Research Article

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# Association of caffeine intake and histological features of chronic hepatitis C

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**Background & Aims**: The severity of chronic hepatitis C (CHC) is modulated by host and environmental factors. Several reports suggest that caffeine intake exerts hepatoprotective effects in patients with chronic liver disease. The aim of this study was to evaluate the impact of caffeine consumption on activity grade and fibrosis stage in patients with CHC.

**Methods**: A total of 238 treatment-naïve patients with histologically-proven CHC were included in the study. Demographic, epidemiological, environmental, virological, and metabolic data were collected, including daily consumption of alcohol, cannabis, tobacco, and caffeine during the six months preceding liver biopsy. Daily caffeine consumption was estimated as the sum of mean intakes of caffeinated coffee, tea, and caffeine-containing sodas. Histological activity grade and fibrosis stage were scored according to Metavir. Patients (154 men, 84 women, mean age: 45 ± 11 years) were categorized according to caffeine consumption quartiles: group 1 (<225 mg/day, $n = 59$), group 2 (225–407 mg/day, $n = 57$), group 3 (408–678 mg/day, $n = 62$), and group 4 (>678 mg/day, $n = 60$).

**Results**: There was a significant inverse relationship between activity grade and daily caffeine consumption: activity grade >A2 was present in 78%, 61%, 52%, and 48% of patients in group 1, 2, 3, and 4, respectively ($p < 0.001$). By multivariate analysis, daily caffeine consumption greater than 408 mg/day was associated with a lesser risk of activity grade >A2 (OR = 0.32 (0.12–0.85). Caffeine intake showed no relation with fibrosis stage.

**Conclusions**: Caffeine consumption greater than 408 mg/day (3 cups or more) is associated with reduced histological activity in patients with CHC. These findings support potential hepatoprotective properties of caffeine in chronic liver diseases.

Keywords: Caffeine; Hepatitis C virus; Chronic hepatitis C.
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*Abbreviations:* ALT, alanine aminotransferase; CHC, chronic hepatitis C; HCV, hepatitis C virus; IVDU, intravenous drug use; BMI, body mass index; SD, standard deviation; IQR, interquartile range.



## Introduction

The natural history of chronic hepatitis C infection is profoundly influenced by a variety of co-factors and co-morbidities that affect both the progression rate and the long-term outcome of infection. These include host parameters such as gender, age at infection, genetic factors, immunosuppression, or the presence of the metabolic syndrome, as well as environmental factors, including excessive alcohol intake or regular cannabis use.

A growing body of evidence suggests that caffeine may have hepatoprotective properties. A large population-based study in the United States has shown that caffeine consumption is associated with a lower risk of elevated serum alanine aminotransferase (ALT) activity in patients at high risk of liver disease [1]. Epidemiological surveys conducted in Europe and Japan also found inverse correlations between coffee drinking and aminotransferases [2–5] or γ-glutamyltransferase [2,3,6–13] serum levels. Coffee and caffeine consumption has been shown to be associated with a reduced risk of fibrosis or cirrhosis in several prospective studies [14–20]. Regular coffee consumption was associated with lower rates of fibrosis progression or clinical outcomes in a large cohort of patients with advanced HCV-related liver disease who failed to respond to peginterferon and ribavirin treatment [21]. A Norwegian population-based study also showed an inverse relationship between coffee intake and rate of death in cirrhotic patients [22]. Finally, several cohort and case-control studies, as well as two recent meta-analyses, suggested an inverse relationship between coffee drinking and the risk of hepatocellular carcinoma in cirrhotic patients [23–31]. Altogether, these data suggest that coffee consumption may reduce liver injury in patients with chronic liver disease. The aim of this study was to evaluate the association of caffeine consumption and severity of histological liver lesions in the specific group of treatment-naive patients with chronic hepatitis C.

# Research Article

## Patients and methods

### Patients

A total of 238 treatment-naive patients with CHC seen in our department (Service d'Hépatologie et de Gastroentérologie, Hôpital Henri Mondor, Créteil) between December 2004 and April 2006 were enrolled into the study if they met the following criteria: (a) positivity for both serum anti-hepatitis C virus (HCV) antibodies (ORTHO™ HCV 3.0 ELISA Test System, Ortho-clinical Diagnostics, Raritan, New Jersey) and serum HCV RNA (Amplicor HCV 2.0 PCR test system, Roche Molecular Systems, Pleasanton, California) documented for at least 6 months; (b) available liver biopsy specimen (15 mm or greater, with at least 6 portal spaces) consistent with CHC; (c) serum ALT levels determined at the time of liver biopsy; (d) absence of co-infection with hepatitis B virus (serum HBsAg positive) or human immunodeficiency virus; (e) absence of previous immunosuppression or antiviral therapy for CHC.

### Data collection

One standardized questionnaire performed prospectively by a physician was used to record, at the time of liver biopsy, caffeine, alcohol, tobacco, and cannabis consumption during the six months preceding biopsy. Questions related to caffeine intake included: the average quantity and frequency of daily consumption of caffeinated coffee, tea (herbal, regular, including ice tea) or cola-type soda (regular, diet) during the six months preceding liver biopsy, as previously reported [1]. Total daily caffeine intake from beverages (mg/day) was estimated by calculating the sum of caffeine from regular coffee (136 mg per cup), regular tea (64 mg per cup), and regular and diet colas and sodas (46 mg per bottle or can) [1]. Alcohol intake was expressed as the daily number of drinks equivalent to 10 g of pure ethanol. Alcohol abuse was defined by an average alcohol intake >30 g/day [32]. Tobacco smoking was recorded as the mean daily number of cigarettes smoked. Cannabis use was assessed by recording the amount (average number of cannabis cigarettes/smoking session) and the frequency (daily, weekly, monthly) of cannabis use over the last 6 months [33]. Demographic, epidemiological, environmental, virological (HCV) and metabolic data were also collected. Serum ALT and fasting glycemia were measured at the time of liver biopsy and hyperglycemia was defined by a glucose level greater than 6.1 mmol/L or a history of diabetes. HCV genotype was determined by a second-generation reverse-hybridization line probe assay (INNO-LiPA HCV II; Innogenetics®, Zwijnaarde, Belgium).

### Liver histopathology

All liver biopsy specimens were fixed in formalin, embedded in paraffin and routinely processed for histological analysis. Histological scoring was performed according to the Metavir scoring system [34–35]. Necroinflammatory activity grade was scored on a scale of 0–3 (A0 = absent; A3 = marked) and fibrosis stage was expressed on a scale of 0–4 (F0 = absent; F4 = cirrhosis). Steatosis was evaluated according to the percentage of hepatocytes containing cytoplasmic fat vacuoles as follows: absent (<5%), mild (5–10%), moderate (11%-29%) and marked (⩾30%) [34–35].

### Statistical analysis

Questionnaires were collected for all patients. Results were expressed as means (SD), medians (IQR), and percentages, as appropriate. Due to the wide range of caffeine intake expressed in milligrams per day, we decided to consider caffeine intake as a qualitative variable. In order to reach the best statistical power, patients were classified in four groups according to daily caffeine use in quartiles: group 1 (<225 mg/day), group 2 (225–407 mg/day), group 3 (408–678 mg/day) and group 4 (>678 mg/day). Expressed in coffee-cup equivalents, caffeine intake is less than 1.5 cup/day in group 1, more than 1.5 but less than 3 cups/day in group 2, at least 3 cups/day but less than 5 in group 3 and at least 5 cups/day in group 4. In our population, the proportion of non-caffeine drinkers was very low ($n = 18$, 7%). Thus, we decided to include these patients in the moderate drinkers group (group 1 <225 mg/day).

Univariate analysis was performed using an overall Chi-squared test and a Chi-square test for a linear trend for categorical data, to identify factors associated with activity grade or fibrosis stage. Factors found to be significant in univariate analysis were tested by stepwise logistic regression analysis to determine factors independently associated with histological activity. Odds ratios were estimated from the model and are presented with their 95% confidence intervals. $p$ Values of less than 0.05 were considered significant.

## Results

### Study population

Table 1 shows baseline characteristics of the study population ($n = 238$). There were 154 men, and 84 women with a mean age at liver biopsy of 45 ± 11 years. Intravenous drug use represented the main source of HCV infection (41%). HCV Genotype 1 (62%) was predominant, followed by HCV genotype 3 (17%). Caffeine consumption (median: 408 mg/day, IQR: 224–680) was mainly related to coffee intake (median: 2 cups per day, IQR: 1–4), whereas tea, colas, or sodas accounted for 0 (0–1) cup per day and 0 (0–0.2) can per day, respectively. Ongoing alcohol abuse, tobacco consumption >15 cigarettes/day [35] and daily cannabis use [37–38] were reported by 17%, 34%, and 25% of patients, respectively. Metavir activity grades ⩾A2 and fibrosis stage ⩾F2 were present in 60% and 39% of patients, respectively. Table 2 depicts the characteristics of patients, ranked by caffeine consumption quartiles. Caffeine consumption was inversely related to age ($p$ <0.001), and correlated with tobacco or cannabis use ($p = 0.005$ and $p = 0.001$, respectively). In contrast, there were no significant differences in rates of alcohol abuse, levels of serum ALT or metabolic features when compared to the caffeine intake level.

### Relationship between caffeine consumption and activity grade of biopsies

The relationship between caffeine intake and histological activity grade is shown in Table 3. Prevalence of a Metavir activity grade >A2 declined gradually, with increasing levels of daily caffeine consumption, from 78% in patients consuming less than 225 mg/day (group 1) to 48% in those with a daily intake greater than 678 mg/day (group 4) ($p$ <0.001, test for linear trend). Other factors related to activity grades higher than A2 included age (at liver biopsy) >40 years (64%, $p = 0.025$), BMI >25 kg/m$^2$ (69%, $p = 0.011$), moderate or marked steatosis (80%, $p$ <0.001), fibrosis stage ⩾F2 (92%, $p$ <0.001), and median serum ALT level (91 IU/ml (55–129), $p$ <0.001) (Table 3). There was no significant relationship between activity grade and either gender, route of transmission, hyperglycemia, or diabetes, daily alcohol intake, tobacco smoking, cannabis use, or HCV genotype.

By multivariate analysis, caffeine consumption >408 mg/day was associated with a lesser risk of clinically significant activity (>A2) for intakes ranging between 408 and 678 mg/day (group 3) (OR = 0.32 [0.12–0.85]) or >678 mg/day (group 4) (0.28 [0.10–0.75]). In addition, a Metavir activity grade >A2 was also independently related to fibrosis stage F2–F4 (OR = 13.3 [5.4–32.7]), moderate-severe steatosis (OR = 2.43 [1.01–5.88]), and serum ALT level (OR = 1.01 [1.00–1.02]) (Table 4).

### Relationship between caffeine consumption and fibrosis

By univariate analysis, advanced fibrosis (⩾F2) significantly correlated with male gender (47% versus 24%, $p$ <0.001), age >40 - years (45% versus 27%, $p = 0.006$), alcohol abuse (60% versus 35%, $p = 0.003$), tobacco smoking >15 cigarettes/day (49% versus 34%, $p = 0.03$), daily cannabis use (54% versus 29% in occasional users and 35% in non-users, $p = 0.019$), HCV genotype 3 (57% versus 35%, $p = 0.011$), BMI >25 kg/m$^2$ (48% versus 32%, $p = 0.009$), moderate-severe steatosis (60% versus 33%,

JOURNAL OF **HEPATOLOGY**

**Table 1. Baseline characteristics of the 238 patients with CHC and caffeine consumption according to individual measures.**

| | | Caffeine consumption (mg/day)[1] median (IQR) |
|---|---|---|
| Male gender, n (%) | 154 (65%) | 242 [116-419] |
| Female gender n (%) | 84 (35%) | 162 [79-274] |
| Age at liver biopsy (years), mean (SD) | 45 (11) | |
| Source of infection, n (%) | | |
| Blood transfusion | 82 (35%) | 196 [105-323] |
| IVDU | 98 (41%) | 245 [116-419] |
| Nosocomial | 26 (11%) | 178 [99-336] |
| Unknown | 32 (13%) | 162 [77-336] |
| Caffeine consumption (mg/day)[1] | | |
| median (IQR) | 408 (224-680) | |
| Tobacco consumption (cig/day)[1] | | |
| median (IQR) | 5 (0-20) | |
| ≤15, n (%) | 158 (66%) | 182 [94-318] |
| >15, n (%) | 80 (34%) | 257 [119-458] |
| Alcohol intake (g/day)[1] | | |
| median (IQR) | 3 (0-14) | |
| <30, n (%) | 198 (83%) | 196 [100-330] |
| ≥30, n (%) | 40 (17%) | 245 [105-448] |
| Cannabis use, n (%)[1] | | |
| None | 145 (61%) | 181 [92-313] |
| Occasional | 34 (14%) | 251 [146-406 |
| Daily | 59 (25%) | 264 [143-474] |
| HCV genotype | | |
| 1 | 148 (62%) | 196 [95-333] |
| 3 | 40 (17%) | 252 [120-418] |
| 2/4/5/6 | 50 (21%) | 221 [175-341] |
| Hyperglycemia or diabetes, n (%) | 14 (6%) | |
| Yes | 14 (6%) | 167 [66-239] |
| No | 224 (94%) | 213 [104-345] |
| BMI (kg/m$^2$) mean (SD) | 25 (4) | |
| ≤25 kg/m$^2$ | 135 (57%) | 215 [104-336] |
| >25 kg/m$^2$ | 103 (43%) | 189 [94-338] |
| Serum ALT level (IU/ml) | | |
| median (IQR) | 69 (44-112) | |
| normal, n (%) | 68 (29%) | 215 [92-390] |
| upper normal | 170 (71%) | 209 [113-331] |
| Metavir activity grade, n (%) | | |
| A1 | 96 (40%) | 241 [124-354] |
| A2 | 132 (55%) | 182 [95-330] |
| A3 | 10 (5%) | 302 [57-625] |
| Metavir fibrosis score, n (%) | | |
| F0 | 13 (6%) | 209 [75-289] |
| F1 | 132 (55%) | 206 [113-333] |
| F2 | 38 (16%) | 229 [138-346] |
| F3 | 24 (10%) | 186 [95-407] |
| F4 | 31 (13%) | 264 [85-418] |
| Steatosis, n (%) | | |
| <5% | 76 (32%) | 171 [100-316] |
| 5-10% | 107 (45%) | 215 [98-334] |
| 11-29% | 19 (8%) | 270 [85-474] |
| ≥30% | 36 (15%) | 200 [138-404] |

IVDU, intravenous drug use; BMI, body mass index; SD, standard deviation; IQR, interquartile range.

[1]During the six month preceding liver biopsy.

# Research Article

**Table 2. Characteristics of the 238 patients with CHC, ranked according to caffeine consumption during the six month preceding liver biopsy.**

| Caffeine consumption[1] (mg/day) | <225[2] (n = 59) | 225-407[2] (n = 57) | 408-678[2] (n = 62) | >678[2] (n = 60) | *p* |
|---|---|---|---|---|---|
| Male gender, n (%) | 37 (63%) | 28 (49%) | 43 (69%) | 46 (7%) | 0.015 |
| Age at liver biopsy (years), mean (SD) | 50 (13) | 48 (12) | 44 (10) | 40 (8) | <0.001 |
| Source of infection, n (%) | | | | | |
| Blood transfusion | 17 (29%) | 28 (49%) | 23 (37%) | 14 (23%) | |
| IVDU | 20 (34%) | 17 (30%) | 28 (45%) | 33 (55%) | 0.033 |
| Nosocomial | 9 (15%) | 5 (9%) | 5 (8%) | 6 (10%) | |
| Unknown | 13 (22%) | 7 (12%) | 6 (10%) | 7 (12%) | |
| Tobacco smoking (cig/day)[1] | | | | | |
| median (IQR) | 0 (0-20) | 0 (0-18) | 8 (0-20) | 20 (4-20) | 0.005 |
| >15, n (%) | 15 (25%) | 14 (25%) | 20 (32%) | 31 (52%) | 0.01 |
| Alcohol intake (g/day) [1] | | | | | |
| median (IQR) | 1 (0-20) | 3 (0-8) | 4 (0-20) | 6 (0-20) | 0.48 |
| ≥30, n (%) | 10 (17%) | 6 (10%) | 13 (21%) | 11 (18%) | |
| Cannabis use[1] | | | | | |
| None | 44 (75%) | 42 (74%) | 34 (55%) | 25 (42%) | |
| Occasional | 5 (8%) | 6 (10%) | 13 (21%) | 10 (16%) | 0.001 |
| Daily | 10 (17%) | 9 (16%) | 15 (24%) | 25 (42%) | |
| HCV genotype, n (%) | | | | | |
| 1 | 35 (59%) | 40 (70%) | 39 (63%) | 34 (56%) | |
| 3 | 10 (17%) | 7 (12%) | 10 (16%) | 13 (22%) | 0.85 |
| 2/4/5/6 | 14 (24%) | 10 (18%) | 13 (21%) | 13 (22%) | |
| Hyperglycemia or diabetes, n (%) | 6 (10%) | 7 (12%) | 4 (6%) | 3 (5%) | 0.46 |
| BMI (kg/m$^2$) mean (SD) | 25 (3.7) | 25 (4.4) | 24 (4.0) | 24 (3.9) | 0.09 |
| >25 kg/m$^2$ | 32 (54%) | 28 (49%) | 18 (29%) | 25 (42%) | 0.03 |
| Serum ALT level (IU/ml) | | | | | |
| median (IQR) | 85 (44-127) | 62 (44-99) | 71 (45-116) | 68 (45-108) | 0.48 |
| normal, n (%) | 15 (25%) | 16 (28%) | 17 (27%) | 20 (33.%) | 0.96 |
| Metavir activity grade, n (%) | | | | | |
| A1 | 13 (22%) | 22 (39%) | 30 (48%) | 31 (52%) | |
| A2 | 43 (73%) | 34 (59%) | 29 (47%) | 26 (43%) | 0.007 |
| A3 | 3 (5%) | 1 (2%) | 3 (5%) | 3 (5%) | |
| Metavir fibrosis stage, n (%) | | | | | |
| F0 | 3 (5%) | 2 (4%) | 3 (6%) | 5 (8.3%) | |
| F1 | 25 (42%) | 38 (67%) | 36 (58%) | 33 (55.0%) | |
| F2 | 11 (19%) | 11 (19%) | 9 (14%) | 7 (11.7%) | 0.24 |
| F3 | 9 (15%) | 3 (5%) | 5 (8%) | 7 (11.7%) | |
| F4 | 11 (19%) | 3 (5%) | 9 (14%) | 8 (13.3%) | |
| Steatosis, n (%) | | | | | |
| <5% | 18 (30%) | 19 (33%) | 19 (31%) | 20 (33%) | |
| 5-10% | 27 (46%) | 27 (48%) | 27 (44%) | 26 (44%) | 0.99 |
| 11-29% | 5 (9%) | 4 (7%) | 4 (6%) | 6 (10%) | |
| ≥30% | 9 (15%) | 7 (12%) | 12 (19%) | 8 (13%) | |

IVDU, intravenous drug use; BMI, body mass index; SD, standard deviation; IQR, interquartile range.
[1]During the six month preceding liver biopsy, divided in quartiles.
[2]<225 mg/day ≈ less than 1.5 cup a day; 225–407 mg/day ≈ more than 1.5 but less than 3 cups a day); 408–678 mg/day ≈ at least 3 cups a day but less than 5; >678 mg/day ≈ at least 5 cups a day).

**Table 3. Univariate analysis of factors associated with Metavir activity grade ⩾A2.**

| | Metavir activity grade ≥A2 n (%) | *p* |
|---|---|---|
| Caffeine consumption[1] | | |
| <225 mg/day (n = 59) | 46 (78) | <0.001* |
| 225-407 mg/day (n = 57) | 35 (61) | |
| 408-678 mg/day (n = 62) | 32 (52) | |
| >678 mg/day (n= 60) | 29 (48) | |
| Age at liver biopsy | | |
| ≤40 years (n = 84) | 42 (50) | 0.025 |
| >40 years (n = 154) | 100 (64) | |
| BMI | | |
| ≤25 kg/m$^2$ (n = 135) | 71 (53) | 0.011 |
| >25 kg/m$^2$ (n = 103) | 71 (69) | |
| Steatosis | | |
| Absent-mild (n = 183) | 98 (54) | <0.001 |
| Moderate-severe (n = 55) | 44 (80) | |
| Metavir fibrosis stage | | |
| F0-F1 (n = 145) | 56 (39) | <0.001 |
| F2-F4 (n = 93) | 86 (92) | |
| Serum ALT level (IU/ml), median (IQR) | 52 (37-76) for A1 (Metavir activity grade) *versus* 91 (55-129) for ≥A2 (Metavir activity grade) | <0.001 |

[1]During the six month preceding liver biopsy.
*Test for linear trend, IQR: interquartile range.

*p* <0.001), and median serum ALT level (99 IU/ml (67–136) versus 56 IU/ml (37–86), *p* <0.001). However, there was no significant relationship between the severity of fibrosis and caffeine consumption, (*p* = 0.08, test for linear trend): severe fibrosis (F2–F4) was found in 52% (31/59), 30% (17/57), 37% (23/62), and 37% (22/60) of patients in group 1, 2, 3, and 4, respectively.

By logistic regression analysis, predictors of fibrosis (⩾F2) were: age at liver biopsy >40 years (OR = 5.8 [2.3–14.5]), male gender (OR = 4.2 [1.9–9.6]), activity grade ⩾ A2 (OR = 22.1 [8.5–56.9]), moderate-severe steatosis (OR = 2.4 [1.1–5.3]), and daily cannabis use (OR = 3.7 [1.4–9.8]).

*Interactions between caffeine consumption and tobacco use*

The relationship between caffeine intake and tobacco smoking is shown in Table 2. The proportion of heavy smokers raised gradually with increasing levels of daily caffeine consumption, from 25% in patients consuming less than 225 mg/day (group 1) to 52% when daily intake was greater than 678 mg/day (group 4) (*p* = 0.005). There was an association between Metavir activity grade ⩾ A2 and tobacco smoking in individuals with low caffeine consumption (<408 mg/day), but not in those with a higher caffeine intake (*p* = 0.007 and *p* = 0.58, respectively). However, by multivariate analysis, tobacco use did not reach significant levels and was not independently associated with Metavir activity grade ⩾2 (Table 4).

**Table 4. Stepwise logistic regression analysis of factors associated with Metavir activity grade ⩾A2.**

| | aOR* | 95% CI | *p* |
|---|---|---|---|
| Caffeine consumption[1] | | | |
| <225 mg/day | 1 | | |
| 225-407 mg/day | 0.74 | 0.28-1.92 | 0.54 |
| 408-678 mg/day | 0.32 | 0.12-0.85 | 0.022 |
| >678 mg/day | 0.28 | 0.10-0.75 | 0.011 |
| Metavir fibrosis stage | | | |
| F0-F1 | 1 | | |
| F2-F4 | 13.3 | 5.4-32.7 | <0.001 |
| Steatosis | | | |
| Absent-mild | 1 | | |
| Moderate-severe | 2.43 | 1.01-5.88 | 0.049 |
| Serum ALT level | 1.01 | 1.00-1.02 | 0.005 |

*Adjusted odd ratio.

## Discussion

Our data show that caffeine consumption is inversely associated with the severity of necroinflammatory lesions in patients with untreated chronic hepatitis C. Indeed, the prevalence of patients with an activity grade >A2 was inversely related to the amount of caffeine taken on a daily basis, (*p* <0.001, test for linear trend). Moreover, by multivariate analysis, daily caffeine consumption greater than 408 mg (3 cups of coffee or more) stood out as an independent predictor of a lesser risk of moderate to marked activity grade, together with age and steatosis grade, two parameters previously described as independent risk factors of activity [36,38]. These findings are in keeping with previous studies suggesting a hepatoprotective effect of caffeine in patients with liver disease of various causes [1,3,5,7,20,21]. However, conflicting data are present in the literature.

In our homogeneous population of patients with chronic hepatitis C, caffeine intake was shown to be independently associated with low-grade activity, but not with fibrosis or ALT levels. Ruhl *et al.* [1] demonstrated that higher caffeine intake was associated with lower ALT levels, and Modi *et al.* [20] reported the impact of caffeine intake on fibrosis but not on activity or ALT levels. Furthermore, we found a cut-off of three or more cups of coffee (more than 407 mg of caffeine per day) while other studies report a lower cut-off of 2 cups per day [1,20]. These conflicting results might be explained by the fact that these are, not very large, cross-sectional studies (except for the Ruhl *et al.* study) and different methods are used for the estimation of caffeine intake or histological analysis (Ishak versus Metavir). These discrepancies suggest that a higher caffeine intake may be a surrogate marker for a confounding factor yet to be determined. Socio-economic status, quality of life, and comorbidities, which may be significantly associated with coffee intake, have not been examined in our cohort. The lack of such information limits the

# Research Article

scope of our study, as any factor associated with coffee drinking could explain our results.

In contrast with previous reports [32,36,38], we found no relationship between histological activity grade and either alcohol abuse or tobacco smoking, raising the question as to a confounding effect of caffeine with respect to these factors. In our cohort, the weak impact of alcohol intake on necroinflammation may be related to the low prevalence of alcohol abuse, as reflected by a median daily consumption below 5 g, and by a low (16.8%) rate of ongoing alcohol intake ($\geqslant$30 g/day). Such a low prevalence of alcohol abuse may reflect changes of lifestyle in patients following a diagnosis of HCV infection. As for tobacco use, our data show a correlation between heavy smoking and caffeine consumption ($p = 0.005$, Table 2), as previously reported [39–40]. There was an association between Metavir activity grade $\geqslant$A2 and tobacco smoking in individuals with low caffeine consumption (<408 mg/day), but not in those with a higher caffeine intake, suggesting a protective role of caffeine in these heavy smokers.

Multivariate analysis of predictors of fibrosis severity revealed known cofactors, including male gender, Metavir activity grade $\geqslant$A2, steatosis severity, and daily cannabis use [33,41–44]. Prior reports have suggested that coffee consumption may reduce the risk of hepatic fibrosis or disease progression [14–21]. Modi *et al.* demonstrated that coffee consumption above a 2 cup equivalent per day was associated with less severe hepatic fibrosis [20]. In a sub-analysis of the HALT-C trial, there was no association between coffee intake and baseline cirrhosis status or Ishak inflammation grade. Nevertheless, there was a trend for a reduced rate of cirrhosis in heavy coffee drinkers ($\geqslant$3 cups a day) as compared to the other categories (31.5% versus 40%, $p = 0.073$) [21]. In the present study, although we found no relationship between fibrosis stage and caffeine intake, necroinflammatory grade was positively related to fibrosis stage and negatively associated with caffeine intake. Taken together, these data suggest that caffeine intake might result in limited fibrosis progression by reducing necroinflammatory liver injury. However, further study is required to validate this hypothesis.

The mechanisms underlying potential hepatoprotective effects of caffeine in patients with chronic hepatitis C remain to be determined. Several reports suggest caffeine and other constituents of coffee, like kahweol and cafesterol, possess antioxidant properties [45–50]. As our study focused on total caffeine intake and not coffee intake, we cannot exclude that other components of coffee may play a role in our findings.

Epidemiological studies support the hypothesis that regular coffee consumption is associated with a substantially lower risk of type 2 diabetes, therefore suggesting that coffee intake may reduce the development of insulin resistance [51]. Interestingly, it has been contended that insulin resistance enhances intrahepatic inflammation in patients with chronic hepatitis C [52]. Freedman *et al.* [21] reported an inverse association between coffee intake and both serum insulin levels and HOMA 2 scores. In this study, the inclusion of data on both insulin levels or HOMA 2 scores to the statistical models attenuated the observed results for caffeine, suggesting that the beneficial effects of caffeine might be partly explained by a modulation of insulin signaling. Nevertheless, whether the beneficial effects of caffeine/coffee on insulin resistance might also account for the reduction in necroinflammatory lesions, found in HCV patients consuming higher quantities of caffeine, remains to be investigated.

In summary, our study uncovers an inverse relationship between caffeine consumption and Metavir activity grade in patients with chronic hepatitis C, suggesting that caffeine intake may lower necroinflammatory injury by an as yet undetermined mechanism. Given the strong relationship between activity grade and fibrosis progression, these results support the hypothesis that caffeine intake may also reduce the progression of liver fibrosis. Additional studies are required to assess whether our findings also apply to other inflammatory chronic liver diseases.

## Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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