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Late HCC Occurrence in Patients Achieving SVR after DAAs Therapy: A Matter of Follow-Up or Something Else?

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Abstract: Background: An unexpected increased of HCC occurrence rate among HCV patients treated with direct acting antivirals (DAAs) combination has been reported. Aim of the study was to evaluate a long-term follow-up to verify disease natural history of HCV patients treated by DAAs who had achieved SVR in view of possible late HCC occurrence. **Methods**: In this prospective multicenter study, all consecutive HCV patients treated with DAAs according to the Italian ministerial guidelines were enrolled between 2015 and 2018. Patients with active HCC on imaging or history of previous treated HCC, HBV or HIV co-infection, or liver transplant recipients were excluded. All patients were followed up every month during treatment and thereafter every 3 months for at least 36 months. An abdominal ultrasound (US) was performed before starting the antiviral therapy (within two weeks). A contrast-enhanced ultrasonography (CEUS), a dynamic computed tomography (CT) scan or dynamic magnetic resonance imaging (MRI) was managed to characterize incidental hepatic lesions. **Results**: Three hundred and six patients completed the 36 weeks follow-up after the end of treatment (median age 67 years, male 55%). All enrolled patients achieved SVR. Sofosbuvir based regimen was administered in 72.5% of the patients, while ribavirin was used in 20%. During the follow-up, a late HCC onset was reported in 20 patients with a cumulative incidence rate of 6.55%. The pattern of HCC occurrence was heterogeneous (median diameter 24 mm). At multivariate and univariate analysis, we found that liver stiffness, diabetes, BMI as well as platelet levels before antiviral therapy were factors related to late HCC occurrence. **Conclusions**: Our data suggest that late HCC occurrence appears despite the SVR. Thus, a long-term regular clinical, laboratory and expert ultrasonography follow-up should carefully be performed in all HCV patients undergoing DAAs.

Keywords: hepatocellular carcinoma; direct acting antivirals; HCV; sustained virological response; liver stiffness

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Introduction

Hepatitis C virus (HCV) infection has been characterized, in the last years, by a significant advancement in treatment schedule with the advent of the new direct-acting antivirals (DAA). These drugs are characterized by a rate of sustained viral response (SVR) higher than 90%, relative few adverse events and short length of treatment reported in clinical trials (1-3). However, in the last few years some concerns have raised according to several reports on HCC occurrence in those cirrhotic patients achieving SVR (4-6) whereas other reports suggest lower risk (7,8). Indeed, among patient with HCV infection and cirrhosis, the risk of hepatocellular carcinoma (HCC) is estimated to be 3% to 7% per year (9-10) while previous reports have shown that cirrhotic patients achieving the SVR, after IFN schedule treatment, had a lower risk of HCC development, with an incidence rate per year of 1.2–1.4% (11-12). Nevertheless, the risk of HCC remains because advanced fibrosis or cirrhosis, which are the most important risk factors for liver cancer, is not completely resolved by antiviral treatment (13). Thus, even if these new antivirals have demonstrated their great value in terms of treatment for the Hepatitis C eradication in the next future some issues still remain to be evaluated, particularly:

(1) Does Viral clearance really mean a disease resolution? That would be mandatory to determine to really weight a significant long term impact on natural history of HCV.
(2) We now have more then 5 years follow-up however still remain unknown a long-term follow-up) more than 10 years about clinical evolution of viral disease (ascites, variceal bleeding, hepato-renal syndrome, hepatic encephalopathy, and hepatocellular carcinoma). Particularly, we do not have decisive findings regarding these outcomes in real life settings and this would be fundamental in the health care system management.

According to our previous research protocol on long-term follow-up of patients undergoing first generation DAAs (referenza WJHEPA), we managed a long-term follow-up to verify disease natural history of patients who underwent second generation DAAs with achieved SVR. Here we will present our data on 48 months real life follow-up.

Methods

Study design and patients' population

From February 2015 to December 2018 a group of 4 Hospital and Academic Centres in Southern Italy (Campania Region) on behalf of CLEO (Italian Society of Hospital Hepatologist) conducted an observational, prospective, real-life study on efficacy and safety of DAAs treatment schedule recruiting all consecutive HCV patients treated with IFN-free DAAs regimens, local ethical committee n674. According to Italian ministerial guidelines for DAAs treatment, the inclusion criteria were HCV-RNA serum positivity, fibrosis stage ≥F3 according to Metavir score (the Italian reimbursement criteria, at the time of enrolment, were applicable only for patients with F3-F4 fibrosis) assessed by liver biopsy or transient elastography (TE) (14). The TE was performed by Fibroscan® (Echosens, Paris, France), according to standard criteria and only reliable tests according to Boursier's criteria were considered optimal for enrolment (15).

In this real-life population study, patients with any of the following features were excluded: active HCC on imaging or history of previous treated HCC, HBV or HIV co-infection, or liver transplant recipients. All patients were followed up every month during treatment and thereafter every 3 months for at least 48 months.

The baseline HCC screening for all patients enrolled in our cohort was performed according to the European guidelines of the European Association for the Study of Liver (EASL) (16).

An abdominal ultrasound (US) was performed before starting the antiviral therapy (within two weeks); each US was performed by two expert experienced operators (defined with more than 5000 exams with certification at SIUMB (Italian Society of Ultrasound Medicine) for each Centre). This approach was managed in order to minimize the bias of a multicenter, unmonitored study. A contrast-enhanced ultrasonography (CEUS), a dynamic computed tomography (CT) scan or dynamic magnetic resonance imaging (MRI) was managed to characterize incidental hepatic lesions. Patients

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showing nodular patterns suggestive of HCC or with uncertain dynamic vascular behaviour at the time of treatment enrolment were excluded from further follow-up.

HCV-RNA was assessed by real-time PCR (COBAS®TaqMan, AmpliPrep, Roche) with a detection limit of 15 IU/mL.

The diagnosis of cirrhosis was based on clinical, biochemical, ultrasonographic, elastographic and, when available, histological features. In particular, for cirrhotic patients, liver function was graded according to the Child-Turcotte-Pugh (CTP) score system.

Demographic characteristics and clinical parameters at baseline, including age, sex, body mass index (BMI), alcohol consumption, tobacco smoking, presence of comorbidities, and biochemical parameters were recorded.

The study was performed according to the Declaration of Helsinki and was approved by the local Ethic Committee (Vanvitelli-Ospedali dei COlli Local Ethical Committee; Approval number: 674); all patients gave informed consent to the study as for previous research protocol.

Antiviral treatment

Eligibility of patients to HCV treatment with IFN-free DAAs regimens was assessed following the priority criteria established in February 2015 by the national Scientific Society and registry of theItalian Medicines Agency Committee (AIFA) (14). The prescribing clinicians, in accordance with national and international guidelines and its updates at that time 39-40, chose the treatment regimen individually.

The treatment duration (12 or 24 weeks) was related to the severity of liver disease, with longer treatments reserved to cirrhotic patients.

Patients were treated with sofosbuvir+ ribavirin, or simeprevir+sofosbuvir ± ribavirin,ordaclatasvir+sofosbuvir ± ribavirin, or ledipasvir+sofosbuvir ± ribavirinorombitasvir/paritaprevir/ritonavir+dasabuvir ± ribavirin(3D).

Then, therapeutic regimens were categorized as follow: sofosbuvir (SOF)-based, ribavirin (RIB)-included and sofosbuvir+ ribavirin. Ribavirin was never dosed under 600mg per day in therapeutic schedule, according to adverse events.

Patients follow-up

Virological response to therapy was assessed by real-time PCR with HCV-RNA detection at the end of treatment, 12 and 24 weeks after the end of treatment. The SVR, defined as the persistent absence of detectable serum HCV-RNA 12 weeks after the end of treatment (SVR12), was assessed for all the enrolled patients. Any relapse of serum HCV-RNA during follow-up was recorded.

At least three ultrasound examinations per year were performed for every enrolled patient during the established follow-up period (at the end of therapy, 12 weeks after the end of therapy and six months later) according to the HCC surveillance program and study design. Any detected liver lesion was evaluated by imaging technique workup (CEUS, or dynamic CT scan or dynamic MRI) according to EASL guidelines (16).

The diagnosed HCC were recorded and scored according to Barcelona Clinic Liver Cancer (BCLC) staging system (17). Once a patient had a diagnosis of HCC, the follow-up was stopped.

Liver stiffness evaluation

TE by FibroScan was carried out using the M probe experienced operators (>1000 exams), according to the manufacturer's instructions. The LSM, expressed in Kilopascal (Kpa), range 2.5-75, was assessed for reliability by the interquartile range (IQR)/median ratios (IQR/M). IQR represents an index of the intrinsic variability of the LSM. Moreover, the operators were blinded to the clinical and biochemical data of the patients. The IQR corresponds to the interval of LSM results containing 50% of the valid measurement between the 25th and 75th percentiles. Advanced fibrosis was defined by a FibroScan ≥ 10 kPa but < 14 kPa. Cirrhosis was defined by a FibroScan ≥ 14 kPa in combination with clinical, laboratory and ultrasound parameters.

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Endpoints of study

Primary endpoint was to evaluate the late occurrence rate of HCC in HCV patients with SVR after DAAs treatment schedule, defined as carcinoma onset after 48 months from SVR. Secondary end point was to evaluate the risk factors associated to late HCC occurrence. As additional end-point, a sub-analysis focused only on cirrhotic patients, to reduce any possible selection bias, assessing difference between Child A and Child B.

Statistical Analysis

Data are shown as either median or range, in the case of continuous variables or number and percentage, for categorical variables. Differences between groups have been analysed by Fisher's exact test or Chi square test for categorical variables. Mann–Whitney U test or Kruskal–Wallis test have instead been performed to compare continuous variables. As multivariate analysis, a logistic regression with the stepwise Wald statistic input was performed.

Finally, a ROC curve analysis was built to measure the real risk of development of HCC based on liver stiffness values (kPa).

P values below 0.05 were considered statistically significant. All analyses were performed with the SPSS software (IBM, Armonk, New York), version 24.

Results

Nine hundred and eighty-five patients completed the treatment and 306 were followed-up for at least 48 months after end of treatment according to study design. Ten patients were excluded from the analysis due to incomplete follow-up data, and one patient died during the antiviral therapy (the causes of death were not related to DAAs).

Data were equally distributed among the single Centers. The baseline demographic characteristics of the enrolled population are reported in Table 1.

Table 1. Baseline characteristics of the entire cohort of study (n = 306).

Parameter	
Age (yrs.), median [IQR]	67 [60 – 73]
Sex, n (%)	
Male	165 (53.9)
Female	141 (46.1)
BMI, median [IQR]	26.1 [24.3 – 28]
Smoke, n (%)	2 (0.7)
Potus, n (%)	2 (0.7)
Diabetes, n (%)	59 (19.3)
Metabolic syndrome, n (%)	22 (7.2)
Number of lesions, n (%)	
0	286 (93.5)
1	15 (4.9)
≥2	5 (1.6)
Portal invasion, n (%)	5 (1.6)
Bright liver, n (%)	22 (7.2)
Liver stiffness (kPa), median [IQR]	21 [16 – 29]
Duration of therapy, median [IQR]	12 [12 – 24]
Platelets, median [IQR]	

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T0	108000 [73000 – 153000]
SVR12	103500 [65250 – 122000]
Genotype, n (%)	
1	243 (79.4)
2	50 (16.3)
3	11 (3.6)
4	2 (0.7)
Child-Pugh Score T0, n (%)	
A	289 (94.4)
B	17 (5.6)
Child-Pugh Score SVR12, n (%)	
A	298 (97.4)
B	8 (2.6)
Therapy, n (%)	
Sofosbuvir	80 (26.1)
Sofosbuvir/Ledipasvir	69 (22.5)
Sofosbuvir/Daclatasvir	23 (7.5)
Sofosbuvir/Simeprevir	50 (16.3)
3D	84 (27.5)
Late HCC, n (%)	20 (6.5)

* Data are expressed ad either number and percentage or median and interquartile range (IQR).

The median age was 67 years, and most patients were male (55.1%). Type 2 diabetes was reported in 13.3% of patients. The great majority of enrolled patients had a genotype 1 HCV infection (77.8%) and an HBV co-infection was observed in less than 2% of population. The liver stiffness median value was 21 Kpa (range 16-29). a cut-off of 22 KPa was associated with a significant increase in the risk of HCC onset (Figure 1). The presence of cirrhosis was recorded in all patients; of which 94.4% having CTP class A and 5.6% showing class B.

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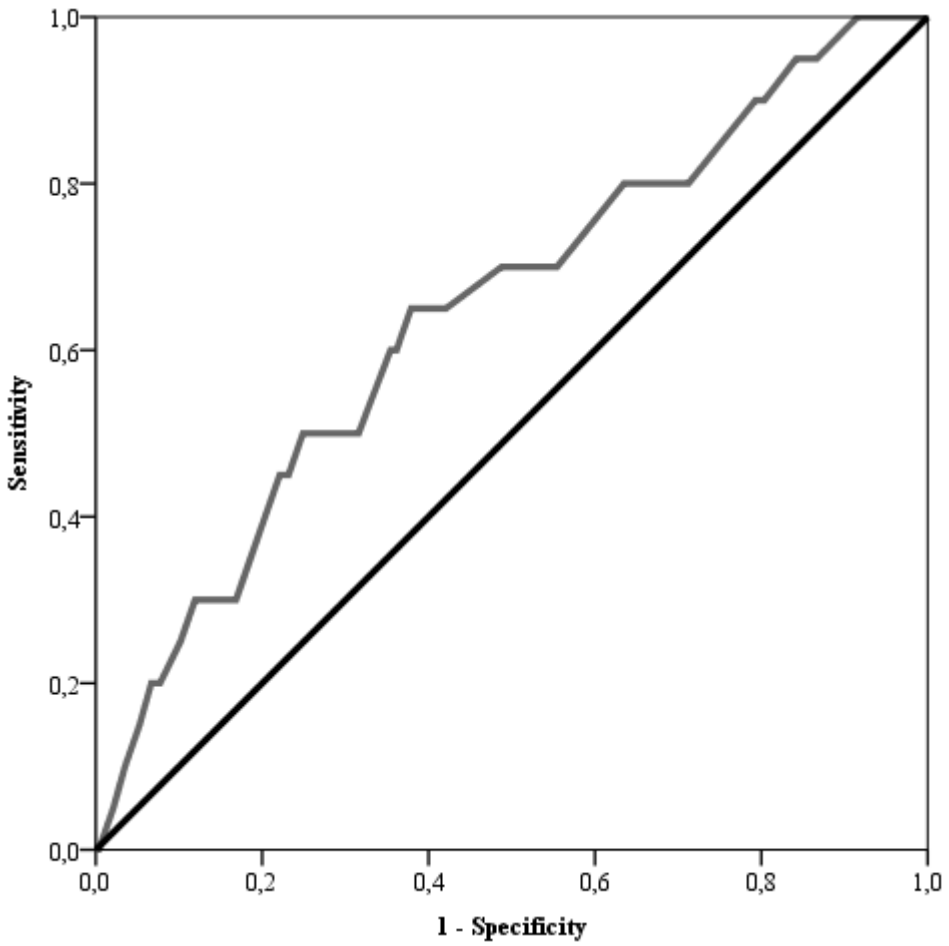

Cut-off	Sensitivity	1 - Specificity
22.9	65%	62.1%

Figure 1. ROC curve describing the discriminant power of the liver stiffness value (kPa) on the risk of developing late HCC in cirrhotic patients [n=306, AUROC score = 0.646, 95% C.I.: 0.519 – 0.774]. The p value for the significance of liver stiffness on the risk of HCC was 0.029 (Kruskal–Wallis test).

A SOF-based regimen was administered in 72.5% out of the patients, while ribavirin was used in 20%. All enrolled patients achieved SVR. During the follow-up, a late HCC onset was reported in 20 patients with a cumulative incidence rate of 6.55%. The pattern of HCC occurrence was heterogeneous: thirty-two patients had a nodular profile, while 3 patients developed infiltrative HCC (4 of them with macro-vascular invasion as portal vein thrombosis). No patient showed extrahepatic metastases. All patients with HCC occurrence did not show any viral relapse achieving SVR. The median diameter of the lesions was 24 mm (range 15-37 mm). None of the patients with HCC was an active alcohol consumer. Three patients (8.5%) were smokers of about 10 cigarettes/day. According to BCLC classification, patients were classified as follow: 13 patients as stage A, 4 patients as stage B, 3 patients as stage C. Among the enrolled patients, 18 out of them (90%) underwent a SOF-based treatment, and 12 of them (60%) were treated without RBV. Based on univariate analysis, CTP B stage (p 0.001), comorbidity of diabetes (p 0.007), presence of cirrhosis (p 0.002), and liver

stiffness value (p 0.0001) were significantly associated with HCC occurrence. At multivariate analysis we found that liver stiffness, diabetes, BMI as well as platelet levels before antiviral therapy were factors related to late HCC occurrence (Table 2).

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Table 2. Baseline Characteristics of Cirrhotic Patients According to late HCC Development: univariate and multivariate analysis (n = 306).

	Univariate Analysis			Multivariate Analysis	
	HCC				
Parameter	**Yes (n = 20)**	**No (n = 286)**	**P**	**O.R. [95% C.I.]**	**p**
Age (yrs), median [IQR]	70 [68.2 – 75]	67 [59.5 – 72]	0.026		
Sex, n (%) *M/F*	15 (75)/5 (25)	150 (52.4)/136 (47.6)	0.050		
BMI, median [IQR]	25 [23.2 – 26.7]	26.2 [24.7 – 28.4]	0.026	0.712 [0.537 – 0.943]	0.018
Smoke, n (%)	2 (10)	0 (-)	0.000		
Potus, n (%)	2 (10)	0 (-)	0.000		
Diabetes, n (%)	9 (45)	50 (17.5)	0.003	0.180 [0.045 – 0.713]	0.015
Metabolic syndrome, n (%)	3 (15)	19 (6.6)	0.162		
Liver stiffness (kPa), median [IQR]	26.5 [18 – 44.5]	20.4 [16 – 28.7]	0.028	1.070 [1.020 – 1.122]	0.006
Duration of therapy (months), median [IQR]	12 [12 – 12]	12 [12 – 24]	0.007		
Platelets, median [IQR] *T0* *SVR*	75000 [48000 – 109500] n.a.	115000 [80000 – 166000] n.a.	0.001 n.a.	0.975 [0.954 – 0.996]	0.019
Genotype, n (%) *1* *2* *3* *4*	 15 (75) 3 (15) 1 (5) 1 (5)	 228 (79.7) 47 (16.4) 10 (3.5) 1 (0.3)	0.095	0.007 [0.000 – 0.417] 0.003 [0.000 – 0.280]	0.017 0.012

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Number of lesions, n (%)			
0	0 (-)	286 (100)	0.000
1	15 (75)	0 (-)	
≥2	5 (25)	0 (-)	
Portal invasion, n (%)	4 (11.4)	0 (-)	0.000
Bright liver, n (%)	1 (5)	21 (16.8)	0.172
Child-Pugh Score T0, n (%)			
A	15 (75)	274 (95.8)	0.000
B	5 (25)	12 (4.2)	
Child-Pugh Score SVR12, n (%)			
A	17 (85)	281 (98.3)	0.000
B	3 (15)	5 (1.7)	
Therapy, n (%)			
Sofosbuvir	16 (80)	64 (22.4)	
Sofosbuvir/Ledipasvir	0 (-)	69 (24.1)	0.000
Sofosbuvir/Daclatasvir	1 (5)	22 (7.7)	
Sofosbuvir/Simeprevir	1 (5)	49 (17.1)	
3D	2 (10)	82 (28.7)	

* Data are expressed ad either number and percentage or median and interquartile range (IQR).

Discussion

The pathogenesis and natural history of HCV are characterized by several factors, including immune system activity with regulatory and effector environments since the early phase of infection (18-19). Chronic infection underlies a persistent activity of immune system with a specific cytokines environment that leads to a liver necro-inflammation and then to HCC onset that may still occur over the years as previously suggested (20-21), due to the continuous damage of inflammation and related fibrosis (22).

Indeed, in interferon-based regimens, the significant reduction of HCC incidence but not its disappearance in those with viral clearance have been associated to several factors including: the cirrhotic persistence architecture, advanced age, presence of latent mutations of HCV, presence of comorbidities such as diabetes, and the consumption of alcohol and tobacco (11-12, 23-24). The approval of second wave DAA is a recent event and it still does not allow a long-term assessment of the impact of SVR on HCC incidence. This is independent, spontaneous, real-life study on a long term follow-up aimed to verify disease natural history after viral clearance. Our findings showed that late HCC may occur after viral clearance and that interestingly, this association with neoplastic onset seems to be strongly occurring in CTP A patients, suggesting that fibrosis could not be the only factor possibly associated to HCC occurrence in this setting of patients. Intriguingly all HCC cases were in those treated with an antiviral SOF-Based regimen, while more enigmatically no HCC developed in those not achieving SVR. Therefore, SVR by DAAs does not seem to completely prevent the occurrence of late HCC recurring. These findings, theoretically, should not occur since the carcinogenic effect of HCV proteins is related to necroinflammation in the presence of active viral replication, which deregulate host cell cycle checkpoints and the virus and immune-mediated oxidative stress leading to DNA mutations in liver cells (25-26). Despite this scientific evidence, mechanisms related to the late HCC occurrence are still unclear even considering possible pathogenetic models that could explain the possible reason of the onset of late HCC in patients with SVR. Indeed, the reason why those who underwent DAAS schedule including SOF seems to be likely

associated to early and late HCC may be related to several factors as the low level of immunosurveillance in patients with advanced fibrosis due to possible downregulation of interferon genes during DAA therapy, and increased cell proliferation. In absence of an appropriate checkpoints, this mechanism could promote tumor development. Therefore, according to previous data (27) and our findings on late HCC occurrence, it seems obvious that further studies are mandatory to better address the potential role of DAA therapy on long term results. In conclusion, HCC is still the most fearsome complication of cirrhosis HCV and that requires both surgical therapies with the possibility of liver transplantation and last generation drugs (28-29). In view of our findings, in addition to previous experiences as well as literature evidence, we have confirmed that the viral clearance by DAAs regimen does not remove the risk of late HCC onset. Therefore, a long-term regular clinical, laboratory and expert ultrasonography follow-up should carefully be performed also on these patients and the current Faster, Higher, Stronger approach to the new antivirals' development should strongly consider possible late adverse events like those we have reported.

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