# Daily Cannabis Smoking as a Risk Factor for Progression of Fibrosis in Chronic Hepatitis C

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**Cannabinoids present in *Cannabis sativa* (marijuana) exert biological effects via cannabinoid receptors CB1 and CB2. We recently demonstrated that CB1 and CB2 receptors regulate progression of experimental liver fibrosis. We therefore investigated the impact of cannabis smoking on fibrosis progression rate in patients with chronic hepatitis C (CHC). Two hundred seventy consecutive untreated patients with CHC of known duration undergoing liver biopsy were studied. Demographic, epidemiological, metabolic, and virological data were recorded, and detailed histories of cannabis, alcohol, and tobacco use over the span of hepatitis C virus infection were obtained. Fibrosis stage, steatosis, and activity grades were scored according to Metavir system. Patients were categorized as noncannabis users (52.2%), occasional users (14.8%), or daily users (33.0%), and the relationship between cannabis use and fibrosis progression rate (FPR) or fibrosis stage was assessed. On multivariate analysis, six factors were independently related to a FPR greater than 0.074 (median value of the cohort): daily cannabis use (OR = 3.4 [1.5-7.4]), Metavir activity grade A2 or higher (OR = 5.4 [2.9-10.3]), age at contamination of more than 40 years (OR = 10.5 [3.0-37.1]), genotype 3 (OR = 3.4 [1.5-7.7]), excessive alcohol intake (OR = 2.2 [1.1-4.5]), and steatosis (OR = 2.0 [1.0-4.1]). Daily cannabis use was also an independent predictor of a rapid FPR (>0.15) (OR = 3.6 [1.5-7.5]). Finally, severe fibrosis (≥F3) was also predicted by daily cannabis use (OR = 2.5 [1.1-5.6]; *P* = .034), independently of Metavir activity grade, excessive alcohol intake, age at liver biopsy, steatosis, and tobacco smoking. In conclusion, daily cannabis smoking is significantly associated with fibrosis progression during CHC. Patients with ongoing CHC should be advised to refrain from regular cannabis use.** (HEPATOLOGY **2005;42:63-71.**)

Chronic hepatitis C (CHC) affects over 170 million people worldwide and is a leading cause of cirrhosis and hepatocellular carcinoma.[1] Progression of fibrosis, however, is highly variable among patients, and while some individuals run a benign clinical course for decades, others rapidly develop end-stage liver disease. Therefore, better understanding of the natural history of CHC is crucial for decision-making in the management of patients and has been the focus of several studies. Well-accepted factors associated with fibrosis progression include sex, older age at exposure, prolonged duration of infection, excessive alcohol drinking, liver necroinflammatory response, and long-term immune supression.[2-8] Other parameters have been recently unearthed or remain controversial, such as steatosis, overweight, diabetes, and tobacco use.[9-12] Nevertheless, progression of fibrosis may occur in the absence of any identified profibrogenic factor, therefore warranting further research in that field.

Cannabis (*Cannabis sativa*, marijuana), the most common recreational drug used in the Western world,[13] is the source of more than 60 cannabinoid compounds that bind two G protein–coupled receptors, CB1 and CB2.[14,15] CB1 receptors predominate in the brain and are responsible for the psychoactive effects of Δ-9-tetrahydrocannabinol, the main active component of cannabis,

*Abbreviations: CHC, chronic hepatitis C; FPR, fibrosis progression rate; HCV, hepatitis C virus; IVDU, intravenous drug use; IQR, interquartile range.*

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*Received November 13, 2004; accepted March 31, 2005.*

*P.G. was supported by INSERM. B.J. was supported by a fellowship from the Ministère de la Recherche et de la Technologie.*

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*Published online in Wiley InterScience (www.interscience.wiley.com).*

*DOI 10.1002/hep.20733*

*Potential conflict of interest: Nothing to report.*

whereas CB2 receptors are mainly expressed in cells of the immune system.[14,15] Expression of both receptors has also been demonstrated in a variety of peripheral tissues.[16] We recently found that CB1 and CB2 receptor expression undergo marked induction in the human liver with cirrhosis.[17-19] We also demonstrated that CB1 receptors strongly enhance experimental liver fibrogenesis, while CB2 receptors exert opposite antifibrogenic effects.[17-19] The present study was therefore undertaken to determine the clinical relevance of these experimental findings. To this aim, we investigated the impact of cannabis smoking on fibrosis progression in patients with CHC.

## Patients and Methods

***Patients.*** Consecutive patients seen in our department between May 2003 and January 2005 were enrolled if they met the following criteria: (1) hepatitis C virus (HCV) infection defined by a positive test for anti-HCV antibodies (ORTHO HCV 3.0 ELISA test system; Ortho Clinical Diagnostics, Raritan, NJ) with detectable serum HCV RNA (Amplicor HCV 2.0 PCR test system; Roche Molecular Systems, Pleasanton, CA), (2) availability of a liver biopsy (>10 mm, enclosing at least 6 portal spaces) consistent with CHC, and (3) identification of a single chronologically determined risk factor of HCV infection. Exclusion criteria included: (1) evidences for other forms of liver diseases (2) coinfection with hepatitis B virus (serum hepatitis B surface antigen–positive) or HIV, (3) previous immunosuppression, (4) previous antiviral treatment, and (5) use of illicit drugs other than cannabis at the time of the study. The study plan was approved by the local institutional review board and informed written consent was obtained from the patients.

***Data Collection.*** Demographic, epidemiological, environmental and metabolic data were collected at time of liver biopsy, including gender, age at infection, source of contamination (intravenous drug use [IVDU], blood transfusion before 1992, or other known nosocomial route of transmission), duration of disease, maintenance treatment by methadone or buprenorphine, and body mass index. Age at infection was estimated from the first exposure to IVDU, blood transfusion or to a known nosocomial route of transmission, as in previous studies.[6,7,20] Duration of infection was estimated as the difference between the date of infection and the date of liver biopsy. Alcohol, cannabis, and tobacco consumptions were recorded in detail over the span of HCV infection. The daily number of drinks equivalent to 10 g of pure ethanol was recorded separately for beer, wine and spirits, weekdays, and weekends. An average daily disease-time alcohol intake was estimated using the ratio of the total number of drinks during HCV infection to the duration of infection; excessive alcohol intake was defined as 30 g/d or more.[4] Disease-time tobacco use was expressed in pack-years, as the average number of packs smoked per day multiplied by the number of years as a smoker, during the course of HCV infection.[12]

Cannabis use was recorded using a standardized questionnaire that was completed at the time of liver biopsy and included the following items: date of onset and duration of cannabis smoking, amount (average amount of cannabis cigarettes/smoking session), and frequency (daily, weekly, monthly). Three categories of patients were defined according to cannabis smoking over the span of HCV infection (disease-time cannabis use): (1) nonsmokers who had no previous history of cannabis use, (2) occasional users who smoked less than one daily cannabis cigarette, and (3) daily users who smoked at least one cannabis cigarette per day (Table 1). Previous history of other illicit drug use was recorded at the same time.

**Table 1. Baseline Characteristics of 270 Patients With Chronic Hepatitis C**

| | |
|---|---|
| Male sex, n (%) | 186 (69.9) |
| Age at exposure (yr), mean (SD) | 24.4 (9.7) |
| Age at liver biopsy (yr), mean (SD) | 43.2 (10.4) |
| Route of transmission | |
| Blood transfusion, n (%) | 104 (38.5) |
| Intravenous drug use, n (%) | 144 (53.3) |
| Nosocomial, n (%) | 22 (8.2) |
| Duration of HCV exposure (yr), mean (SD) | 18.8 (7.8) |
| Disease-time tobacco use (pack-yr), median (IQR) | 10 (0–19) |
| Disease-time alcohol intake | |
| g/d, median (IQR) | 10 (1–33) |
| ≥30 g/d (%) | 75 (27.8) |
| Methadone/buprenorphine use, n (%) | 25 (9.3) |
| HCV genotype*, n (%) | |
| 1 | 157 (58.8) |
| 2 | 20 (7.5) |
| 3 | 66 (24.7) |
| 4/5 | 24 (9.0) |
| Fasting glucose level ≤6.1 mmol/L, n (%) | 249 (92.2) |
| Body mass index (kg/m$^2$), mean (SD) | 24.4 (4.2) |
| Steatosis, n (%) | |
| Absent | 74 (27.4) |
| Mild | 120 (44.4) |
| Moderate | 28 (10.4) |
| Marked | 48 (17.8) |
| Metavir activity grade, n (%) | |
| A1 | 116 (43.0) |
| A2 | 142 (52.6) |
| A3 | 12 (4.4) |
| Metavir fibrosis stage, n (%) | |
| F0 | 13 (4.8) |
| F1 | 154 (57.0) |
| F2 | 46 (17.0) |
| F3 | 21 (7.8) |
| F4 | 36 (13.3) |
| Fibrosis progression rate (Metavir U/yr), median (IQR) | 0.074 (0.05–0.14) |
| Fibrosis progression rate >0.15 (%) | 64 (23.7) |

*Information missing in 3 patients.

Fasting serum glucose was assayed at the time of liver biopsy; hyperglycemia was defined as a serum glucose level greater than 6.1 mmol/L or as a previous history of diabetes.[21] HCV genotype was determined using INNO-LiPA HCV II (Innogenetics, Zwijnaarde, Belgium).

***Liver Histopathology.*** Liver biopsy specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin safran. Histological features were analyzed according to the Metavir scoring system by a single pathologist.[22] Steatosis was graded as absent, mild (<10% of hepatocytes), moderate (10% to 30% of hepatocytes), or marked (>30% of hepatocytes).[22] Necroinflammatory lesions were scored by the activity index on a scale of 0 to 3 (A0 to A3) with significant histological activity defined as A2 or higher. Fibrosis was staged on a scale of 0 to 4 (F0 to F4, F4 defining cirrhosis). Two types of fibrosis end points were considered. The primary outcome variable was fibrosis progression rate (FPR), defined by the ratio of the fibrosis stage in Metavir units to the duration of infection in years as in previous studies,[6] and the secondary variable was a fibrosis stage of F3 or F4 (defined as severe fibrosis).

***Statistical Analysis.*** Descriptive statistics are shown as the mean ± SD, medians and interquartile ranges (IQRs), or percentages, as appropriate. Comparisons between groups were made using the Kruskall Wallis test or Mann-Whitney test for quantitative data and the $\chi^2$ test or Fischer exact test for qualitative data. All tests were two-tailed. The median fibrosis progression rate for the cohort was 0.074 (IQR: 0.05-0.14). Two thresholds were selected for analysis of FPR: (1) the median value of FPR in the study population (0.074) and (2) a threshold of 0.15, which allowed to check the relevance of the former analysis in the subgroup of rapid fibrosers (FPR >0.15). Factors related to FPR were first identified by univariate analysis: for this purpose, quantitative data were transformed in qualitative or ordinal variables, based either on clinical relevance or on mean value of the parameter. Stepwise logistic regression analysis was used to explore the independence of factors related to FPR by univariate analysis. All factors with a $P$ value of .05 or less at univariate analysis were tested in the model. Odds ratios were estimated from the model and given with their 95% confidence intervals. The relationship of daily cannabis use to fibrosis stage (treated as a categorical variable, <F3 or ≥F3) was also explored via multivariate analysis using a fixed model that took into account all variables retained either by forward or backward procedures in stepwise logistic regression analysis.

Analyses were performed using BMDP statistical software (BMDP Statistical Software, Los Angeles, CA) and Stata (Stata Corporation, College Station, TX).

## Results

### *Study Population*

Table 1 shows the main features of the 270 patients. There were 186 men and 84 women with a mean age at infection of 24.4 years and a mean disease duration of 18.8 years. Contamination was related to blood transfusion, IVDU, or nosocomial exposure in 38.5%, 53.3%, and 8.2% of patients, respectively. Genotype 1 (58.8%) predominated in the study population, followed by genotype 3 (24.7%). Moderate to marked steatosis and Metavir activity grade A2 or higher were present in 28.2% and 57.0% of patients, respectively, and severe fibrosis (≥F3) was found in 21.1% of patients. The median FPR of the cohort overall was 0.074 Metavir U/yr (IQR: 0.05-0.14).

Table 2 depicts the characteristics of patients according to cannabis smoking. One hundred forty one patients (53.2%) had no previous history of cannabis use; occasional cannabis smoking was reported in 40 patients (14.8%), with a median disease-time cannabis consumption of 8 (IQR: 4-10) cigarettes/mo in this group, and 89 patients (33.0%) were daily cannabis users with a median disease-time cannabis use of 60 (IQR: 30-122) cigarettes/mo ($P < .001$ (see Table 2). Compared with noncannabis users, occasional and daily cannabis smokers were significantly more often males (88.8% and 80.0% vs. 53.2%), with a younger age at exposure (21.1 and 20.6 vs. 27.6 years), and had a significantly shorter disease duration (17.1 and 16.7 vs. 20.4 years). An IVDU route of transmission was significantly more frequent in daily and occasional cannabis smokers (93.3% and 87.5%, respectively, vs. 18.4% in cannabis nonusers), and accordingly, prevalence of genotype 3 was significantly higher in these patients (see Table 2). Excessive alcohol intake was significantly more frequent in occasional and daily cannabis smokers (35.0% and 48.3%, respectively) compared with noncannabis users (12.8%) (see Table 2). Disease-time tobacco smoking was also significantly higher in cannabis users compared with nonusers. In contrast, there were no significant differences for demographic, epidemiological, environmental, metabolic, and virological features between daily and occasional cannabis smokers. Regarding liver histology, there were no significant differences for steatosis and Metavir activity grades between groups. However, fibrosis stage and median FPR were significantly higher in daily cannabis users compared with occasional users and nonusers. Finally, the proportion of rapid fibrosers (FPR >0.15) was significantly greater in daily cannabis users (33.7%) compared with occasional users (15.0%) and nonusers (19.9%).

**Table 2. Characteristics of Patients According to Cannabis Use**

| | Noncannabis Users (n = 141) | Occasional Cannabis Users (n = 40) | Daily Cannabis Users (n = 89) | P* |
|---|---|---|---|---|
| Cannabis use (cigarettes/month), median (IQR) | 0 | 8 (4–10) | 60 (30–122) | <.001 |
| Male sex, n (%) | 75 (53.2) | 30 (80.0) | 79 (88.8) | <.001 |
| Age at exposure (yr), mean (SD) | 27.6 (12.0) | 20.6 (3.8) | 21.1 (4.4) | <.001 |
| Age at liver biopsy (yr), mean (SD) | 48.0 (11.1) | 37.2 (6.5) | 38.3 (6.3) | <.001 |
| Route of transmission | | | | |
| Blood transfusion, n (%) | 95 (67.4) | 4 (10.0) | 5 (5.6) | |
| Intravenous drug use, n (%) | 26 (18.4) | 35 (87.5) | 83 (93.3) | <.001 |
| Nosocomial exposure, n (%) | 20 (14.2) | 1 (2.5) | 1 (1.1) | |
| Duration of HCV exposure (yr), mean (SD) | 20.4 (8.9) | 16.7 (6.9) | 17.1 (5.4) | .02 |
| Disease-time tobacco use (pack-yr), median (IQR) | 0 (0–15) | 13 (8–20) | 15 (10–22) | <.001 |
| Disease-time alcohol intake | | | | |
| g/d, median (IQR) | 3 (1–13) | 18 (4–42) | 27 (10–49) | <.001 |
| ≥30 g/d (%) | 18 (12.8) | 14 (35.0) | 43 (48.3) | <.001 |
| Methadone/buprenorphine use, n (%) | 1 (1.2) | 4 (10.0) | 20 (22.5) | .10† |
| HCV genotype, n (%)‡ | | | | |
| 1 | 90 (64.7) | 25 (62.5) | 42 (47.7) | |
| 2 | 18 (13.0) | 0 (0) | 2 (2.3) | |
| 3 | 17 (12.2) | 12 (30.0) | 37 (42.0) | <.001 |
| 4, 5 | 14 (10.1) | 3 (7.5) | 7 (8.0) | |
| Fasting glycemia ≤6.1 mmol/L, n (%) | 125 (88.7) | 39 (97.5) | 85 (95.5) | .15 |
| Body mass index (kg/m$^2$), mean (SD) | 25.4 (4.9) | 23.3 (2.5) | 23.4 (3.8) | .002 |
| Steatosis, n (%) | | | | |
| Absent | 39 (27.7) | 12 (30.0) | 23 (25.8) | |
| Mild | 60 (42.5) | 23 (57.5) | 37 (41.6) | .24 |
| Moderate | 18 (12.8) | 2 (5.0) | 8 (9.0) | |
| Marked | 24 (17.0) | 3 (7.5) | 21 (23.6) | |
| Metavir activity grade, n (%) | | | | |
| A1 | 65 (46.1) | 18 (45.0) | 33 (37.1) | |
| A2 | 71 (50.4) | 21 (52.5) | 50 (56.2) | .53 |
| A3 | 5 (3.6) | 1 (2.5) | 6 (6.7) | |
| Metavir fibrosis stage, n (%) | | | | |
| F0 | 8 (5.7) | 3 (7.5) | 2 (2.2) | |
| F1 | 84 (59.6) | 28 (70.0) | 42 (47.2) | |
| F2 | 26 (18.4) | 4 (10.0) | 16 (18.0) | .004 |
| F3 | 7 (5.0) | 1 (2.5) | 13 (14.6) | |
| F4 | 16 (11.3) | 4 (10.0) | 16 (18.0) | |
| Fibrosis progression rate (Metavir U/yr), median (IQR) | 0.06 (0.04–0.11) | 0.07 (0.05–0.12) | 0.11 (0.07–0.17) | .001 |
| Fibrosis progression rate >0.15 (%) | 28 (19.9) | 6 (15.0) | 30 (33.7) | .02 |

*$P$ value of the global test. Significant results of two-by-two comparisons are reported in the text.
†Occasional versus daily smokers.
‡Information missing in 3 patients.

### *Factors Associated With FPR*

***Predictors of a FPR Greater Than 0.074.*** FPR was first analyzed according to the median value of the cohort (0.074). Daily cannabis use was significantly associated with a FPR greater than 0.074 by univariate analysis (68.5% in daily users compared with 42.5% and 39.7% in occasional users and nonusers, respectively; $P < .001$). Other factors related to a FPR greater than 0.074 included male sex (53.8%; $P = .04$), age at contamination of more than 40 years (69.6%; $P = .002$), alcohol intake of 30 g/d or more (69.3%; $P = .001$), genotype 3 (74.2 %; $P < .001$), hyperglycemia (71.4%; $P = .038$), moderate to severe steatosis (72.4%; $P < .001$), and Metavir activity grade A2 or higher (67.5%; $P < .001$). Conversely, route of transmission, tobacco smoking, maintenance treatment by methadone/buprenorphine, and body mass index were not associated with FPR (Table 3).

Multivariate analysis identified six factors independently related to FPR (Table 4): daily cannabis use (OR = 3.4 [95% CI 1.5-7.4]), age at exposure (21-40 yr, OR = 2.4 [95% CI 1.2-4.8]; >40 yr, OR =10.5 [95% CI 3.0-37.1]), Metavir activity grade A2 or higher (OR = 5.4

**Table 3. Univariate Analysis of Factors Associated With Fibrosis Progression Rate >0.074 Metavir U/yr**

| | Fibrosis Progression Rate >0.074 U/yr, n (%) | P |
|---|---|---|
| Sex | | |
| Male (n = 186) | 100 (53.8) | |
| Female (n = 84) | 34 (40.5) | .04 |
| Age at exposure (yr) | | |
| ≤20 (n = 111) | 46 (41.4) | |
| 21–40 (n = 136) | 72 (52.9) | .023 |
| >40 (n = 23) | 16 (70) | |
| Route of transmission | | |
| Blood transfusion (n = 104) | 42 (40.4) | |
| IVDU (n = 144) | 82 (56.9) | .034 |
| Nosocomial (n = 22) | 10 (45.5) | |
| Genotype* | | |
| 1 (n = 157) | 66 (42.0) | |
| 2 (n = 20) | 7 (35.0) | |
| 3 (n = 66) | 49 (74.2) | <.001† |
| 4/5 (n = 24) | 11 (45.8) | |
| Disease-time cannabis use | | |
| Nonsmokers (n = 141) | 56 (39.7) | |
| Occasional smokers (n = 40) | 17 (42.5) | <.001‡ |
| Daily smokers (n = 89) | 61 (68.5) | |
| Disease-time alcohol intake | | |
| <30 g/d (n = 195) | 82 (42.1) | |
| ≥30 g/d (n = 75) | 52 (69.3) | .001 |
| Disease-time tobacco (pack-yr) | | |
| None (n = 80) | 34 (42.5) | |
| 0–10 (n = 59) | 32 (54.2) | |
| 11–20 (n = 87) | 43 (49.4) | .39 |
| >20 (n = 44) | 25 (56.8) | |
| Methadone/buprenorphine treatment | | |
| Absent (n = 245) | 118 (48.2) | |
| Present (n = 25) | 16 (64.0) | .13 |
| Body mass index (kg/m$^2$) | | |
| ≤27 (n = 212) | 106 (48.3) | |
| >27 (n = 58) | 28 (50.0) | .82 |
| Fasting serum glucose (mmol/L) | | |
| ≤6.1 (n = 249) | 119 (47.8) | |
| >6.1 (n = 21) | 15 (71.4) | .038 |
| Steatosis | | |
| Absent to mild (n = 194) | 79 (40.7) | |
| Moderate to severe (n = 76) | 55 (72.4) | <.001 |
| Metavir activity grade | | |
| A1 (n = 116) | 30 (25.9) | |
| A2–A3 (n = 154) | 104 (67.5) | <.001 |

*Information missing in 3 patients.
†Due to differences between genotype 3 and other genotypes.
‡Due to differences between daily cannabis smokers and occasional and noncannabis smokers.

[95% CI 2.9-10.3]), alcohol intake exceeding 30 g/d (OR = 2.2 [95% CI 1.1-4.5]), moderate to severe steatosis (OR = 2.0 [95% CI 1.0-4.1]), and genotype 3 (OR = 3.4 [95% CI 1.5-7.7]) (see Table 4). In contrast, there was no significant link between occasional cannabis use and FPR.

***Predictive Factors in Rapid Fibrosers (FPR >0.15).*** In a second step, we investigated predictors of a rapid FPR, as defined by a value greater than 0.15 in previous studies.[6] Univariate analysis showed that rapid fibrosers were significantly more frequently daily cannabis users (33.7%) compared with occasional users (15.0%) and nonusers (19.9%) ($P = .02$). Other factors associated with a FPR greater than 0.15 included age at exposure ($P < .001$), genotype 3 ($P < .04$), excessive alcohol intake ($P = .003$), hyperglycemia ($P = .003$); moderate to severe steatosis ($P < .001$) and Metavir activity grade A2 or higher ($P < .001$). Multivariate analysis showed that FPR >0.15 was independently related to daily cannabis smoking (OR = 3.3 [95% CI 1.5-7.5]; $P = .007$), age at exposure (21-40 yr, OR = 6.6 [95% CI 2.8-15.1], $P < .001$; >40 yr, OR = 10.6 [95% CI 2.8-39.5]), genotype 3 (OR = 2.3 [95% CI 1.1-4.9]; $P = .03$), Metavir activity grade A2 or higher (OR = 3.0 [95% CI 1.4-6.4]; $P = .007$), and moderate to severe steatosis (OR = 2.2 [95% CI 1.1-4.5]; $P = .03$). These results were consistent with those observed for a FPR threshold of 0.074.

### *Relationship Between Fibrosis Stage and Cannabis Smoking*

To further define the impact of daily cannabis smoking on fibrosis, we investigated the relationship between daily cannabis use and the presence of severe fibrosis as defined by a Metavir fibrosis stage of F3 or more. Occasional users and nonusers were grouped together, because occasional cannabis use was not identified as a predictor of FPR via

**Table 4. Stepwise Logistic Regression Analysis of Factors Associated With Fibrosis Progression Rate >0.074 U/yr (n = 267)**

| | OR | 95% CI | P |
|---|---|---|---|
| Disease-time cannabis use | | | |
| None | 1 | | |
| Occasional | 1.3 | 0.5–3.3 | .57 |
| Daily | 3.4 | 1.5–7.4 | .005 |
| Age at contamination (yr) | | | |
| ≤20 | 1 | | |
| 21–40 | 2.4 | 1.2–4.8 | .01 |
| >40 | 10.5 | 3.0–37.1 | <.001 |
| Metavir activity grade | | | |
| <A2 | 1 | | |
| ≥A2 | 5.4 | 2.9–10.3 | <.001 |
| HCV genotype | | | |
| 1 | 1 | | |
| 2 | 1.0 | 0.3–3.1 | .95 |
| 3 | 3.4 | 1.5–7.7 | .005 |
| 4/5 | 1.2 | 0.4–3.6 | .69 |
| Disease-time alcohol intake | | | |
| <30 g/d | 1 | | |
| ≥30 g/d | 2.2 | 1.1–4.5 | .03 |
| Steatosis | | | |
| Absent to mild | 1 | | |
| Moderate to severe | 2.0 | 1.0–4.1 | .05 |

**Table 5. Logistic Regression Analysis of Factors Associated With Severe Fibrosis (Metavir of F3 or More) in 270 Patients**

| | OR | 95% CI | P |
|---|---|---|---|
| Disease-time cannabis use | | | |
| None and occasional | 1 | | |
| Daily | 2.3 | 1.1–4.8 | .034 |
| Age at liver biopsy (yr) | | | |
| ≤40 | 1 | | |
| >40 | 2.2 | 1.0–4.8 | .035 |
| Metavir activity grade | | | |
| <A2 | 1 | | |
| ≥A2 | 5.6 | 2.3–13.8 | <.001 |
| Disease-time alcohol intake | | | |
| <30 g/d | 1 | | |
| ≥30 g/d | 2.1 | 1.0–4.2 | .05 |
| Steatosis | | | |
| Absent to mild | 1 | | |
| Moderate to severe | 2.0 | 0.97–4.0 | .06 |
| Disease-time tobacco (pack-yr) | | | |
| ≤10 | 1 | | |
| 11–20 | 1.6 | 0.7–3.6 | .23 |
| >20 | 3.1 | 1.2–7.8 | .02 |

multivariate analysis. Age at liver biopsy was entered in the model to control differences in age at infection and disease duration between groups (see Table 2). Multivariate analysis tested factors associated with severe fibrosis upon univariate analysis. Among factors associated with a fibrosis stage of F3 or more, steatosis, tobacco use, and alcohol intake were close to significant levels. Because stepwise logistic regression yielded discrepant results with respect to these three parameters, when performed forward or backward, analysis was conducted using a fixed model that included all variables retained at least once by forward or backward logistic regression. Logistic regression analysis controlling for age at liver biopsy, alcohol intake, steatosis, tobacco smoking, and Metavir activity grade indicated that daily cannabis use was an independent predictor of severe fibrosis (OR = 2.3 [95% CI 1.1-4.8]) (Table 5). Figure 1 illustrates the relationship of fibrosis stage to daily cannabis use according to age at liver biopsy and alcohol drinking habits and shows that in patients with no excessive alcohol intake, the prevalence of Metavir fibrosis stage F3 or higher was significantly higher in daily cannabis users than in occasional users and nonusers, whatever the age at liver biopsy ($P = .02$, Mantel Haenszel test) (see Fig. 1). In contrast, in excessive drinkers, the impact of ethanol consumption hindered the effect of daily cannabis use on FPR ($P = .34$, Mantel Haenszel test) (see Fig. 1).

Because daily and occasional cannabis users shared similar demographic, epidemiological, metabolic, and virological features (see Table 2), we also compared the prevalence of severe fibrosis in these 2 groups (Fig. 2). After controlling for alcohol intake, the proportion of patients with fibrosis stage F3 or higher was significantly higher in daily cannabis users than in occasional users both in nondrinkers and in excessive drinkers ($P = .048$, Mantel-Haenszel test).

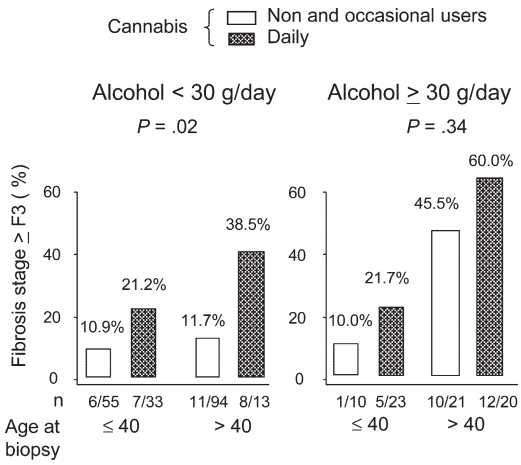


Fig. 1. Relationship between severe fibrosis and daily cannabis use according to daily alcohol intake, after adjustment for age at liver biopsy (*P* value of Mantel Haenszel test).

## Discussion

The present study investigates the impact of cannabis use on the natural history of CHC in a large series of patients with untreated CHC and known disease dura-

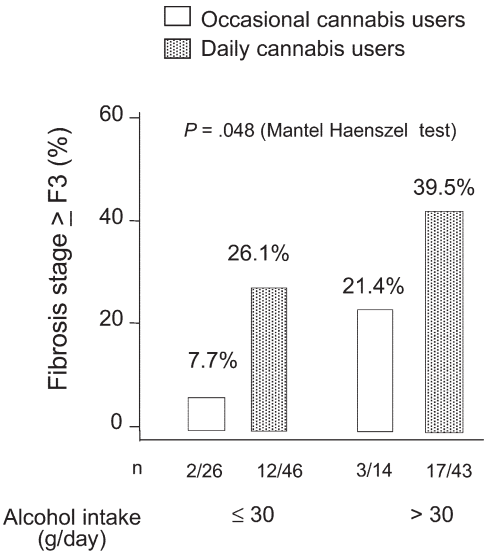


Fig. 2. Prevalence of severe fibrosis in daily and occasional cannabis smokers, after adjustment for daily alcohol intake (*P* value of Mantel Haenszel test).

tion. Using multivariate analysis, we identify daily cannabis smoking as an independent predictor of FPR, in contrast to occasional cannabis use. In keeping with these results, severe fibrosis (≥F3) is also independently related to daily cannabis use.

Major factors previously incriminated in fibrosis progression during CHC were identified as predictors of FPR and fibrosis stage, in addition to daily cannabis smoking, as expected. Older age at infection and chronic excessive alcohol intake are consistently considered primary determinants of fibrosis progression,[4,6,7,20,23,24] and the relationship between fibrosis stage and necroinflammatory grade has been documented in both longitudinal and cross-sectional studies.[3,12,25,26] Steatosis is also a recognized factor associated with severe fibrosis[11,27,28] and emerged as an independent predictor of FPR in our study, while being close to significance for the prediction of severe fibrosis. An impact of genotype 3 was found in analyses based on FPR, independently of steatosis, and was absent when considering fibrosis stage. This finding is rather unusual, because the majority of previous studies found no effect of viral genotype on fibrosis progression.[29] We considered the possibility of a confounding effect related to an interaction between daily cannabis use and genotype 3. However, in daily users of cannabis, the proportion of patients with a FPR greater than 0.074 or greater than 0.15 was not significantly different in patients with genotype 3 compared with patients who had other genotypes (data not shown; $P = .411$ and .583, respectively). In addition, there was no impact of genotype 3 on the prevalence of severe fibrosis in daily cannabis users ($P = .411$). Overall, discrepant results obtained with respect to viral genotype deserve additional investigation in further studies.

Our study closely investigated possible confounders of cannabis impact. Arguably, the shorter duration of HCV infection in cannabis users compared with nonusers may result in overestimation of FPR in daily cannabis users. However, daily cannabis use was also independently related to fibrosis stage. Moreover, occasional cannabis smoking did not emerge as an independent predictor of FPR, although this group of patients had similar disease duration compared with daily users. As reported in several studies, prevalence of excessive alcohol intake was high in cannabis users.[13] Nevertheless, it should be noted that there was a significant relationship between fibrosis stage and daily cannabis use in the subgroup of patients with low disease-time alcohol intake. This finding therefore allows us to rule out a confounding effect of alcohol on cannabis impact. Ongoing use of illicit drugs other than cannabis is another potential confounding factor that was excluded at enrollment. An influence of maintenance treatment by methadone or buprenorphine in former IVDU was investigated and ruled out via univariate analysis. Finally, tobacco smoking was also taken into account, given the conflicting results of recent studies.[12,30]

Several limitations of the study must be acknowledged. As in several previous reports,[6,9,24,31] fibrosis progression rate was calculated from a single liver biopsy and estimated disease duration. Potential inaccuracy in the presumed date of infection[32] was limited by exclusive enrollment of patients with a previous history of a single, well-identified route of exposure. The assumption of linearity of FPR has recently been disputed in a report suggesting late acceleration of fibrogenesis.[23] However, our findings are strongly supported by the fact that daily cannabis use was also identified as an independent predictor of fibrosis stage. Disease-time cannabis history recording is also subject to potential inaccuracy. Therefore, this possible bias was minimized by categorizing patients according to the pattern of cannabis use (none, occasional, or daily) rather than a quantitative estimation of usage. Uncertainties in quantification of disease-time alcohol intake were also controlled by grouping patients according to two types of behavior.

Life prevalence of cannabis use has increased steadily over the past 30 years in the Western world.[33,34] A recent survey from the National Institutes of Health also shows that within a period of 10 years, there has been a significant increase in cannabis use among 45- to 64-year-old men and women.[35] In our study, cannabis use was recorded using a standardized questionnaire covering the span of HCV infection. Daily and occasional use of cannabis was reported in 32% and 17% of patients, respectively, and predominantly involved former IVDU, as expected. These findings are in keeping with the notion that prolonged cannabis use for up to 20 years predominantly occurs in near-daily and daily users.[36-38]

There have been great advances in the understanding of mechanisms of action of plant-derived cannabinoids in recent years. Biological effects are elicited by two G protein–coupled cannabinoid receptors, CB1 and CB2, that also bind endogenous lipidic cannabinoid ligands with autocrine and paracrine effects.[14,15] Although the central properties of cannabinoids such as mood regulation, stimulation of appetite, and analgesia are best known, the peripheral effects of the compounds are becoming increasingly recognized.[16] In this respect, it has been shown that endogenous activation of the cannabinoid system plays a role in the pathogenesis of portal hypertension associated with cirrhosis via

CB1-dependent splanchnic vasodilation.[39,40] We recently demonstrated that the cannabinoid system is involved in experimental liver fibrogenesis.[17-19] Along this line, results of the present study are in keeping with our experimental data demonstrating the profibrogenic role of CB1 receptors.[18,19] Indeed, we found a strong induction of CB1 receptor expression in samples of human livers with cirrhosis, predominating in liver fibrogenic cells. Moreover, we showed that mice genetically invalidated for the CB1 receptor display reduced fibrosis following chronic intoxication with carbon tetrachloride compared with wild-type littermates. These data suggest that CB1-receptor antagonism may open new therapeutic avenues in the treatment of liver fibrosis.[19]

In conclusion, we show a significant relationship between daily cannabis use and fibrosis progression in patients with ongoing CHC. We believe that patients with ongoing CHC should be strongly advised to abstain from daily cannabis use. This recommendation might be particularly beneficial in difficult-to-treat patients.

*Acknowledgment:* The authors thank Chantal Gricourt and Fatiha Medkour for skillful technical assistance.

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