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Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals

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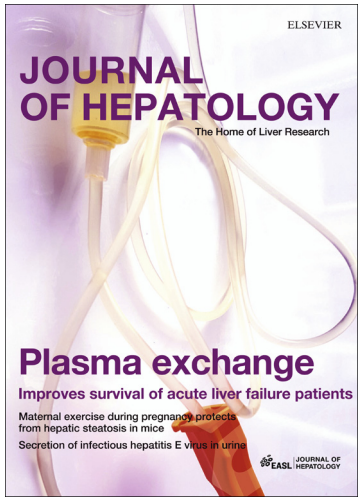

Early Occurrence and Recurrence of Hepatocellular Carcinoma in HCV-related Cirrhosis Treated with Direct Acting Antivirals

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EARLY OCCURRENCE AND RECURRENCE OF HEPATOCELLULAR CARCINOMA IN HCV-RELATED CIRRHOSIS TREATED WITH DIRECT ACTING ANTIVIRALS.

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Keywords: Hepatitis C; Hepatitis C Virus; Direct-Acting Antivirals; Interferon-free therapy; Liver Fibrosis; Cirrhosis; Sustained Virological Response; Hepatocellular Carcinoma; HCC recurrence; HCC occurrence; HCC development; HCC prevention; Cancer immunosurveillance.

Abbreviations: HCC (hepatocellular carcinoma); DAA (direct-acting antivirals); HCV (hepatitis C virus); SVR (sustained virological response); AIFA (Italian Medicines Agency); CEUS (contrast-enhanced ultrasonography); CT (computerized tomography); MRI (magnetic resonance imaging); RFA (radiofrequency ablation); TACE (trans-arterial chemoembolization); PEI (percutaneous ethanol injection); ROC (receiver operating characteristics); kPa (kilo Pascal); MELD (model for end stage liver disease); HBsAg (hepatitis B surface antigen); BMI (body mass index); SVR12 (sustained virological response 12 weeks after treatment completion); SOF (sofosbuvir); SMV (simeprevir); 3D (ombitasvir, paritaprevir with ritonavir, and dasabuvir); RBV (ribavirin); DCV (daclatasvir); LDV (ledipasvir); AFP (α-fetoprotein); BCLC (Barcelona Clinic Liver Cancer).

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LAY SUMMARY

New direct-acting antivirals are able to eradicate HCV infection in over 90% of patients with advanced liver disease. Unfortunately, the occurrence of liver cancer is not reduced in effectively treated cirrhotic patients. In addition, patients previously

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treated for HCC have still a high risk of tumour recurrence in the short term, despite DAA treatment.

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ABSTRACT

Background & Aims: Hepatocellular carcinoma (HCC) represents a serious complication of HCV-related cirrhosis. New direct-acting antivirals (DAA) cure HCV infection in over 90% of patients. Aim of this study was to evaluate the early occurrence and recurrence of HCC in cirrhotic patients treated with DAA.

Methods: We analysed 344 consecutive cirrhotic patients, without HCC, who were treated with DAA, and followed for 24 weeks. Fifty-nine patients had previous HCC.

Results: DAA therapy induced sustained virological response in 91% of patients. During 24-week follow-up, HCC was detected in 26 patients (7.6%, 95% CI: 4.99-10.84): 17 of 59 patients (28.81%, 95% CI: 17.76-42.07) with previous HCC and 9 of 285 patients (3.16%, 95% CI: 1.45-5.90) without previous HCC. Child-Pugh Class B, more severe liver fibrosis, lower platelet count, and previous HCC were significantly associated with HCC development, at univariate analysis. At multivariate analysis, Child Pugh class ($p= 0.03$, OR: 4.18, 95% CI: 1.17-14.8) and history of HCC ($p< 0.0001$, OR: 12.0, 95% CI: 4.02-35.74) resulted independently associated with HCC development. Among the 59 patients with previous HCC, younger age and more severe liver fibrosis were significantly associated with HCC recurrence, both at univariate and at multivariate analysis.

Conclusions: In patients with HCV-related cirrhosis, DAA-induced resolution of HCV infection does not seem to reduce occurrence of HCC, and patients previously treated for HCC have still a high risk of tumour recurrence, in the short term. For these reasons, all cirrhotic patients should be closely monitored and followed during and after antiviral therapy.

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INTRODUCTION

Hepatitis C virus (HCV) infection is responsible for chronic hepatitis C, a necro-inflammatory process of the liver that progresses towards liver cirrhosis in about 20-30% of patients (1, 2). When liver cirrhosis is established, liver cancer may occur at an average 3.5% annual rate (3, 4). During the past decades, treatment of chronic hepatitis C with pegylated interferon and ribavirin led to cure of HCV infection in about 50% of treated patients (5, 6). A sustained virological response (SVR), as undetectable HCV RNA after therapy end, has been associated with a reduced risk of developing hepatocellular carcinoma (HCC) (7, 8, 9).

The recent introduction of new antiviral drugs, directly targeting HCV replication, allowed achieving SVR rates in over 90% of treated patients, irrespective of the liver fibrosis stage (10, 11, 12). This has raised the hope of a drastic decline in HCC occurrence, and even a decline in recurring HCC in those patients who experienced liver cancer in the past, and went through effective surgical or ablative treatment of the neoplastic lesions.

The aim of this study was to evaluate the early occurrence of HCC in cirrhotic patients without history of liver cancer and the recurrence of HCC in cirrhotic patients with a history of previously treated HCC, systematically followed during and after treatment with direct acting antivirals (DAA).

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PATIENTS AND METHODS

In this retrospective cohort study, we analysed data from all the consecutive patients with advanced liver fibrosis who were prospectively enrolled for treatment with DAAs at our centres in the Bologna area, Italy, between March and September 2015. Data were first retrieved from the electronic regional registry database (Piattaforma SOLE). Then, all the additional data were obtained from the individual patient records.

Eligibility of each patient for treatment of hepatitis C with DAAs was assessed following the priority criteria established by the national registry of the Italian Medicines Agency committee (AIFA). Approved priority criteria for treatment included: patients with Child-Pugh class A or B liver cirrhosis, without history of previous HCC or with history of complete response to surgical resection or loco-regional ablation of previous HCC, and patients with a METAVIR F3 fibrosis score, assessed by liver histology or transient elastography result > 10 kPa. When possible alternative treatment options were available for the same indication, the choice of therapy was left to the clinician's discretion.

At the end of November 2015, the database included 448 patients with HCV (+) advanced liver disease, without concomitant HIV infection or active alcohol consumption, and with no evidence of HCC, who had been treated with different regimens of DAAs. Since our analysis was restricted to patients with liver cirrhosis, 82 patients were excluded because of a METAVIR score of F3 or a transient elastography result of less than 12 kPa. Nine patients were excluded because of

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incomplete HCC treatment response before starting antiviral therapy. Finally, 11 patients were not included in the present analysis because of incomplete follow-up data, including three patients who died during antiviral therapy, and two patients because of decompensated liver disease (Child-Pugh class C) (Figure 1).

Before starting antiviral therapy, all patients without history of previous HCC underwent abdomen ultrasound. If a possible focal lesion was detected in the liver, the diagnostic work up was completed with contrast-enhanced ultrasonography (CEUS), and subsequent computerized tomography (CT) scan or magnetic resonance imaging (MRI) was performed to exclude the presence of HCC. On the other hand, all patients with a history of HCC underwent CT-scan or MRI, besides ultrasound, to exclude recurrent HCC. At the end of antiviral therapy, 12 and 24 weeks thereafter, patients repeated abdominal ultrasound evaluation. Again, any suspected focal lesion of the liver was re-evaluated with CEUS and CT scan or MRI to assess the occurrence or the recurrence of HCC.

Virological response to therapy was assessed by quantitative HCV RNA detection, using real-time PCR with a limit of detection of 15 IU/mL.

Comparisons between means or medians were made by unpaired t test or Mann Whitney test, with 2-tail p value, as appropriate. Analyses of contingency tables were made by Fisher's exact test or chi-square test, with two-sided p value, as appropriate. Logistic regression was used to analyse the association between binary outcomes and multiple exposure variables, to control for confounding.

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RESULTS

We performed our analysis on the 344 consecutive adult patients, with HCV-related liver cirrhosis, who completed a full course of antiviral therapy with different DAA regimens between April and November 2015, and were systematically followed for 24 weeks after treatment completion. The principle baseline characteristics of the study population are reported in Table 1. In particular, most patients were males (60.2%), mean aged 63 years, with prevalent HCV genotype 1 infection (68.9%), and previously unsuccessfully treated with peg-interferon plus ribavirin in 191 (55.5%). Only a small proportion of patients had HBV coinfection (2%). About severity of liver disease, 39 patients (11.3%) were classified as Child-Pugh class B at the start of DAA treatment, and measurement of liver stiffness by transient elastography resulted a mean kPa value of 23.6

Fifty-nine patients had a history of previous HCC: a single nodule smaller than 5 cm in diameter in 42 patients, up to 3 nodules smaller than 3 cm in diameter in 14, and 2 nodules with diameter between 3 and 5 cm in one patient. No extrahepatic manifestations or macrovascular invasion was recorded. Medical data records did not allow an exact classification in 2 subjects. HCC had been treated with surgical resection in 19 cases, resection and radiofrequency ablation (RFA) in 2, resection and trans-arterial chemoembolization (TACE) in 2, RFA in 18, RFA and TACE in 4, percutaneous ethanol injection (PEI) in 6, TACE in 5. Previous HCC treatment data were not complete in 3 patients. The median interval between previous HCC treatment and beginning of antiviral therapy with DAA was 376 days (range: 45 – 2,706). At the start of DAA therapy, all patients resulted without active HCC.

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SVR, defined as undetectable HCV RNA at 12-week post-treatment follow-up visit, was achieved in 314 of the 344 patients (91%), confirming the high antiviral efficacy of DAA even in cirrhotic patients.

Analysis of baseline characteristics for patients with and without previous HCC showed that patients with history of HCC were older, more frequently affected by diabetes, and more frequently treated with antiviral therapy in the past (Supplementary Table 1). Interestingly, they did not differ in terms of liver function stage, as assessed by Child Pugh class, and liver stiffness, as assessed by transient elastography.

During the 24-week post treatment evaluation, HCC was detected, and confirmed by at least two independent imaging techniques, in 26 patients (7.6%, 95% CI: 4.99-10.84): single nodule in 21 and multinodular in 5. HCC developed in 17 of 59 patients (28.81%, 95% CI: 17.76-42.07) with history of previous HCC and in 9 of 285 patients (3.16%, 95% CI: 1.45-5.90) without history of liver cancer.

We analysed the clinical characteristics of patients who developed HCC in comparison with those of patients who resulted without HCC (Table 2). Based on univariate analysis, Child-Pugh Class B (p= 0.02, OR: 3.29, 95% CI: 1.28-8.43), a more severe liver fibrosis assessed by transient elastography (p= 0.01), and a lower platelet count (p= 0.02) were significantly associated with HCC development. Using a receiver operating characteristics (ROC) curve, for optimum sensitivity to specificity weighing (1:1), a convenient cut-off value of 21.3 kPa for liver stiffness measurement was identified to better discriminate between individuals with and without HCC (sensitivity: 0.76, specificity: 0.57). Patients with kPa values > 21.3 were significantly more prone to develop HCC (p= 0.005, OR: 4.24, 95% CI: 1.50-11.97). History of

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previous HCC resulted the strongest baseline characteristic associated with HCC development (p< 0.0001, OR: 12.41, 95% CI: 5.19-29.65). Interestingly, neither HCV genotype nor therapeutic DAA regimen correlated to HCC occurrence, as well as known risk factors such as body mass index and diabetes.

Logistic regression was used to control for the confounding effects of multiple causal variables on developing HCC. At multivariate analysis, two variables resulted independently associated with HCC development: Child Pugh class (p= 0.03, OR: 4.18, 95% CI: 1.17-14.8) and history of HCC (p< 0.0001, OR: 12.0, 95% CI: 4.02-35.74).

Analysis of patients without history of previous HCC did not show significant characteristics in patients who developed HCC after DAA (supplementary table 2). This could have been influenced by the small number of patients with the neoplastic outcome, in comparison with the large number of those who did not experience the neoplastic event.

Subsequent analysis on the 59 patients with history of previous HCC, who had a high risk of HCC recurrence, showed that baseline characteristics were relatively homogeneous (Table 3). At univariate analysis, a younger age (p= 0.04) and a more severe liver fibrosis, assessed by transient elastography, (p= 0.05) were significantly associated with HCC recurrence. Using a ROC curve, for optimum sensitivity to specificity weighing (1:1), a convenient cut-off value of 21.5 kPa for liver stiffness measurement was identified to better discriminate between individuals with and without HCC recurrence (sensitivity: 0.91, specificity: 0.52). Patients with kPa values > 21.5 were significantly more prone to experience HCC recurrence (p= 0.01, OR: 11.91, 95% CI: 1.33-106.78). Even at multivariate analysis, these two variables

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resulted independently associated with HCC recurrence: age (p= 0.02, OR: 0.82, 95% CI: 0.69-0.97) and liver stiffness (p< 0.03, OR: 1.19, 95% CI: 1.01-1.39). Again, even in patients with history of previous HCC, neither HCV genotype nor therapeutic DAA regimen correlated to HCC recurrence, as well as known risk factors for HCC such as body mass index or diabetes.

HCC recurrence was not influenced by the treatment modality of previous HCC, and the disease-free interval was similar in those patients who experienced recurrent liver cancer. In addition, the proportion of recurrence was not different between patients who had been treated with potentially curative treatment (surgery and RFA) and those previously treated with potentially non-curative treatment (TACE), and the corresponding intervals since previous HCC treatment were also similar. Finally, baseline α-fetoprotein (AFP) levels were not significantly different between patients with and without HCC recurrence. In addition, this serologic marker of liver carcinogenesis did not help to detect early HCC recurrence, since at the time of recurrence only 2 of 17 patients (11.8%) had AFP values > 50 ng/mL.

The 17 patients who experienced HCC recurrence after DAA treatment were individually characterized, in order to better evaluate the characteristics potentially associated with HCC recurrence (Table 4). Previous HCC status was within Milan criteria in all but one. Barcelona Clinic Liver Cancer (BCLC) system was used as staging system for HCC (13). According to it, 5 patients corresponded to BCLC stage 0, 11 patients to BCLC stage A, and only 1 patient to BCLC stage B. Previous HCC consisted of a single nodule in 12 patients, and 2 nodules in 5. The maximum diameter of the neoplastic lesions, the kind of HCC treatment, the interval between previous HCC treatment and DAA therapy, and the Child-Pugh class for each patient

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are also reported in the table. As stated above, no previous HCC had imaging aspects of extrahepatic manifestations or macrovascular invasion.

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DISCUSSION

Hepatocellular carcinoma is a possible serious complication in the natural history of chronic hepatitis C. The risk of developing HCC increases as liver fibrosis advances and in patients with advanced liver disease and cirrhosis the annual incidence rate averages 3.5% (3, 14). Liver carcinogenesis is a long multistep process requiring chronic hepatic inflammation, progressive liver fibrosis, initiation of neoplastic clones, and progression of the malignant clones in a carcinogenic tissue environment (15). HCV-induced inflammation is supposed to be a strong promoter of tumour development and resolution of HCV infection should translate in a reduced incidence of HCC, even in patients with liver cirrhosis. This is indeed the case in cirrhotic patients who have been successfully treated with peg-interferon and ribavirin (16).

Unfortunately, in the past, the adverse events of interferon and ribavirin did not allow to treat the more demanding patients, those with advanced liver cirrhosis. Therefore, the real benefit of antiviral therapy in HCV-related cirrhosis has waned, and the incidence of HCC has increased during the last decade (17).

The recent introduction of effective direct-acting antivirals against HCV has allowed an all-oral interferon-free therapy for almost all patients, including those with decompensated liver disease and awaiting liver transplantation, arising a confident hope the natural history of HCV-related liver cirrhosis could be changed.

Data exist to support a decrease in liver function worsening after DAA therapy (11, 18), but no data on the consequences of DAA-induced HCV eradication on HCC occurrence have been reported so far. In addition, the good safety profile of DAAs

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even allow managing cirrhotic patients who went through a successfully treated HCC. In these patients, the spontaneous risk of HCC recurrence after curative therapy is relatively high over time, and resolving HCV-related inflammation may lessen this residual ominous event.

Based on this background, in November 2015 we planned to retrospectively collect the clinical data of a large homogeneous cohort of cirrhotic patients who had been consecutively and prospectively assigned to DAA therapy between March and September 2015, at our centres. The aim was to evaluate whether effective DAA therapy affected the early occurrence of new HCC in patients without history of HCC and the early recurrence of HCC in those effectively treated for the neoplastic disease.

The first positive finding of our study was that 91% of the 344 cirrhotic patients achieved SVR. Patients with and without a history of previous HCC responded equally well to the antiviral treatment. This high response rate prompted us to feel confident both in a low occurrence rate of new HCC and in a lower HCC recurrence. Unfortunately, this was not the case during the 24-week post-treatment follow-up observation.

Even during this relatively short period, a significant number of HCC was detected at the planned abdominal ultrasound visits, and confirmed at the following radiological evaluation. HCC was found in 26 patients, representing a 7.6% of the entire study population. New HCC were not equally distributed between patients with and without history of previous HCC, since they were found in 28.8% of the former, and in 3.2% of the latter (Figure 2).

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Without a control group, it is not easy to assume whether these figures are high and alarming. Therefore, we decided to compare these findings with those of a historic population of similar untreated cirrhotic patients, previously followed at our centres (unpublished data). The cumulative HCC occurrence rate was 3.2% at 1-year, an amount comparable to other experiences in our country (3, 14), and equivalent to what found in the present study. Even if the observation period was shorter than 1-year in some patients, we cannot assume an increased occurrence rate of HCC in DAA-treated cirrhotic patients.

Interestingly, near all our patients had SVR. Therefore, cirrhotic patients with SVR after interferon and ribavirin therapy could be considered for comparison of our findings. Unfortunately, because of the contraindications to the use of interferon that are present in cirrhotic patients, the populations of those treated with interferon were different in background characteristics and not fairly comparable to the DDA-treated ones (19). Different studies have reported a reduced occurrence of HCC in interferon-treated patients, with a residual annual incidence lower than 2% (20). In our study, despite SVR, we failed to observe a reduction in the natural HCC occurrence. Therefore, we postulate that SVR after DAA is not associated with a reduced incidence of HCC in cirrhotics.

Recurrence of HCC occurred in 28.8% of cirrhotic patients with a history of previous liver cancer, grouped in the short period of our follow-up. Liver cancer recurrence after surgical resection or radiofrequency ablation is not uncommon (21). This may be due to a mistaken definition of cured HCC, leading to reappearance of an incompletely resected or ablated tumour, or to recurrence of a new HCC in the cirrhotic liver. Due to the strict eligibility criteria for DAA therapy fixed by the national

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registry of the Italian Medicines Agency committee, only patients with history of complete response to surgical resection or loco-regional therapy of previous HCC were included in our centres. In addition, all but one patients had previous HCC within Milan criteria, without extrahepatic manifestations or macrovascular invasion, and were in the BCLC very early stage 0 or early stage A. Complete response to HCC treatment was assessed in each patient by different imaging evaluations performed by expert radiologists. The median time from HCC treatment and beginning of DAA therapy was 376 days. This allowed radiologists to perform multiple evaluations in most patients, with confirmation of complete response before DAA therapy. Reassessing all the medical records, we found only two of the fifty-nine patients in whom the radiological definition of complete remission of liver cancer before starting DAA therapy could have been questionable. During follow-up, one of these two patients had a recurrence.

After surgical resection or radiofrequency ablation, HCC recurrence at 1-year may be expected in about 20% of patients (22). In addition, HCC had been treated with TACE in 5 of our patients. Therefore, our finding of a 28.8% recurrence rate in patients with a median disease-free interval of 376 days was not unexpected. This is particularly true if we consider the median disease-free interval between HCC treatment and the end of DAA-therapy (475 days), that puts our patients in a high risk period for recurrence of the initial tumour. More difficult to explain is the reason for a sudden and simultaneous HCC recurrence, within few months since DAA treatment, in seventeen patients, who had a median HCC-free interval of 446 days at the start of DAA therapy. Similar results from an authoritative group have been accepted for publication very recently (23), supporting that our findings may not be influenced by significant biases. Due to these results, we postulate that DAA therapy

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in cirrhotic patients with previous HCC may accelerate the recurrence of liver cancer, even if it probably does not increase the absolute HCC recurrence rate.

Interpretation of the biological mechanisms responsible for our findings is not straightforward. Inflammation is considered to play a critical role in tumorigenesis, and an inflammatory microenvironment is an essential component of all tumours. In addition, many environmental causes of cancer and risk factors, including HCV infection, are associated with some form of chronic inflammation (24). Therefore, an active treatment able to resolve chronic inflammation should lead to suppression of its promoting role in tumorigenesis.

In chronic hepatitis C, DAA therapy can rapidly reduce liver inflammation, since elevated transaminase levels revert to normal within few weeks in most patients. If chronic inflammation was the major tumour promoter, this could determine a significant decrease in HCC development. Our data probably indicate the opposite; therefore, a different interpretation is necessary.

In tumours arising in the context of chronic inflammation, such as HCC, the net effect of the immune system seems stimulation of tumour growth and progression. However, cancer cells express “non-self” antigens, and tumour clones may be subject to immunosurveillance and killing by activated T and NK cells (25). Therefore, an effective cancer immunosurveillance process may prevent cancer development. It is not known whether this is the case for HCC arising in HCV-related cirrhosis, but it is known that DAA therapy is associated with a rapid decrease in NK cell activation and a normalization of NK cell cytotoxic effector functions (26). In addition, DAA-induced viral load decline reduces serum levels of NK cell-stimulating cytokines and causes correction of the altered NK cell phenotype (27). These

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immunological changes could be responsible for a reduced immunosorveillance of neoplastic clone growing and spreading.

Additional findings emerging from our study are that patients who experienced HCC recurrence were younger and had a more severe liver stiffness. This may corroborate the hypothesis of a less efficient immunosurveillance associated with a more advanced liver fibrosis. If this immunosurveillance is further reduced after DAA therapy, this could allow the growth of liver cancer, especially in younger patients, in whom neoplastic lesions are generally more aggressive.

In conclusion, our study indicates that in patients with HCV-related cirrhosis, DAA-induced resolution of HCV infection does not seem to reduce the occurrence of HCC, in the short term. In addition, patients previously treated for HCC have still a high risk of tumour recurrence, despite DAA treatment. For these reasons, all cirrhotic patients should be closely monitored, during and after antiviral therapy, implementing or continuing HCC surveillance, despite resolution of HCV infection. Finally, when possible, antiviral treatment should be started early, before the development of liver cirrhosis.

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Author names in bold designate shared co-first authorship.

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FIGURE LEGENDS

Figure 1

Flow chart of the study population

Figure 2

Proportion of patients who developed HCC after DAA therapy, according to overall study population, patients without history of HCC (occurrence), and patients with history of previous HCC (recurrence).

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Table 1. Baseline characteristics of patients from the entire study population

Males, n. (%)	207 (60.2)
Age, yrs. (median, range)	63 (29-85)
HCV genotype, n. 1 2 3 4	 237 40 38 29
Antiviral Treatment, n. Naive Experienced	 153 191
Child-Pugh A / B, n.	305 / 39
MELD score (mean, SEM)	8.6 (0.1)
Liver stiffness, kPa (mean, SEM)	23.6 (0.8)
HBsAg positive, n. (%)	7 (2.0)
History of previous HCC, n. (%)	59 (17.2)

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Table 2. Baseline characteristics of patients according to development of HCC after DAA therapy

	Without HCC after DAAs (n= 318)	With HCC after DAAs (n= 26)	p
Males, n. (%)	189 (59.4)	18 (69.2)	0.40
Age, yr (median, range)	64 (37-86)	57.5 (47-81)	0.32
Diabetes, n. (%)	62 (19.5)	6 (23.1)	0.61
BMI, median (range)	25.6 (17-40)	25.2 (21-30)	0.37
Child-Pugh class B, n. (%)	32 (10.1)	7 (26.9)	0.02
Liver stiffness, Kpa (mean, SEM)	23.1 (0.8)	28.1 (2.5)	0.01
Liver stiffness Kpa			0.005
<21.3	134	5	
>21.3	101	16	
HCV Genotype			0.15
1	224	13	
2	35	5	
3	34	4	
4	25	4	
SOF + SMV	135	7	0.22
3D	54	2	
SOF + RBV	52	10	
SOF + DCV	51	6	
SOF + LDV	23	1	
DCV + SMV	1	0	
SVR12			0.26
Yes	292	22	
No	26	4	
Previous antiviral treatment			0.07
Naive	146	7	
Experienced	172	19	
History of previous HCC			0.0001
Yes	42	17	
No	276	9	
Albumin, g/dL (mean SEM)	3.86 (0.03)	3.76 (0.09)	0.11
Bilirubin, mg/dL (mean, SEM)	1.03 (0.07)	1.20 (0.14)	0.07
Platelets, x1000/mm3 (mean, SEM)	124.4 (3.9)	102.3 (13.7)	0.02

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Table 3. Baseline characteristics of patients with history of previous HCC according to HCC recurrence after DAA therapy.

	Without HCC after DAAs (n= 42)	With HCC after DAAs (n= 17)	P
Males, n. (%)	28 (66.6)	12 (70.6)	1.0
Age, yr (median, range)	73 (48-84)	56 (48-81)	0.04
Diabetes, n. (%)	14 (33.3)	6 (35.2)	1.0
BMI, median (range)	25.5 (19-34)	25.0 (21-30)	0.64
Child-Pugh class B, n. (%)	5 (11.9)	5 (29.4)	0.13
Liver stiffness, Kpa (mean, SEM)	23.2 (1.8)	29.2 (2.3)	0.05
Liver stiffness, Kpa			
<21.5	13	1	0.01
>21.5	12	11	
HCV Genotype			
1	34	9	
2	2	4	0.09
3	4	2	
4	2	2	
SOF + SMV	17	3	
3D	7	2	0.26
SOF + RBV	5	7	
SOF + DCV	8	5	
SOF + LDV	4	0	
DCV + SMV	1	0	
SVR12			
Yes	38	15	1.0
No	4	2	
Antiviral treatment			
Naive	16	2	0.06
Experienced	26	15	
Albumin, g/dL (mean, SEM)	3.73 (0.08)	3.75 (0.11)	0.76
Bilirubin, mg/dL (mean, SEM)	0.90 (0.09)	1.03 (0.12)	0.18
Platelets, x1000/mm3 (mean, SEM)	114.5 (9.5)	113.2 (20.1)	0.36
AFP, ng/mL (mean, SEM)	12.8 (2.6)	25.6 (10.2)	0.18
Previous HCC			
Single	30	12	0.73
Multiple	9	5	
Days since HCC treatment (median, range)	360 (45-2706)	446 (50-1301)	0.88
Previous HCC treatment			
Surgery (± RFA/TACE)	15	8	
RFA (± TACE)	16	6	0.83

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PEI	5	1	
TACE	3	2	

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Table 4. Previous HCC history and baseline characteristics of the 17 patients who experienced HCC recurrence after DAA

Patient	Previous HCC nodules (n.)	Previous HCC maximum nodule diameters (mm)	Previous HCC treatment	Days since HCC treatment	BCLC stage	Child-Pugh
1	1	22	Surgery	1301	A	A
2	1	20	PEI	373	0	A
3	1	25	Surgery	475	A	A
4	1	13	RFA	202	A	B
5	1	20	RFA	260	0	A
6	2	16, 12	TACE	664	A	A
7	2	25, 7	Surgery	50	A	A
8	2	25, 22	RFA	1016	A	B
9	2	30, 10	RFA (+TACE)	99	A	A
10	1	20	Surgery	237	0	A
11	1	50	Surgery	95	A	A
12	1	17	RFA	482	A	B
13	1	15	Surgery	970	0	A
14	1	38	Surgery	700	A	B
15	1	15	RFA	483	0	A
16	2	46, 28	TACE	778	B	B
17	1	23	Surgery	406	A	A

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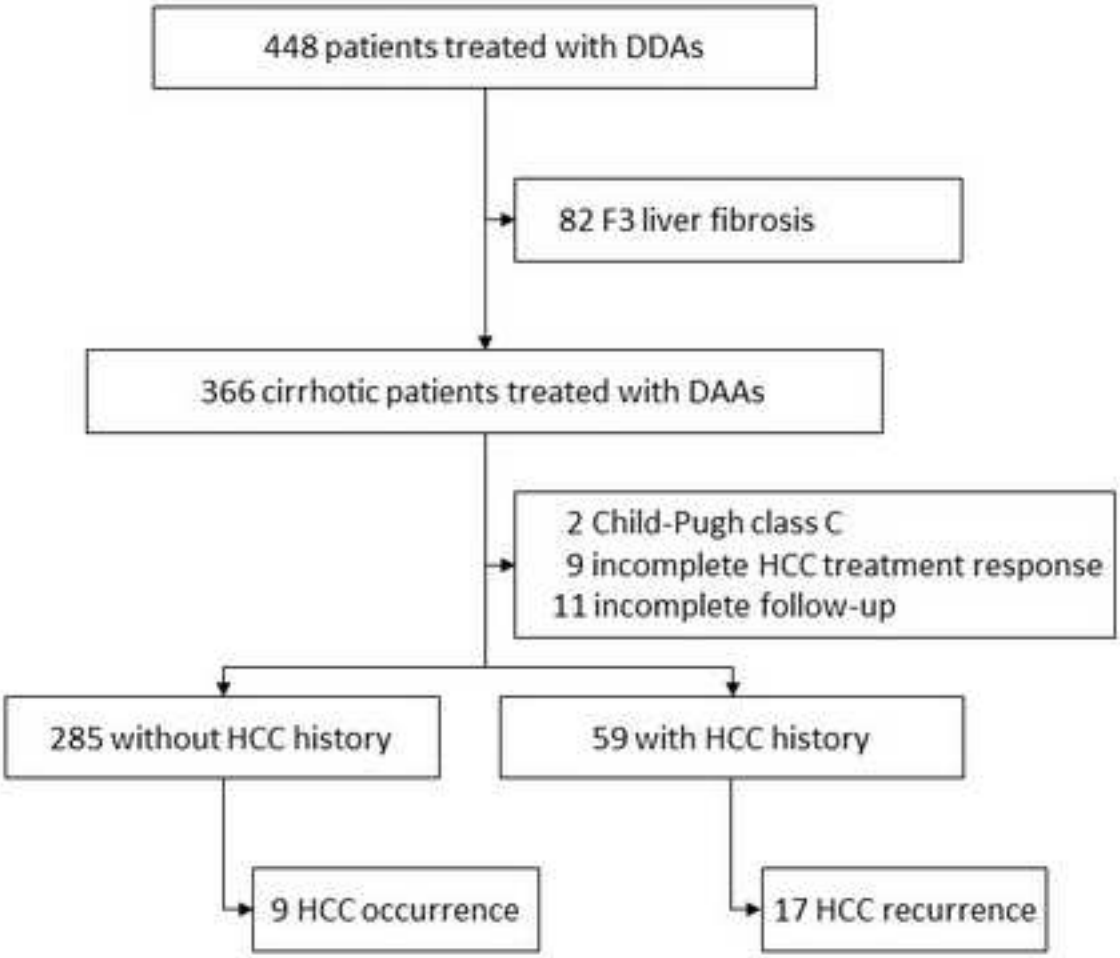

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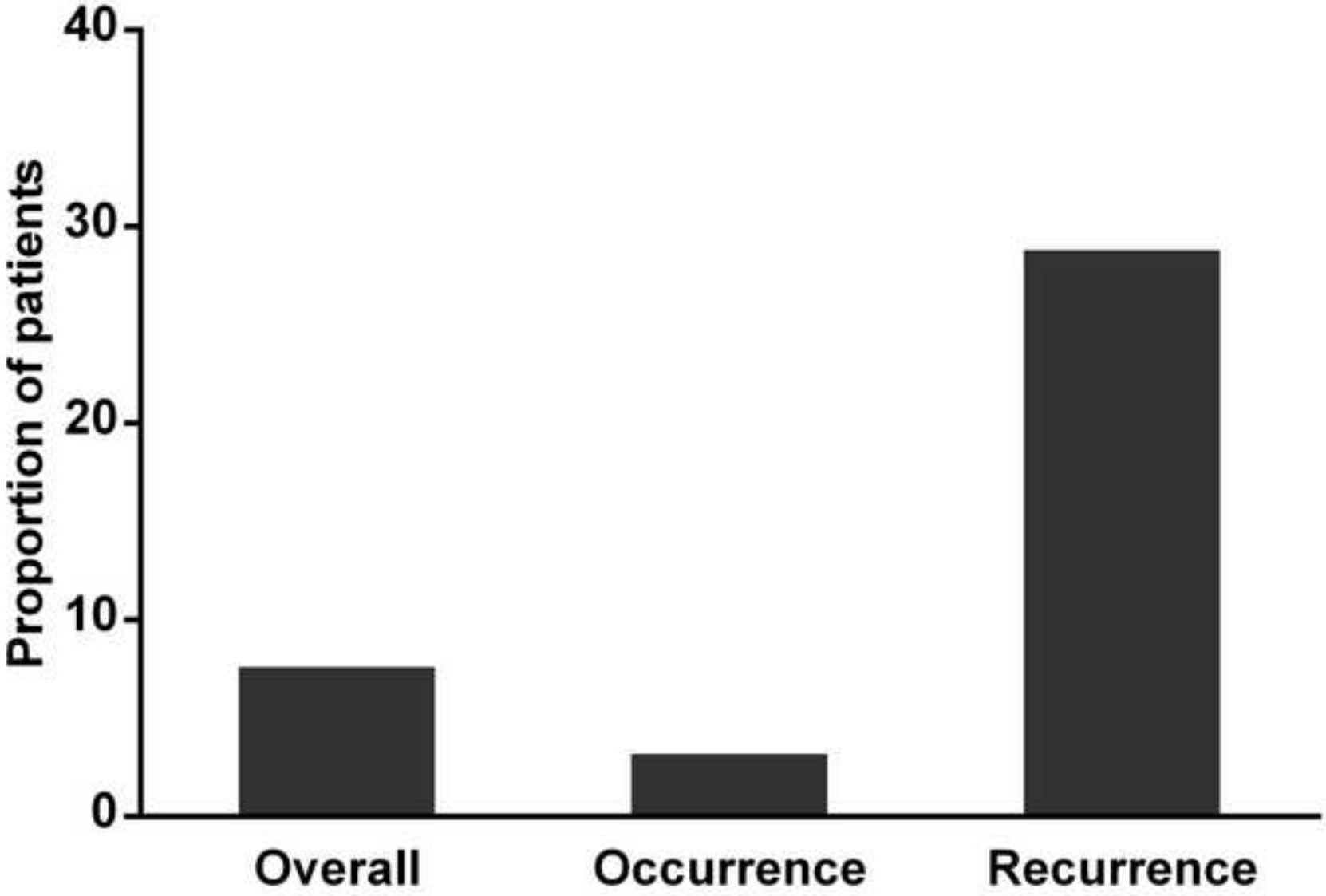

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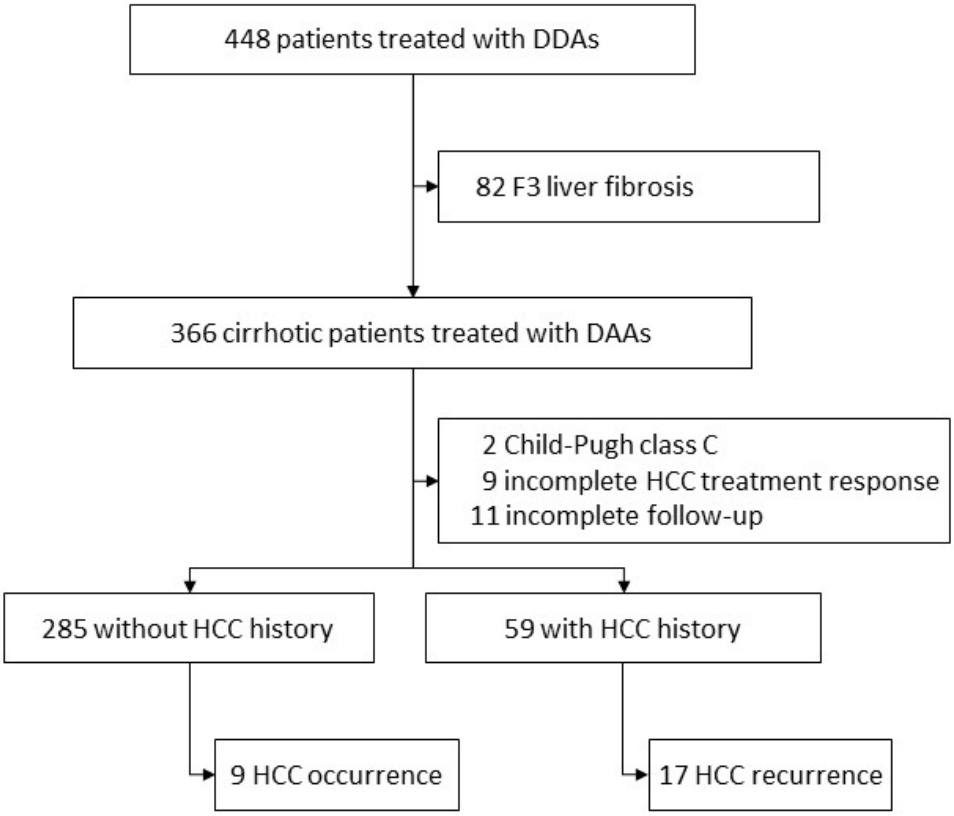
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