

Fibrosis Progression in Human Immunodeficiency Virus/Hepatitis C Virus Coinfected Adults: Prospective Analysis of 435 Liver Biopsy Pairs

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Human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfection is associated with progressive liver disease. However, the rate of progression is variable and the ability to differentiate patients with stable versus progressive HCV disease is limited. The objective of this study was to assess the incidence of and risk factors for fibrosis progression in a prospective cohort of coinfecting patients. Overall, 435 liver biopsy pairs from 282 patients without cirrhosis were analyzed. Biopsies were scored according to the METAVIR system by a single pathologist blind to biopsy sequence. Fibrosis progression was defined as an increase of at least one METAVIR fibrosis stage between paired biopsies. The majority of patients were African American (84.8%), male (67.7%), and infected with HCV genotype 1 (93.4%). On initial biopsy, no or minimal fibrosis was identified in 243 patients (86%). The median interval between biopsies was 2.5 years. Fibrosis progression was observed in 97 of 282 (34%) patients and 149 of 435 (34%) biopsy pairs. After adjustment, greater body mass index (adjusted odds ratio [aOR]: 1.04 per 1 unit increase), diabetes (aOR: 1.56), and hepatic steatosis (aOR: 1.78) at the time of initial biopsy were marginally associated with subsequent fibrosis progression. Between biopsies, elevated serum aspartate and alanine aminotransferase (AST, ALT) (aOR AST: 3.34, ALT: 2.18 for >25% values >100 U/L versus <25% values >100 U/L) were strongly associated with fibrosis progression. *Conclusion:* Fibrosis progression is common among HIV/HCV coinfecting patients; these data suggest that progression can be rapid. Persistent elevations in serum transaminase levels may serve as important noninvasive markers to identify subsets of patients who are more likely to progress and thus warrant closer monitoring and consideration of HCV treatment. (HEPATOLOGY 2013; 00:000–000)

Due to shared modes of transmission, 15%–30% of individuals with human immunodeficiency virus (HIV) infection are coinfecting with hepatitis C virus (HCV).^{1,2} In the era of antiretroviral therapy (ART), chronic HCV infection leads to progressive liver disease, resulting in end-stage liver disease, hepatocellular carcinoma, and death in some, but not all coinfecting patients.^{3–5} While the variable

progression of HCV disease is well recognized, the rate and risk factors for progressive liver disease in HIV/HCV coinfecting patients are incompletely understood. Several studies conducted shortly after the availability of highly active ART suggest that effective treatment of HIV may be associated with decreased risk of liver disease progression.^{6–10} However, the contributions of other potentially modifiable (e.g., obesity) and

Abbreviations: AIDS, acquired immune deficiency syndrome; ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet-ratio index; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; SVR, sustained virologic response.

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Received March 2, 2013; accepted September 10, 2013.

Supported by grants K24DA00432, DA-11602, DA-16065, and DA-13806 from the National Institute on Drug Abuse, AA016893 from the National Institute on Alcohol Abuse and Alcoholism, K23 AT002862 (TTB) from the National Center for Complementary and Alternative Medicine, grant HS 07-809 from the Agency for Healthcare Policy and Research and the Clinical Research Unit at the Johns Hopkins Medical Institutions, M01RR-02719. This publication was made possible by grant number UL1 RR 025005 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH.

unmodifiable (e.g., age) factors to the worsening of hepatic fibrosis have not been determined. Greater understanding of such factors may have important implications for the clinical management of HIV/HCV coinfecting patients. For example, current HCV treatment guidelines for HIV-infected patients recommend treatment of those patients at the greatest risk for developing liver disease. Some, but not all, expert guidelines recommend HCV treatment for HIV-infected patients independent of biopsy stage based on an assumption of rapidly progressive disease in this population.¹¹⁻¹⁷ The identification of factors associated with progression may help to refine clinical decision-making as well as identify potentially modifiable exposures. Accordingly, the objective of this study was to determine the incidence of and risk factors for fibrosis progression in a prospective cohort of coinfecting adults who underwent serial liver biopsy with the aim of identifying coinfecting patients with no or minimal fibrosis who are at risk for progressive liver disease over a relatively short period of time.

Patients and Methods

Study Population. This prospective cohort study evaluated 289 HIV/HCV coinfecting adults who received medical care in an urban HIV clinic in Baltimore, Maryland, from July 1993 until December 2008. Treatment for HIV and/or HCV was provided by healthcare providers according to published practice guidelines.^{18,19} Individuals with at least two liver biopsies as part of their medical care were included in the study. A total of 282 patients had an initial noncirrhotic biopsy and were assessed. Of these individuals, 124 had more than two liver biopsies including 97 patients with three biopsies, 25 with four biopsies, and two with five biopsies. In total, these 282 patients contributed 435 liver biopsy pairs to the analysis. For all patients, demographic, clinical, and laboratory data were abstracted from patient charts and a laboratory database by trained personnel. Data on injection drug use and alcohol abuse were ascertained based on physician diagnosis, chart review, and self-reports.

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

Liver Histology. A transcutaneous liver biopsy was performed using an 18G needle. Liver tissue was fixed in 10% formalin, and paraffin-embedded sections were stained with hematoxylin-eosin and trichrome stains. Biopsies were scored according to the METAVIR and the modified histological activity index scoring system by a single pathologist (M.T.) blind to biopsy sequence. 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. Steatosis was 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.²⁰ All biopsies were deemed adequate for inclusion based on expert opinion by the hepatopathologist (M.T.) including assessment of size and number of portal tracts. The median length of the first biopsy of a pair was 12.0 mm (interquartile range [IQR] 10.0, 14.0 mm), while the median length of the second biopsy of a pair was 13.0 mm (IQR 10.0, 15.0 mm). The median number of portal tracts for the first biopsy of a pair was 10 (IQR 8, 13), and for the second biopsy of a pair was 11 (IQR 9, 14).

Statistical Analysis. Significant fibrosis progression was defined as an increase of at least one METAVIR stage between the biopsies. The proportion of patients who had fibrosis progression was equivalent when analyzed using the Ishak scoring system (Supporting Table 1). Subsequently, the remaining analysis was done using the METAVIR scoring system alone. To characterize changes over time, we used 435 biopsy pairs contributed by 282 individuals such that the unit of

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DOI 10.1002/hep.26741

Potential conflict of interest: Nothing to report.

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analysis was the biopsy pair and not the individual (e.g., for a patient with three biopsies, biopsy 1 → 2 was analyzed as one pair and biopsy 2 → 3 as a second pair). Univariate and multivariate logistic regression with generalized estimating equation were used to assess determinants of fibrosis progression in order to account for the correlation of biopsy pairs from within the same individuals. Variables associated with fibrosis progression in univariate analysis with $P < 0.15$ were considered for multivariate models. Time between biopsies was included as a covariate in the models as a categorical variable (<2, 2-2.9, 3-3.9, 4+ years). Further variables that had been previously identified as predictors of progression (gender, race, and age) were forced into models regardless of statistical significance. A series of models were built, first including fixed covariates and covariates at first biopsy and then including covariates between serial biopsies. Due to collinearity, separate models were built for laboratory values measured between serial biopsies.

Predictors of interest included fixed characteristics, characteristics at the time of the first biopsy, and characteristics between the serial biopsies. Fixed characteristics included demographics, HCV genotype, and history of alcohol or injection drug use. Characteristics at the time of the first biopsy (within six months) included diabetes, body mass index (BMI), CD4 count, HIV-RNA level, ever and cumulative ART exposure up to the first biopsy, HCV-RNA level, HCV treatment before initial biopsy, cumulative HCV treatment, duration of HCV infection (estimated by age at first injection), median AST and ALT levels, hepatic steatosis, histological necroinflammatory activity, and METAVIR score. BMI was categorized according to the standard classification system as follows: normal 18.5-24.99, overweight 25-29.99, obese ≥ 30 . The presence of diabetes was determined by clinical diagnosis.

Predictors between biopsies included any ART use between biopsies, cumulative ART use between biopsies, change in CD4 cell count and HIV viral load, HCV treatment, change in AST and ALT levels, and change in BMI. HIV viral load and CD4 cell count between biopsies were analyzed as the proportion of CD4 cell counts that were < 200 cells/ μL and the proportion of HIV RNA measurements that were undetectable (< 400 copies/mL). ALT and AST were examined as the cumulative proportion of ALT and AST levels more than 2.5 times the upper limit of normal reference range (AST 37 U/L; ALT 40 U/L). BMI change was defined as greater than a one unit increase or decrease in BMI.

Analyses were performed using SAS v. 9.1 software (SAS Institute, Cary, NC).

Approval. This study was approved by the Johns Hopkins Medicine Institutional Review Boards and written informed consent was obtained for all participants.

Results

Study Population. The demographic and clinical characteristics of the study population at initial biopsy are shown in Table 1. The median age was 44.5 years (IQR 40.5, 48.7). The majority of individuals were African American (84.8%), male (67.7%), and infected with HCV genotype 1 (93.4%). A history of injection drug use (76.6%) and alcohol abuse (48%) were frequently reported. The median BMI was 25.4 (IQR 22.5, 29.2). At the time of first biopsy most patients were receiving ART (69.2%) and had been for a median duration of 1.9 years (IQR 0, 4.3). Only 28% of patients had never received ART. The median CD4 cell count was 386 cells/ μL and 15.9% had CD4 cell counts < 200 cells/ μL . The majority of patients (55.9%) had an HIV RNA level below the limit of detection. The median ALT and AST were 47 U/L (IQR 31, 75) and 46 U/L (IQR 33, 71), respectively. AST levels exceeding 100 U/L were observed in 14.7% (40 of 272) of patients at the time of the first biopsy and were associated with clinical history of alcohol abuse and the absence of ART (Supporting Table 2). The median HCV-RNA level was 700,000 IU/mL (IQR 500,000-1,530,000). Most patients (279 of 282, 99%) had not received HCV treatment before initial biopsy.

On initial biopsy, no or minimal fibrosis (METAVIR stage 0 or 1) was identified in 243 patients (86%), whereas 31 patients (11%) had METAVIR stage 2, and eight patients (2.8%) had METAVIR stage 3. With regard to necroinflammatory activity, 173 patients (65.8%) had a score < 5 with 90 patients (34.2%) having a score of 5 or greater. Hepatic steatosis (any grade) was observed in 50 patients (12.8%).

Incidence of Fibrosis Progression. The median interval between biopsies was 2.5 years (IQR 2-3.2 years). Fibrosis progression was observed in 97 of 282 (34%) patients between their first and second liver biopsy. Among the 435 biopsy pairs, fibrosis progression was observed in 149 (34%), with 39 biopsy pairs (8.9%) demonstrating an increase of two or more METAVIR fibrosis stages. Notably, fibrosis progression was detected in 45% of 179 pairs in which the initial

Table 1. Baseline Characteristics of 282 HIV/HCV Coinfected Adults

Characteristics at Initial Biopsy	Overall (N = 282)*	One Biopsy Pair (N = 158)*	Multiple Biopsy Pairs* (N = 124)	P Value
Male sex	191 (67.7)	103 (65.2)	88 (71.0)	0.30
Age, median (IQR)	44.5 (40.0, 48.7)	44.6 (39.9, 49.2)	44.0 (40.3, 47.5)	0.22
African-American race	239 (84.8)	135 (85.4)	104 (83.9)	0.72
Injection drug use, active or inactive	216 (76.6)	124 (78.5)	92 (74.2)	0.40
Alcohol abuse at or before initial biopsy [†]	129 (48.0)	74 (49.7)	55 (45.8)	0.53
Body mass index, median (IQR)	25.4 (22.5-29.2)	25.0 (22.3, 28.8)	25.8 (22.6, 29.7)	0.26
Diabetes mellitus	43 (17.8)	17 (13.8)	26 (22)	0.10
CD4 cell count (cells/ μ l), median (IQR)	386 (267-550)	374 (237, 541)	423 (289, 566)	0.20
<200	43 (15.9)	29 (19.2)	14 (11.8)	
200-349	68 (25.2)	37 (24.5)	31 (26.1)	
\geq 350	159 (58.9)	85 (56.3)	74 (62.2)	0.25
HIV-RNA level < 400 (copies/ml)	148 (55.9)	71 (48.3)	77 (65.3)	0.006
Previous ART	204 (72.3)	115 (72.8)	89 (71.8)	0.85
On ART	195 (69.2)	109 (69.0)	86 (69.4)	0.95
Median cumulative time on ART (IQR)	1.9 (0, 4.3)	1.7 (0, 4.3)	2.4 (0, 4.3)	0.77
HCV Genotype 1	253 (93.4)	140 (93.3)	113 (93.4)	0.99
HCV-RNA level, median (IQR)	700,000 (500,000, 1,530,000)	700,000 (500,000, 2,705,105)	700,000 (500,000, 1,255,000)	0.09
HCV treatment before initial biopsy	3 (1.1)	3 (1.9)	0 (0.0)	0.12
ALT (U/l), median (IQR)	47 (31, 75)	46 (30, 80)	48 (31, 74)	0.94
AST (U/l), median (IQR)	46 (33, 71)	54 (35, 80)	43 (32, 69)	0.06
APRI				
<0.5	123 (45.2)	61 (40.1)	62 (51.7)	
0.5-1.4	111 (40.8)	66 (43.4)	45 (37.5)	
\geq 1.5	38 (14.0)	25 (16.5)	13 (10.8)	0.13
FIB4				
\leq 1.45	132 (48.7)	64 (42.1)	68 (57.1)	
1.46-3.24	106 (39.1)	66 (43.4)	40 (33.6)	
\geq 3.25	33 (12.2)	22 (14.5)	11 (9.2)	0.04
No steatosis	232 (87.2)	129 (85.4)	103 (89.6)	0.32
Modified histological activity index				
<5	173 (65.8)	85 (56.7)	88 (77.9)	
5 or greater	90 (34.2)	65 (43.3)	25 (22.1)	0.0003
METAVIR score				
0	125 (44.3)	60 (38.0)	65 (52.4)	
1	118 (41.8)	69 (43.7)	49 (39.5)	
2	31 (11.0)	22 (13.9)	9 (7.3)	
3	8 (2.8)	7 (4.4)	1 (0.8)	0.02

ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; ART, antiretroviral therapy; AST, aspartate aminotransferase; FIB4, non-invasive marker of liver fibrosis consisting of ALT, AST, platelet count and age; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; Unless otherwise indicated, data shown are numbers (proportion).

*Other than for demographic characteristics, numbers do not add up to total due to missing data.

[†]Alcohol abuse was defined as clinical diagnosis of alcoholism or abuse.

biopsy of the pair revealed no fibrosis (METAVIR stage 0; Table 2). While the majority of those with progression had stage 1 fibrosis on the second biopsy, 14 biopsy pairs (7.8%) had progression of two or more METAVIR fibrosis stages.

Correlates of Progression at Baseline. Among the 435 biopsy pairs, 149 (34%) had progression of one or more METAVIR stages between biopsies and are referred to as "progressors" (Table 3). Markers of increased hepatic inflammation measured by median AST and ALT as well as noninvasive measures of liver disease (AST-platelet ratio index [APRI] and FIB4 index) were associated with subsequent fibrosis progression. Measures of obesity (BMI, $P = 0.02$), diabetes ($P = 0.01$), and hepatic steatosis ($P = 0.01$) at the time of the first biopsy were associated with progression of

fibrosis on the subsequent liver biopsy. Compared to nonobese patients, those with BMI >30 at the time of the initial biopsy had higher AST levels, higher prevalence of steatosis, higher histological activity indexes, and were more likely to be male (Supporting Table 3). The modified histological activity index was not related to fibrosis progression. Measures of biopsy quality including the length of the specimen were not associated with fibrosis progression. The median length was 12 mm among both nonprogressors (IQR 10, 14) and progressors (IQR 9, 14) with a Wilcoxon rank sum test P value of 0.13. Similarly, measures of HIV disease (CD4 cell count, HIV-RNA, ART exposure) at the time of initial biopsy were not significantly different among progressors and nonprogressors. After adjustment for baseline factors in multivariate analysis, AST level >100

Table 2. METAVIR Fibrosis Stage of 435 Biopsy Pairs Contributed by 282 HIV/HCV Coinfected Adults*

Biopsy Pair Part 1 Fibrosis Stage	Biopsy Pair Part 2 Fibrosis Stage					
	0	1	2	3	4	
0	100	65	3	9	2	179
1	20	130	26	8	8	192
2	1	7	19	15	9	51
3	0	1	4	4	4	13
	121	203	52	36	23	435

*Shaded regions represent subjects in whom fibrosis stage was at least 1 METAVIR stage greater than on prior biopsy.

IU/mL at the time of the first liver biopsy was independently associated with subsequent fibrosis progression for all biopsy pairs (adjusted odds ratio [aOR] 2.12, 95% confidence interval [CI] 1.06-4.26) but not for biopsy pairs restricted to those with minimal fibrosis (stage 0 or 1) at first biopsy (aOR 1.54, 95% CI 0.63-3.72) (Table 4).

Among pairs METAVIR stage 0 or 1 ($n = 371$) on initial biopsy, we also characterized the accuracy of elevated AST level (>100 U/L) at the time of the initial liver biopsy in prediction of fibrosis progression. The sensitivity of this threshold was low (13%), with a specificity of 93%. The positive predictive value of AST >100 U/L at baseline was 53%, whereas the negative predictive value was 68%.

Correlates of Progression Between Serial Biopsies. The univariate correlations of exposures between biopsies and progression were similar to those measures at baseline (Table 3). Antiretroviral therapy and suppression of HIV replication between biopsies were not associated with fibrosis progression. The change in CD4 cell count and the proportion of measured HIV-RNA values <400 copies/mL median IQR were also not statistically different between the two groups. Treatment for HCV infection with interferon plus ribavirin was prescribed in between 90 biopsy pairs, 58 (20.5%) of the nonprogressors, and 32 (21.5%) of the progressors ($P = 0.81$). Of the 90 biopsy pairs, HCV treatment resulted in durable or transient viral response in 17 instances (three in sustained virologic response [SVR] and 14 with relapse). None of the treated progressors achieved SVR, whereas SVR was achieved in three out of 58 treated nonprogressors ($P = 0.19$).

Similar to baseline correlates, measures of hepatic inflammation were significantly different between progressors and nonprogressors between biopsies. The median AST and ALT levels between biopsies were significantly higher among biopsy pairs with fibrosis progression compared to those without fibrosis progression ($P = 0.0004$ for ALT and $P < 0.0001$ for AST). In

addition, between biopsies, pairs with fibrosis progression had a significantly greater proportion of ALT and AST values >2.5 times the upper limit of normal compared with those without progression ($P = 0.0002$ for ALT and $P < 0.0001$ for AST). Time between biopsies was not associated with progression. After adjustment for baseline and between biopsy factors in multivariate analysis, the proportion of AST level >100 IU/mL between biopsies was independently associated with subsequent fibrosis progression for all biopsy pairs and for biopsy pairs restricted to those with minimal fibrosis (stage 0 or 1) (Table 4). Additional sensitivity analyses were performed to assess the impact of restricting analysis to only the first biopsy in the pair, a change of at least two METAVIR stages, biopsy length, and number of portal tracts and the results were not significantly changed (Supporting Table 4).

Discussion

Effective ART has substantially reduced the incidence of acquired immune deficiency syndrome (AIDS)-related death among HIV-infected adults; among those coinfecting with HCV, liver disease has emerged as an important cause of morbidity and mortality. While HIV infection has been consistently associated with more rapid progression of hepatic fibrosis, the mechanisms underlying this association are incompletely understood. In this context, our data related to the incidence and correlates of progressive hepatic fibrosis among 282 HIV/HCV coinfecting adults who underwent serial fibrosis staging resulting in 435 paired liver biopsies provide several insights into liver disease progression in HIV/HCV coinfecting patients.

The majority of coinfecting patients in our prospective cohort had minimal fibrosis on initial liver biopsy. Nonetheless, over a median follow-up time of 2.5 years we observed fibrosis progression (≥ 1 METAVIR stage) in one-third of individual patients between the first and second liver biopsy ($n = 282$) and among one-third of all biopsy pairs ($n = 435$). While the majority of histologic change was limited to one METAVIR stage, progression of two or more stages was found in $\sim 9\%$ of biopsy pairs. Further, nearly 45% of patients with no evidence of hepatic fibrosis on the initial biopsy had at least stage 1 fibrosis on subsequent liver biopsy. This observed incidence of fibrosis progression over a relatively short time interval is consistent with our earlier observations and those reported in other HIV/HCV coinfecting patient cohorts in which progressive disease was noted in 17% to 50% of paired histologic evaluations.^{3,9,10,21,22} However, the

Table 3. Correlates of Fibrosis Progression Among 435 Biopsy Pairs Contributed by 282 HIV/HCV Coinfected Adults

	Nonprogressors (N = 286)*	Progressors (N = 149)*	P Value
Characteristics at first biopsy			
Male sex	195 (68.2)	102 (68.5)	0.95
Age, median (IQR)	45.0 (40.8, 49.2)	45.6 (41.5, 49.7)	0.37
African-American race	239 (83.6)	129 (86.6)	0.41
Injection drug use, history of	217 (75.9)	114 (76.5)	0.88
Alcohol abuse at or before initial biopsy [†]	126 (46.2)	69 (47.6)	0.78
Body mass index, median (IQR)	25.0 (22.5, 29.1)	26.1 (23.5, 30.7)	0.02
Diabetes mellitus	42 (16.4)	36 (27.3)	0.01
Median CD4 cell count (IQR)	384 (253, 563)	421 (284, 542)	0.39
Nadir CD4 cell count/ μ l median (IQR)	113 (40, 232)	134 (36, 225)	0.87
HIV-RNA level < 400 (copies/ml)	158 (58.7)	79 (56.0)	0.60
Previous ART	220 (76.9)	109 (73.2)	0.38
On ART	209 (73.1)	104 (69.8)	0.47
Median cumulative time on ART (IQR)	3.2 (0.2, 5.4)	2.7 (0, 5.9)	0.83
HCV Genotype1	250 (91.2)	143 (97.3)	0.02
HCV-RNA level, median (IQR)	806,500 (500,000, 2,580,000)	700,000 (500,000, 1,865,000)	0.31
Age at first injection (n = 198)			0.12
12-16 years	19 (14.5)	13 (19.7)	
17-20 years	35 (26.7)	26 (39.4)	
21-24 years	43 (32.8)	16 (24.2)	
25+ years	34 (26.0)	11 (16.7)	
ALT (U/l), median (IQR)	47 (30, 70)	47 (33, 82)	0.08
AST (U/l), median (IQR)	43 (32, 64)	49 (37, 78)	0.003
Proportion with AST (U/l) > 2.5 x ULN	22 (8.0)	25 (16.9)	0.006
Platelet count x 1000 median (IQR)	213 (172, 255)	194 (157, 239)	0.03
APRI, median (IQR)	0.5 (0.4, 0.8)	0.7 (0.4, 1.1)	0.002
<0.5	133 (48.4)	54 (36.5)	
0.5-1.5	121 (44.0)	65 (43.9)	
>1.5	21 (7.6)	29 (19.6)	0.0006
Median FIB4 (IQR)	1.5 (1.1, 1.9)	1.8 (1.2, 2.4)	0.001
\leq 1.45	136 (49.6)	57 (38.8)	
1.46-3.24	116 (42.3)	66 (44.9)	
\geq 3.25	22 (8.0)	24 (16.3)	0.01
No steatosis	245 (89.4)	116 (80.6)	0.01
Modified histological activity index			
<5	184 (67.9)	96 (66.7)	
5 or greater	87 (32.1)	48 (33.3)	0.80
METAVIR score < 2	250 (87.4)	121 (81.2)	0.08
Characteristics between serial biopsies			
ART exposure, median years (IQR)	2.1 (0.9, 2.9)	2.1 (0.8, 3.0)	0.68
ART use	231 (80.8)	122 (81.9)	0.78
ART regimen			
PI	179 (62.6)	98 (65.8)	0.51
NNRTI	129 (45.1)	73 (49.0)	0.44
Other ART	242 (84.6)	123 (82.6)	0.58
Change in CD4 cell count/ μ l median (IQR)	19 (-92, 144)	24 (-102, 161)	0.65
Proportion of CD4 cell count/ μ l below 200, median (IQR)	0 (0, 17)	0 (0, 18)	0.71
0	185 (65.8)	99 (67.8)	
1-50	57 (20.3)	26 (17.8)	
>50	39 (13.9)	21 (14.4)	0.83
Proportion of measured HIV-RNA values < 400 copies/ml, median (IQR)	73 (30, 100)	63 (21, 100)	0.16
100	101 (36.5)	46 (32.2)	
75-99	37 (13.4)	18 (12.6)	
25-74	75 (27.1)	43 (30.1)	
<25	64 (23.1)	36 (25.2)	0.80
ALT U/l, median of individual values (IQR)	40 (29, 61)	52 (34, 73)	0.0004
Proportion of ALT values > 2.5 x ULN (100 U/l)	0 (0, 6)	0 (0, 24)	0.0002
0	197 (70.6)	76 (52.8)	
1-24	44 (15.8)	32 (22.2)	
\geq 25	38 (13.6)	36 (25.0)	0.001
AST U/l, median of individual values (IQR)	43 (32, 56)	55 (38, 82)	<0.0001
Proportion of AST values > 2.5 x ULN (100 U/l)	0 (0, 7)	4 (0, 32)	<0.0001
0	191 (68.5)	69 (47.9)	

Table 3. Continued

	Nonprogressors (N = 286)*	Progressors (N = 149)*	P Value
1-24	59 (21.2)	33 (22.9)	
≥ 25	29 (10.4)	42 (29.2)	<0.0001
Received HCV treatment in between biopsies	58 (20.5)	32 (21.5)	0.81
HCV treatment resulting in viral response in between biopsies	14 (24.1)	3 (9.4)	0.09
SVR	3 (5.2)	0 (0)	0.19
Relapse	11 (19.0)	3 (9.4)	0.23
HCV treatment resulting in viral non-response in between biopsies	44 (75.9)	29 (90.6)	0.09
Change in body mass index	-0.3 (-1.4, 1.3)	-0.1 (-1.5, 1.4)	0.79
Decrease	89 (33.6)	49 (35.3)	
Stable	102 (38.5)	46 (33.1)	
Increase	74 (27.9)	44 (31.7)	0.54

ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet-ratio index; ART, antiretroviral therapy; AST, aspartate aminotransferase; FIB4, non-invasive marker of liver fibrosis consisting of ALT, AST, platelet count and age; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range. NNRTI: non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SVR, sustained virologic response; ULN, upper limit of normal reference range. Unless otherwise indicated, data shown are numbers (proportion).

*Other than for demographic characteristics, numbers do not add up to total due to missing data.

†Alcohol abuse was defined as clinical diagnosis of alcoholism or abuse.

precision of these prior estimates of progression was limited by small sample size.^{9,21,22} For example, Schiavini et al.⁹ calculated a rate of fibrosis progression of 50% based on analysis of 36 paired liver biopsies. Taken together, these data indicate that progression of HCV disease can occur in HIV/HCV coinfecting persons with minimal fibrosis on initial staging. Since most patients had been HCV-infected for many years prior to this first biopsy, this observation suggests that fibrosis progression may be nonlinear in this patient population and underscores the need for serial monitoring in such patients (Supporting Figure 1). Importantly, among persons with minimal disease on first

biopsy, the progression was generally limited to one METAVIR stage; as such, serial monitoring allows for the detection of individuals with progressive disease prior to the onset of clinical liver disease such as hepatocellular carcinoma or end-stage liver disease.

We also identified baseline and time-varying factors associated with fibrosis progression between biopsy pairs. Interestingly, HCV genotype 1 was associated with an increased risk of progression in some models. While it is possible that this reflects biological differences in disease related to HCV diversity, our cohort was relatively homogeneous with respect to patient (largely African American) and viral (largely genotype 1)

Table 4. Multivariate Analysis of Correlates of Fibrosis Progression Among 435 Biopsy Pairs Contributed by 282 HIV/HCV Coinfecting Adults*†

	Crude OR (95% CI)	Model 1	Model 2	Model 3
Female	0.99 (0.65, 1.50)	1.08 (0.67, 1.76)	1.10 (0.67, 1.81)	1.25 (0.74, 2.11)
White race	0.79 (0.48, 1.30)	1.23 (0.71, 2.14)	1.24 (0.72, 2.14)	1.20 (0.65, 2.20)
Age (per 5 years)	1.05 (0.92, 1.20)	1.11 (0.95, 1.28)	1.10 (0.95, 1.29)	1.14 (0.98, 1.32)
Body mass index(per 1 unit increase)	1.04 (1.00, 1.09)	1.04 (0.99, 1.09)	1.05 (1.00, 1.09)	1.04 (0.99, 1.08)
Diabetes	1.91 (1.18, 3.10)	1.56 (0.90, 2.68)	1.41 (0.81, 2.46)	1.46 (0.84, 2.54)
HCV genotype 1	3.43 (1.23, 9.54)	3.29 (1.20, 9.01)	2.72 (0.93, 7.92)	3.35 (1.15, 9.74)
Steatosis‡	2.04 (1.17, 3.56)	1.78 (0.95, 3.33)	1.63 (0.86, 3.09)	1.73 (0.92, 3.25)
AST > 2.5 x ULN at first biopsy	2.34 (1.27, 4.30)	2.12 (1.06, 4.26)	0.99 (0.43, 2.29)	1.47 (0.69, 3.12)
Proportion of ALT values > 2.5 x ULN (100 U/l)				
0	1			1
1-24	1.89 (1.10, 3.23)			1.79 (0.99, 3.25)
≥ 25	2.46 (1.47, 4.10)			2.18 (1.20, 3.96)
Proportion of AST values > 2.5 x ULN (100 U/l)				
0	1		1	
1-24	1.55 (0.95, 2.51)		1.56 (0.91, 2.67)	
≥ 25	4.01 (2.32, 6.92)		3.34 (1.77, 6.31)	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; HIV, human immunodeficiency virus; ULN, upper limit of normal reference range.

*Models include adjustment for time between biopsy.

†The first column includes univariate odds ratios. In each of the other three columns, only the variables listed for each model are included in that specific model.

‡Defined as patients with grade 2 (5-30%), grade 3 (30-60%) or grade 4 (>60%) steatosis.

characteristics. As such, this finding requires validation in other settings. Our data confirm the relationship of chronically elevated serum liver enzyme levels, namely AST and ALT levels, and fibrosis progression. While the biologic mechanism underlying the observed relationship of AST level and disease was not directly measured, elevated serum AST levels >100 U/L in our cohort were associated with alcohol abuse and failure to be on ART and may reflect the impact of these factors on disease progression. Similar to our prior study, elevated serum AST at baseline and between histologic assessment were independently associated with progression; HIV/HCV coinfecting patients for whom measured AST levels were always <100 U/L were significantly less likely to have evidence of fibrosis progression on the next liver biopsy. Thus, AST level may represent an inexpensive, routinely obtained biomarker to identify persons at greater risk of progressive disease.

Interestingly, we found that higher AST levels were associated with clinical history of alcohol abuse and the lack of treatment with antiretrovirals. Although alcohol is clearly related to HCV disease pathogenesis, accurate assessment of alcohol intake in clinical cohorts may be challenging due to underreporting by patients. The observation that patients with elevated AST levels are at greater risk of progression may represent the effect of undetected alcohol exposure; novel alcohol biomarkers such as phosphatidylethanol or carbohydrate-deficient transferrin may be useful to further assess the contribution of underreported alcohol exposure.^{23,24} Despite the observation that ART exposure was associated with a lower likelihood of having high AST levels, we did not detect an association of ART, HIV RNA suppression, or CD4 cell count with fibrosis progression. In contrast, we recently observed that the receipt of ART was independently associated with a 66% reduction in the risk of HCV-related clinical outcomes including end-stage liver disease, hepatocellular carcinoma, or liver-related death.²⁵ Taken together, these findings suggest that while the treatment or prevention of HIV disease may reduce liver inflammation and clinical outcomes, ART alone is not sufficient to prevent fibrosis progression in coinfecting patients.

We also found that markers of metabolic derangement at initial biopsy were associated with fibrosis progression, although the statistical significance did not persist in all models after multivariate analysis. The impact of obesity and its associated complications, namely hepatic steatosis and diabetes, have appropriately become focal points of investigation in the ART era during which time the metabolic profile of HIV-infected patients has shifted.²⁶ Our finding of steatosis

as a predictor of fibrosis progression is in concordance with recent investigations on this topic that have found the presence of steatosis to be strongly associated with advanced fibrosis.²⁷⁻³⁰ In fact, Gaslightwala and Bin²⁷ found in their investigation of 154 coinfecting patients that fibrosis progression rates increased in a linear fashion with the grade of hepatic steatosis. Similarly, diabetes and insulin resistance are additional complications of obesity that have been identified as independent predictors of cirrhosis.^{31,32} Prospective studies are needed to investigate strategies to modify obesity and to assess the impact on the risk of fibrosis progression in HIV/HCV coinfecting patients. In the absence of such prospective data, our findings suggest that measures to facilitate weight loss should be a priority in obese coinfecting patients and those with a normal BMI should strive to maintain this.

While the major strength of our study is the prospective assessment of histologic disease progression in a large sample of coinfecting patients, there are several limitations to our findings. First, our cohort consists primarily of African American patients infected with HCV genotype 1; our findings may not be generalizable to more diverse patient populations including those infected with other HCV genotypes. Second, our patients who underwent serial liver biopsies were engaged in medical care and were willing to undergo multiple liver biopsies; this patient population may differ from coinfecting patients who were not referred for care. Noninvasive methods of fibrosis assessment such as liver elastography may be a useful tool to overcome this potential bias. Third, few HCV/HIV coinfecting patients who were successfully treated for HCV underwent serial liver biopsy; while not unexpected, this limits our ability to assess the impact of HCV eradication on disease progression. Finally, studies based on liver biopsy are subject to sampling error and misclassification. To limit misclassification, biopsies were read as pairs by a single expert hepatopathologist who was blind to biopsy sequence. The criteria for adequacy of the biopsy specimen used in our cohort may also represent a limitation since we did not apply specific criteria for adequacy based on length or number of portal tracts. However, sensitivity analysis in which such criteria were applied did not change our findings. Finally, while it is possible that some patients with apparent fibrosis progression reflect sampling error, only 33 of 256 biopsy pairs with fibrosis on the first biopsy of the pair had evidence of fibrosis regression (12.8%), suggesting that misclassification was not common.

In conclusion, approximately one-third of HIV/HCV coinfecting patients experienced fibrosis progression of at least one METAVIR stage over a relatively short period

of time, including patients with no or minimal fibrosis on first biopsy and those taking ART. Patients with persistent liver enzyme elevation, particularly of serum AST, were more likely to progress, suggesting that this simple measurement may be useful in identifying coinfecting patients at greater risk for HCV disease progression. The association of obesity and its related complications with fibrosis progression underscores the potential importance of this modifiable risk factor. However, our limited ability to accurately predict progression in most patients, underscores the need for additional research to understand the basis for variable HCV disease progression in HIV-infected patients.

References

- Sulkowski MS, Thomas DL. Hepatitis C in the HIV-infected person. *Ann Intern Med* 2003;138:197-207.
- Sherman KE, Rouster SD, Chung RT, Rajcic N. Hepatitis C virus prevalence among patients infected with human immunodeficiency virus: a cross-sectional analysis of the US adult AIDS Clinical Trials Group. *Clin Infect Dis* 2002;34:831-837.
- Sulkowski MS, Mehta SH, Torbenson MS, Higgins Y, Brinkley SC, de Oca RM, et al. Rapid fibrosis progression among HIV/hepatitis C virus-co-infected adults. *AIDS* 2007;21:2209-2216.
- Rosenthal E, Poiree M, Pradier C, Perronne C, Salmon-Ceron D, Geffray L, et al. Mortality due to hepatitis C-related liver disease in HIV-infected patients in France (Mortavic 2001 study). *AIDS* 2003;17:1803-1809.
- Bica I, McGovern B, Dhar R, Stone D, McGowan K, Scheib R, et al. Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. *Clin Infect Dis* 2001;32:492-497.
- Benhamou Y, DiMartino V, Bochet M, Colombet G, Thibault V, Liou A, et al. Factors affecting liver fibrosis in human immunodeficiency virus-and hepatitis C virus-coinfected patients: impact of protease inhibitor therapy. *HEPATOLOGY* 2001;34:283-287.
- Benhamou Y, Bochet M, Di Martino V, Charlotte F, Azria F, Coutellier A, et al. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfecting patients. *The Multivir Group. HEPATOLOGY* 1999;30:1054-1058.
- Brau N, Salvatore M, Rios-Bedoya CF, Fernandez-Carbia A, Paronetto F, Rodriguez-Orengo JF, et al. Slower fibrosis progression in HIV/HCV-coinfected patients with successful HIV suppression using antiretroviral therapy. *J Hepatol* 2006;44:47-55.
- Schiavini M, Angeli E, Mainini A, Zerbi P, Duca PG, Gubertini G, et al. Risk factors for fibrosis progression in HIV/HCV coinfecting patients from a retrospective analysis of liver biopsies in 1985-2002. *HIV Med* 2006;7:331-337.
- Macias J, Berenguer J, Japon MA, Giron JA, Rivero A, Lopez-Cortes LE, et al. Fast fibrosis progression between repeated liver biopsies in patients coinfecting with human immunodeficiency virus/hepatitis C virus. *HEPATOLOGY* 2009;50:1056-1063.
- Alberti A, Clumeck N, Collins S, Gerlich W, Lundgren J, Palu G, et al. Short statement of the first European Consensus Conference on the treatment of chronic hepatitis B and C in HIV-coinfected patients. *J Hepatol* 2005;42:615-624.
- Tien PC. Management and treatment of hepatitis C virus infection in HIV-infected adults: recommendations from the Veterans Affairs Hepatitis C Resource Center Program and National Hepatitis C Program Office. *Am J Gastroenterol* 2005;100:2338-2354.
- Soriano V, Puoti M, Sulkowski M, Mauss S, Cacoub P, Cargnel A, et al. Care of patients with hepatitis C and HIV co-infection. *AIDS* 2004;18:1-12.
- Soriano V, Martin-Carbonero L, Garcia-Samaniego J. Treatment of chronic hepatitis C virus infection: we must target the virus or liver fibrosis? *AIDS* 2003;17:751-753.
- Lagging M, Wejstal R, Uhnoo I, Gerden B, Fischler B, Friman S, et al. Treatment of hepatitis C virus infection: updated Swedish Consensus recommendations. *Scand J Infect Dis* 2009;41:389-402.
- Soriano V, Miro JM, Garcia-Samaniego J, Torre-Cisneros J, Nunez M, del Romero J, et al. Consensus conference on chronic viral hepatitis and HIV infection: updated Spanish recommendations. *J Viral Hepat* 2004;11:2-17.
- Shamoun DK, Anania FA. Which patients with hepatitis C virus should be treated? *Semin Gastrointest Dis* 2000;11:84-95.
- Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. Available at: <http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>
- Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. Diagnosis, management and treatment of hepatitis C: an update. *HEPATOLOGY* 2009;49:1335-1374.
- Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999;94:2467-2474.
- Bonnard P, Lescure FX, Amiel C, Guiard-Schmid JB, Callard P, Gharakhanian S, et al. Documented rapid course of hepatic fibrosis between two biopsies in patients coinfecting by HIV and HCV despite high CD4 cell count. *J Viral Hepat* 2007;14:806-811.
- Bani-Sadr F, Lapidus N, Bedossa P, De Boever CM, Perronne C, Halfon P, et al. Progression of fibrosis in HIV and hepatitis C virus-coinfected patients treated with interferon plus ribavirin-based therapy: analysis of risk factors. *Clin Infect Dis* 2008;46:768-774.
- Stewart SH, Reuben A, Brzezinski WA, Koch DG, Basile J, Randall PK, et al. Preliminary evaluation of phosphatidylethanol and alcohol consumption in patients with liver disease and hypertension. *Alcohol Alcohol* 2009;44:464-467.
- Imbert-Bismut F, Naveau S, Morra R, Munteanu M, Ratziu V, Abella A, et al. The diagnostic value of combining carbohydrate-deficient transferrin, fibrosis, and steatosis biomarkers for the prediction of excessive alcohol consumption. *Eur J Gastroenterol Hepatol* 2009;21:18-27.
- Limketkai BN, Mehta SH, Sutcliffe CG, Higgins YM, Torbenson MS, Brinkley SC, et al. Relationship of liver disease stage and antiviral therapy with liver-related events and death in adults coinfecting with HIV/HCV. *JAMA* 2012;308:370-378.
- Keithley JK, Duloy AM, Swanson B, Zeller JM. HIV infection and obesity: a review of the evidence. *J Assoc Nurses AIDS Care* 2009;20:260-274.
- Gaslightwala I, Bin EJ. Impact of human immunodeficiency virus infection on the prevalence and severity of steatosis in patients with chronic hepatitis C virus infection. *J Hepatol* 2006;44:1026-1032.
- Sterling RK, Contos MJ, Smith PG, Stravitz RT, Luketic VA, Fuchs M, et al. Steatohepatitis: risk factors and impact on disease severity in human immunodeficiency virus/hepatitis C virus coinfection. *HEPATOLOGY* 2008;47:1118-1127.
- Marks KM, Petrovic LM, Talal AH, Murray MP, Gulick RM, Glesby MJ. Histological findings and clinical characteristics associated with hepatic steatosis in patients coinfecting with HIV and Hepatitis C virus. *J Infect Dis* 2005;192:1943-1949.
- Sulkowski MS, Mehta SH, Torbenson M, Afdhal NH, Mirel L, Moore RD, et al. Hepatic steatosis and antiretroviral drug use among adults coinfecting with HIV and hepatitis C virus. *AIDS* 2005;19:585-592.
- Loko MA, Bani-Sadr F, Winnock M, Lacombe K, Carrieri P, Neau D, Morlat P, et al. Impact of HAART exposure and associated lipodystrophy on advanced liver fibrosis in HIV/HCV-coinfected patients. *J Viral Hepat* 2011;18:307-314.
- Ryan P, Berenguer J, Michelaud D, Miralles P, Bellon JM, Alvarez E, et al. Insulin resistance is associated with advanced liver fibrosis and high body mass index in HIV/HCV-coinfected patients. *J Acquir Immune Defic Syndr* 2009;1:109-110.