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Hepatitis C virus genotype 3 and the risk of severe liver disease in a large population of drug users in France

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Abstract

Although risk factors for cirrhosis in chronic hepatitis C virus (HCV) infection have been identified, the role of HCV-genotype 3 remains controversial, and limited data are available in drug users. The aim of the study was to assess risk factors for severe liver disease (cirrhosis/hepatocellular carcinoma) in HCV-infected drug users between 2001 and 2007 in France.

Patients who reported drug use and who had been referred for HCV infection to hepatology centres from a national surveillance system were identified. The severity of liver disease was assessed clinically and histologically (Metavir score). Factors associated with sever liver disease were analysed after estimating missing values by multiple imputation.

Of the 4065 drug users naive to anti-HCV treatment who were referred to the 26 participating centres, 8.0% had severe liver disease, 25.7% were infected with HCV-genotype 3. Factors associated independently with an increased risk of severe liver disease were HCV-genotype 3 (adjusted odds ratio, multiple imputation, (aOR_{MI}) = 1.6, [95%CI: 1.2-2.1]), HIV infection (aOR_{MI} = 1.8, [1.2-2.8]), male sex (aOR_{MI} = 2.0, [1.4-2.8]), age over 40 years (aOR_{MI} = 2.1, [1.6-2.9]), history of excessive alcohol consumption (aOR_{MI} = 2.8, [2.1-3.7]) and duration of infection ≥18years (aOR_{MI} = 2.9, [2.0-4.3]).

This analysis shows that HCV-genotype 3 is associated with severe liver disease in drug users, independently of age, sex, duration of infection, alcohol consumption and co-infection with HIV. These results are in favour of earlier treatment for drug users infected with HCV-genotype 3 and confirm the need for concomitant care for excessive alcohol consumption.

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Keywords: alcohol, cirrhosis, drug users, HCV genotype 3, HIV.

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Introduction

In France, the prevalence of anti-HCV in injecting drug users ranges from 55% to 60% [Jauffret-Roustide et al., 2009; Meffre et al., 2010]. In addition, injecting drug users accounted for more than two thirds of the patients co-infected with HIV and HCV [Larsen et al., 2008].

Factors associated with progression of liver fibrosis have been well established, and are related to host factors (age at contamination, male sex), comorbidities (co-infection with HIV, heavy alcohol consumption), metabolic conditions (obesity, steatosis, diabetes mellitus) and duration of infection [Mallat et al., 2008; Massard et al., 2006]. Furthermore, co-infection with HIV and a history of excessive alcohol consumption are linked to death at an earlier age in patients infected chronically with HCV as shown in a recent study on mortality related to chronic hepatitis C in France [Marcellin et al., 2008]. Viral induced steatosis linked to HCV genotype 3 has also emerged as a cofactor for progression of liver fibrosis in patients infected with HCV [Rubbia-Brandt et al., 2004]. HCV genotype 3 is also the most frequent genotype found among drug users infected with HCV in France, accounting for more than one third [Payan et al., 2005]. Excessive alcohol consumption is common in this population, too [Campbell et al., 2006]. However, limited data are available on factors associated with progression to liver fibrosis in former or current injecting drug users infected by HCV.

In addition, with new antiviral treatment options and efficacy, recommendations on antiviral treatment of drug users infected with HCV have been expanded in France [Dhumeaux et al., 2003] since the 2002 consensus conference on treatment of HCV infection.

In France, a surveillance system was set up to monitor changes over time of the epidemiological and clinical characteristics of patients infected with HCV at the first referral to hepatology reference centres; nearly 3900 cases of chronic hepatitis C, of whom 35% acknowledged drug use, have been recorded each year since 2001 by this system. The aim of the present study was to assess risk factors associated with severe liver disease in patients infected with HCV who had reported drug use using the data collected by this surveillance system.

Methods

<u>Study population and inclusion criteria</u>

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Details of the methods have been described previously [Delarocque-Astagneau et al., 2005]. Briefly, this surveillance system based on voluntary participation of 26 of the 30 existing hepatology reference centres was set up in 2000 to monitor changes over time of the epidemiological and clinical characteristics of patients infected with HCV at the time of their first referral. Hepatology reference centres are located in University hospitals throughout France. To be included, a case was defined as a patient with anti-HCV antibodies who was referred (first contact as an in- or out-patient) to any participating reference centre. Patients can be referred by their general practitioner, by a specialist or by self-referral. Patients included in the analysis were those who reported having injected or snorted drugs at least once in their lifetime. To ascertain the first referral, patients with no prior histological evaluation of their liver disease at referral were selected for the analysis. The surveillance system obtained ethical approval from the French data protection authority (CNIL).

Data collection

A standardized notification form was used in all centres to collect data routinely, including risk factors for HCV transmission, the last HCV negative test (if available), date and the reasons for anti-HCV testing, HCV RNA serum status, HCV genotype, co-infections with HIV (anti-HIV serostatus) and hepatitis B virus (hepatitis B surface antigen serostatus), history of excessive alcohol consumption defined as more than 210g of pure ethanol per week for women and 280g for men, and the number of years with excessive consumption. The severity of liver disease was assessed either by liver biopsy (Metavir scores [Bedossa and Poynard, 1996]) and/or by clinical evaluation including clinical examination, biochemical tests and imaging (mainly abdominal ultrasound). The clinical evaluation was graded as follows: repeated normal ALT values defined as ALT levels below the upper limit of normal at three consecutive tests within six months, chronic hepatitis C, cirrhosis (compensated or not) and hepatocellular carcinoma. Cirrhosis at clinical evaluation was defined by the fibrosis Metavir score F4 when a liver biopsy was carried out or by the association of clinical signs, laboratory findings and ultrasound images used by all participating centres [Schuppan and Afdhal, 2008]. The diagnostic criteria used for hepatocellular carcinoma were those established during the Barcelona-2000 EASL Conference [Bruix et al., 2001]).

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The form was modified in 2004 to include data on non-invasive assessment of liver fibrosis and on antiviral therapy.

HCV genotyping was carried out by the referral laboratories of the participating centres. Most of these laboratories were involved in the French ANRS panel quality control study in 2000 [Lefrere et al., 2004] and the same standardized genotyping assays (Inno LiPA assay) or other methods (sequencing, primer-specific PCR) during the study period were used.

The database was checked regularly for duplicate reporting in and between participating reference centres.

The suspected year of HCV infection was defined by the year of the last HCV negative test performed during the drug-use period or year of first drug injection. Time from the suspected year of infection to the year of referral to the reference centre was used as a surrogate of the duration of HCV infection. The median duration of infection was used to create a qualitative variable (≥ 18 years; < 18 years) in the univariate and multivariate analysis.

Patients included in the analysis were drug users with HCV RNA in the serum who were naive to anti-HCV treatment.

The outcome of severe liver disease was defined as cirrhosis (compensated or not) or hepatocellular carcinoma at the first referral, based on the clinical assessment.

Statistical analysis

Factors associated with severe liver disease

Univariate analysis was carried out to assess the characteristics associated with severe liver disease at clinical evaluation. Variables with p values <0.2 in univariate analysis were entered into the multivariate analysis. STATA® 9.2 statistical package was used for the analysis (Stata Corporation, College Station, Texas, USA).

First, the general approach for multivariate analysis was used including only patients who had no missing data in any of the variables (complete case analysis). However, six out of the eight variables included in the multivariate model comprised 10% to 27% of missing values resulting in a loss of power and precision, complication in data handling and analysis and potentially biased odds ratios (OR) due to differences between the observed and unobserved data [Little and Rubin, 2002].

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Therefore, missing values were estimated from the observed values using multiple imputation (imputation by chained equations, ice procedure STATA® 9.2) [Royston, 2007]. Several complete data sets (m=30) were created. Each data set was analysed separately, and the results were combined to obtain the odds ratios [Little & Rubin, 2002]. The sensitivity of the odds ratios to departures from the assumed missing mechanism was tested for three variables (alcohol consumption, co-infection with HIV and HCV genotype 3) using a weighting approach developed by Carpenter *et al.* [Carpenter et al., 2007].

Results

Of the 5004 drug users who were referred for HCV infection between 2001 and 2007 and who were naive to anti-HCV treatment, 4236 were HCV RNA positive and were included in the analysis. The remaining 768 had either a negative (n = 455) or an unspecified (n = 313) result for HCV RNA.

Characteristics of the study population

Of the 4236 patients positive for HCV RNA, 77% were male; the median age was 39 years and the median estimated duration of HCV infection was 18 years (Table I). Co-infections with HBV and HIV were diagnosed in 2% and 6%, respectively. A history of excessive alcohol consumption was reported for 43 %. Among the 1154 patients who reported previous heavy drinking in the past with an available duration of excessive consumption, 67% had drunk heavily for more than five years. HCV genotypes were mainly genotype 1 (36%) and 3 (25%), the distribution of HCV genotypes remained stable over the study period.

A liver biopsy was carried out in 53.6% of the drug users included in 2001-2003 and in 27.1% of those included in 2004-2007. In the latter period, 29.2% had an evaluation of fibrosis by non-invasive methods only.

A clinical evaluation of liver disease was available for 4065 (96%) patients with HCV RNA, and severe liver disease was diagnosed in 8% of the patients. Cirrhosis was diagnosed in 301 patients and hepatocellular carcinoma in 10 patients. Of these 311 patients, 236 (76%) had an available HCV

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genotyping result: HCV genotypes 1 and 3 were found in 48% and 42%, respectively. Of the 164 patients with severe liver disease at clinical evaluation who underwent a liver biopsy, the Metavir score F4 was diagnosed for 94% including three out of the four patients with hepatocellular carcinoma (F3 for the remaining patient).

In univariate analysis the following variables were associated with a severe liver disease at referral (Table II): inclusion after 2003, male sex, age >40 years at referral, estimated duration of infection ≥18 years, history of excessive alcohol intake, co-infection with HIV and HCV genotype 3. Time between the first HCV positive test and referral was not significant.

In both the complete-case and the multiple imputation (MI) analyses, the independent factors associated with severe liver disease at clinical evaluation were male sex (adjusted OR_{MI} (aOR_{MI}): 2.0; 95% CI, 1.4-2.8), age > 40 years at referral (aOR_{MI}: 2.1; 95% CI, 1.6–2.9), duration of HCV infection ≥ 18 years (aOR_{MI}: 2.9; 95% CI, 2.0-4.3), history of excessive alcohol consumption (aOR_{MI}: 2.8; 95% CI, 2.1-3.7) and HCV genotype 3 (aOR_{MI}: 1.6; 95% CI, 1.2-2.1) (Table III). In the multiple imputation analysis, co-infection with HIV (aOR_{MI}: 1.8; 95% CI, 1.2-2.8) was associated significantly with severe liver disease. No significant interactions were found.

The univariate analysis for the factors associated with the F4 Metavir score, limited to the patients who had a liver biopsy (n=1725), showed similar results although the 95% confidence intervals were wider. However, co-infections with HIV and HBV were not significant statistically (p>0.25; data not shown).

Discussion

This multi-site study documents factors associated with severe liver disease in a large group of patients infected with HCV who reported drug use. The findings confirm the role of excessive alcohol consumption and co-infection with HIV and suggest that HCV genotype 3 is associated with an increased risk of severe liver disease independently of age, sex, duration of infection and excessive alcohol consumption. To our knowledge this is the first study to address the risk factors of severe liver disease in a population of drug users infected with HCV at first referral.

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Clinical studies have demonstrated a significant association between HCV genotype 3 infection and the presence of steatosis [Cross et al., 2009; Hui et al., 2002; Rubbia-Brandt et al., 2004]. In patients infected with HCV genotype 3, fat accumulation in the liver is correlated with the level of HCV replication [Adinolfi et al., 2001] and disappears following successful antiviral therapy [Castera et al., 2004]. Although there have been several studies providing evidence for the influence of steatosis on the progression of fibrosis regardless of the HCV genotype [Cross et al., 2009; Fartoux et al., 2005; Hu et al., 2004] or in patients infected with HCV genotype 3 [Castera et al., 2003; Castera et al., 2004; Rubbia-Brandt et al., 2004], the issue remains controversial. Indeed, other studies failed to show any association between steatosis and liver fibrosis [Asselah et al., 2003; Perumalswami et al., 2006; Ryder et al., 2004], including one study on a large cohort of patients infected with HCV [Perumalswami et al., 2006]. However, the comparability of the studies is questionable, as there were differences in the study populations and the risk factors studied. Furthermore, another study showed that steatosis had no association with fibrosis in patients infected with HCV genotype 3 [Bugianesi et al., 2006] suggesting other causes for the progression of fibrosis such as insulin resistance or viral induced liver inflammation.

Numerous studies have shown that a past or present history of excessive alcohol consumption was associated with more rapid progression to fibrosis [Tolstrup et al., 2009; Westin et al., 2002]. As expected in this particular population of patients who reported drug use, cirrhosis at the time of first referral was associated strongly with excessive alcohol consumption in the past. The definition of excessive alcohol consumption used in this study corresponds to more than 30g/day for women and 40g/day for men compared with 50g/day for both sexes in many other studies. Of those who acknowledged excessive alcohol consumption in the past, more than half reported that the period lasted at least five years, allowing the development of fibrosis. In addition, more than one third of the patients who reported excessive alcohol consumption in the past, acknowledged current excessive consumption. This is consistent with the results reported by Campbell *et al.* [Campbell et al., 2006], who found that in a population of drug users infected with HCV, knowledge about the detrimental effects of alcohol consumption related to HCV infection did not appear to influence alcohol use.

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The analysis of the mortality data in the Swiss hepatitis C cohort showed recently that mortality was increased for individuals who reported drinking more than 40g alcohol per day. HCV genotype 3 was associated also with a higher mortality although it did not remain significant in the multivariate analysis [Prasad et al., 2009].

HIV infection is known to modify the natural history of HCV by accelerating the rate of progression of fibrosis and the development of advanced fibrosis both before [Poynard et al., 2003] and since [Thein et al., 2008] the highly active antiretroviral therapy era. Interestingly, a study has shown advanced liver fibrosis to be associated with HCV genotype 3 in a population of patients co-infected with HIV and HCV [Barreiro et al., 2006]. Surprisingly, in the study by Barreiro *et al*. [2006], neither past nor current excessive alcohol consumption remained associated significantly with advanced liver fibrosis. However, the assessment of liver fibrosis in this study was based on a non-invasive method (elastometry) only.

Among the limitations of the present study is its cross-sectional design. Indeed, it is difficult to use cross-sectional data to estimate longitudinal parameters. However, although collected at the same time as the outcome of interest (severe liver disease), most of the variables assessed in this study reflect exposures that occurred prior to the outcome. Therefore, this study could be considered as a retrospective cohort, which is less subject to bias than a purely cross-sectional study [Rothman and Greenland, 1998]. Others factors shown to be associated with progression of fibrosis were not collected, such as daily cannabis smoking [Hezode et al., 2005] and insulin resistance related to the patient characteristics such as obesity and type 2 diabetes mellitus, and to the virus [Cua et al., 2008; Moucari et al., 2008; Petta et al., 2008]. However, Moucari *et al.* [2008] provided evidence that insulin resistance was associated with HCV genotypes 1 and 4 in contrast to patients with HCV genotypes 2 and 3. This lower rate of insulin resistance in patients with HCV genotype 3 was shown also in another study [Hui et al., 2003]. The validity of the method used in the present study for assessing the severity of liver disease could be called into question since it was not based on histology. However, the classification used for clinical evaluation of the liver disease is likely to be homogeneous within the participating reference centres. Furthermore, as the indication for liver biopsy may be related to certain risk factors or the intention to begin a course of antiviral therapy, the

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use of the clinical stage for assessment of severe liver disease appeared more suited to the purpose of the study objectives. Also, the proportion of patients managed in a reference centre who had a liver biopsy decreased dramatically from 45% in 2001 to 18% in 2006 [Delarocque-Astagneau et al., 2009]. Indeed, since 2002, physicians in France have been treating patients infected with HCV genotypes 2 and 3 or with HIV co-infection without performing a liver biopsy, in accordance with the 2002 French recommendations on HCV treatment [Dhumeaux et al., 2003]. The univariate analysis of the factors associated with the F4 Metavir score limited to patients who had a liver biopsy showed similar results, but the 95% confidence intervals were wider and co-infection with HIV was not significant statistically.

The present analysis was confronted with missing values as it was based on surveillance data. In order to keep all cases in the analysis, a multiple imputation method was applied to the data. Multiple imputation is a well established method for the analysis of data sets with missing values. As the multivariate analysis will include all cases, multiple imputation provides consistency in results [Little & Rubin, 2002]. Thus, the association between HCV genotype 3 and severe liver disease found in the complete case analysis was reinforced by the multiple imputation analysis. Also, HIV infection was associated significantly with severe liver disease after multiple imputation analysis. In addition, the sensitivity analysis performed on HCV genotype 3, co-infection with HIV and alcohol consumption allowed to be confident of the robustness of the estimations [Carpenter et al., 2007][data not shown]. A recent analysis of the data from the Swiss hepatitis C cohort found HCV genotype 3 as a factor associated with progression of liver fibrosis, which reinforced the validity of the results [Bochud et al., 2009].

These results suggest that in patients infected chronically with HCV who reported drug use, early therapeutic intervention should be recommended for those patients who are infected with HCV genotype 3 and who acknowledge excessive alcohol consumption in the past, since progression to cirrhosis may be more rapid. Therefore, integrated treatment of HCV and alcohol addiction is essential. Thus, collaboration between healthcare providers who specialize in the management of addictions and those who specialize in the management of HCV infection should be promoted.

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Table I: Main characteristics of HCV RNA positive drug users at first referral to hepatology reference centres in France, 2001-2007.

Characteristic	All patients (n = 4236)		HCV genotype 3 (n = 1077)		other HCV genotypes (n = 1986)	
Sex ratio (female/male)	1 / 3		1 / 3		1 / 3	
Age, years	39	(10)	39	(11)	40	(10)
Duration of HCV infection[a], years,	18	(12)	18	(12)	19	(12)
ALT level[b], xULN	1.5	(1.5)	2.0	(1.9)	1.5	(1.2)
History of excessive alcohol intake[c]						
Missing	467	(11.0)	119	(11.1)	212	(10.7)
No	1952	(46.1)	499	(46.3)	908	(45.7)
Yes	1817	(42.9)	459	(42.6)	866	(43.6)
HIV serostatus						
Missing	702	(16.6)	162	(15.0)	289	(14.6)
Negative	3284	(77.5)	868	(80.6)	1 557	(78.4)
Positive	250	(5.9)	47	(4.4)	140	(7.0)
HBsAg serostatus						
Missing	685	(16.2)	150	(13.9)	285	(14.3)
Negative	3466	(81.8)	906	(84.1)	1 660	(83.6)
Positive	85	(2.0)	21	(2.0)	41	(2.1)
HCV genotype						
Missing	1173	(27.7)				
1 non a, b	407	(9.6)				
1a	694	(16.4)				
1b	439	(10.3)				
2	117	(2.8)				
3 non a	317	(7.5)				
3a	760	(17.9)				
4	325	(7.7)				
5	4	(0.1)				
Clinical stage at 1st referral						
Missing	171	(4.1)	31	(2.9)	58	(2.9)
Normal ALT values	713	(16.8)	116	(10.8)	356	(17.9)
Chronic hepatitis	3000	(70.8)	815	(75.6)	1419	(71.4)
Cirrhosis[d]	301	(7.1)	97	(9.0)	132	(6.7)
Hepatocellular carcinoma	10	(0.2)	3	(0.3)	4	(0.2)
Acute hepatitis	41	(1.0)	15	(1.4)	17	(0.9)
Liver biopsy at 1st referral	1765	(41.7)	405	(37.6)	992	(49.9)
Fibrosis Metavir [Bedossa and Poynard, 1996] score at liver biopsy	1725		396		976	
F0	138	(8.0)	25	(6.3)	81	(8.3)
F1	735	(42.6)	136	(34.3)	455	(46.6)
F2	480	(27.8)	112	(28.3)	264	(27.1)
F3	195	(11.3)	62	(15.7)	94	(9.6)
F4	177	(10.3)	61	(15.4)	82	(8.4)

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Note. Data are no. (%) of patients or median values (interquartile range), unless otherwise indicated. HCV, hepatitis C virus; IQR, interquartile range; SD, standard deviation; ALT, alanine aminotransferase; ULN, upper limit of normal; HIV, human immunodeficiency virus; HBsAg, hepatitis B surface antigen;

[a] available for 3626 (85.6%) HCV RNA+ drug users, for 932 (86.5%) infected with genotype 3, for 1673 (84.2%) infected with other genotype;

[b] available for 3938 (93.0%) HCV RNA+ drug users, for 1023 (95.0%) infected with genotype 3, for 1906 (96.0%) infected with other genotype;

[c] >210 g/week for women and >280 g/week for men (g= gram of ethanol)

[d] including decompensated cirrhosis

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Table II: Factors associated with severe liver disease at clinical evaluation in HCV RNA positive drug users at first referral to hepatology reference centres in France, 2001-2007

Factors	Patients (n = 4065)	% SLD	Univariate Analysis OR (95% CI)	p
Period of inclusion				
2001-2003	2211	6.8	1	
2004-2007	1854	8.7	1.3 (1.0-1.6)	0.02
Sex				
Female	929	4.0	1	
Male	3136	8.7	2.3 (1.6-3.3)	<0.001
Age				
≤ 40 years	2302	3.7	1	
> 40 years	1763	12.8	3.8 (2.9-4.9)	<0.001
Time between 1st HCV+ test and referral				
< 1 year	1683	6.8	1	
≥ 1 year	1987	7.7	1.1 (0.9-1.5)	0.3
Missing	395	10.6		
Duration of HCV infection at referral[a]				
< 18 years	1636	3.0	1	
≥ 18 years	1843	12.5	4.7 (3.4-6.5)	<0.001
Missing	586	7.7		
History of excessive alcohol intake[b]				
No	1881	4.1	1	
Yes	1740	12.5	3.3 (2.5-4.3)	<0.001
Missing	444	3.6		
HBsAg				
Negative	3366	7.8	1	
Positive	83	13.2	1.8 (0.9-3.4)	0.07
Missing	616	6.0		
HIV				
Negative	3180	7.7	1	
Positive	240	12.9	1.8 (1.2-2.7)	0.004
Missing	645	5.6		
HCV genotype 3				
No	1928	7.0	1	
Yes	1046	9.6	1.4 (1.1-1.8)	0.02
Missing	1091	6.9		

Note. SLD, severe liver disease (cirrhosis, hepatocellular carcinoma); aOR, odds ratio; CI, confidence interval; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; HIV, human immunodeficiency virus;

[a] Time from suspected year of infection to year of referral to the reference centre. Suspected year of HCV infection is defined as year of the last HCV negative test performed during the drug-use period or year of first drug injection

[b] >210g/week for women and >280g/week for men (g = gram of ethanol)

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Table III: Factors independently associated with severe liver disease at clinical evaluation in HCV RNA positive drug users at first referral to hepatology reference centres in France, 2001-2007

	Multivariate analysis			
	Complete case (n = 2294)		Multiple imputation (n = 4065)	
Factors	aOR (95% CI)	p	aOR (95% CI)	p
Sex				
Female	1		1	
Male	2.0 (1.2-3.1).	0.005	2.0 (1.4-2.8)	<0.001
Age				
≤ 40 years	1		1	
> 40 years	2.4 (1.6-3.6)	<0.001	2.1 (1.6-2.9)	<0.001
Duration of HCV infection at referral[a]				
< 18 years	1		1	
≥ 18 years	2.9 (1.8-4.6)	<0.001	2.9 (2.0-4.3)	<0.001
History of excessive alcohol intake[b]				
No	1		1	
Yes	2.6 (1.8-3.6)	<0.001	2.8 (2.1-3.7)	<0.001
HCV genotype 3				
No	1		1	
Yes	1.4 (1.0-1.9)	0.05	1.6 (1.2-2.1)	0.003
HIV serostatus[c]				
Negative	-	-	1	
Positive	-	-	1.8 (1.2-2.8)	0.005

Note. severe liver disease (cirrhosis, hepatocellular carcinoma); aOR, adjusted odds ratio; CI, confidence interval; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; HIV, human immunodeficiency virus;

[a] Time from suspected year of infection to year of referral to the reference centre. Suspected year of HCV infection is defined as year of the last HCV negative test performed during the drug-use period or year of first drug injection

[b] >210g/week for women and >280g/week for men (g = gram of ethanol)

[c] HIV serostatus did not remain significant in the complete case analysis

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