

Risk of hepatocellular carcinoma and fibrosis evolution in hepatitis C patients with severe fibrosis or cirrhosis treated with direct acting antiviral agents

C. Hamoir, Y. Horsmans, P. Stärkel, G. Dahlqvist, S. Negrin Dastis, N. Lanthier

Service d'Hépatito-Gastroentérologie, Cliniques universitaires Saint- Luc, UCLouvain, Brussels, Belgium.

Abstract

Background and study aims : Cirrhosis associated to chronic hepatitis C virus (HCV) is one of the leading cause of hepatocellular carcinoma (HCC). The goal of our study was to evaluate first the risk and determinants of HCC and second the evolution of fibrosis in patients treated for HCV with advanced fibrosis stages who achieved sustained virological response (SVR) after direct-acting antivirals (DAA) treatment.

Patients and methods : We conducted a prospective study on HCV patients with F3 or F4 Metavir fibrosis scores treated with DAA between October 2014 and February 2017. The annual incidence rate for HCC was calculated. We used Cox regression model in order to identify factors associated with HCC. Transient elastography (TE) was performed 12 and 24 months after the end of DAA treatment and non-invasive liver fibrosis biomarkers were performed twice a year during follow-up.

Results : 143 patients with severe fibrosis or cirrhosis were enrolled in the study. 6 patients developed HCC. The annual incidence rate of HCC in our cohort was 2.7 per 100 patients. Risk factors associated with HCC after DAA were genotype 2 and steatosis. Overall TE values significantly decreased after DAA treatment with a median value prior to treatment of 16.9 kPa to a median of 10.8 kPa 24 months after the end of the treatment. Biological fibrosis scores also significantly decreased following viral eradication.

Conclusions : DAA treatment does not seem to be associated with HCC promotion after HCV eradication in patients with severe fibrosis stages. DAA-induced SVR is associated with a reduced estimation of fibrosis. (*Acta gastroenterol. belg.*, 2021, 84, 25-32).

Keywords : hepatitis C, direct-acting antiviral agents, hepatocellular carcinoma, fibrosis, cirrhosis, transient elastography.

Abbreviations

HCV : hepatitis C virus
 HCC : hepatocellular carcinoma
 DAA : direct-acting antivirals
 SVR : sustained virological response
 TE : transient elastography
 APRI : aspartate transaminase to platelet ratio index
 FIB-4 : fibrosis 4
 CAP : controlled attenuation parameter
 IFN : interferon
 LSM : liver stiffness measurement

Introduction

Hepatitis C virus (HCV) infection is a public health challenge throughout the world with an estimated number of at least 170 million HCV carriers worldwide (1). Despite the increasing prevalence of metabolic dysfunction-associated fatty liver disease (2, 3), chronic infection

with HCV remains a major cause of end-stage liver disease, liver transplantation, hepatocellular carcinoma (HCC) and liver related death in the Western world. In Belgium, based on a recent meta-analysis, we estimated an HCV seroprevalence of 1% (4). It is generally a slow progressive disease characterized by persistent hepatic inflammation leading to the development of cirrhosis in approximately 10-30% of patients over 20-30 years of HCV infection (5). Once cirrhosis is established, there is a 1-5 % annual risk of hepatocellular carcinoma (HCC) and a 3-6% annual risk of hepatic decompensation (5).

The treatment of chronic HCV infection has experienced a revolution with the discovery and the availability of the new direct-acting antiviral agents (DAA). With those treatments, sustained virological response (SVR) rates, defined as undetectable HCV RNA 12 weeks after completion of therapy for hepatitis C, reaches more than 90% with very few reported side effects compared with previous therapies (6). The introduction of DAA also allows treatment of patients with even decompensated cirrhosis. The expected long-term outcome of DAA-induced SVR is to reduce fibrosis and decrease complications of chronic hepatitis C, including HCC development (7).

Previous studies on interferon based therapies have demonstrated that liver fibrosis and even cirrhosis may regress in HCV patients once SVR is achieved (8). In patients treated by DAA who achieved SVR, a significant regression of liver stiffness values obtained by transient elastography (TE) and fibrosis markers (fibrosis-4 score and aspartate aminotransferase-platelet ratio index) have recently been described (9).

The high efficacy of DAA have also been shown in large real-life cohorts reporting improvements in disease severity (Child-Pugh score, model for end stage liver disease) in some patients after treatment (10). These encouraging results have raised hope for a subsequent decline of HCC occurrence and recurrence. However, the positive impact of DAA therapy on HCC occurrence or recurrence is still debated. Indeed, studies suggested a potential unexpected incidence of HCC in treated

Correspondence to : Pr. Yves Horsmans, Cliniques universitaires Saint-Luc, UCLouvain, 10 avenue Hippocrate, 1200 Bruxelles. Phone : +3227642824. Fax : +327648927.

E-mail : yves.horsmans@uclouvain.be

Submission date : 08/09/2020

Acceptance date : 30/09/2020

patients, both in those without a prior history of cancer and in those who have been successfully treated for HCC and were disease-free for various periods of time (6, 11-15). Conversely, other studies suggested no increased risk and even a reduction of recurrence or occurrence of HCC in patients with SVR after DAA therapy (7, 16-26).

The goal of our study was to evaluate first the occurrence of HCC and second the evolution of fibrosis in our patients treated in an academic hospital for hepatitis C with severe fibrosis stages (F3 and F4 Metavir scores) who achieved SVR after DAA treatment

Methods

Patients

In this prospective cohort study, we recorded the data from all the consecutive patients with severe liver fibrosis who were prospectively enrolled for treatment with DAA in our center between October 2014 and February 2017.

Patient inclusion criteria were the presence of chronic hepatitis C in patients older than 18 years and Metavir scores of F3 or above assessed either by liver histology or combination of liver stiffness measurement (LSM) \geq 9.5 kPa obtained by TE associated with one concordant biological fibrosis score \geq F3 (Fibrotest \geq 0.59, AST to Platelet ratio Index (APRI) \geq 1 or Fibrosis-4 (FIB-4) \geq 2.1). Those eligibility criteria followed the priority criteria for drug reimbursement established by the Belgian medicines refund committee (CRM) during the study period, as explained in a recent observational study published in this Journal (27). Patients with history of HCC who had complete response to surgical resection or loco-regional ablation of previous HCC before starting DAA were also included.

LSM were performed using TE (FibroScan®, Echosens, Paris, France) according to current European Association for the Study of the Liver (EASL) guidelines using the either the M or XL probe according to the automatic probe selection tool based on the subcutaneous fat thickness.

Drug regimens and treatment duration (12 or 24 weeks) were chosen by the clinicians according to the patients' clinical characteristics, literature recommendations and current Belgian Association for the Study of the Liver (BASL) and EASL guidelines.

Virological response to therapy was assessed by quantitative HCV RNA detection, using real-time PCR (Roche cobas® HCV Test, Indianapolis, USA) with a limit of detection of 30 U/ml.

Clinical and radiological follow-up

Baseline clinical characteristics and laboratory data were registered in all patients before starting antiviral therapy. Follow-up included clinical and laboratory data according to routine clinical practice. All patients underwent liver imaging (either ultrasound, computerised

tomography scan or magnetic resonance imaging) to exclude the presence of active HCC before starting DAA and then twice a year during follow-up.

Alcohol abuse was defined as intake of >30 g alcohol per day for men and > 20 g alcohol per day for women. Diabetes was defined as taking one or more medication(s) for diabetes. Steatosis was defined by the presence of visible steatosis in $> 5\%$ of hepatocytes on liver biopsy or a controlled attenuation parameter (CAP) value > 215 dB/m on FibroScan® (28). High cholesterol was defined by total cholesterol rate > 190 mg/dL or when taking one or more cholesterol lowering drugs. We defined hypertension when the patient was treated for high blood pressure. Renal function was classified according to Kidney Disease Improving Global Outcomes (KDIGO) nomenclature and chronic renal failure was defined as glomerular filtration rate < 60 ml/min (KDIGO grade 2).

In order to evaluate fibrosis evolution after DAA treatment, TE was performed 12 and 24 months after the end of DAA treatment. Non-invasive liver fibrosis biomarkers (APRI and FIB-4) were performed twice a year during follow-up.

Ethics statement

Written informed consent was obtained from each patient. The study protocol was in concordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committee of Saint-Luc Hospital.

Statistical analysis

All analyses were conducted using SPSS 24 software (SPSS software [IBM Corp. 2011. IBM SPSS Statistics for Windows. Armonk, NY, USA : IBM Corp]), with graphs drawn using Graphpad Prism 7.0 (GraphPad Software, La Jolla, CA, USA). Continuous variables were expressed as mean \pm one standard deviation (SD) or median with interquartile ratio (IQR), and categorical variables as counts and percentages. Categorical variables were analyzed using Chi-squared test or Fisher's exact test. Continuous variables were analyzed using unpaired t-test or Mann-Whitney U-test, according to statistical distribution. The data were subjected to the Kolmogorov-Smirnov normality test and Bartlett's test for homogeneity of variance. Values were log₁₀-transformed when appropriate. Means or medians were compared across groups using one way ANOVA with F test (Bonferroni correction applied to p-values in multiples comparisons) or with Kruskal Wallis test, respectively. We analyzed Fibroscan results in tertiles for comparison in different groups. We generated Kaplan-Meier curves to illustrate and compare the cumulative incidence rates of HCC by race, genotype and presence of steatosis and used the log-rank test to evaluate the differences between these curves. In order to identify factors associated with HCC, a Cox logistic regression model was built as follows :

all the variables with p value < 0.10 in the univariable analyses were entered into a multivariable model with a backward elimination procedure, based on the likelihood ratio. The results were expressed as hazard ratio (HR) with 95% confidence intervals [95%CI]. Finally, we calculated the annual incidence rates for HCC in the global cohort.

Results

Patients' characteristics

At the end of February 2017, the database included 168 adult patients who have been treated with different regimens of DAA for HCV-related chronic liver disease. Overall 25 patients were excluded from the study: 14 due to the absence of severe fibrosis; 2 with a viral load > 30 UI/ml after the end of the treatment; and 9 because of insufficient follow-up < 3 months) after the end of DAA (Figure 1) leaving a total study population of 143 patients.

Principal baseline characteristics of the study population are summarized in Table 1. Forty-six patients were classified as fibrosis stage 3 (F3 or severe fibrosis) and 97 patients as fibrosis stage 4 (F4 or cirrhosis). Fifteen patients who had a history of HCC in the past, were considered as cured either with liver transplantation or local ablative treatment without recurrence in the past 6 months. DAA treatment was given in association with PEG-IFN in 3 patients.

Median follow-up time after end of DAA was 13 months (IQR: 8-21). Five patients died during follow-up: one patient who developed HCC and four without HCC. Five patients underwent liver transplantation during follow-up: two patients who developed HCC during follow-up and three without HCC (Table 1).

Risk of HCC during follow-up

Our first question was to evaluate the incidence and risk factors of HCC after DAA treatment in our population.

The annual incidence rate of HCC in our cohort was evaluated at 2.7 per 100 patients. Six patients developed HCC during follow-up (Table 1). The median time to HCC occurrence was 12 months (IQR: 7-21). We analysed the clinical characteristics of patients who developed HCC in comparison with patients without HCC (Table 1). The two groups had similar age at the time of DAA initiation (mean 60.5 years for the total study population) (Table 1). The significant differences between patients who developed HCC or not after treatment were the ethnicity, the presence of steatosis, the genotype and a history of HCC without liver transplantation (Table 1). The risk of developing HCC after DAA treatment tended also to be higher in patients with a history of previous treatment for HCV although it did not reach statistical significance (Table 1). During follow-up, 5 patients received a liver

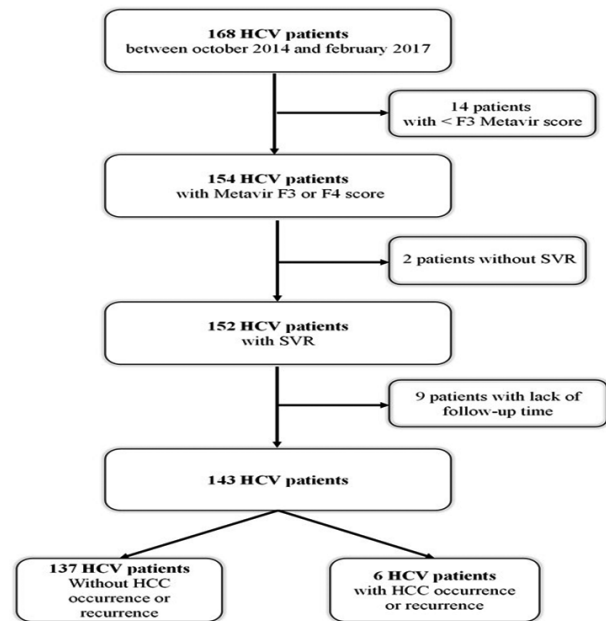


Figure 1. — Flow chart of the study population.

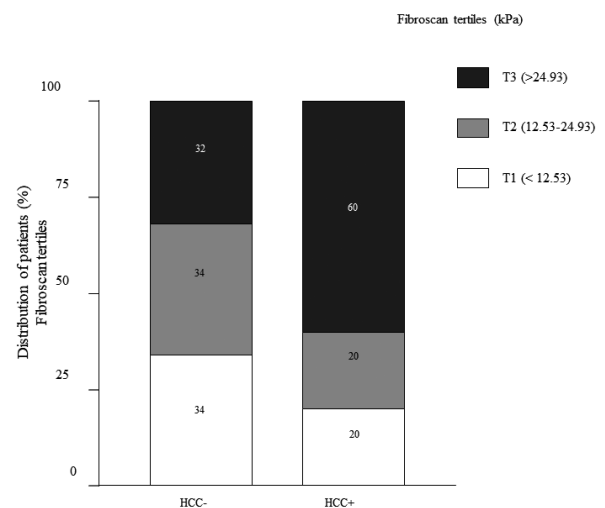


Figure 2. — Distribution of patients with or without incident hepatocellular carcinoma according to liver stiffness measurement (transient elastography – TE) tertile.

transplantation, 2 (33%) patients in the group with HCC and 3 (2%) patients in the other group (indication: end-stage liver disease) (Table 1). During follow-up, 5 patients died (heart failure, multisystemic cryoglobulinemia, acute degenerative neurologic disorder, multifocal HCC with atrial thrombosis and unknown death cause for the last patient), 1 (17%) patient in the group with HCC and 4 (3%) patients in the other group (Table 1).

For comparison between groups, TE results were divided in equal tertiles. Tertile 3 TE results corresponded to the highest LSM scores. Sixty percent of the patients who developed HCC had tertile 3 FibroScan values whereas only 32% of the patients without HCC had the highest TE scores (Table 2 and Figure 2). However, these results were not statistically significant ($p=0.43$).

Table 1. — Baseline characteristics of the global population and of patients with or without incident hepatocellular carcinoma during follow-up

Characteristics	Global Population N= 143	HCC N=6	Without HCC N=137	<i>p val</i>
Age, yrs	60.5 ± 11.2	64.2 ± 6.6	60.3 ± 11.3	0.41
Male gender, n (%)	83 (58)	4 (67)	79 (58)	0.66
BMI kg/m ²	25.8 (23-29.3)	25.6 (21.9-31.6)	25.8 (23- 29.3)	0.91
Ethnicity, n (%)				
Caucasian	100 (70)	2 (33)	98 (72)	0.042
African	22 (15)	2 (33)	20 (14)	0.22
Middle Eastern	13 (9)	2 (33)	11 (8)	0.025
Others	8 (6)	0 (0)	8 (6)	0.60
Steatosis, n (%)	16 (11)	3 (50)	13 (9)	0.002
Cholesterol, n (%)	36 (25)	1 (17)	35 (31)	0.45
Hypertension, n (%)	73 (51)	3 (50)	50 (52)	0.91
Renal failure, n (%)	16 (11)	0 (0)	16 (12)	0.37
Diabetes, n (%)	22 (15)	1 (17)	21 (15)	0.93
Alcohol, n (%)	24 (17)	1 (17)	23 (17)	0.99
Tobacco, n (%)	33 (23)	2 (33)	31 (23)	0.54
Genotype, n (%)				0.001
1a - 1b	90 (63)	2 (33)	88 (64)	
2	12 (8)	3 (50)	9 (7)	
3	14 (10)	0 (0)	14 (10)	
4	25 (18)	1 (17)	24 (18)	
5 - 6	2 (1)	0 (0)	2 (1)	
Previous antiviral treatment, n (%)	95 (66)	6 (100)	89 (65)	0.07
Albumin (g/l)	40.2 ± 5.9	38.3 ± 6.6	40.3 ± 5.9	0.44
Bilirubin (mg/dl)	0.9 ± 0.8	0.8 ± 0.3	0.9 ± 0.9	0.86
Platelets (n/μl)	149356 ± 60730	138167 ± 113139	149846 ± 58091	0.65
MELD score	7 (7-9)	11 (6.5-12.5)	7 (7-9)	0.28
AFP (μg/l)	7.1 (4.3-14.7)	5.5 (3.6-26.4)	7.3 (4.3-13.5)	0.69
F4 Metavir Score n (%)	97 (68)	5 (83)	92 (67)	0.66
APRI	1.4 (0.8-2.5)	1.4 (0.7-2.8)	1.4 (0.8-2.5)	0.77
FIB-4	3.5 (2.3-6.1)	5.48 (3.5-8.4)	3.49 (2.3-6)	0.2
Previous HCC, n (%)	15 (10)	2 (33)	13 (9)	0.06
Previous HCC without transplant, n (%)	8 (6)	2 (33)	6 (4)	0.003
Previous transplant n (%)	12 (8)	0 (0)	12 (9)	0.45
HIV, n (%)	6 (4)	1 (17)	5 (4)	0.12
Transplant post treatment, n (%)	5 (3)	2 (33)	3 (2)	< 0.001
Death, n (%)	5 (3)	1 (17)	4 (3)	0.07

BMI : body mass index ; AFP : alpha-fetoprotein ; MELD : model for end-stage liver disease ; APRI : AST to platelet ratio index ; FIB-4 : fibrosis-4 ; HCC : hepatocellular carcinoma.

Table 2. — Transient elastography (TE) baseline values: global results and tertiles. Comparison between patients with or without incident hepatocellular carcinoma during follow-up

Transient Elastography Baseline	Global Population N = 117	HCC N = 5	Without HCC N = 112	<i>p val</i>
TE, kPa	17.1 (11.8-7.7)	28 (14.9-40.6)	16.8 (11.8-26.9)	0.27
TE tertile, n (%)				0.43
<12.53 (T1)	39 (33.3%)	1 (20%)	38 (33.9%)	
12.53-24.93 (T2)	39 (33.3%)	1 (20%)	38 (33.9%)	
>24.93 (T3)	39 (33.3%)	3 (60%)	36 (32.1%)	

HCC : hepatocellular carcinoma.

We found a significant difference in HCC occurrence in non-Caucasian patients in comparison with Caucasian patients (*log rank p-value* = 0.044, Figure 3A), in genotype 2 HCV patients in comparison with other genotypes (*log rank p-value* < 0.001, Figure 3B)

and in patients with steatosis in comparison with patients without steatosis (*log rank p-value* = 0.020, Figure 3C).

In our multivariate model, HCC occurrence was independently associated with genotype and steatosis (Table 3). Patients with genotype 2 HCV had higher risk

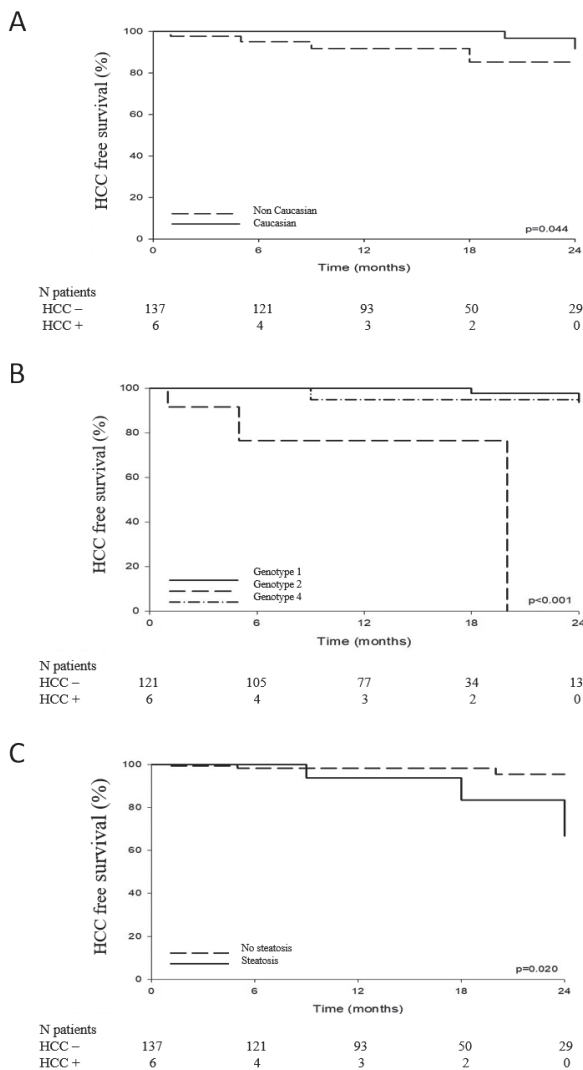


Figure 3. — Survival free of hepatocellular carcinoma according to ethnicity (A), genotype (B) and steatosis (C).

of HCC than patients with other genotypes (adjusted HR compared with genotype 1 = 280, 64, 95% CI = 7.83-10055). The risk of HCC was also higher in patients with steatosis than in patients without (adjusted HR=14.93, 95% CI = 1.08-206.16).

Regression of fibrosis after DAA treatment

Our second question was the evolution of liver fibrosis after DAA treatment in our population.

Overall TE values of the included patients significantly decreased after DAA treatment with a median value prior to treatment of 16.9 kPa (IQR : 11.7-27.4) to a median of 9.9 kPa (IQR : 6.8-21.3. $p < 0.0001$) 12 months after the end of the treatment and a median of 10.8 (IQR : 6.55-15.48. $p = 0.006$) 24 months after the end of the treatment (Figure 4A).

In accordance to TE measurement decline, biological fibrosis scores APRI and FIB-4 also significantly decreased after DAA treatment (Figure 4B).

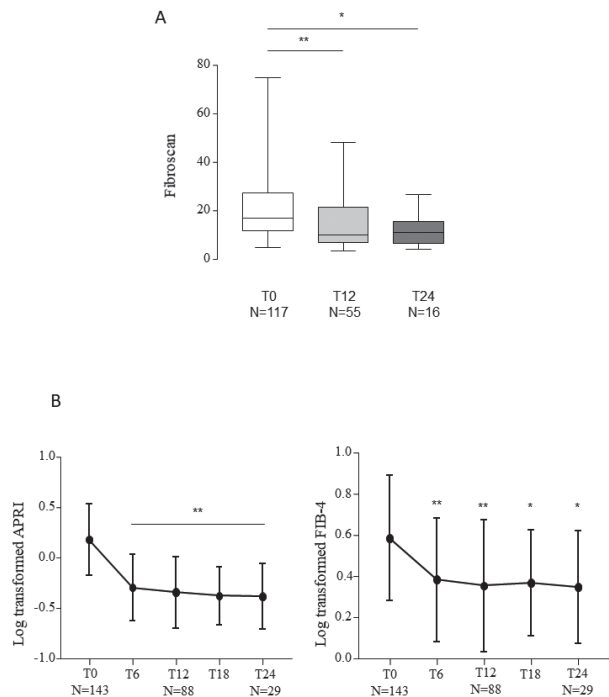


Figure 4. — Evolution of non-invasive markers of fibrosis after DAA treatment. Transient elastography results with liver stiffness measurement pre-treatment, 12 months and 24 months after the end of the treatment. * $p = 0.006$; ** $p < 0.0001$. Decrease in biological fibrosis scores APRI and FIB-4 in repeated measurements post-DAA. * $p < 0.01$; ** $p < 0.0001$. APRI : AST to platelet ratio index ; FIB-4 : Fibrosis-4

Discussion

The major findings of our study based on patients treated for hepatitis C with high fibrosis stages who achieved SVR after DAA treatment were first an annual incidence rate of HCC of 2.7% ; secondly three identified risk factors of HCC after DAA treatment : ethnicity, genotype 2 and presence of steatosis ; and finally significant fibrosis regression after DAA treatment.

Since the arrival of new DAA treatments with high SVR, hope for a decline of hepatocellular carcinoma as observed with interferon therapy has emerged. The first aim of our study was thus to evaluate the risk of HCC after DAA treatment which continues to be a matter of debate since Reig et al. have published their study results in 2016 (6). The discussion remains passionate : a publication suggested increased HCC incidence in the DAA era than in the IFN era among liver transplant registrants with hepatitis C virus infection (29). On the other hand, Vaziri et al. demonstrated an association between DAA and reduced listing rates for HCV-related cirrhosis and HCC in the United Kingdom (30). Moreover, Ioannou et al. showed in a study of 62534 patients a 71% reduction in HCC risk associated with DAA-induced SVR (25).

In our HCV population treated with DAA treatment, we observed an annual incidence rate of HCC of 2.7 per

Table 3. — Factors associated with incident hepatocellular carcinoma (HCC)

Univariate and Multivariate Cox regression model for HCC				
Variable	Univariate		Multivariate	
	HR (95% CI)	p val	HR (95% CI)	p val
Age	1.03 (0.95-1.11)	0.47		
Gender (male=1)	1.28 (0.23-7.01)	0.78		
BMI	1.05 (0.89-1.23)	0.58		
Ethnicity (Non-caucasian=1)	4.85 (0.88-26.58)	0.07	11.08 (0.98-125.48)	0.052
Genotype (Reference genotype 1)		0.001		0.003
Genotype 2	47.06 (6.11-362.36)	<0.001	280.64 (7.83-10055)	0.002
Genotype 4	2.67 (0.24-29.73)	0.43	1.43 (0.08-26.50)	0.81
Previous HCV treatment	33.81 (0.02-77000)	0.37		
MELD score	1.24 (0.93-1.65)	0.15		
Past history HCC (non-transplant)	74.28 (5.65-977)	0.001		
HIV	6.12 (0.71-52.96)	0.10		
Steatosis	5.49 (1.10-27.46)	0.038	14.93 (1.08-206.16)	0.04
Diabetes	0.75 (0.09-6.48)	0.79		
Alcohol	1.15 (0.13-9.92)	0.90		
Tobacco	1.70 (0.31-9.29)	0.54		

Results from univariate and multivariate models Cox logistic regression model. All the variables with p value < 0.10 in the univariable analyses were entered into a multivariable model with a backward elimination procedure, based on the likelihood ratio. Results expressed as hazard ratio (HR) with 95% confidence intervals (95%CI). BMI : body mass index ; HCV : hepatitis C virus ; HCC : hepatocellular carcinoma.

100 patients which seems lower than the rate of 3-8 % in untreated patients described in older reports (5). A review published by Waziry et al. summarizing the evidence on HCC occurrence and recurrence following DAA or IFN-based therapy over the last 17 years showed a similar annual occurrence rate of HCC (2.96%) in patients treated by DAA (31). Moreover, in their meta-regression analysis after adjusting for follow-up and age, DAA therapy was not associated with higher occurrence or recurrence in comparison with IFN-based therapy (31). The comparison between HCC risk after DAA treatment and IFN-based therapy has several drawbacks. In fact, there are obvious differences in populations assessed between studies about DAA or IFN treatments : age of patients, genotype, severity of the disease, follow-up duration or time chosen for starting follow-up. Furthermore, the duration of the different treatments (IFN and DAA) and most importantly the eradication rate are markedly different making any direct comparison rather hazardous.

In a second phase, with a Cox logistic regression model, we identified two independent risk factors of HCC after DAA treatment : genotype 2 and steatosis.

We observed among our patients that HCV genotype 2 was significantly associated with HCC compared to patients with non-2 genotypes. To the best of our knowledge, there is no other study in the literature describing genotype 2 as a risk factor for HCC. A study from Taiwan showed that HCV genotype 1b increases cumulative lifetime risk of HCC (32). Kanwal et al. from USA described an increased risk of HCC in veterans with HCV genotype 3 (33). A recent study suggested that HCV genotype 6 increased the risk for HCC among Southeast Asian patients with liver cirrhosis (34). One might hypothesize that HCV genotype 2 as a HCC risk factor could be a distinctive feature of our Belgian population.

We also showed that steatosis is a risk factor for HCC in HCV patients even after eradication of HCV. Globally, this finding confirms previous reports of two different forms of hepatic steatosis in patients with chronic HCV infection (35-39). In patients with HCV genotype 3, steatosis could be induced by the virus itself through a direct cytopathic effect. In patients with other genotypes, steatosis is considered to be linked with classical metabolic syndrome risk factors and/or with alcohol (35). In our population, we did not find any difference in BMI, cholesterol levels, arterial hypertension, diabetes mellitus and alcohol intake between patients who developed HCC after treatment and those who did not. We cannot thus affirm that in our population, steatosis was associated with metabolic comorbidities or was directly linked with the virus itself. Nevertheless, none of the patients who developed HCC in our study was infected by genotype 3 hepatitis C virus and genotype 2 virus is not known to induce directly liver steatosis.

In addition, we found a statistically significant ethnicity effect in our analyses. Non-Caucasian patients had a higher risk of developing HCC compared with Caucasian which is consistent with previous reports suggesting a role of ethnicity as a risk factor for HCC in patients with chronic HCV and cirrhosis (22, 40).

In a third phase, we observed a liver stiffness improvement one and two years after the end of DAA treatment. In the same way, fibrosis scores APRI and FIB-4 significantly decreased after treatment. Previous studies on interferon therapies have demonstrated that liver fibrosis may regress in a large percentage of HCV patients once SVR is achieved (8). Moreover, recent studies showed a liver stiffness improvement also after new DAA treatment (9, 41, 42). These studies demonstrated a decrease of liver stiffness for all stages of fibrosis. We showed improvement of TE results

particularly in patients with advanced fibrosis stages. Various non-invasive imaging methods for evaluating liver fibrosis including liver stiffness evaluation have shown good correlation with the stage of fibrosis observed on a biopsy. However, liver stiffness may be affected by factors others than fibrosis as, for example, inflammatory activity (43, 44). Tachi et al. suggested that the early reduction in liver stiffness evaluation with elastography performed 24 weeks after treatment might due to the resolution of liver inflammation rather than to true fibrosis regression and is indeed related to the presence of moderate or severe liver inflammation before treatment (41).

It must be outlined that our study suffers from several limitations : the relative low number of patients, the low number of events (HCC), the limited time of follow-up, the absence of paired liver biopsy and the lack of control group.

In conclusion, DAAs do not seem to be associated with HCC promotion after HCV eradication in patients with severe fibrosis or cirrhosis stages (F3 and F4 Metavir scores). We confirmed that DAA-induced SVR is associated with a reduction of liver stiffness even in severe fibrosis stages. Nevertheless, HCC surveillance should be continued after eradication of HCV in patients with severe liver fibrosis since HCV eradication is not associated with HCC disappearance. Future large scale studies might provide refined guidelines for HCC surveillance after HCV eradication and fibrosis regression.

Authors contributions

Coralie Hamoir : data collection, data analysis, data curation, writing (original draft, review and editing)

Yves Horsmans : conceptualization, methodology, patients' care, data analysis, supervision, writing (review and editing)

Peter Stärkel : conceptualization, patients' care, writing (review and editing)

Géraldine Dahlqvist : patients' care, writing (review)

Sergio Negrin Dastis : conceptualization, patients' care, writing (review)

Nicolas Lanthier : conceptualization, methodology, ethics committee document preparation and submission, patients' care, data analysis, validation, supervision, writing (original draft, review and editing).

Disclosures

YH received financial support from AbbVie, MSD and Gilead Sciences industries. NL received consultancy fees and grant support from Gilead and travel grants from AbbVie, Gilead and MSD. PS received grant support and consultancy fees from Gilead and AbbVie.

References

1. MOHD HANAFIAH K., GROEGER J., FLAXMAN A. D., WIERSMA S. T. Global epidemiology of hepatitis C virus infection : new estimates of age-specific antibody to HCV seroprevalence. *Hepatology*, 2013, **57** : 1333-1342.

2. FRANQUE S., LANTHIER N., VERBEKE L., REYNAERT H., VAN STEENKISTE C., VONGHIA L. et al. The Belgian Association for Study of the Liver Guidance Document on the Management of Adult and Paediatric Non-Alcoholic Fatty Liver Disease. *Acta Gastroenterol. Belg.*, 2018, **81** : 55-81.
3. LANTHIER N., FRANQUE S. NASH : a welfare disease with emerging questions and adequate answer attempts. *Acta Gastroenterol. Belg.*, 2020, **83** : 339.
4. MUYLDERMANS G., BIELEN R., BOTTERMAN R., BOURGEOIS S., COLLE L., DERESSA B. et al. Hepatitis C virus (HCV) prevalence estimation in the adult general population in Belgium : a meta-analysis. *Acta Gastroenterol. Belg.*, 2019, **82** : 479-485.
5. WESTBROOK R. H., DUSHEIKO G. Natural history of hepatitis C. *J. Hepatol.*, 2014, **61** : S58-68.
6. REIG M., MARINO Z., PERELLO C., INARRAIRAEGUI M., RIBEIRO A., LENS S. et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J. Hepatol.*, 2016, **65** : 719-726.
7. VIRLOGEUX V., PRADAT P., HARTIG-LAVIE K., BAILLY F., MAYNARD M., OZIEL G. et al. Direct-acting antiviral therapy decreases hepatocellular carcinoma recurrence rate in cirrhotic patients with chronic hepatitis C. *Liver Int.*, 2017, **37** : 1122-1127.
8. SHIRATORI Y., IMAZEKI F., MORIYAMA M., YANO M., ARAKAWA Y., YOKOSUKA O. et al. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann. Intern. Med.*, 2000, **132** : 517-524.
9. BACHOFNER J. A., VALLI P. V., KROGER A., BERGAMIN I., KUNZLER P., BASERGA A. et al. Direct antiviral agent treatment of chronic hepatitis C results in rapid regression of transient elastography and fibrosis markers fibrosis-4 score and aspartate aminotransferase-platelet ratio index. *Liver Int.*, 2017, **37** : 369-376.
10. FOSTER G. R., IRVING W. L., CHEUNG M. C., WALKER A. J., HUDSON B. E., VERMA S. et al. Impact of direct acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis. *J. Hepatol.*, 2016, **64** : 1224-1231.
11. CONTI F., BUONFIGLIOLI F., SCUTERI A., CRESPI C., BOLONDI L., CARACENI P. et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J. Hepatol.*, 2016, **65** : 727-733.
12. YANG J. D., AQEL B. A., PUNGPAPONG S., GORES G. J., ROBERTS L. R., LEISE M. D. Direct acting antiviral therapy and tumor recurrence after liver transplantation for hepatitis C-associated hepatocellular carcinoma. *J. Hepatol.*, 2016, **65** : 859-860.
13. RAVI S., AXLEY P., JONES D., KODALI S., SIMPSON H., MCGUIRE B. M. et al. Unusually High Rates of Hepatocellular Carcinoma After Treatment With Direct-Acting Antiviral Therapy for Hepatitis C Related Cirrhosis. *Gastroenterology*, 2017, **152** : 911-912.
14. KOZBIAL K., MOSER S., SCHWARZER R., LAFERL H., AL-ZOAIRY R., STAUBER R. et al. Unexpected high incidence of hepatocellular carcinoma in cirrhotic patients with sustained virologic response following interferon-free direct-acting antiviral treatment. *J. Hepatol.*, 2016, **65** : 856-858.
15. CARDOSO H., VALE A. M., RODRIGUES S., GONCALVES R., ALBUQUERQUE A., PEREIRA P. et al. High incidence of hepatocellular carcinoma following successful interferon-free antiviral therapy for hepatitis C associated cirrhosis. *J. Hepatol.*, 2016, **65** : 1070-1071.
16. POL S. et al. Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma : Data from three ANRS cohorts. *J. Hepatol.*, 2016, **65** : 734-740.
17. TORRES H. A., VAUTHEY J. N., ECONOMIDES M. P., MAHALE P., KASEB A. Hepatocellular carcinoma recurrence after treatment with direct-acting antivirals : First, do no harm by withdrawing treatment. *J. Hepatol.*, 2016, **65** : 862-864.
18. ZAVAGLIA C., OKOLICSANYI S., CESARINI L., MAZZARELLI C., PONTECORVI V., CIACCIO A. et al. Is the risk of neoplastic recurrence increased after prescribing direct-acting antivirals for HCV patients whose HCC was previously cured? *J. Hepatol.*, 2017, **66** : 236-237.
19. KOBAYASHI M., SUZUKI F., FUJIYAMA S., KAWAMURA Y., SEZAKI H., HOSAKA T. et al. Sustained virologic response by direct antiviral agents reduces the incidence of hepatocellular carcinoma in patients with HCV infection. *J. Med. Virol.*, 2017, **89** : 476-483.
20. HUANG C. F., YEH M. L., HUANG C. I., LIN Y. J., TSAI P. C., LIN Z. Y. et al. Risk of hepatitis C virus related hepatocellular carcinoma between subjects with spontaneous and treatment-induced viral clearance. *Oncotarget*, 2017, **8** : 43925-43933.
21. BIELEN R., MORENO C., VAN VLIJBERGHE H., BOURGEOIS S., MULKAY J. P., VANWOLLEGHEM T. et al. The risk of early occurrence and recurrence of hepatocellular carcinoma in hepatitis C-infected patients treated with direct-acting antivirals with and without pegylated interferon : A Belgian experience. *J. Viral. Hepat.*, 2017.
22. KANWAL F., KRAMER J., ASCH S. M., CHAYANUPATKUL M., CAO Y., EL-SERAG H. B. Risk of Hepatocellular Cancer in HCV Patients Treated With Direct Acting Antiviral Agents. *Gastroenterology*, 2017.

23. CHEUNG M. C., WALKER A. J., HUDSON B. E., VERMA S., MCLAUCHLAN J., MUTIMER D. J. *et al.* Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis. *J. Hepatol.*, 2016, **65** : 741-747.
24. MINAMI T., TATEISHI R., NAKAGOMI R., FUJIWARA N., SATO M., ENOOKU K. *et al.* The impact of direct-acting antivirals on early tumor recurrence after radiofrequency ablation in hepatitis C-related hepatocellular carcinoma. *J. Hepatol.*, 2016, **65** : 1272-1273.
25. IOANNOU G. N., GREEN P. K., BERRY K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J. Hepatol.*, 2017.
26. CALVARUSO V., CABIBBO G., CACCIOLA I., PETTA S., MADONIA S., BELLIA A. *et al.* Incidence of Hepatocellular Carcinoma in Patients With HCV-Associated Cirrhosis Treated With Direct-Acting Antiviral Agents. *Gastroenterology*, 2018, **155** : 411-421 e414.
27. BOURGEOIS S., MULKAY J. P., LASSER L., ROBAEYS G., BASTENS B., DELWAIDE J. *et al.* A multicentre, observational study on demographic and disease characteristics of patients seeking care for chronic hepatitis C in Belgium in 2016. *Acta Gastroenterol. Belg.*, 2019, **82** : 43-52.
28. MYERS R. P., POLLETT A., KIRSCH R., POMIER-LAYRARGUES G., BEATON M., LEVSTIK M. *et al.* Controlled Attenuation Parameter (CAP) : a noninvasive method for the detection of hepatic steatosis based on transient elastography. *Liver Int.*, 2012, **32** : 902-910.
29. KWONG A. J., KIM W. R., FLEMMING J. A. De Novo Hepatocellular Carcinoma Among Liver Transplant Registrants in the Direct Acting Antiviral Era. *Hepatology*, 2018, **68** : 1288-1297.
30. VAZIRI A., GIMSON A., AGARWAL K., ALDERSLEY M., BATHGATE A., MACDONALD D. *et al.* Liver transplant listing for hepatitis C-associated cirrhosis and hepatocellular carcinoma has fallen in the United Kingdom since the introduction of direct-acting antiviral therapy. *J. Viral Hepat.*, 2018.
31. WAZIRY R., HAJARIZADEH B., GREBELY J., AMIN J., LAW M., DANTA M. *et al.* Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy : A systematic review, meta-analyses, and meta-regression. *J. Hepatol.*, 2017.
32. LEE M. H., YANG H. I., LU S. N., JEN C. L., YOU S. L., WANG L. Y. *et al.* Hepatitis C virus genotype 1b increases cumulative lifetime risk of hepatocellular carcinoma. *Int. J. Cancer*, 2014, **135** : 1119-1126.
33. KANWAL F., KRAMER J. R., ILYAS J., DUAN Z., EL-SERAG H. B. HCV genotype 3 is associated with an increased risk of cirrhosis and hepatocellular cancer in a national sample of U.S. Veterans with HCV. *Hepatology*, 2014, **60** : 98-105.
34. LEE M. H., HSIAO T. I., SUBRAMANIAM S. R., LE A. K., VU V. D., TRINH H. N. *et al.* HCV Genotype 6 Increased the Risk for Hepatocellular Carcinoma Among Asian Patients With Liver Cirrhosis. *Am. J. Gastroenterol.*, 2017, **112** : 1111-1119.
35. WU J. M., SKILL N. J., MALUCCIO M. A. Evidence of aberrant lipid metabolism in hepatitis C and hepatocellular carcinoma. *HPB (Oxford)*, 2010, **12** : 625-636.
36. TAKUMA Y., NOUSO K., MAKINO Y., SAITO S., TAKAYAMA H., TAKAHARA M. *et al.* Hepatic steatosis correlates with the postoperative recurrence of hepatitis C virus-associated hepatocellular carcinoma. *Liver Int.*, 2007, **27** : 620-626.
37. PEKOW J. R., BHAN A. K., ZHENG H., CHUNG R. T. Hepatic steatosis is associated with increased frequency of hepatocellular carcinoma in patients with hepatitis C-related cirrhosis. *Cancer*, 2007, **109** : 2490-2496.
38. LONARDO A., ADINOLFI L. E., RESTIVO L., BALLESTRI S., ROMAGNOLI D., BALDELLI E. *et al.* Pathogenesis and significance of hepatitis C virus steatosis : an update on survival strategy of a successful pathogen. *World J. Gastroenterol.*, 2014, **20** : 7089-7103.
39. CLAREMBAU F., BALE G., LANTHIER N. Cirrhosis and insulin resistance : current knowledge, pathophysiological mechanisms, complications and potential treatments. *Clin. Sci. (Lond)*, 2020, **134** : 2117-2135.
40. EL-SERAG H. B., KRAMER J., DUAN Z., KANWAL F. Racial differences in the progression to cirrhosis and hepatocellular carcinoma in HCV-infected veterans. *Am. J. Gastroenterol.*, 2014, **109** : 1427-1435.
41. TACHI Y., HIRAI T., KOJIMA Y., ISHIZU Y., HONDA T., KUZUYA T. *et al.* Liver stiffness reduction correlates with histological characteristics of hepatitis C patients with sustained virological response. *Liver Int.*, 2017.
42. OGASAWARA N., KOBAYASHI M., AKUTA N., KOMINAMI Y., FUJIYAMA S., KAWAMURA Y. *et al.* Serial changes in liver stiffness and controlled attenuation parameter following direct-acting antiviral therapy against hepatitis C virus genotype 1b. *J. Med. Virol.*, 2017.
43. LANTHIER N., STARKEL P., HORSMANS Y. New imaging assisted methods for liver fibrosis quantification : Is it really favorable to classical transient elastography? *J. Hepatol.*, 2015, **63** : 765-766.
44. MACALUSO F. S., MAIDA M., CAMMA C., CABIBBO G., CABIBI D., ALDUINO R. *et al.* Steatosis affects the performance of liver stiffness measurement for fibrosis assessment in patients with genotype 1 chronic hepatitis C. *J. Hepatol.*, 2014, **61** : 523-529.