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Incidence and risk factors for steatosis progression in adults coinfectd with HIV and hepatitis C virus

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Abstract

Background and Aims—Hepatic steatosis is a common histological finding in patients that are co-infected with HIV and hepatitis C virus (HCV), although little is known about its natural history. We prospectively examined the natural history of steatosis in patients co-infected with HIV and HCV that attended an urban HIV clinic.

Methods—The study cohort consisted of 222 co-infected patients (87% African American, 94% with HCV genotype 1 infection) who had at least 2 liver biopsies performed between 1993 and 2008. Biopsies were scored by a single pathologist; samples were classified as having trivial (< 5% of hepatocytes affected) or significant (>5%) levels of fat (steatosis). We characterized progression to significant levels of fat among patients whose first biopsy samples had no or trivial levels of fat, and regression among those with significant fat, using logistic regression.

Results—Initial biopsies from most patients (88%) had no or trivial amounts of fat. Among second biopsy samples, 74% had no or trivial fat and 13% had significant amounts of fat. The strongest risk factors for steatosis progression were alcohol abuse and overweight/obesity; cumulative exposure to anti-retroviral therapy between biopsies and high counts of CD4+ T cells were associated with reduced progression of steatosis. Among the 28 patients whose initial biopsy had significant fat levels, most (75%) regressed.

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Shruti Mehta: Statistical analysis, Analysis and interpretation of data. Manuscript revision

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Yvonne Higgins: Acquisition of data. Manuscript revision.

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Michael Torbenson: Acquisition of data. Manuscript revision.

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Mark Sulkowski: Study concept and design. Analysis and interpretation of data. Manuscript revision. Obtained funding.

Conclusions—Antiretroviral therapy and high counts of CD4+ T cells are associated with reduced progression of steatosis in patients co-infected with HIV and HCV. Efforts to diagnose and prevent steatosis should focus on persons with high body mass index and excessive alcohol intake.

Keywords

pathology; cirrhosis; AIDS; liver disease

Introduction

Hepatitis C virus (HCV) infects approximately 15–30% of individuals with human immunodeficiency virus (HIV) infection.^{1,2} Compared to those without HIV disease, co-infected persons experience more rapid progression of liver fibrosis, leading to cirrhosis, hepatocellular carcinoma and end-stage liver disease.³ Liver disease has emerged as a significant cause of morbidity and mortality in HIV-infected patients in the era of effective antiretroviral therapy (ART).^{4,5}

Hepatic steatosis is a common histological finding in HIV/HCV co-infected individuals, occurring in about 30–70% of patients.^{6–15} Several studies have shown that steatosis is more frequent and severe in co-infected individuals compared to those with HCV-monoinfection.^{7,8} Hepatic steatosis in HIV/HCV co-infected individuals has particular clinical importance as it has been associated with presence of significant fibrosis^{6–10,14–15} and thus may contribute to the more rapid progression of liver disease. The etiology of steatosis in co-infected patients is multifactorial, related to both host and viral factors, similar to those observed in HCV-monoinfected patients.^{16,17} Studies have identified Caucasian race, obesity, hyperglycemia, hyperlipidemia, lipodystrophy, and HCV viral load as risk factors for steatosis^{6,7,9,10,12–15}, but these data derive primarily from cross-sectional studies. Further, in patients infected with HCV genotype 3, the virus is thought to cause steatosis through a direct cytopathic effect.¹⁸

Beyond HIV itself, antiretroviral therapy (ART) may also predispose individuals to the development of steatosis through various mechanisms. Protease inhibitors (PIs) are associated with risk factors for steatosis such as insulin resistance and hyperlipidemia.^{19,20} Certain nucleoside reverse-transcriptase inhibitors (NRTIs) may lead to the abnormal deposition of triglycerides in hepatocytes as a result of mitochondrial toxicity.^{21,22} The presence of steatosis has been associated with NRTIs such as stavudine and didanosine in HIV/HCV co-infected patients.^{9,10,15}

Although several cross-sectional studies have shown that steatosis is prevalent in co-infected patients^{6–15}, the natural history of hepatic steatosis is incompletely understood due to limited prospective observations. Having previously characterized the prevalence and risk factors for steatosis in a cross-sectional study of 112 HIV/HCV co-infected persons¹⁵, the study objective was to prospectively examine the natural history of steatosis by examining subsequent liver biopsies performed in these and additional HIV/HCV co-infected patients attending an urban HIV clinic.

Methods

Study population

The study population consists of 222 HCV/HIV co-infected patients who had at least two liver biopsies between July 1993 and December 2008 and were followed prospectively in the Johns Hopkins University HIV clinic cohort in Baltimore, Maryland USA. Baseline data

on 112 of these patients were previously published.¹⁵ Of these 222 individuals, 79, 19, and 2 underwent three, four, and five liver biopsies, respectively; thus, contributing more than one biopsy pair to the analysis (e.g., one person with three liver biopsies yielded two pairs: biopsy 1 and 2; biopsy 2 and 3). Overall, the study population provided 345 biopsy pairs for prospective analysis. Demographic, clinical, and laboratory data were abstracted from patient charts and a laboratory database. Data on injection drug use and alcohol abuse were ascertained based on physician diagnosis and self-reports through audio computer-assisted self interview. The Alcohol Use Disorders Identification Test (AUDIT), a validated screening tool which consists of 10 multiple choice questions designed to identify individuals at risk for alcohol abuse and dependence, was used to determine current alcohol use. A total score of 8 or more on this test is indicative of hazardous and harmful alcohol use and possible alcohol dependence.²³

Laboratory testing

Patients had standard laboratory assessments performed by licensed clinical laboratories, including a CD4 cell count, plasma HIV RNA level, complete blood count, serum chemistry panels, and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. HCV testing was performed using a third-generation enzyme immunoassay. HCV RNA and genotype testing was performed using reverse-transcriptase polymerase chain reaction.

Liver histology

A transcutaneous liver biopsy was performed using an 18-gauge needle. Liver tissue was fixed in 10% formalin, and paraffin-embedded sections were stained with hematoxylin-eosin and trichome stains. Slides, which were deemed adequate based on specimen size and number of portal tracts, were evaluated by a single pathologist (M. Torbenson) who was blinded to biopsy sequence. The median length of the first biopsy of a pair was 12.0 millimeters (interquartile range (IQR) 10.0, 14.0 millimeters), while the median length of the second biopsy was 13.0 millimeters (IQR: 11.0, 15.0 millimeters). Biopsies were scored based on the percentage of hepatocytes affected according to a 5-point scale as follows: Grade 0: none; 1: < 5% fat; 2: 5- < 30% fat; 3: 30–60% fat; 4: > 60% fat.²⁴ Liver tissue was also scored for activity grade and fibrosis stage according to the METAVIR system; the scale to classify fibrosis was as follows: F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis with few septa; F3 = numerous septa without cirrhosis; F4 = cirrhosis.

All procedures and protocols for this study were reviewed and approved by the Johns Hopkins Institutional Review Board. Written informed consent was obtained for all participants.

Statistical Analysis

The prevalence of hepatic steatosis (grades 2–4) at first biopsy among all 222 individuals was ascertained. χ^2 and Wilcoxon rank sum tests were used to compare categorical and continuous characteristics, respectively, across individuals with no or trivial fat versus significant fat at first biopsy. To characterize changes over time, we used all 345 biopsy pairs from 222 individuals such that the unit of analysis was the biopsy pair not the individual. Two separate analyses were conducted: 1) among biopsy pairs in which the first biopsy had no or trivial fat, we identified factors associated with progression to significant fat (grade 2, 3, or 4 on second biopsy); and 2) among biopsy pairs in which the first biopsy had significant fat, we characterized regression to a lower grade (e.g. grade 4 to 3, grade 3 to 2, grade 2 to 1 or 0).

Univariate and multivariate logistic regression was used to identify predictors of steatosis progression for the first analysis. As individuals could contribute multiple paired biopsies to

the analysis, generalized estimating equations with robust variance estimation were used. In addition, we also performed the analysis of fat progression restricted to the first biopsy pair per individual in the 222 individuals in our study. Predictors of interest included fixed characteristics, characteristics at the time of the first biopsy, and characteristics between the two biopsies. Fixed predictors included demographic characteristics, HCV genotype, and lifetime history of alcohol and injection drug use. Characteristics at the time of the first biopsy (within six months) included hyperglycemia (defined as a random blood glucose level > 200 mg/dl), diabetes, fibrosis (METAVIR score 2, 3 or 4), CD4 count, HIV viral load, ever and cumulative ART exposure up to the first biopsy, and body mass index (BMI). BMI was categorized according to the standard WHO classification system as follows: normal 18.5–24.99, overweight 25–29.99, obese ≥ 30 . The presence of diabetes was determined by either clinical diagnosis or self-report. Predictors between biopsies included any ART use between biopsies, cumulative ART use between biopsies, HCV treatment, change in fibrosis, change in HIV viral load and CD4 count, and change in BMI. Change in fibrosis was defined as any increase or decrease in METAVIR score between biopsies. HIV viral load and CD4 cell count between biopsies were analyzed as the proportion of CD4 cell counts that were $< 200 \times 10^6$ cells/l and the proportion of HIV RNA measurements that were undetectable (< 400 copies/ml). BMI change was defined as greater than a one unit increase or decrease in BMI. We also analyzed change in CD4 cell counts and BMI between biopsies as continuous variables using area under the curve (AUC). Variables found to be associated ($p < 0.1$) with progression in the univariate analyses were included in the multivariate model. We also included age, sex and race in the model regardless of statistical significance as they were deemed a priori to be important determinants of steatosis. Two-sided p -values < 0.05 were considered statistically significant.

The limited number of patients with significant fat precluded regression analysis of factors associated with decreasing fat among those with significant fat at first biopsy so χ^2 testing was used to explore associations between factors of interest and decreasing fat. All statistical analyses were performed using STATA, version 9 (StataCorp LP, College Station, Texas).

Results

Study Population

The demographic and clinical characteristics of the study population at initial biopsy are shown in Table 1. The median age was 44.3 years (IQR 40.5, 48.9); 66% were men; 87% were African American; 94% had HCV genotype 1 infection. While the median body mass index (BMI) was 25.0 (IQR 22.4, 29.0), 29% of patients were overweight and 19% were obese. Twenty-five participants (14%) had diabetes. The majority (73.4%) of patients had a history of injection drug use; 46% had a prior clinical diagnosis of alcoholism or alcohol abuse. However, few patients reported active alcohol use on direct questioning as ascertained by AUDIT. At the time of first biopsy, 80% of patients were taking ART. 54% and 36% were taking PI and non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens, respectively. Stavudine was used by 21% of patients at the time of initial biopsy. 57% of patients had an HIV RNA level < 400 copies/ml, and 56% had a CD4 count > 350 cells/ μ l. The median cumulative exposure to ART before first biopsy was 4.5 years (IQR 1.3, 7.1).

Steatosis at baseline

Among the 222 individuals in the study, 136 (61%) had no fat on initial biopsy. 58 patients (26%) had fat involving $< 5\%$ of hepatocytes; 20 (9%) had fat in 5–29% of hepatocytes and 8 (4%) had fat in $> 30\%$ of hepatocytes (Table 1). Compared to patients with no or trivial steatosis ($n = 194$), patients with significant steatosis ($n = 28$) were less likely to be African-

American (75% vs. 89%) and male (46% vs. 69%). They had a higher median BMI (26.8 vs. 24.7 kg/m²) and a higher prevalence of obesity (36.0% vs. 17.1%). While no association with antiretroviral therapy or any specific antiretroviral drug was detected, patients with significant fat were more likely to have uncontrolled HIV replication (HIV RNA > 10,000 copies/mL) than those with no or trivial fat (44% vs. 22%). The prevalence of diabetes was higher among those with significant fat compared to those with no or trivial fat (25% vs. 12%), although this was not statistically significant ($p = 0.09$). There were no differences detected with respect to age, HCV genotype, hyperglycemia, current or past alcohol abuse, injection drug use, or fibrosis.

Change in hepatic steatosis over time

The median time between biopsies was: first and second ($n = 222$), 2.82 years (IQR 2.20, 3.45); second and third ($n = 100$), 2.09 years (1.94, 2.72); third and fourth ($n = 21$), 2.00 years (1.88, 2.30); fourth and fifth ($n = 2$), 2.02 years (1.94, 2.11).

The changes in fat across multiple liver biopsies for the 222 individual patients are shown in Figure 1. 165 individuals (74%) had trivial or no fat that remained stable; 29 (13%) had progression to significant fat, while 7 (3%) maintained significant fat. 21 individuals (10%) showed regression of significant fat.

Predictors of progression of hepatic steatosis

From these 222 patients, there were 345 unique liver biopsy pairs; 88% (302) had no or trivial fat on the initial biopsy of the pair; of these, 274 (91%) had no progression of steatosis and 28 (9%) had progression to significant fat. Univariate and multivariate predictors of progression to significant fat are shown in Table 2. Interestingly, an increase in fibrosis stage between biopsy pairs was also associated with progression of steatosis (OR, 2.22; 95% CI, 0.99, 4.96). As shown in Figure 3, fibrosis progression of at least 1 METAVIR stage was detected in 46% of biopsy pairs in which fat progression was observed. In multivariate analysis, independent predictors of hepatic steatosis progression in persons with no or trivial fat on the initial biopsy were a history of alcohol abuse [adjusted OR (AOR), 3.68; 95% CI, 1.41–9.63], cumulative ART exposure between biopsies (AOR, 0.60; 95% CI, 0.36–0.99), and BMI > 25 and BMI > 30 (compared with normal BMI, AOR for overweight, 3.97; 95% CI, 1.20–13.06; AOR for obesity, 7.17; 95% CI, 2.26–22.74), and CD4 count at first biopsy (compared with CD4 < 200, AOR for CD4 200–350, 0.18; 95% CI, 0.04–0.77; AOR for CD4 > 350, 0.19; 95% CI, 0.06–0.62). When AUC for changes in BMI and CD4 counts were analyzed as continuous variables, our inferences were the same. History of injection drug use and percentage of viral load measurements below the level of detection in between biopsies were marginally associated with progression. No association was found between fat progression and age, gender, race, diabetes, ART use prior to first biopsy, HCV genotype, fibrosis at initial biopsy, or HCV treatment between biopsies. When the analysis was restricted to only those first biopsy pairs, the results were the same (data not shown).

Predictors of regression of hepatic steatosis

Of the 43 unique biopsies with significant fat at first biopsy, 28 (65%) had a decrease in fat at second biopsy, 12 (28%) remained stable, and 3 (7%) had an increase in fat at second biopsy (Fig. 2). Table 3 illustrates the characteristics of biopsy pairs where fat decreased compared to those with stable fat. There were no statistically significant differences with respect to gender, race, alcohol abuse, injection drug use, HIV viral load, ART use at first biopsy or between biopsies, obesity, and HCV treatment between biopsies. A higher percentage of individuals who had a fat decrease vs. no change had a CD4 count > 350 cells/ μ l at first biopsy but this was not statistically significant ($p = 0.07$). There was some

suggestion that those with stable significant fat were more likely to have persistent immunosuppression (greater percentage of CD4 cell counts <200 cells/ μ l), but this trend was not statistically significant ($p = 0.08$). Those with a decrease in fat were marginally more likely to have no change in fibrosis ($p = 0.06$).

Discussion

In this investigation, the majority of patients had no (61%) or trivial (28%) fat on initial liver biopsy. This finding is markedly lower than estimates obtained from prior cross-sectional studies, which range from 23%–32%.^{6,7,11,12} The likely explanation for this difference is the composition of our study population as well as the modification of the specific type of antiretroviral drugs used over time with markedly diminished prescription of agents, such as stavudine and didanosine which have been linked with impairment of mitochondrial DNA replication. Further, while prior studies have shown that Caucasian race and HCV genotype 3 are associated with steatosis^{6,7,10,12,14,15}, African-Americans comprised 87% of patients in our study and 94% were infected with HCV genotype 1. The strong associations between steatosis and HCV genotype, race, and other factors underscore the importance of carefully measuring these covariates in related research studies.

During prospective follow-up of nearly three years, the majority (74%) of individuals with no or trivial fat on initial biopsy did not progress and most (75%) individuals with significant fat on initial biopsy experienced regression. Our findings are consistent with the observations of Rodriguez-Torres and colleagues who examined changes in hepatic steatosis in patients during HCV treatment with interferon alfa or peginterferon alfa-2a with or without ribavirin in the AIDS Pegasys Ribavirin International Co-infection Trial (APRICOT).¹³ They found that for most treated patients, steatosis remained stable over approximately 72 weeks with a range from 56 to 115 weeks, which was shorter than the median follow-up time in our study of about 147 weeks.¹³

Nonetheless, progression to significant hepatic fat was observed in approximately 13% of individuals in our cohort. Importantly, longer exposure to antiretroviral therapy between biopsy pairs and higher CD4 cell count at the initial biopsy were protective against fat progression. There are several potential mechanisms for why ART use may be protective against progression to steatosis. One possibility is that HIV infection promotes steatosis by facilitating HCV replication in hepatocytes, which may have a direct cytopathic effect on cells.²⁶ Another possibility is that HIV acts synergistically with HCV to disrupt lipid metabolism.⁸ Thus, direct inhibition of HIV viral replication could decrease steatosis, particularly if the antiretroviral agent used have minimal impact on mitochondrial replication and function.

We did not detect any association of fat progression and exposure to any specific antiretroviral agent or class of ART. While prior cross-sectional studies, including our own, identified stavudine as a risk factor for hepatic steatosis, we were unable to assess the role of stavudine on fat progression in this study due to its infrequent use after the first biopsy. While ~20% of patients were receiving stavudine at the time of first biopsy, the use of this agent decreased to only 6% of patients at the second biopsy as the Department of Health and Human Services Guidelines for the use of antiretroviral therapy in adults designated stavudine as a “non-preferred” agent in 2008.²⁷ Further, the current HIV treatment guidelines recommend the use of a nucleos(t)ide backbone of tenofovir plus emtricitabine or lamivudine which has been shown to have less inhibition of mitochondrial function and is associated with a more favorable lipid profile and less lipodystrophy than stavudine.²⁸ Although the limited sample size (345 biopsy pairs) may not permit the detection of more

subtle effects of ART, our data suggest that treatment of HIV infection according to current expert guidelines is unlikely to strongly contribute to the development of steatosis.

Interestingly, steatosis progression was strongly associated with a history of alcoholism and alcohol abuse. These data suggest that for some co-infected patients, hepatic steatosis may actually be a manifestation of under-reported alcohol exposure rather than reflective of non-alcoholic fatty liver disease. Our data also highlight the difficulty in assessing alcohol consumption in studies of this nature. Although ~ 50% of patients had the clinical diagnosis of alcoholism or alcohol abuse recorded in the medical record, few patients reported excessive (or any) alcohol exposure on direct questioning with AUDIT. The fact that this and prior studies have not found an association between current alcohol abuse and the presence of steatosis^{8-11,14-15} may be due to the underreporting of alcohol use by patients, particularly when aware of participation in a study focused on liver outcomes. Clinicians evaluating fatty liver disease in HIV-infected patients should remain cognizant about the likelihood of under-reporting of alcohol consumption.

Not unexpectedly, one of the strongest predictors of steatosis progression was obesity. This finding correlates with the results of many cross-sectional studies which show that a higher BMI is associated with the presence as well as severity of steatosis.^{6,7,9,12,14,29} While much of the study population had normal BMI, 48% of individuals were either overweight or obese. Indeed, the prevalence of overweight/obesity in our study population was similar to that observed in a large cohort of HIV-HCV co-infected adults screened for a randomized controlled trial in the United States.³⁰ While less than 15 years ago, HIV was a progressively fatal disease characterized by severe wasting, the advent of HAART has transformed HIV into a chronic disease with an increase in the prevalence of obesity.³¹ These data underscore the importance of measures to prevent and treat obesity in HIV/HCV co-infected patients since the development or worsening of hepatic steatosis in these patients may be associated with liver fibrosis progression.

The major strengths of our study are the prospective, longitudinal design using paired liver biopsy data which were scored in a blinded fashion by a single expert hepatopathologist as well as the collection of detailed demographic, clinical, and laboratory data for our large cohort of co-infected individuals attending an urban HIV clinic. The longitudinal nature of the study makes it possible to examine the temporal relationship when assessing risk factors for steatosis progression unlike the prior cross-sectional studies. However, there are several important limitations. The majority of our patients were African Americans with HCV genotype 1, which is not representative of the general population of co-infected individuals. Thus, our findings may not be generalizable to all populations, especially Caucasian patients with a higher prevalence of HCV genotype 3. Furthermore, the relatively low prevalence of individuals with significant fat at baseline limited the power of our study to detect factors associated with steatosis regression. In addition, information on certain variables related to metabolic syndrome risk factors such as fasting glucose and lipid measurements were not consistently available. Finally, the sampling error inherent of liver biopsy is another limitation of our study. While the effect of biopsy length on accuracy of steatosis grading is less clear, biopsy length has been found to be an important determinant in the accuracy of fibrosis staging.³² Importantly, our sample included biopsy pairs subjects for which the tissue sample was determined to be adequate by a single, experienced hepatopathologist.

In summary, the majority of HIV/HCV coinfecting patients had no or trivial steatosis on initial liver biopsy and most (91%) did not experience progression of steatosis over time. While fat progression between biopsy pairs was uncommon, fibrosis progression was observed more frequently in conjunction with increase in steatosis. Fat progression was associated with The clinical diagnosis of alcoholism and a body mass index greater than 25;

importantly, exposure to contemporary antiretroviral medications and higher baseline CD4 cell count appeared to be protective against progressive steatosis. These data suggest that, efforts to diagnose and prevent steatosis should be focused on persons with high body mass and excessive alcohol intake.

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Figure 1.
Patterns of fat across multiple liver biopsies (N=222).

Figure 2.
Steatosis progression at subsequent biopsy by fat score at the initial biopsy of the biopsy pair.

Figure 3.
Relationship of the change of and steatosis grade and fibrosis stage (n=222).

Table 1

Characteristics of study population at study entry according to steatosis grade at first biopsy.

Characteristic, n (%)	Total N = 222	No or trivial fat ** N = 194	Significant fat N = 28	P value *
Age (years), median (IQR)	44.3 (40.5, 48.9)	44.8 (40.5, 49.1)	44.3 (40.7, 48.3)	0.62
Male gender	147 (66)	134 (69)	13 (46)	0.02
African American Race	194 (87)	173 (89)	21 (75)	0.06
HCV genotype 1	207 (94)	180 (93)	27 (96)	0.52
BMI (kg/m ²)				
< 25 (Normal)	106 (52)	98 (54)	8 (32)	0.05
25 - < 30 (Overweight)	60 (29)	52 (29)	8 (32)	
>= 30 (Obese)	40 (19)	31 (17)	9 (36)	
Hyperglycemia	5 (2)	4 (2)	1 (4)	0.59
Diabetes	25 (14)	19 (12)	6 (25)	0.09
Triglycerides (mg/l), median (IQR)	141 (101, 235)	132 (95, 228)	215 (153, 266)	0.08
AST, median (IQR)	46 (32, 72)	44 (32, 70)	63 (44, 88)	0.12
ALT, median (IQR)	46 (29, 73)	45 (28, 73)	47 (35, 65)	0.54
History of alcohol abuse	102 (46)	89 (46)	13 (46)	0.96
AUDIT category				
< 8	141 (93)	121 (93)	20 (95)	0.71
>= 8	10 (7)	9 (7)	1 (5)	
History of injection drug use	163 (73)	142 (73)	21 (75)	0.84
CD4 count (cells/μl)				
< 200	34 (16)	31 (17)	3 (11)	0.64
200–350	58 (28)	51 (28)	7 (26)	
> 350	115 (56)	98 (54)	17 (63)	
HIV RNA (copies/ml)				
< 400	114 (57)	102 (58)	12 (48)	0.05
400–10000	36 (18)	34 (19)	2 (8)	
> 10000	50 (25)	39 (22)	11 (44)	
Previous ART	190 (86)	168 (87)	22 (79)	0.26
Duration of ART exposure before biopsy (years), median (IQR)	4.50 (1.34, 7.06)	5.00 (1.46, 7.13)	2.60 (0.58, 5.84)	0.09
On ART at first biopsy	177 (80)	140 (72)	17 (61)	0.21
On PI at first biopsy	120 (54)	108 (56)	12 (43)	0.20
On NNRTI at first biopsy	80 (36)	71 (37)	9 (32)	0.65
On NRTI at first biopsy	172 (78)	154 (79)	18 (64)	0.07
On stavudine at first biopsy	46 (21)	40 (21)	6 (21)	0.92
On didanosine at first biopsy	26 (12)	22 (11)	4 (14)	0.65
Number of biopsies				0.28
2	122 (55)	102 (53)	20 (71)	

Characteristic, n (%)	Total N = 222	No or trivial fat** N = 194	Significant fat N = 28	P value *
> 2	100 (45)	92 (47)	8 (29)	
HCV treatment prior to first biopsy	3 (1)	2 (1)	1 (4)	0.28
Follow-up time in study (years), median (IQR)	4.14 (2.44, 5.41)	4.43 (2.71, 5.55)	3.09 (2.21, 4.45)	0.02
Steatosis grade				
0	136 (61)			
1	58 (26)			
2	20 (9)			
3	7 (3)			
4	1 (1)			
Fibrosis stage				
0	101 (45)	91 (47)	10 (36)	0.46
1	88 (40)	76 (39)	12 (43)	
2	22 (10)	17 (9)	5 (18)	
3	5 (2)	5 (3)	0 (0)	
4	6 (3)	5 (3)	1 (4)	

HCV, hepatitis C virus; BMI, body mass index; AUDIT, Alcohol Use Disorders Identification Test; ART, antiretroviral therapy; HAART, highly active antiretroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; IQR, interquartile range

* χ^2 and Wilcoxon rank sum tests were used to compare the categorical and continuous characteristics, respectively

** Trivial fat was defined as fat involving < 5% of hepatocytes

Table 2

Determinants of hepatic steatosis progression over time among HIV/HCV co-infected individuals with no/trivial fat at initial biopsy.

Characteristic	Crude Odds Ratio (95% Confidence Interval)	Adjusted Odds Ratio (95% Confidence Interval)
Predictors at initial biopsy		
Age (per 5 yr)	0.94 (0.72, 1.22)	1.13 (0.74, 1.70)
Female gender	1.57 (0.71, 3.48)	0.79 (0.31, 2.02)
Caucasian race	0.28 (0.04, 2.19)	0.04 (0.01, 0.30)
HCV genotype 1	0.64 (0.08, 5.00)	
BMI		
Normal	1	1
Overweight	2.27 (0.86, 6.00)	4.00 (1.22, 13.07)
Obese	3.30 (1.18, 9.23)	5.79 (1.82, 18.40)
Diabetes	1.68 (0.61, 4.64)	
History of alcohol abuse	2.60 (1.17, 5.81)	4.00 (1.48, 10.80)
History of injection drug use	2.39 (0.82, 6.99)	3.49 (0.86, 14.23)
AST (per 20)	1.07 (0.99, 1.16)	
ALT (per 20)	1.07 (1.01, 1.13)	1.13 (1.04, 1.22)
CD4 count (cells/μl)		
<200	1	1
200–350	0.31 (0.10, 0.97)	0.19 (0.04, 0.86)
>350	0.41 (0.16, 1.02)	0.18 (0.06, 0.57)
HIV RNA (copies/ml)		
<400	1	
400–10000	2.42 (0.97, 6.00)	
>10000	1.07 (0.37, 3.11)	
Cumulative ART use (years)	1.02 (0.91, 1.14)	
Cumulative PI use (years)	0.99 (0.86, 1.15)	
Cumulative NNRTI use (years)	1.11 (0.93, 1.32)	
Cumulative NRTI use (years)	1.03 (0.92, 1.15)	
Fibrosis	0.64 (0.18, 2.28)	
Predictors between biopsies		
On ART	0.63 (0.24, 1.67)	
Cumulative ART use (years)	0.75 (0.54, 1.05)	0.56 (0.35, 0.90)
On PI	0.87 (0.39, 1.94)	
Cumulative PI use (years)	0.83 (0.61, 1.13)	
On NNRTI	0.92 (0.41, 2.06)	
Cumulative NNRTI use (years)	0.92 (0.63, 1.35)	
On NRTI	0.78 (0.30, 2.05)	

Characteristic	Crude Odds Ratio (95% Confidence Interval)	Adjusted Odds Ratio (95% Confidence Interval)
Cumulative NRTI use (years)	0.83 (0.62, 1.13)	
% CD4 measurements < 200 cells/ μ l		
< 25%	1	
25–75%	1.44 (0.51, 4.04)	
> 75%	2.08 (0.71, 6.04)	
% HIV RNA measurements < 400 copies/ml		
< 25%	2.33 (0.86, 6.26)	1.93 (0.62, 6.02)
25–75%	1.94 (0.72, 5.23)	2.26 (0.80, 6.39)
>75%	1	1
100% (vs. < 100%)	0.51 (0.20, 1.32)	
Change in BMI		
Decrease	1.83 (0.68, 4.90)	0.95 (0.26, 3.48)
Stable	1	1
Increase	1.41 (0.49, 4.05)	1.04 (0.31, 3.55)
HCV treatment	0.83 (0.28, 2.46)	
Change in fibrosis		
Increase	2.22 (0.99, 4.96)	
Stable	1	
Decrease	1.91 (0.51, 7.19)	

HCV, hepatitis C virus; BMI, body mass index; ART, antiretroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor

Table 3

Characteristics of participants with significant fat at initial biopsy with no change in fat vs. fat decrease at subsequent biopsy.

Characteristic, n (%)	Stable fat, N = 12	Fat decrease, N = 28	p-value
Predictors at initial biopsy			
Female gender	4 (33)	12 (43)	0.57
African American race	7 (58)	23 (82)	0.11
HCV genotype 1	12 (100)	27 (96)	0.51
Obese	2 (18)	9 (36)	0.40
Hyperglycemia	1 (9)	2 (8)	0.89
Diabetes	2 (22)	6 (24)	0.91
History of alcohol abuse	8 (67)	12 (43)	0.17
History of injection drug use	8 (67)	22 (79)	0.43
CD4 count > 350 cells/ μ l	4 (36)	17 (61)	0.07
HIV RNA < 400 copies/ml	6 (55)	10 (40)	0.20
On ART	9 (75)	21 (75)	1.00
On PI	9 (75)	13 (46)	0.10
On NNRTI	1 (8)	9 (32)	0.11
On NRTI	9 (75)	20 (71)	0.82
Fibrosis	2 (16.7)	8 (28.6)	0.43
Predictors between biopsies			
On ART	10 (83)	23 (82)	0.93
On PI	9 (75)	17 (61)	0.39
On NNRTI	2 (17)	13 (46)	0.08
On NRTI	9 (75)	22 (79)	0.80
> 75% of CD4 measures < 200 cells/ μ l	3 (27)	1 (4)	0.08
> 75% HIV RNA measures < 400 copies/ml	6 (50)	11 (65)	0.36
BMI increase	2 (18)	7 (28)	0.50
HCV treatment	5 (42)	11 (39)	0.89
Change in fibrosis			
Stable	3 (25)	17 (61)	
Increase	8 (67)	11 (39)	
Decrease	1 (8)	0 (0)	0.06

HCV, hepatitis C virus; ART, antiretroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; BMI, body mass index