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Time association between hepatitis C therapy and hepatocellular carcinoma emergence in cirrhosis: Relevance of non-characterized nodules

Graphical abstract

Highlights

- The risk of HCC in patients with HCV cirrhosis treated with DAAs persists despite viral cure.
- The presence of indeterminate nodules before starting DAA is associated with a 3 times greater risk of HCC.
- A time association between DAA therapy and developing HCC reflects increased short-term HCC risk.
- DAA therapy elicits a mechanism that primes the emergence of HCC early during follow-up.

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Lay summary

In this cohort of cirrhotic patients, interferon-free therapies achieved a high rate of sustained virologic response (>95%); however, we reported a risk of *de novo* hepatocellular carcinoma of 3.73 per 100 person-years and a clear-cut time association with antiviral therapy. The time association between starting direct-acting antivirals and developing hepatocellular carcinoma, together with the association with the presence of non-characterized nodules at baseline ultrasound, suggests that antiviral therapy elicits a mechanism (probably immune-related) that primes the growth and clinical recognition of hepatocellular carcinoma early during follow-up. As a result, short-term liver cancer risk is significantly increased.

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Time association between hepatitis C therapy and hepatocellular carcinoma emergence in cirrhosis: Relevance of non-characterized nodules

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Background & Aims: Despite direct-acting antivirals being highly effective at eradicating hepatitis C virus infection, their impact on the development of hepatocellular carcinoma (HCC) remains controversial. We analyzed the clinical and radiological outcome of cirrhotic patients treated with interferon-free regimens to estimate the risk of developing HCC.

Methods: This was a retrospective multicenter study focusing on cirrhotic patients treated with direct-acting antivirals until December 2016. Clinical and radiologic characteristics were collected before the start of antiviral therapy, at follow-up and at HCC development. Diagnosis of HCC was centrally validated and its incidence was expressed as HCC/100 person-years.

Results: A total of 1,123 patients were included (60.6% males, 83.8% Child-Pugh A) and 95.2% achieved a sustained virologic response. Median time of follow-up was 19.6 months. Seventy-two patients developed HCC within a median of 10.3 months after starting antiviral treatment. HCC incidence was 3.73 HCC/100 person-years (95% CI 2.96–4.70). Baseline liver function, alcohol intake and hepatic decompensation were associated with a higher risk of HCC. The relative risk was significantly increased in patients with non-characterized nodules at baseline 2.83 (95% CI 1.55–5.16) *vs.* absence of non-characterized nodules. When excluding these patients, the risk remained increased.

Conclusion: These data expose a clear-cut time association between interferon-free treatment and HCC. The mechanisms involved in the increased risk of HCC emergence in the short term require further investigation.

Lay summary: In this cohort of cirrhotic patients, interferon-free therapies achieved a high rate of sustained virologic response (>95%); however, we reported a risk of *de novo* hepatocellular carcinoma of 3.73 per 100 person-years and a clear-cut time association with antiviral therapy. The time association between starting direct-acting antivirals and developing hepatocellular carcinoma, together with the association with the presence of non-characterized nodules at baseline ultrasound, suggests that antiviral therapy elicits a mechanism (probably immune-related) that primes the growth and clinical recognition of hepatocellular carcinoma early during follow-up. As a result, short-term liver cancer risk is significantly increased.

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Keywords: *De novo* hepatocellular carcinoma; Incidence; HCV; Direct-acting antivirals; Cirrhosis.
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Introduction

Elimination of hepatitis C virus (HCV) infection is now a feasible goal through the availability of oral direct-acting antivirals (DAAs).[1] Treatment is safe and it achieves a high rate of sustained

virologic response (SVR) both in cirrhotic and non-cirrhotic patients.[1] Progressive liver function impairment is halted in patients that achieve SVR and long-term follow-up studies should define the impact of successful treatment on the incidence of hepatocellular carcinoma (HCC). Malignant transformation occurs years before clinical recognition and thus, even if the viral infection is solved, the progression of transformed clones may still take place and ultimately, result in clinically detectable HCC.[2–5] Hence, the elimination of viral infection should prevent new oncogenic events and HCC incidence is likely to decrease after several years. However, the current controversial issue is not whether HCC incidence will vanish in the long-term, but whether there is a time-associated peak in HCC incidence after DAA therapy.[6] Such a peak would mimic the increased HCC recurrence associated with DAAs that was reported some time ago and is still a matter of debate.[7–12] The suggested mechanism for an increased recurrence risk was an imbalance in immune surveillance related to a sudden change in hepatic inflammation, which may also occur in patients without a prior diagnosis of HCC.[11,13–16] The absence of randomized controlled trials prevents a direct comparison between treated and untreated patients. Thus, any insight into this issue should come from careful description of HCC development in large cohorts of patients treated with DAA.[11] Comparison should be attempted with existing data of almost contemporary cohorts of untreated patients. Any comparisons must consider that impaired liver function does not impede DAA therapy, as was the case for interferon-based regimens. In that sense, the prospective HALT-C[17] and EPIC trials[18] reported the HCC risk in 2 well-followed homogeneous cohorts of untreated HCV-viremic patients with compensated cirrhosis, in which HCC development was the main endpoint. The prospective CIRVIR study in France has also provided the follow-up data in a population of HCV cirrhotic patients undergoing HCC screening by ultrasound (US).[19,20] Hence, they offer valuable comparative information. Unfortunately, none of the registration studies assessing the efficacy and safety of DAAs included prospective US examination to detect HCC and follow-up was too short in some studies.[11] Therefore, they are not useful to attempt any comparison.

Our aim was to estimate the risk of HCC in a cohort of cirrhotic patients treated with DAAs, followed over a long period of time and to shed light on the unanswered question of whether DAA-based antiviral treatment of hepatitis C changes the short-term risk for *de novo* HCC.

Patients and methods

This is a retrospective, observational, multicenter study from 9 tertiary reference hospitals in Spain. All consecutive cirrhotic patients of the different hospitals starting an all-oral interferon (IFN)-free regimen before 30 October 2015 were considered. Cases were recruited in each hospital registry and were centralized in Hospital Clinic. The end of follow-up was set as 31 December 2016.

The inclusion criteria, patient characterization, hepatitis C treatment and ethics are described in the supplementary material.

Clinical assessment of evolutionary events and follow-up

Patients were followed until 31 December 2016 or until the date of one of the following evolutionary events: death, liver transplantation or loss to follow-up. Patients' status was established as alive or dead at the end of follow-up. HCC occurrence was the main outcome of the study; liver cancer characterization is described in the supplementary material and methods section.

Radiology and pathology evaluation

The presence of liver cancer was ruled out by abdominal imaging assessment in all patients before entering the study. The detection of benign entities or non-characterized nodules together with their size and pattern were registered. Non-characterized nodules were defined as nodules ≤10 mm or nodules >10 mm but in which HCC diagnosis was ruled out before starting DAA by contrast-enhanced ultrasound, computed tomography, magnetic resonance or biopsy.

Baseline nodules and those appearing during follow-up were evaluated in each center following their clinical practice and ultimately sent to Hospital Clinic for a central evaluation (AD, EB, CA). The imaging dates, type of images and the description of liver nodules at each time-point of the radiologic evaluation were registered.

Radiologic assessment was divided into baseline (pre-DAA images [pre-DAAi]) and follow-up (post-DAA images [post-DAAi]) images. For those patients who did not develop liver nodules after starting DAA, we registered the first post-DAAi (defined as the first imaging study performed after DAA initiation) and the last post-DAAi (defined as the last radiologic evaluation at the time of the database lock) ruling out the presence of nodules. For those patients who developed liver nodules after starting DAA, the first and successive post-DAAi to assess tumor progression or response were registered. Two pathologists with more than 10 years' experience in HCC (AD, MS) performed the central pathology evaluation of biopsies. Central radiologists and pathologists were blinded to the patient clinical evolution.

Time points for the analysis and the methods for liver cancer characterization are described in the supplementary material.

Statistics

Statistical analysis was performed by 2 statisticians (VS, JR). Quantitative variables were expressed as median and interquartile range [IQR 25th-75th percentiles]; in case of follow-up, median and IQR were estimated by Kaplan-Meier method and December 31st 2016 was defined as censoring date in case of absence of date for loss to follow-up or end of follow-up. Categorical variables were described as absolute frequencies and percentages (%). Main analyses, estimation of incidence of HCC, and their 95% CIs were performed using Poisson models, unadjusted in order to establish a crude estimation of HCC incidence, and including potential clinical factor modifiers of this incidence in order to obtain an adjusted estimation of incidence of HCC. These models were also performed for comparing subgroups according to external criteria described in HALT-C[17] and EPIC[18] studies. These estimations of predicted individual incidence rates are expressed as HCC/100 person-years (HCC/100 PY). Evaluation of changes in estimation of risk of HCC incidence compared by factors are also shown graphically. They depict the magnitude of the incidences and their 95% CIs accompanied by the estimation of relative risk (RR) and its 95% CI. Additionally, expected cumulative probability functions were obtained from estimated values of incidence rate obtained from unadjusted models previously established. Other comparisons between groups (presence of HCC) for quantitative or ordinal variables were assessed by Mann-Whitney *U* test. Fisher's exact test was used to compare categorical variables. p values ≤0.05

were considered statistically significant and all calculations were performed with SAS software, version 9.4. Due to the observational pragmatic nature and characteristics of this study, no formal sample size calculation was performed and no methods for multiplicity were used.

For further details regarding the materials and methods used, please refer to the Supplementary information.

Results

During the inclusion period, 1,825 HCV-infected patients with cirrhosis were treated with all-oral DAAs. Referral bias was avoided by the exclusion of 22 patients with *de novo* HCC who received DAA therapy in other centers. A total number of 1,250 met the inclusion/exclusion criteria and were considered for the study in the sites. After central review, 1,123 cirrhotic patients were finally included (Fig. S1). Patients with more than 8 months (6 months ± 2 months) between pre-DAAi and the first post-DAAi were considered as "*patients without adherence to adequate HCC screening*" and represented 26.5% of the cohort (n = 297). Four patients did not have follow-up imaging after starting DAA treatment because of liver transplantation (n = 3) or death (n = 1) (Fig. 1).

Baseline characteristics

The baseline cohort characteristics are depicted in Table 1. Cirrhosis diagnosis was mainly based on the presence of portal hypertension or radiological signs of cirrhosis (48.7%), previous liver decompensation (21,1%) or LSM ≥14 KPa (26.7%). Most patients were male (60.5%), with a median age of 59.3 years (IQR 52.4–68.4) and 941 patients (83.8%) were classified as Child-Pugh A. LSM was performed in 81.9% of the cohort; median LSM was 21.3 KPa (IQR 16.1–30.9). HVPG measurement was performed in 25.7% of the patients, and the median value was 14 mmHg (IQR 10.5–18); 71.5% of the patients (n = 803) had CSPH at baseline.

Baseline imaging (pre-DAAi) did not identify nodules in 995 patients (89% of the cohort). In the remaining 128 cases, 4% (n = 48) presented benign nodules and 7% (n = 80) presented non-characterized nodules (Table 1 and Tables S1, S3).

Follow-up times and evolutionary events

The median clinical follow-up from DAA initiation to end of follow-up was 19.6 months (IQR 16.8–23.3). The median radiologic follow-up (from pre-DAAi to the last available post-DAAi or HCC development) was 12.33 months (IQR 9.04–15.94). During that time, 72 patients (6.4%) developed HCC and 31 patients (2.7%) died (Fig. 1).

The median time between the pre-DAAi and the date of DAA initiation was 2.57 (IQR 1.24–4.1) months; 8.9% of patients (n = 100) had a pre-DAAi performed more than 6 months before starting DAA (Table 1).

The median time between pre-DAAi and the first screening post-DAAi was 6.6 (IQR 5.82–8.16) months.

Fig. 1. Flow-chart of the included patients. This figure describes the flow-chart of the all patients consider for this study. DAA, direct-acting antivirals; HCC, hepatocellular carcinoma; LT, liver transplantation.

Table 1. Baseline and antiviral therapy characteristics of the cohort (n = 1,123). *Available in 920 patients; **Available in 289 patients; ***Available in 1,006 patients; ¥CSPH: considered if HVPG ≥10 mmHg, LSM ≥21 KPa, previous liver decompensation or presence of esophageal varices at baseline; ^Referred to patients receiving SOF plus RBV. †(See additional data in Table S1).

Variables	Values
Age (years)	59.3 (52.4–68.4)
Gender (male)	680 (60.55)
Alcohol (yes)	243 (21.75)
Diabetes mellitus (Yes)	212 (18.89)
Viral co-infection (HBV/HIV)	11 (1.02)/47 (5.23)
Diagnosis of cirrhosis	
LSM (≥14 kPa)	300 (26,7)
Signs of cirrhosis/portal hypertension	547 (48.7)
Liver decompensation	237 (21.1)
Liver biopsy	39 (3,5)
LSM (kPa)*	21.3 (16.1–30.9)
LSM ≥ 21 kPa (%)	487 (43.37)
HVPG**	14 (10.5–18)
Gastroesophageal varices***	550 (49)
Child-Pugh (A/B/C)	941 (83.79)/161 (14.34)/21 (1.87)
MELD score	7 (6–9)
CSPH¥	803 (71.5)
Viral genotype 1	894 (79.6)
Viral load HCV RNA (log_{10}) (IU/ml)	6.08 (5.62–6.44)
Previous non-responders to IFN therapy	593 (52.8)
ALT (IU/L)	77 (46–115)
Bilirubin (mg/dl)	1 (0.7–1.5)
Albumin (g/L)	40 (36–43)
Platelet count ($\times 10^9$)	106 (73–148)
INR	1.12 (1.04–1.24)
DAA therapy	
SOF/LDV	322 (28.67)
OBV/PTV/r ± DBV	249 (22.17)
SOF^	42 (3.74)
SOF/SMV	311 (27.69)
SOF/DCV	154 (13.71)
SMV/DCV	20 (1.78)
Others	25 (2.23)
Use of RBV	851 (75.78)
Time from PreDAAi to DAA initiation (months)	2.57 (1.24–4.1)
Time PreDAAi-DAA > 6 months	100 (8.9%)
PreDAAi data	
No nodules	995 (89%)
Benign nodules	48 (4%)
Non-characterized nodules	80 (7%)

CSPH, clinically significant portal hypertension; DAAs, direct-acting antivirals; DBV, dasabuvir; DCV, daclatasvir; HBV, hepatitis B virus; HIV, human immunodeficiency virus; HVPG, hepatic venous pressure gradient; IFN, interferon; INR, international normalized ratio; LDV, ledipasvir; LSM, liver stiffness measurement; MELD, model for end-stage liver disease; OBV/PTV/r, ombitasvir/paritaprevir/ritonavir; Pre-DAAi, radiologic image pre-DAA; RBV, ribavirin; SMV, simeprevir; SOF, sofosbuvir.

Antiviral treatment and virologic response

Most patients (65.1%) received a 12-week DAA regimen and 851 patients (75.8%) received concomitant ribavirin. SVR was achieved in 95.2% of cases (n = 1,070). Non-response (4.8%) was classified as virologic failure in 40 cases, 3 patients discontinued treatment early due to adverse events and 8 patients were lost to follow-up. Treatment failure was significantly more frequent in patients receiving initial suboptimal regimens (*i.e.* faldaprevir/deleobuvir or simeprevir/daclatasvir) or in those patients with more advanced liver disease.[1] SVR in Child-Pugh B/C was 90.5% *vs.* 97% in Child-Pugh A patients.

Assessment of HCC risk in DAA-treated patients: estimated incidences

In order to evaluate HCC risk and to avoid bias due to heterogeneous adherence to the HCC screening, the HCC risk assessment was done in 4 steps:

HCC incidence regardless of the time of pre-DAAi and/or adherence to HCC screening

Crude estimation was 3.73 HCC/100 PY (95% CI 2.96–4.70) in the whole cohort. Estimation by Child-Pugh was 2.93 HCC/100 PY in CP-A (95% CI 2.20–3.90), 7.81 HCC/100 PY in CP-B (95% CI 5.19–11.76) and 5.73 HCC/100 PY (95% CI 2.42–4.65) in the limited cohort of 23 CP-C patients. The estimated HCC incidence differed according to the pre-DAAi information: the RR of developing HCC was 2.83 (95% CI 1.55–5.16) in patients with non-characterized nodules compared to patients without nodules and 0.36 (0.05–2.62) in those patients with benign nodules compared to those with no nodules (Fig. 2).

In CP-A patients, this incidence was 1.46 (95% CI 0.21–10.36), 2.67 (95% CI 1.94–3.67) and 7.24 (95% CI 3.62–14.48) HCC/100 PY in patients with benign nodules, no nodules or non-characterized nodules, respectively.

HCC incidence in patients with adequate adherence to HCC screening

Crude estimation in those patients who were under HCC screening (n = 826) was 3.83 HCC/100 PY (95% CI 2.92–5.03) (Fig. 3). Estimations by Child-Pugh were 2.93 HCC/100 PY in CP-A (95% CI 2.09–4.13), 8.14 HCC/100 PY in CP-B (95% CI 5.06–13.09) and 8.82 HCC/100 PY (95% CI 2.21–35.28) in the limited cohort of just 2 CP-C patients. The estimated HCC incidence differed according to the pre-DAAi information. The RR of developing HCC was 2.93 (95% CI 1.50–5.72; $p = 0.0016$) between patients without nodules and patients with non-characterized nodules, and 0.52 (95% CI 0.07–3.77) between patients with no nodules and those with benign nodules.

Fig. 2. Incidence and relative risk of developing HCC in the whole cohort and according to the imaging information before starting direct-acting antivirals. This figure describes the incidence and RR of developing HCC in the whole cohort and according to the imaging information before starting direct-acting antivirals. HCC, hepatocellular carcinoma; PY, person-years; RR, relative risk.

Fig. 3. Incidence according to the adherence to HCC screening program. This figure describes the incidence and RR of developing HCC in those patients who were under HCC screening program and in those who did not be under screening program. HCC, hepatocellular carcinoma; PY, person-years; RR, relative risk.

In CP-A patients (n = 692), the incidence was 2.03 (95% CI 0.29–14.41), 2.63 (95% CI 1.79–3.87) and 6.77 (95% CI 3.04–15.06) HCC/100 PY in patients with benign nodules, no nodules or non-characterized nodules, respectively.

HCC incidence in patients without adequate adherence to HCC screening

Crude estimation in these patients (n = 297) was 3.48 HCC/100 PY (95% CI 2.24–5.39) (Fig. 3). Estimations by Child-Pugh were 2.93 HCC/100 PY in CP-A (95% CI 1.74–4.95), 7.02 HCC/100 PY in CP-B (95% CI 2.16–15.64) and it was not estimated in the 7 CP-C patients due to the absence of HCC events. The estimated HCC incidence differed according to the pre-DAAi information. The RR of developing HCC was 2.38 (95% CI 0.55–10.24) between patients without nodules and patients with non-characterized nodules.

In CP-A patients (n = 249), this risk was 2.75 (95% CI 1.56–4.85) and 9.16 (95% CI 2.29–36.63) HCC/100 PY in patients with no nodules or non-characterized nodules, respectively.

Relative risk of developing HCC adjusted by baseline variables

The RR of developing HCC between Child C *vs.* Child A patients was 1.95 (95% CI 0.47–8.04; $p = 0.3540$) and between Child B *vs.* Child A patients 2.66 (95% CI 1.62–4.39; $p < 0.0001$). Regarding patients with CSPH, RR was 2.11 (95% CI 1.11–4.01; $p = 0.0224$) as compared to those without it. Finally, the RR of HCC in patients with pre-DAA non-characterized nodules was 2.91 (95% CI 1.60–5.31; $p = 0.0005$) compared to those without nodules/benign nodules at that time-point (Table 2).

Table 2. HCC incidence in the cohort (n = 1,123) and according to baseline characteristics.

Variable	HCC (n)	Whole group	Incidence (HCC/100PY)	95% CI	Contrast	RR	95% CI	*p* value
Whole cohort	72	1,123	3.73	2.96–4.70				
Child-Pugh								
A	47	941	2.93	2.20–3.90				
B	23	161	7.81	5.19–11.7	B/A	2.66	1.62–4.39	0.0001
C	2	21	5.73	1.43–22.9	C/A	1.95	0.47–8.04	0.3540
PreDAAi								
Benign or no nodules	59	1,043	3.29	2.55–4.24				
Non-characterized nodules	13	80	9.57	5.56–16.5	Non-characterized/ benign or no nodule	2.91	1.60–5.31	0.0005
LSM (KPa)*								
LSM <21 kPa	14	433	1.92	1.14–3.25				
LSM ≥21 kPa	33	487	3.91	2.78–5.50	LSM ≥21/<21	2.03	1.09–3.80	0.0260
CSPH								
No CSPH	11	320	2.06	1.14–3.73				
CSPH	61	803	4.36	3.39–5.61	CSPH yes/no	2.11	1.11–4.01	0.0224
Diabetes								
No	56	910	3.56	2.74–4.62				
Yes	16	212	4.49	2.75–7.33	DM yes/no	1.26	0.72–2.20	0.4118
Alcohol								
No	47	874	3.11	2.34–4.14				
Yes	25	243	6.10	4.12–9.03	Alcohol yes/no	1.96	1.21–3.19	0.0065
History of decompensation								
No	41	861	2.8	2.06–3.80				
Yes	31	262	6.67	4.69–9.48	Decomp. yes/no	2.38	1.50–3.80	0.0003
History of decompensation and LSM (KPa)**								
No and LSM <13.6	2	77	1.59	0.40–6.34				
No and LSM from 13.6 to ≤21	12	324	2.21	1.26–3.89	No decompensation and LSM (13.6–21)/no decompensation and LSM (<13.6)	1.39	0.31–6.23	0.664
Yes or LSM >21	55	674	4.68	3.59–6.10	Yes (decompensation) and LSM > 21/no decompensation and LSM (<13.6)	2.95	0.72–12.09	0.132

CSPH, clinically significant portal hypertension; DAA, direct-acting antivirals; Decomp, History of Decompensation; HCC, hepatocellular carcinoma; LSM, liver stiffness measurement; pre-DAAi, Pre-DAA images; RR, relative risk. *p* value calculated using the Wald test.

*According to Lens *et al.* Gastroenterology 2017 Nov;153(5):1273–1283.

**According to Llop *et al.* J Hepatol. 2012 Jan;56(1):103–8.

Table 3. HCC incidence in Child-Pugh A patients (n = 941) and according to baseline characteristics.

Variable	HCC (n)	Whole group	Incidence (HCC/100PY)	95% CI		Contrast	RR	95% CI	*p* value
preDAAi									
No nodules	38	837	2.67	1.94	3.67				
Non-characterized	8	63	7.24	3.62	14.48	Benign nodules/ No nodules	0.55	0.08–3.98	0.5512
Benign nodules	1	41	1.46	0.21	10.36	Non-characterized/ No nodules	2.71	1.27–5.81	0.0103
LSM (kpa)*									
LSM <21 KPa	14	417	2.01	1.19	3.39				
LSM ≥21 KPa	21	389	3.13	2.04	4.81	LSM ≥ 21/<21	0.64	0.33–1.26	0.1972
CSPH									
No	11	317	2.08	1.15	3.76				
Yes	36	624	3.35	2.42	4.65	Yes/no	1.61	0.82–3.17	0.1656
Diabetes									
No	37	758	2.86	2.07	3.94				
Yes	10	182	3.27	1.76	6.08	DM yes/no	1.15	0.57–2.30	0.7028
Alcohol									
No	35	748	2.75	1.97	3.82				
Yes	12	187	3.77	2.14	6.64	Alcohol yes/no	1.37	0.71–2.65	0.3417
History of decompensation									
No	37	822	2.64	1.91	3.65				
Yes	10	119	4.96	2.67	9.23	Yes/no	1.88	0.93–3.78	0.0766
History of decompensation and LSM (KPa)**									
No decomp and LSM < 13.6	2	77	1.59	0.40	6.34				
No decomp and LSM from 13 to ≤ 21	12	324	2.21	1.26	3.89	No and LSM (13.6–21)/ no and LSM (<13.6)	1.39	0.31–6.23	0.6640
Yes (decomp) and/or LSM > 21	30	492	3.55	2.48	5.07	Yes and LSM > 21/ no and LSM (<13.6)	2.24	0.53–9.36	0.2706

CSPH, clinically significant portal hypertension; DAA, direct-acting antivirals; Decomp, History of Decompensation; HCC, hepatocellular carcinoma; pre-DAAi, Pre-DAA images; RR, relative risk. *p* value calculated using the Wald test.
*According to Lens *et al.* Gastroenterology 2017 Nov;153(5):1273–1283.
**According to Llop *et al.* J Hepatol. 2012 Jan;56(1):103–8.

The RR when considering CP-A patients only (83.8% of the cohort) is depicted (Table 3). The incidence of HCC in CP-A patients with non-characterized nodules at pre-DAAi was 7.24 HCC/100 PY (95% CI 3.62–14.48), compared with 2.77 HCC/100 PY (95% CI 1.29–5.93; $p = 0.0087$) in CP-A patients without non-characterized or benign nodules at pre-DAAi. Finally, the RR of HCC development was 2.71 (95% CI 1.27–5.81; $p = 0.0103$) between patients with non-characterized nodules at pre-DAAi in comparison with those without nodules and 0.55 (95% CI 0.08–3.98; $p = 0.5512$) between patients without nodules and with benign nodules.

Characteristics of liver nodules during the follow-up

A total of 152 patients developed *de novo* liver nodules that were suspicious of HCC (Fig. 1). HCC diagnosis was confirmed in 72 patients (Table 4), supporting a raw 3.73 HCC/100 PY incidence. Fifty-four of them (75%) were diagnosed according to non-invasive criteria. Thirty-seven liver nodules without specific radiologic profiles were biopsied: 18 were HCC. The remaining nodules (n = 80) corresponded to pancreatic metastasis (n = 1), neuroendocrine tumor (n = 1), lymphoma (n = 1) and non-malignant entities such as dysplastic nodules, regenerative nodules or hamartomas (n = 77). The median times from DAA initiation and from DAA finalization to HCC diagnosis were 10.3 (IQR 5.7–15.9) and 6.6 (IQR 2.2–11.9) months, respectively.

Table 4 presents the main characteristics at diagnosis of the 72 patients with HCC, including the first therapy received. Out of the 72 patients described, 13 had presented with non-characterized nodules on US before starting DAAs and 6 of them developed the HCC in the same non-characterized nodule that were seen before starting DAAs. More than 50% of patients were classified as BCLC 0/A, of whom 59.2% received potentially curative treatments. However, after a median follow-up of 4.13 (IQR 3.19–8.00) months, 21% (n = 15) of these patients developed HCC recurrence/progression and 2 patients presented spontaneous HCC regression.

Fourteen patients died within the following 6.68 (IQR 3.15–9.69) months after HCC diagnosis. At the time of DAA initiation, 7 of them were classified as CP-A, 6 as CP-B and 1 as CP-C. At the time of first recurrence 42.9% were BCLC-A, 18.8% B, 6.1% C and 28.6% BCLC-D.

Risk of HCC according to DAA virologic response

The HCC rate was 15.67 HCC/100 PY in non-SVR patients (4.8%) *vs.* 3.06 HCC/100 PY in patients achieving an SVR (95.2%) (Tables S4). A direct follow-up comparison between these 2 groups is not valid due to the confounding effect of the different baseline characteristics at the time of DAA treatment (37.7% CP-B/C patients *vs.* 15.1 % in non-SVR *vs.* SVR patients, respectively).

Mortality

Thirty-one patients (13 CP-A, 17 CP-B and 1 CP-C) died during follow-up (Fig. 1): 1.60 deaths/100 PY (95% CI 1.13–2.28). The median time from DAA initiation and DAA finalization to death was 14.99 (IQR 7.68–19.16) and 11.94 (IQR 4.13–15.77) months, respectively. The main cause of death was liver decompensation/sepsis in 12 patients (6 of them with concomitant HCC); in the remaining 19 patients, the registered causes were: HCC progression (n = 8), intestinal ischemia (n = 2), liver decompensation/no sepsis (n = 1), pancreatic cancer (n = 1), aortic

Table 4. HCC and patient characteristics (n = 72) at the time of diagnosis, including first treatment received.*

Variable	Categories	
Pathology (n = 18)		
	Well differentiated	9 (16.98%)
	Moderately differentiated	7 (13.21%)
	Poorly differentiated	2 (3.77%)
Child-Pugh		
	A	52 (73.24%)
	B	14 (19.72%)
	C	5 (7.04%)
ECOG PS		
	0	60 (84.50%)
	1	5 (7.04%)
	≥2	6 (8.45%)
Clinical ascites		
	No	57 (80.28%)
	Yes	14 (19.72%)
Use of diuretics		
	No	49 (69.01%)
	Yes	22 (30.99%)
Encephalopathy		
	No	67 (94.37%)
	Yes	4 (5.63%)
BCLC		
	0	20 (28.17%)
	A	32 (45.07%)
	B	5 (7.04%)
	C	3 (4.23%)
	D**	11 (15.49%)
AFP, ng/dl* **		
	Median (IQR) [max–min]	7.5 (4.6–37.8) [1.2–18,791]
First HCC treatment received		
	Spontaneous regression	2 (2.81%)
	Follow-up****	1 (1.40%)
	Liver transplantation waiting list	1 (1.40%)
	Liver transplantation	5 (7.04%)
	Resection	6 (8.50%)
	Percutaneous treatment	30 (42.27%)
	Laparoscopic radiofrequency/ microwave ablation	1 (1.40%)
	TACE	8 (11.26%)
	Sorafenib	3 (4.22%)
	Radioembolization	2 (2.81%)
	Symptomatic	11 (15.49%)
	Clinical trial	1 (1.40%)

AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer classification; ECOG PS; Eastern Cooperative Oncology Group performance status; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization.
*The HCC diagnosis in 1 patient was done in the pathology at the time of liver transplantation.
**The baseline Child-Pugh score of these 11 patients were: 2 Child-Pugh-A, 8 Child-Pugh-B, and 1 Child-Pugh-C.
***Available in 58 out of 72 patients with HCC.
****Due to the small size of the lesion, the center decided to follow-up the patient instead of treating.

dissection (n = 1), valvular heart disease (n = 1) and unknown in 5 patients (none of them presented HCC at the last follow-up evaluation). Death due to HCC represented 45% of the global mortality (14/31). Among patients who developed HCC, tumor progression was the cause of death in (14/72) 19.4% of cases.

The incidence of death according to the baseline CP-score was 0.81 deaths/100 PY (95% CI 0.47–1.40) and 5.47 deaths/100 PY (95% CI 3.44–8.68) in CP-A and CP-B/C, respectively. Four out of the 13 CP-A patients had a history of previous decompensation, 10 presented baseline CSPH and none of them presented non-characterized nodules in pre-DAAi.

Additional information about this cohort is reported in the supplementary material.

HCC incidence in potential candidates to IFN-therapies

A direct comparison between the risk of developing *de novo* HCC between our DAA-treated cohort and the historical IFN-treated cohorts is not valid. However, we estimated the increase of risk, by RR estimation, of developing HCC in patients from our cohort who met the inclusion/exclusion criteria of the HALT-C[17] or the EPIC[18] trials, respectively. These patients were defined as "*patients potentially eligible for IFN-based therapy*". Three hundred ninety-three (35%) of our patients could have been selected for HALT-C, and 563 (50.1%) could have been EPIC candidates. The estimated incidence of developing HCC was 2.53 HCC/100 PY (95% CI 1.57–4.07) for HALT-C candidates and 2.26 HCC/100 PY (95% CI 1.49–3.43) for EPIC candidates. The RR between those patients who did not meet the HALT-C and EPIC criteria *vs.* those who met these criteria in our cohort were 1.70 (95% CI 1.003–2.98) in HALT- C and 2.30 (95% CI 1.40–3.82) EPIC, respectively. Fig. 4 shows the graphical comparison between the empirical accumulative probability in our subcohort of patients who met the HALT-C and EPIC criteria and the empirical accumulative probability of HCC development according to the HALT-C and EPIC data. Fig. 5 shows the sensitivity analysis but only in patients who were *potentially eligible for IFN-based therapy* and did not have nodules in the pre-DAAi (n = 353 in HALT-C and n = 511 in EPIC). In addition, we evaluated the risk in those patients who met the EPIC and HALT-C criteria, but excluded those with a high-risk profile for HCC development, such as those with varices and/or platelet counts and age beyond their cut-off for HCC risk.

Discussion

Our study suggests that there is an increased emergence of HCC early during follow-up in patients with cirrhosis treated with DAA. The peak in new cancer cases during follow-up, which

Fig. 4. Incidence and relative risk of developing HCC in the whole cohort and dividing the cohort according to HALT-C and EPIC criteria. This figure represents the incidence and RR of developing HCC in the whole cohort and also the incidence and RR of developing HCC in those patients from our cohort who meet the HALT-C and EPIC criteria. HCC, hepatocellular carcinoma; PY, person-years; RR, relative risk.

Fig. 5. Empirical cumulative probability function of developing HCC according to the HCV treatment received. Comparison of the empirical CPF of developing HCC between patients potentially eligible for IFN-based therapy but who were treated with DAAs in our cohort and patients potentially eligible for IFN-based therapy according to (A) the data reported in the original manuscript of EPIC and (B) the data reported in the original manuscript of HALT-C. CPF, cumulative probability function; DAA, direct-acting antiviral; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon.

appears to be time-related to DAA therapy, is relevant in patients that were shown to have undetermined nodules prior to treatment initiation. Some of these cases could have corresponded to HCC that was not diagnosed, although this would be an infrequent circumstance as study centers were already involved in studies regarding HCC detection and diagnosis. Thus, in the majority of patients these undetermined nodules most likely correspond to minute low or high-grade dysplastic foci or macro-regenerative nodules in a cirrhotic liver. It could be argued that those who developed HCC and had non-characterized nodules could belong to the strata of patients without proper screening. However, the results do not change when excluding patients without proper screening. Thus, such a flaw is discarded.

At the same time, we show that the risk of HCC development is increased in patients who had already developed portal hypertension. This finding is not novel, as it is well known that the risk of HCC increases with the progression of cirrhosis. However, it is important to put our data in perspective by comparing the observed incidence with that reported in the CIRVIR cohort prior to DAA availability[21] which was 1.9 (95% CI 1.3–2.8) at 1st year follow-up, as well as that reported in the HALT-C[17] and EPIC trials,[18] with the appropriate exclusion of those patients not sharing the inclusion criteria for these studies assessing the impact of long-term IFN administration. The probability of developing HCC in this sub-group of patients was higher than in those patients who were included in EPIC or HALT-C: 2.06 HCC/100 PY (95% CI 1.3–3.27) in patients who met EPIC without varices, platelets ≥150.000/mm^3 and age <58 years-old and 2.02 HCC/100 PY (95% CI 1.12–3.65) in patients who met HALT-C without platelets ≥150.000/mm^3 and age <68 years-old (Fig. 5).

Finally, we also analyzed the risk of HCC development in a cohort of Spanish patients treated with IFN.[22] The incidence of HCC in that cohort was 2.09 HCC/100 PY for the whole cohort, 0.65 HCC/100 PY in the SVR patients and 1.31 in non-SVR patients. Thus, the estimation of HCC/100 PY in our cohort may seem similar, but the incidence in SVR patients is clearly higher in our study. Indeed, the division of HCC risk according to SVR is misleading as risk associated with DAA therapy should not be determined according to the achievement of SVR during follow-up as this success is not known at baseline.

It has been suggested that development of HCC in DAA-treated patients would represent the emergence of already existing clones and would have thus appeared irrespective of treatment and its success. This is surely the case, but the key point is to what extent DAA therapy is associated with an increased rate of HCC emergence early during follow-up after therapy. Our data support this possibility and suggest that some clones are primed to grow and become overt cancers in a minority, but clinically relevant proportion of patients.

Up to now, none of the cohort investigations have been designed to assess the incidence of HCC after DAA therapy. Studies to assess the efficacy of DAA did not have HCC screening in their methodology and/or did not have enough follow-up to secure a minimal time between DAA therapy and US screening.[11,15] Registry studies suffer from the same retrospective and flawed design.[23–27] In most studies, the profile of the population at baseline is not well registered, the follow-up may not include HCC screening and a variable proportion of the population may be lost to follow-up.[28,29] This is a major issue as registration of events may affect those individuals lost to follow-up. All these limitations are frequently dismissed, as it is far easier to uphold an optimistic message about the benefits of a treatment that has represented a major breakthrough, than to raise concerns about potential unexpected adverse events. Viral eradication will have a major impact on public health and control the negative consequences of HCV infection in the community. However, in patients with compensated cirrhosis and normal quality of life, the decision may be more complex, particularly if an US examination in the months prior to treatment has shown the presence of undetermined nodules. Such observation should prompt a delay of treatment while securing a proper diagnosis of the detected nodules. If they are unequivocally diagnosed with HCC, anticancer therapy should be

instituted and the decision to initiate DAA therapy should be delayed to later stages but retaining the concern regarding a potential priming of HCC recurrence. If diagnosis is not established, physicians and patients should take a shared decision according to their individualized assessment.[7,8,10]

The major challenge affects patients with minute, undetermined nodules that may turn into overt HCC during or early after DAA therapy. Data from Italy[30] show that evident nodules classified by biopsy as low or high-grade dysplasia may become HCC during follow-up. Our cohort includes undetermined nodules <10 mm that were not biopsied according to current recommendations.[31] They could correspond to dysplastic nodules or to mere distorted architecture of a cirrhotic liver, and attending physicians did not suspect HCC at all. Despite this difference with the Iavarone *et al.* study,[30] the HCC incidence is higher and this suggests that the emerging tumors were the result of the evolution of dysplastic nodules and of the growth of already existing subclinical dormant clones of transformed hepatocytes. The mechanisms involved in the transition from dysplasia to HCC or the growth of dormant clones are not known but immune surveillance is surely involved.[3,5,32–34] The issue of immune surveillance was already raised when first describing an increased recurrence rate. It fits with the description of hepatitis B virus[35–38] or herpes virus[39] reactivation associated with DAA therapy. Interestingly, 2 of our patients diagnosed with HCC by biopsy presented tumor regression. This further suggests that immune surveillance may have failed initially, but afterwards been recovered and prime regression. However, in most patients this is not observed and despite HCC detection at a potentially treatable stage, the evolution is dismal with tumor recurrence and excessive death rate in a cohort of patients without major liver failure.[11,40]

Undetermined nodules have to be seen as the tip of the iceberg regarding the presence of HCC. The sensibility of US to detect HCC is limited and it misses those minute HCCs that already exist at a microscopic level and may be encountered at explant or necropsy. This is common to all malignancies for which there is a lengthy period of subclinical evolution without image recognition. Initially, we would detect the first wave of HCCs representing those closer to clinical emergence, while later on we would encounter those which take months to become apparent. Ultimately, all clones may emerge and few would vanish because of immune recovery.

Our data are not unique. Cohort studies in France,[20] the UK[26,27] and Italy[8,41] have already exposed a slight increased incidence of HCC early during follow-up. The relative risk of developing HCC after IFN *vs.* DAA in the French cohort was 0.59 [0.07–4.70]. Thus, increased risk is not ruled out and longer follow-up may provide more accuracy. The accurate incidence in the UK cohort is not robust as per intention-to-treat principle. Foster *et al.* reported 5.4% within the first 6 months of starting DAA therapy, whereas Cheung analysis started at the end of treatment and not at DAA initiation. Finally, Romano *et al.*[41] reported an incidence of 0.97 per 100 person-years (95% CI 0.73–1.26) but 24.5% of the patients were F3 and the rest Child-Pugh A (61%) or B (9%). Thus, the incidence appears higher than expected. Several confounders such as the absence of proper HCC screening prior to DAA, advanced liver disease with already known increased HCC risk or existing comorbidities such as alcohol intake, smoking or metabolic syndrome may affect all investigations. Thus, it is key to design prospective investigations where all these confounders are properly assessed. Until such detailed investigations are in place the controversy regarding the association between DAA therapy and HCC detection will not be over. Some may argue that a randomized controlled trial would be the sole approach able to provide a final answer, but this is currently seen as unfeasible and we will have to rely on additional robust observational studies involving a large number of patients.

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Conflicts of interest

ZM: Speaker fees from Abbvie, Gilead, Janssen, MSD; Advisor for Gilead. AD: Nothing to disclose. SL: Speaker fees from Abbvie, Gilead, MSD, Janssen, Advisor for Gilead and Janssen. VS: Nothing to disclose. EB: Nothing to disclose. CP: Nothing to disclose. JLC: Speaker Fee for Abbvie, Gilead science, MSD. Advisor for Abbvie, Gilead science, MSD, Bayer. MV: Speaker fees from Bayer. Consultancy for BMS, Sirtex, BTG and Bayer. MR: Consultant and Speaker fees from Gilead. Speaker fees from MSD and Abbvie. CRdL: Speaker fees from Bayer. SLL: Nothing to disclose. XT: Speaker fees from Abbvie, Gilead and MSD. Advisor for Gilead and Abbvie. AG: Speaker fees from Gilead. Advisor for Bayer and Abbvie. MS: nothing to disclose. MRM: Speaker fees from Gilead, AbbVie, Merck, BMS and Janssen. Advisor for BMS, Merck, Gilead and AbbVie. BM: Speaker fees from Bayer, advisor for Bayer. JLL: Nothing to disclose. SC: Nothing to disclose. JAC: Speaker fees from Abbvie, Gilead and MSD. Advisor for AbbVie. MI: Speaker fees from Bayer. BS: Speaker, advisory or consulting fees from Adaptimmune, Astra Zeneca, Bayer, BMS, BTG, Lilly, Medimmune, MSD, Sirtex and Terumo. RV: Nothing to disclose. MS: Speaker fees from Bayer. CA: Speaker fees from Bayer. JR: Consultancy for Boehringer Ingelheim, Smith & Nephew SAU, FERRER INTERNACIONAL and Novartis. Speaker fees from Boehringer Ingelheim, Novartis, Spherium Biomed, SOLTI, BCNSCIENCE, ROVI and SFJ Pharmaceuticals. JB: Consultancy for Gilead, Abbvie, Kowa, Bayer, BTG, Arqule, Terumo, BMS, Boehringer Ingelheim, Lilly, Kowa, Novartis, OSI, Roche and Onxeo. XF: Unrestricted Grant Support from Abbvie and Gilead. Advisor for Abbvie, and Gilead. MR: Speaker fees from Gilead, BMS, BTG, Lilly and Bayer, advisor for Bayer and BMS.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

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Supplementary data

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Author names in bold designate shared co-first authorship

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