLD.[22] Another study using computer tomography showed steatosis in 37% of 225 patients.[21] The prevalence of HIV-associated NAFLD in our study population was 38%. Therefore, the findings are consistent with prior reports. Patients with HIV-associated NAFLD in our study had higher rates of NASH compared to patients with age–sex-matched primary NAFLD and compared to the general population.[1, 3, 31] Although there were more Hispanic patients in the HIV group, our findings remained statistically significant when adjusted for ethnicity.[32, 33] There is a paucity of data on this topic in the existing literature, with small pathological studies reporting rates of NASH between 20% and 89%. Crum-Cianflone *et al.* showed NASH in 20% of 20 patients with biopsy-proven NAFLD.[22] Higher prevalence was seen in studies by Mohammed *et al.* and Ingiliz *et al.* who found NASH in 54.8% of 26 patients and 89% of 18 patients with NAFLD respectively.[34, 35] However, detailed description of clinical, biochemical and histological characteristics of HIV-associated NAFLD has not been previously reported.

Through detailed histological assessment, we identified additional features of advanced disease as well as ongoing liver injury in HIV-associated NAFLD compared to primary NAFLD. The HIV-associated NAFLD group had significantly higher NAS scores and more lobular inflammation and acidophil bodies. These are novel findings in this population. Laboratory evaluation

supported our pathological findings, with more significant transaminitis and higher APRI and FIB-4 scores in HIV-associated NAFLD. These makers were selected because they are readily available and have been evaluated in HIV-infected patients.[29]

We found similar BMI and metabolic risk factors, with the exception of higher triglyceride levels, in HIV-associated NAFLD compared to age and sex matched controls. Indeed, our findings are consistent with recent studies showing increasing obesity in the HIV population, and especially in HIV-infected women.[23, 24] The increase in triglycerides levels is also consistent with recent studies [22, 34] and is likely multifactorial, due to direct effect of certain antiretrovirals, indirect association with lipodystrophy or the reflection of metabolic syndrome in the context of increased BMI. Lipodystrophy, a syndrome associated with cART, is characterised by an increase in visceral adiposity, insulin resistance, hypertriglyceridaemia and low HDL.[25, 26, 36, 37] Acquired and genetic lipodystrophy is known to be associated with secondary NAFLD.[10]

The only HIV-specific factor correlating with advanced pathology in our study was duration of HIV infection. Longer duration was associated with NASH, even when adjusted for age. We speculate that either HIV infection itself or longer duration of ART use could explain this finding. Although hepatic steatosis has been linked to NRTI use,[38] and multiple metabolic abnormalities have been linked to PIs,[39] we saw no differences in disease severity based on cART regimen. In the existing literature, findings are mixed. Exposure to NRTIs was identified as a risk factor for hepatic steatosis in two studies,[21, 40] but not in others.[22, 41]

Strengths and limitations

The following are the key strengths of our study. We conducted a standardised, detailed, histological research evaluation of all biopsies by a liver pathologist who was blinded to clinical as well as case–control status. In contrast to prior studies, liver biopsies were scored using the well-validated NASH CRN Histologic Scoring System[30] and were used as the gold standard for the diagnosis of both NAFLD and NASH. Clinical and biochemical data were also collected through a systematic chart review by a single trained investigator. Cases and controls were identified from the same pathology database and matching was randomised. No significant differences were seen in the indication for liver biopsy, history of transaminitis or presence of fatty liver on imaging when comparing cases and controls (Table S2). To date, this is the first systematic assessment and detailed pathological description of HIV-associated NAFLD. With the emergence of novel therapies for the treatment of HIV-associated NAFLD,[37] a detailed description of liver histology of HIV-associated NAFLD is the quintessential next step in the eventual development of histological end-points to assess treatment response in HIV-associated NAFLD and NASH. However, we would like to acknowledge several limitations of our study. The main weaknesses of this study include its' retrospective design and cross-sectional nature. The relatively small sample size did not allow us to examine whether HIV-associated NAFLD is more likely to be associated with a higher fibrosis stage than primary NAFLD. It was, however, associated with significantly higher APRI and FIB-4 scores (non-invasive biomarkers for advanced fibrosis) than primary NAFLD. Larger studies are needed to examine the association between HIV-associated NAFLD and the stage of fibrosis. This is also a single centre study in a tertiary referral setting so there is a possibility of a referral bias. We do not think that referral bias was different between the two groups because there were no significant differences in the indications for liver biopsy between the HIV-associated NAFLD and primary NAFLD. An additional limitation was that biopsies were reviewed by a single pathologist, so inter-rater agreement could not be assessed.

CONCLUSION

HIV-associated NAFLD patients have significantly higher rates of NASH and increased severity of liver disease than age–sex-matched patients with primary NAFLD. This is the first study to provide a detailed clinical, biochemical and histopathological description of HIV-associated NAFLD. The age–sex-matched case–control design allows the clinicians to appreciate the differences between HIV-associated NAFLD and primary NAFLD using a standardised and validated histological scoring system. As new therapies emerge for HIV-associated NAFLD, improvement in liver histology will be an important treatment end-point. Therefore, detailed characterisation and understanding of the histopathology of HIV-associated NAFLD is a key step for initiating prospective studies to better characterise the natural history of HIV-associated NAFLD, and to develop histological end-points to assess treatment response.

AUTHORSHIP

Guarantor of the article: Dr. Loomba.

Author contributions: Drs. Vodkin and Loomba performed the research, Drs. Vodkin, Valasek and Ms. Bettencourt collected and analysed the data, Dr. Loomba designed the research study, Dr. Vodkin wrote the initial

draft of the paper, and Dr. Valasek, Ms. Bettencourt, Dr. Cachay and Dr. Loomba provided critical input on the manuscript, Dr. Cachay, Valasek, and Ms. Bettencourt contributed to the design of the study. All authors approved the final version of the manuscript.

ACKNOWLEDGEMENTS

Declaration of personal interests: None.

Declaration of financial interests: This study was funded in part by the American Gastroenterological Association (AGA) Foundation – Sucampo – ASP Designated Research Award in Geriatric Gastroenterology and by a T. Franklin Williams Scholarship Award; Funding provided by: Atlantic Philanthropies, Inc, the John A. Hartford Foundation, the Association of Specialty Professors, and the American Gastroenterological Association and grant K23-DK090303.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow diagram. Study design, selection of cases and controls, and reasons for exclusion.

Table S1. Clinical and biochemical characteristics of HIV-associated NAFLD.

Table S2. Indications for liver biopsy for HIV-associated and primary NAFLD.

Appendix S1. HIV-associated NAFLD vs. Primary NAFLD Study standard Pathology Evaluation Form.

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