Published in final edited form as:
Clin Gastroenterol Hepatol. 2008 January ; 6(1): 69–75. doi:10.1016/j.cgh.2007.10.021.

Influence of Cannabis Use on Severity of Hepatitis C Disease

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Abstract

Background—Complications of hepatitis C virus (HCV) infection are primarily related to the development of advanced fibrosis.

Methods—Baseline data from a prospective community-based cohort study of 204 persons with chronic hepatitis C virus (HCV) infection were used for analysis. The outcome was fibrosis score on biopsy and the primary predictor evaluated was daily cannabis use.

Results—The median age of the cohort was 46.8 years, 69.1% were male, 49.0% were Caucasian, and the presumed route of infection was injection drug use in 70.1%. The median lifetime duration and average daily use of alcohol were 29.1 years and 1.94 drink equivalents per day. Cannabis use frequency (within prior 12 months) was daily in 13.7%, occasional in 45.1%, and never in 41.2%. Fibrosis stage, assessed by Ishak method, was F0, F1–2 and F3–6 in 27.5%, 55.4% and 17.2% of subjects, respectively. Daily compared to non-daily cannabis use was significantly associated with moderate to severe fibrosis (F3–6 versus F1–2) in univariate [OR = 3.21 (95% CI, 1.20–8.56), p = 0.020] and multivariate analyses (OR = 6.78, (1.89–24.31), p=0.003). Other independent predictors of F3–6 were ≥11 portal tracts (compared to <5, OR = 6.92 (1.34–35.7), p=0.021] and lifetime duration of moderate and heavy alcohol use [OR per decade = 1.72 (1.02–2.90), p=0.044].

Conclusion—We conclude that daily cannabis use is strongly associated with moderate to severe fibrosis and that HCV-infected individuals should be counseled to reduce or abstain from cannabis use.

Keywords

fibrosis; alcohol; viral load; marijuana; cirrhosis

Introduction

Hepatitis C virus (HCV) infection is a major public health concern and the burden of disease related to cirrhosis and liver cancer is predicted to increase in the next decade (1). Understanding the factors that influence disease progression and the development of cirrhosis may provide opportunities for intervention. Male gender, older age at the time of HCV infection, duration of HCV infection, heavy alcohol consumption and coinfection with

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human immunodeficiency virus (HIV) have been identified as risk factors for fibrosis progression (2–5)

Cannabis (*Cannabis sativa*) has a long history of use for medicinal and recreational purposes and is commonly used throughout the world (6). Cannabis is the source of over 60 cannabinoid compounds, including δ^9-tetrahydrocannabinol (δ^9-THC), which is primarily responsible for the psychoactive effects of the plant (7). Cannabinoid compounds bind to G protein-coupled receptors called CB1, which predominate in the central nervous system, and CB2, which are expressed mainly by immune cells (7). Cannabinoid receptor expression is upregulated in cirrhotic livers compared to normal livers. In liver specimens, CB receptors appear localized to stellate cells and myofibroblasts—the cell types central to fibrosis production, (8. 9). In cannabinoid receptor knockout mice, CB1 receptor inactivation promotes fibrosis development while CB2 receptor activation exerts an inhibitory effect (8) and animal studies show CB1 receptor antagonism reduces fibrosis (9). These studies suggest cannabinoids may have an important, but as yet undefined, role in hepatic fibrosis

In the United States, the prevalence of cannabis use among adults is estimated to be approximately 4.0% and has increased in certain population subgroups including 18–29-year olds (10). Among individuals with chronic HCV infection, the prevalence of cannabis use has not been carefully studied, and there is a paucity of epidemiologic studies evaluating the effect of cannabis on liver fibrosis (11). Given the prevalence of cannabis use, the biological basis for its effect on liver fibrosis and the lack of epidemiologic studies on this topic, we sought to investigate the effect of cannabis on fibrosis severity in a U.S. cohort with chronic HCV infection.

METHODS

Study Population

Consecutive subjects with a diagnosis of chronic HCV infection were recruited from the University of California at San Francisco and community-based sources in Northern California between 2001 and 2004. Clinics serving HIV-infected populations were encouraged to refer to the study, with the goal of having 25% HCV-HIV coinfected subjects in the cohort to insure representation of coinfected subjects in the final cohort Subjects were included if they were at least 18 years old, English-speaking and had HCV RNA detectable in serum or plasma. Subjects were excluded if they had a history of HCV treatment for longer than 3 months and other chronic liver diseases including hepatitis B. Of the 328 individuals who had completed an in-person interview at study entry, 124 were excluded for the following reasons: lack of HCV viremia (n = 28), HCV treatment greater than 3 months (n = 12), hepatitis B infection (n = 1) and lack of the baseline liver biopsy requirement (n = 83). The remaining 204 individuals who formed the study cohort completed all the study requirements including an in-person interview, virologic testing and a liver biopsy. The local institutional review board approved the study, and all subjects provided their informed written consent.

Study Procedures

Subjects underwent an in-person interview that collected information about demographics, risk factors for HCV infection and use of cannabis, alcohol, and other substances. Interviewers and subjects were blinded to the hypothesis regarding cannabis use and fibrosis. Subjects were asked about the frequency of their current (within 12 months of enrollment) cannabis use. The response categories were everyday, three or four times a week, one or two times a week, seven to eleven times a month, one to three times a month, three to six times, twice, once or never in the last 12 months. Lifetime alcohol use was

assessed in detail using a validated questionnaire (12). A standard drink was estimated to contain 10 grams of alcohol and was equivalent to 12 oz of beer, 1 oz liquor or 4 oz of wine. The duration, quantity, frequency and type (beer, liquor or wine) of alcohol consumed were recorded. Only 15.2% of subjects were aware of their liver biopsy results at the time of interview.

Laboratory Testing—Subjects were considered to have chronic HCV infection if they were HCV antibody positive and had HCV viral load ≥1000 IU/mL or an identified HCV genotype. If HCV antibody was unavailable or negative, subjects were considered to have chronic HCV infection if they had HCV viral load ≥1000 IU/mL and/or a HCV genotype result (n = 16). The window to capture all HCV test results to verify HCV status was ±15 months from the date of enrollment.

Testing for HCV antibody was performed using the enzyme-linked immunosorbent assay (Abbott HCV EIA 2.0, Abbott Laboratories Diagnostics Division, Abbott Park, IL). HCV RNA was quantified (in IU/mL) by branched DNA assay (VERSANT® HCV RNA 3.0 bDNA Assay, Bayer HealthCare LLC Diagnostics Division, Pittsburgh, PA) or polymerase chain reaction (PCR) (COBAS AMPLICOR™ HCV MONITOR Test, v2.0, Roche Molecular Systems, Inc., Pleasanton, CA). For subjects with HCV RNA above quantitation limits of the assays, repeat testing of a diluted sample was performed (n = 6). If a sample was not available for repeat testing, the median viral load of the subjects who had a viral load above the upper limit of quantitation was used (n = 5). HCV genotyping was performed using the Linear Array Hepatitis C Virus Genotyping Test (Roche Molecular Systems, Inc., Pleasanton, CA) or Versant® HCV Genotype (LiPA) Assay (Bayer Diagnostics, Tarrytown, NY).

Subjects were considered HIV positive if they had positive HIV antibody. If HIV antibody was unavailable, subjects were considered HIV positive if they had HIV viral load ≥1000 copies/ml (n = 11). If laboratory evidence was not available, HIV positive status was confirmed by review of medical records (n = 11) or subject self-report (n = 2).

Liver Biopsy—All subjects in the study cohort underwent a liver biopsy at or near the time of study entry; 89% of biopsies were collected within 6 months prior to or after enrollment (range 0 to 24 months). A single pathologist, blinded to clinical data, scored each biopsy for necroinflammation using the Knodell method (scale 0–18) (13), steatosis using the Brunt criteria (14), and fibrosis using the Ishak method (scale F0 to F6) (15). To control for inaccuracy in assessing the stage of fibrosis that was related to biopsy adequacy (16, 17), all biopsies were evaluated for length (cm) and the number of portal tracts.

Statistical Analysis

Descriptive statistics are expressed as percentages for categorical data and medians with interquartile range (IQR, 25% and 75% percentiles) for continuous data. Differences between the subjects included and excluded from the analysis and daily and non-daily cannabis users were assessed using the Chi-square and Fisher's exact tests for categorical data and the Mann-Whitney U test for continuous data. A p-value of ≤0.05 was considered statistically significant.

Age at HCV infection was estimated using the year of first exposure to intravenous drug use, occupational needlestick exposure or blood transfusion before 1992, whichever occurred first. If exposure to multiple risk factors occurred in the same year, injection drug use was considered the source of infection. Duration of HCV infection was estimated as the difference between the age at enrollment and age at HCV infection. Frequency of reported current cannabis use within 12 months of enrollment was dichotomized as daily versus non-

daily or none for analysis. For dose-response analysis of cannabis and fibrosis severity, the categories of cannabis use were (i) daily (included daily and nearly daily), (ii) weekly (included three or four times a week, one or two times a week, seven to eleven times a month), (iii) monthly (included one to three times a month) and (iv) rare/never (included three to six times, twice, once and never). Lifetime alcohol consumption was expressed as duration of any use, duration of moderate to heavy use (2 or more drink equivalents per day on average in women and 4 or more drink equivalents per day on average in men), average daily use, and total number of drinks.

The associations between the predictor variables and fibrosis severity were analyzed with logistic regression. Separate models were developed to predict the odds of having mild fibrosis (F1–2) compared to no fibrosis (F0), excluding F3–F6, and the odds of having moderate to severe fibrosis (F3–6) compared to mild fibrosis (F1–2), excluding F0. Multivariate models were examined to estimate effects while controlling for important potential confounders. Predictor variables with a p-value ≤ 0.10 were evaluated in multivariate models. In multivariate models, biopsy adequacy, as measured by the number of portal tracts, was controlled for in all analyses. The analysis was performed using SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

Characteristics of Study Population

The baseline characteristics of the screened subjects who were included (n = 204) in the study were similar to excluded subjects (n=124), except for a higher percentage of daily cannabis users in the included group (13.7% vs. 6.45%, p = 0.041). The median age of the study population was 46.8 years (IQR 41.1–51.0), 69.1% were male, 49.0% were Caucasian, and 32.3% were African-American. The majority of subjects were infected with genotype 1 (78.7%) and 21.1% were HIV coinfected. Injection drug use was the most common risk factor for HCV acquisition (70.1%), and the median estimated duration of HCV infection was 25.5 years (IQR 19.0–34.0). Daily or nearly daily cannabis use was reported in 13.7%. The median lifetime duration and average daily use of alcohol were 29.1 years and 1.94 drink equivalents per day.

The characteristics of daily and non-daily cannabis users were similar (Table 1), except daily cannabis users had a significantly lower median body mass index than that of non-daily cannabis users (25.2 vs. 26.4 kg/m^2, p = 0.007), were more likely to have medically prescribed cannabis (57.1% vs. 8.79%, p <0.001), and more frequently HIV coinfected (39.3% vs. 18.2%, p = 0.011). Non-users of cannabis and non-daily users were similar in all baseline characteristics (data not shown) and therefore pooled for subsequent analyses. Among those with medically prescribed cannabis, the proportion of daily users among those who were HIV positive and negative was similar (69.2% versus 63.6%, p=1.0). Among HIV-positive patients, median CD4 counts among daily and non-daily cannabis users were similar (368/mm^3 and 441/mm^3, p=1.0)

The proportion of subjects with fibrosis scores of 0, 1–2, and 3–6 was 27.5%, 55.4%, and 17.2%, respectively. The median necroinflammation score was 5 (IQR 3–7). The median AST was 48.0 U/L (normal range 16–41 U/L) and ALT was 56.0 U/L (normal range 12–59 U/L). The biopsy specimen length was 2 cm or greater in 45.8% of biopsies, and the number of portal tracts were <5 in 26.7%, 5 to 10 in 52.0% and ≥11 in 21.3% of biopsies. Longer biopsies and greater number of portal tracts were associated with higher fibrosis scores (see below).

Predictors of Mild Fibrosis (F1–2) Compared to No Fibrosis (F0)

The variables associated with mild fibrosis in univariate analysis were HCV viral load (OR = 1.79 per $\log_{10}$ increase, p = 0.008), steatosis (OR = 2.17, p = 0.041), necroinflammatory score (>5 compared to ≤5, OR = 23.02, p=,0.001) and number of portal tracts (5 to <11 portal tracts compared to <5, OR = 2.80, p = 0.005, and ≥11 portal tracts compared to <5, OR = 10.6, p = <0.001) (Table 2). HIV coinfection (OR = 2.42, p = 0.054) and biopsy length 1 – <2 cm compared to <1 cm, (OR = 0.14, p = 0.070) were of borderline significance. An association between daily (compared to non-daily) cannabis use and mild fibrosis was not apparent (OR = 0.65, p = 0.380). There was no association between mild fibrosis and age at enrollment, estimated age and duration of HCV infection, gender, race, body mass index, HCV genotype, source of HCV infection or lifetime alcohol use. In multivariate analysis, the only independent predictors of mild fibrosis were HCV viral load (OR = 1.85 per $\log_{10}$ increase, p = 0.009), necroinflammatory score >5 (OR = 19.01, p < 0.001) and number of portal tracts (Table 3).

Predictors of Moderate to Severe Fibrosis (F3–6) Compared to Mild Fibrosis (F1–2)

In univariate analysis, there was a significant association between daily (compared to non-daily) cannabis use and moderate to severe fibrosis in univariate analysis (OR = 3.21, p = 0.020) (Table 4). Other significant predictors were age at enrollment (OR = 2.84 per 10 years, p = 0.002), lifetime duration of alcohol use (OR = 1.83 per 10 years, p = 0.029), lifetime duration of moderate to heavy alcohol use (OR = 1.79 per 10 years, p = 0.006), and necroinflammatory score (>5 compared to ≤5, OR 3.39, p = 0.005). In multivariate analysis, controlling for biopsy adequacy, there was a stronger association evident between daily cannabis use and moderate to severe fibrosis (OR = 6.78, p = 0.003) (Table 5). There did not appear to be a dose-response relationship when “less than daily use” was broken down into weekly (N=24) and monthly (N=27) and compared to rarely or never categories (N=125) (OR= 0.69, 95% CI: 0.12 to 3.8 for weekly; OR = 1.44, 95% CI: 0.37 to 5.7 for monthly). However, the confidence intervals were so wide that a possible dose-response pattern could not be excluded.

Other independent predictors of moderate to severe fibrosis were lifetime duration of moderate to heavy alcohol use (OR = 1.72 per 10 years, p = 0.044) and number of portal tracts (≥11 compared to <5) (OR = 6.92, p = 0.021). Age at enrollment was of borderline significance (OR = 2.19 per 10 years, p = 0.064) (Table 5). Necroinflammation was of borderline significance also (OR = 2.60 for score >5 versus ≤5, p = 0.067) and did not add significantly to the multivariate model. Gender, race, body mass index, HCV viral load and genotype, HIV coinfection, source of HCV infection, and biopsy length were not significantly associated with moderate to severe fibrosis stage in univariate ormultivariate analysis. The effect of cannabis and the results of the multivariate models were similar with HIV coinfected subjects excluded (data not shown). The association between daily cannabis use and moderate to severe fibrosis was similar when subjects using medically-prescribed cannabis were excluded (OR = 5.65, 95% CI: 1.07, 29.89, P=0.042)

Potential interactions between cannabis use and moderate to heavy alcohol use were examined. In the multivariate model, the effect was in the direction of substantial synergy but the confidence intervals were wide and the association did not achieve statistical significance (p = 0.40).

DISCUSSION

Understanding the factors influencing HCV disease severity, especially those that are potentially modifiable, is of great importance in patient management. The strong

recommendation for HCV-infected persons to limit or abstain from alcohol use (18, 19) reflects the consistent association between heavy alcohol use and more severe fibrosis and greater risk of cirrhosis (2, 20–22). Similarly, steatosis has recently been identified as an important factor associated with fibrosis severity (23). Metabolic, virologic and alcohol-related contributions to fatty liver are recognized, and at least some of these factors can be modifiable. Based on our results, we recommend that cannabis be added to the list of modifiable risk factors for HCV disease severity. We have shown that daily cannabis use is an independent risk factor for moderate to severe fibrosis and one of substantial magnitude, with daily cannabis users having a nearly sevenfold higher odds of moderate to severe fibrosis compared to non-daily users. HCV-HIV coinfected subjects were significantly more likely to use cannabis daily and to have a prescription for medical cannabis than HCV monoinfected subjects. The recommendation to avoid cannabis use may be especially important for HCV/HIV coinfected persons given that fibrosis progression is already enhanced in this group (24).

Our results support the findings of a French study of liver clinic patients with chronic HCV infection, which found that daily cannabis smoking was an independent risk factor for severe fibrosis (≥F3 on the METAVIR scale) and rapid fibrosis progression (>0.15 METAVIR units/year) (11). This study assumed a linear model of progression and did not examine predictors of mild fibrosis and moderate to severe fibrosis, as was done in our study. It is of interest that we found a strong association between daily cannabis use and having moderate to severe fibrosis compared to mild fibrosis, but little association was apparent between cannabis use and the presence of mild fibrosis compared to no fibrosis. This suggests that there may be a different or minimal effect of cannabis in early versus later stage disease. Cannabis may have little or no influence on the initiation of fibrosis, but once fibrosis is present, it may be an important cofactor in fibrosis progression. Further studies are needed to confirm this apparent difference in association by stage of fibrosis. Pending such studies, the safest recommendation to patients would be to reduce or avoid daily cannabis use, regardless of the stage of disease.

Fibrosis results from an imbalance in the profibrogenic and antifibrogenic factors expressed in the setting of chronic liver injury (25). Studies in human livers and mouse models of fibrosis demonstrate upregulated expression of the cannabinoid receptors, CB1 and CB2, in chronic liver injury compared to normal controls (8, 9). Immunohistochemical staining of human specimen with cirrhosis shows localization of the CB receptors to hepatic myofibroblasts (8, 9). In experimental models of fibrosis, CB1 receptor activation is associated with profibrogenic effects whereas CB2 receptor activation is associated with antifibrogenic effects (8, 9). In, studies of liver injury in mice, blockade of the CB1 receptor by a CB1 antagonist or use of CB1 knockout is associated with lesser fibrosis than control animals (9). Antagonism of the CB1 receptor has been associated with reduced expression of the TGFβ1, a cytokine central to fibrosis production, and decreased stellate cell proliferation and increased apoptosis, all of which would be predicted to reduce fibrosis (9). Additionally, CB1 receptor antagonism has been associated with increased levels of adiponectin (26), an adipokine with antifibrotic properties in animal models (27). Thus, there are several potential mechanisms by which enhanced CB1 receptor expression or activity may lead to increased fibrosis. In terms of the CB2 receptor, activation is associated with antiproliferative and apoptotic effects in myofibroblasts and activated stellate cells, and in a mouse model of chronic liver injury, CB2 receptor blockade is associated with enhanced liver fibrosis compared to control mice (8). While studies suggest tetrahydrocannabinol binds equally to CB1 and CB2 receptors, whether this is true in the setting of chronic liver injury due to HCV is unknown. Dysregulation of ligand binding to the CB1 and/or CB2 receptors or post-binding alterations may result in a situation favoring fibrogenesis.

Similar to other studies evaluating the factors associated with severe fibrosis, we found that duration of moderate to heavy alcohol use was an independent predictor of moderate to severe fibrosis (2, 21, 28, 29). Definitions of “heavy” alcohol use vary across different studies and not all studies use gender-specific cut-offs. We used the Lifetime Drinking History to carefully evaluate lifetime alcohol use and defined moderate to heavy use as 2 or more drink equivalents per day on average in women and 4 or more drink equivalents per day on average in men. For every 10 years of alcohol use at these levels, the odds of having moderate to severe fibrosis compared to mild fibrosis increased by nearly 2-fold. Alcohol use at levels below these cutoffs did not appear to be associated with a substantially increased risk of fibrosis in our models. Our results are consistent with a recent metaanalysis of 20 studies including 15,000 HCV-infected persons, in which heavy alcohol intake, defined by a range of at least 210–560 g of alcohol per week, had a pooled relative risk of cirrhosis of 2.33 (95% CI, 1.67–3.26) (4). There remains a paucity of data on the effects of infrequent and light alcohol intake on HCV disease progression (30), particularly in persons with minimal fibrosis.

This is the first study to evaluate the relationship between alcohol and cannabis use. This relationship is critical to understand for two reasons. First, concurrent use or abuse of alcohol and marijuana is not an uncommon behavior. Second, those who are moderate and heavy users of alcohol may substitute cannabis for alcohol in efforts to reduce alcohol intake, particularly once they learn of their HCV diagnosis. The risks from daily cannabis use and moderate and heavy lifetime alcohol use, which we defined as an average daily intake of 2 or more drink equivalents for women and 4 or more drink equivalents in men, had a suggestion of synergy but with very wide uncertainty in our model of moderate to severe fibrosis.

HCV viral load has not been shown consistently to have an effect on fibrosis severity (3). Whether the relationship between HCV viral load and disease severity is dependent upon the stage of fibrosis is unknown. In our study, HCV viral load was the only factor with a statistically significant association with mild fibrosis compared to no fibrosis. Two recent paired biopsy studies of individuals with chronic HCV infection and predominantly mild fibrosis identified HCV viral load as an independent predictor of fibrosis progression (29, 31). These results suggest that the relationship between HCV viral load and risk of fibrosis progression warrants reevaluation, with a focus on those persons with minimal or mild HCV disease. If the association between HCV viral load and disease severity is confirmed in this subgroup of HCV-infected persons, this may become an additional factor influencing decisions related to the urgency of undertaking antiviral therapy.

Having a greater number of portal tracts was associated with greater odds of detecting mild and moderate to severe fibrosis. This finding is in keeping with the increasing body of literature that stresses the importance of biopsy adequacy (as measured by length and the number of portal tracts) in the assessment of fibrosis (16, 17, 32).

Limitations of this study must be acknowledged. First, the cross-sectional design limits our ability to establish a temporal relationship between cannabis use and fibrosis stage. It is possible that having moderate to severe fibrosis may lead to increased usage of cannabis. However, the majority of subjects were non-cirrhotic and HCV infection is largely asymptomatic until cirrhosis and decompensation occur, making this a less likely explanation for the association. Secondly, we lack detailed information on the quantity, duration and method of cannabis use. Such information may have allowed us to better characterize the dose-effect relationship between cannabis and fibrosis severity. However, one of the challenges in studying cannabis is the lack of a standardized “product” and future studies may benefit from the inclusion of biologic markers of cannabis dose. The finding of

a higher rate of cannabis use in the included versus excluded subjects raises the issue of whether there was a selection bias. This is not likely as the study cohort was initially assembled to study the effects of alcohol use on HCV disease progression without any regard to cannabis intake. The strengths of our study include the use of a community-based cohort rather than a tertiary referral cohort, prospective collection of alcohol and other substance use, and a detailed assessment of liver biopsy adequacy. Additionally, our approach of evaluating predictors of severity in mild compared to more severe disease offers potential insights into the factors influencing disease progression at different stages of disease.

In summary, we found that daily cannabis use was significantly associated with the presence of moderate to severe fibrosis compared to mild fibrosis in persons with chronic HCV infection. Furthermore, daily cannabis use and moderate to heavy alcohol use appeared to have at least multiplicative effects on the odds of severe fibrosis. Based on our results, we would advise that individuals with chronic HCV infection be counseled to reduce or abstain from cannabis use.

Acknowledgments

This research was supported by the National Institute on Alcohol Abuse and Alcoholism (1R01 AA012879) and the UCSF Liver Center (P30 DK 26743). JI was supported by the Doris Duke Charitable Foundation. The study was carried out in part in the General Clinical Research Center, Moffitt Hospital, University of California, San Francisco, with funds provided by the National Center for Research Resources, 5 M01 RR-00079, U.S. Public Health Service. We would like to acknowledge the contributions of study personnel, especially Megan Burns and Katherine Welker, and study participants.

Abbreviations

AST	aspartate aminotransferase
ALT	alanine aminotransferase
BMI	body mass index
CI	confidence interval
GGT	gamma glutamyl transpeptidase
HCV	hepatitis C virus
HIV	human immunodeficiency virus
IQR	interquartile range, Q1 and Q3
OR	odds ratio

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Table 1

Comparison of Baseline Characteristics of Daily and Non-Daily Cannabis Users

VARIABLE	N	Daily Cannabis Use N=28	Non-Daily Cannabis Use N= 176)	P VALUE
Age at enrollment (yrs.), median (IQR)	204	45.1 (40.3–49.7)	46.8 (41.7–51.1)	0.355
Male (%)	203	67.9	69.3	0.905
African-American (%)	198	28.6	32.9	0.647
BMI (kg/m^2), median (IQR)[†]	**189**	**25.2 (21.5, 26.5)**	**26.4 (23.7, 30.0)**	**0.007**
HCV viral load (log_{10} IU/ml), median (IQR)	194	6.25 (5.79, 6.54)	5.92 (5.44, 6.42)	0.165
HCV genotype 1 (%)[††]	169	81.8	78.2	0.702
HIV positive (%)	**204**	**39.3**	**18.2**	**0.011**
Duration of HCV infection, median(IQR)[§]	172	24.0 (15.0, 34.0)	26.0 (20.0, 34.0)	0.322
Medical cannabis prescribed (%)	**204**	**57.1**	**8.79**	**<0.001**
Lifetime drinking duration (yrs.), median (IQR)	202	27.9 (22.4, 32.0)	29.7 (25.0, 35.0)	0.119
Lifetime moderate to heavy drinking duration (yrs.), median(IQR) [†††]	179	16.3 (8.50, 27.0)	18.0 (11.0, 25.8)	0.616
Necroinflammation, median (IQR)	204	5.00 (3.00, 6.00)	5.00 (4.00, 7.00)	0.245
Steatosis (%)	204	25.0%	35.8%	0.265
Fibrosis, median (IQR)	204	1.00 (0.00, 3.00)	1.00 (0.00, 2.00)	0.651
Biopsy length (cm), median (IQR)	204	1.75 (1.40, 2.25)	1.90 (1.40, 2.40)	0.276
Portal tracts < 5 (%)	204	35.7	25.3	0.416
Portal tracts 5 – < 11 (%)		50.0	52.3	
Portal tracts ≥11 (%)		14.3	22.4	

[†] 7.35% missing data,

[††] 17.2% missing data,

[†††] 12.3% missing data,

[§] 84.3% with an identifiable risk factor

Table 2

Predictors of Mild Fibrosis (F1–2) Compared to No Fibrosis (F0) – Univariate Analysis

VARIABLE	N	OR (95% CI)	P VALUE
Age at enrollment (per 10 yrs)	169	1.30 (0.82, 2.06)	0.258
Age at HCV infection (per 10 yrs)	142	1.21 (0.75, 1.95)	0.425
Duration of HCV infection (per 10 yrs)	142	1.10 (0.77, 1.57)	0.607
Male vs. female sex	168	0.88 (0.44, 1.77)	0.726
Caucasian vs. African-American race	137	1.09 (0.52, 2.25)	0.825
BMI (kg/m^2)	138	1.02 (0.96, 1.08)	0.613
HCV viral load (IU/ml; per log_{10} increase)	**168**	**1.79 (1.16–2.75)**	**0.008**
HCV genotype 1 vs. all others	139	0.95 (0.41, 2.23)	0.911
HIV positive vs. negative	**169**	**2.42 (0.99, 5.93)**	**0.054**
IVDU vs. other modes of transmission	169	1.01 (0.51, 2.01)	0.970
Cannabis use (daily vs. non-daily)	169	0.65 (0.24, 1.71)	0.380
Lifetime drinking duration (per 10 yrs)	148	0.99 (0.64, 1.52)	0.952
Lifetime moderate to heavy drinking duration (per 10 yrs)†	148	1.04 (0.73, 1.49)	0.816
Necroinflammation (>5 versus ≤5)	**169**	**23.02 (5.35, 99.01)**	**<0.001**
Steatosis (1–3 versus 0)	**169**	**2.17 (1.03, 4.56)**	**0.041**
Biopsy length	**169**		
1 – <2 vs. <1 cm		**0.14 (0.02, 1.17)**	**0.070**
≥2 vs. <1 cm		**0.34 (0.04, 2.85)**	**0.320**
Number of portal tracts	**169**		
5 – <11 vs. <5		**2.80 (1.36, 5.75)**	**0.005**
≥11 vs. <5		**10.6 (2.83, 39.4)**	**<0.001***

†Defined as 2 or more drink equivalents per day on average for women and 4 or more drink equivalents per day on average for men.

Table 3

Multivariate Associations with Having Mild Fibrosis (F1–2) Compared to No Fibrosis (F0)*

VARIABLE	OR (95% CI)	P-VALUE
HCV viral load (IU/ml; per $\log_{10}$ increase)	1.99 (1.18, 3.36)	0.010
Number of portal tracts		
5 – <11 vs. <5	2.42 (1.06, 5.53)	0.035
≥11 vs. <5	5.76 (1.37, 24.28)	0.017
Cannabis use (daily vs. non-daily)	1.01 (0.32, 3.17)	0.99
Necroinflammation (>5 versus ≤5)	19.01 (4.20, 85.96)	0.0002

* N=167 (N=56 with F0 and N=111 with F1–2)

Hosmer-Lemeshow test, p=0.21

Table 4

Univariate Analysis of Predictors of Moderate to Severe (F3–6) Compared to Mild Fibrosis (F1–2)

VARIABLE	N	OR (95% CI)	P-VALUE
Age at enrollment (per 10 yrs)	**148**	**2.84 (1.47–5.48)**	**0.002**
Age at HCV infection (per 10 yrs)	121	1.25 (0.73–2.13)	0.422
Duration of HCV infection (per 10 yrs)	121	1.25 (0.81–1.91)	0.313
Male vs. female sex	147	1.66 (0.69–4.02)	0.257
Caucasian vs. African-American race	116	0.73 (0.29–1.80)	0.488
BMI (kg/m^2)	137	0.98 (0.90–1.05)	0.536
HCV viral load (IU/mL; per log_{10} increase)	147	0.96 (0.59,1.56)	0.875
HCV genotype 1 vs. all others	123	1.46 (0.50–4.28)	0.492
HIV positive vs. negative	148	0.72 (0.29–1.83)	0.496
IVDU vs. other modes of transmission	148	1.87 (0.75–4.68)	0.181
Cannabis use (daily vs. non-daily)	**148**	**3.21 (1.20–8.56)**	**0.020**
Lifetime drinking duration (per 10 yrs)	**147**	**1.83 (1.06–3.16)**	**0.029**
Lifetime duration of moderate to heavy drinking (per 10 yrs)†	**127**	**1.79 (1.18–2.71)**	**0.006**
Necroinflammation (>5 versus ≤5)	**148**	**3.39 (1.46, 7.88)**	**0.005**
Steatosis (1–3 versus 0)	148	1.42 (0.676, 3.06)	0.367
Biopsy length	148		
1 – <2 vs. <1 cm		1.66 (0.33–8.49)	0.541
≥2 vs. <1 cm		1.24 (0.24–6.33)	0.795
Number of portal tracts	148		
5 – <11 vs. <5		1.58 (0.48–5.18)	0.453
≥11 vs. <5		2.77 (0.79–9.67)	0.111

†Defined as 2 more drink equivalents per day on average for women and 4 or more drink equivalents per day on average for men.

Table 5

Multivariate Analysis of Predictors of Moderate to Severe (F3–6) Compared to Mild Fibrosis (F1–2)*

VARIABLE	OR (95% CI)	P-VALUE
Cannabis use (daily vs. non-daily)	6.78 (1.89–24.3)	0.003
Lifetime duration of moderate to heavy drinking (per 10 yrs.)†	1.72 (1.02–2.90)	0.044
Number of portal tracts		
5 – <11 vs. <5	3.23 (0.71–14.7)	0.130
≥11 vs. <5	6.92 (1.34–35.7)	0.021
Age at enrollment (per 10 yrs.)	2.19 (0.95–5.05)	0.064

†Defined as 2 or more drink equivalents per day on average for women and 4 or more drink equivalents per day on average for men.

*N=125 (N=95 with F1–2 and N=35 with F3–6)

Hosmer-Lemeshow test, p=0.83