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Dynamics of liver stiffness predicts complications in patients with HCV related cirrhosis treated with direct-acting antivirals

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ABSTRACT

Background: Direct acting antivirals(DAAs) are effective in reducing inflammatory ant fibrotic markers in patients with chronic hepatitis C virus(HCV) infection and to prevent liver-related complications. Two-dimensional shear wave elastography(2D-SWE) is an effective technique for the assessment of liver fibrosis.

Aim: To evaluate changes in liver stiffness(LS) in HCV cirrhotic patients undergoing DAA therapy and to identify non-invasive parameters that predict the occurrence of liver-related events.

Methods: We enrolled 229 patients who received DAAs between January 2015 and October 2018. Ultrasound parameters and laboratory data were assessed before treatment and 24(T1) and 48(T2) weeks after end of treatment. Patients were followed up every 6 months to evaluate the development of HCC and other liver related complications. Multiple Cox regression analysis was used to determine parameters associated with the development of complications.

Results: Model for End-stage Liver Disease(MELD) score(HR 1.16; CI 95% 1.01–1.33; $p = 0.026$) and a change in LS at T2(1-year Delta LS) < 20%(HR 2.98; CI 95% 1.01–8.1; $p = 0.03$) were independently associated with HCC risk. One-year Delta-LS <20% was independently associated with the development of ascites(HR 5.08; CI 95% 1.03 - 25.14; $p = 0.04$).

Conclusions: Dynamic changes of 2D-SWE-measured LS after DAA therapy may be a useful tool to identify patients who are at higher risk of liver related complications.

1. Introduction

Direct-acting antiviral agents (DAAs) have become the new standard of care for patients with chronic hepatitis C virus (HCV) infection with an overall eradication rate of over 90% [1]. The optimal drug safety profile allows HCV treatment in different stages of chronic liver disease from mild fibrosis to advanced cirrhosis [1].

In patients with advanced liver disease, the fibrotic rearrangement of the liver tissue is responsible for both vascular disturbances involved in the development of portal hypertension (PH) and microenvironmental changes associated with the occurrence of hepatocellular carcinoma (HCC) [1]. For this reason, the current clinical practice guidelines for the treatment of hepatitis C recommend the evaluation of liver fibrosis to support decisions on treatment timing and patients follow-up [1].

Moreover, the achievement of a sustained virologic response (SVR) has been shown to prevent liver-related complications in patients with HCV-related cirrhosis [2].

However, the first reports concerning HCC risk after the achievement of SVR with DAAs have shown conflicting results: some studies demonstrated a protective effect of SVR on HCC development [3,4], whereas others underlined a relationship between DAAs treatment and HCC recurrence [5].

Finally, a recent metanalysis concluded that there are no substantial differences in HCC development in patients treated with

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antivirals [6]. However, the heterogeneity of data, the retrospective design and the short follow-up term of many studies do not allow to draw definitive conclusions. Moreover, because of these limitations, it is not possible to identify predictors of HCC onset. Even less information is available about PH-related events, such as ascites, hepatorenal syndrome, infections and gastrointestinal bleeding. Some authors have suggested that in patients with cirrhosis and SVR after DAA therapy the improvement in liver function and PH is associated with a decrease in liver-related events and an overall increase in survival rates [7]. Nevertheless, it is important to early identify patients who are at higher risk for liver complications.

Ultrasound elastography provides a non-invasive approach for assessing liver stiffness (LS) and determining the stage of liver fibrosis [8]. Different shear wave-based elastographic methods are available. Transient elastography (TE) is the oldest and most validated one. Currently, it is recommended for the non-invasive staging of hepatic fibrosis in the clinical practice guidelines of the European Association for the Study of the Liver [1,9].

Thereafter, the integration of ultrasound-based elastography into conventional ultrasound machines and the evolution from mono-dimensional to bidimensional techniques has favored the use of non-invasive assessment of liver fibrosis in the clinical practice. Among these techniques, bidimensional shear wave elastography (2D-SWE) has the advantage to fuse in real time B-mode imaging with a stiffness map, allowing the exact localization of the optimal sampling region [10].

In the context of the non-invasive assessment of liver fibrosis in patients with hepatitis C, several studies have revealed that LS decreases after SVR compared with the pre-treatment value [11–13].

Although increasing evidence for the prognostic value of non-invasive LS assessment has been reported, only few studies have evaluated longitudinal changes in hepatic fibrosis and liver disease outcomes after DAA treatment, particularly in patients with severe liver damage at baseline [14] and most of them were performed with TE [15]. It has been suggested that this information may help to recognize patients with residual risk for developing complications, thus individualizing follow-up [11].

Moreover only one study has assessed the relationship between LS measured using 2D-SWE and the occurrence of post-SVR HCC [16]. However this study has a retrospective design and did not evaluated the occurrence of other liver related events.

The purpose of this prospective study was to investigate whether changes in LS evaluated by 2D-SWE were associated with the development of liver related events in a large cohort of HCV patients with cirrhosis treated with DAAs The secondary aim was to identify non-invasive parameters able to predict the occurrence of HCC and PH-related complications.

2. Patients and methods

2.1. Patients

Between January 2015 and October 2018, all cirrhotic patients who were started on DAAs therapy for HCV infection were evaluated for participation in this prospective study. Patients were consecutively enrolled at the Hepatology outpatient clinic of Fondazione Policlinico Universitario “A. Gemelli” IRCCS of Rome, according to the following inclusion criteria: diagnosis of cirrhosis according to histologic criteria and/or unequivocal ultrasound signs of cirrhosis, presence of detectable HCV-RNA before the start of DAA treatment and undetectable HCV RNA in serum or plasma 12 weeks after the end of DAA therapy. Patients were excluded if they had Child C cirrhosis, human immunodeficiency virus and hepatitis B virus coinfection, other liver disease (autoimmune hepatitis, vascular disease of the liver, and biliary tract disorders), prior or current history of liver decompensation and active HCC at the time of enrollment. Additional exclusion criteria were development of HCC before achieving SVR, prior liver transplant, assumption of antiviral or immunosuppressive therapies during the 6 months prior to enrollment.

The study protocol was approved by the Ethical Review Board of Fondazione Policlinico Universitario “A. Gemelli” IRCCS and conformed to the ethical guidelines of the Declaration of Helsinki. All patients provided written informed consent before inclusion.

2.2. Study protocol

The patients’ clinical data, including demographics (age, gender, body mass index), HCV genotype, type of DAA therapy, anamnestic data regarding alcohol consumption, dyslipidemia, hypertension, and diabetes were recorded at the start of DAA therapy. Patients were clinically evaluated and underwent laboratory testing, standard abdominal ultrasound examination and LS measurement within 3 months before the start of the DAA treatment (baseline) and 24 (T1) and 48 (T2) weeks after the end of the therapy. Data on laboratory parameters such as platelet count, renal function, aminotransferases, international normalized ratio, albumin, bilirubin, Child-Pugh score, Model for End-Stage Liver Disease (MELD) score, AST to platelet ratio (APRI), Fibrosis-4 score (FIB-4) were collected. SVR was defined as undetectable HCV RNA at 12 weeks after the end of treatment (EOT).

During follow-up, any liver-related event was registered as for standard clinical practice at our centre. Liver-related events were defined as occurrence of ascites, variceal bleeding, hepatorenal syndrome (HRS), spontaneous bacterial peritonitis (SBP) or other infections, hepatic encephalopathy (HE), portal vein thrombosis (PVT) or HCC. We evaluated also the increase in the staging of esophageal varices in patient who underwent follow-up esophagogastroduodenoscopy.

In cases with a nodular lesion detected using ultrasonography the patient underwent contrast-enhanced magnetic resonance imaging and/or contrast-enhanced computed tomography for further characterization and HCC diagnosis according to the typical hemodynamic signs [17].

Follow-up time was calculated from EOT to the occurrence of events, death or February 2021, however only liver-related events occurring after SVR were considered. Mortality was also registered as liver-related or non-liver-related. In patients who were lost during follow-up, the last day they were known to be alive was registered.

2.3. 2D-SWE examinations

2D-SWE was performed by using the Aixplorer® US system (Supersonic Imagine S.A., Aix-en-Provence, France) equipped with a convex broadband probe (SC6–1) by a single experienced sonographer (MEA) with 8 years of experience in elastographic techniques, who was unaware of clinical data. After at least 6 h fasting with patient in the supine decubitus position and the right arm in maximal abduction, the probe was placed in the right intercostal space that provided the best view of the right liver lobe. A B-mode scanning was performed to select the most appropriate area of the right lobe at least 5 cm in diameter, free of large vessels, and 2 cm below the liver capsule. Measurements were obtained by positioning a 15-mm-diameter region of interest (ROI) in the center of a color map with complete and homogeneous filling, obtained during a breath hold. The mean value of three measurements was calculated in each patient and expressed in kilopascals (kPa).

Table 1
Demographic and clinical data of the study population.

Characteristic	All patients (n = 229)
Age, years	67 (11)
Mean (SD)	
Sex, n (%)	136 (59.4%) /93 (40.6%)
Male/Female	
BMI, kg/m^2	25.5 (3.8)
Mean (SD)	
Comorbidities, n (%)	
Hypertension	114 (49.8)
Diabetes	55 (24.0)
Dyslipidemia	38 (16.6)
History of Alcohol Consumption, n (%)	28 (12.2)
Child-Pugh Score, n (%)	
A	220 (96.1)
B	9 (3.9)
C	0 (0)
MELD	
Mean (SD)	8.9 (2.7)
HCV infection years prior to treatment	
Mean (SD)	17.2 (7.6)
Previous Therapies, n (%)	
Naive	132 (57.9)
Non-responder	64 (28.1)
Relapser	32 (14.0)
Therapy, n (%)	
Sofosbuvir + Ribavirin	37 (16.2)
Simeprevir/Sofosbuvir ± Ribavirin	40 (17.5)
Sofosbuvir/Velpatasvir ± Ribavirin	26 (11.4)
Daclatasvir/Sofosbuvir ± Ribavirin	23 (10.0)
Elbasvir/Grazoprevir	8 (3.5)
Ledipasvir/Sofosbuvir ± Ribavirin	46 (20.1)
Paritaprevir/Ritonavir/Ombitasvir/Dasabuvir ± Ribavirin	46 (20.1)
Glecaprevir/Pibrentasvir	3 (1.2)

n, number of patients; SD standard deviation; BMI, body mass index; HCV, hepatitis C virus; MELD, Model for End-stage Liver Disease.

2.4. Statistical analysis

Data analysis was performed using STATA® Software (Version 14.0, Stata Corporation; College Station, TX, USA). Kolmogorov-Smirnov test was used to assess variables' normality. Continuous variables were reported as mean ± Standard Deviation (SD), categorical data using frequency counts (percentage). To identify differences between patients who developed HCC and others, categorical variables were compared using the χ^2 or the Fisher exact tests and continuous variables by the unpaired Student's t-test. Differences between variables at various time-points were evaluated using paired Student's t-test.

All variables with a p <0.05 at univariate analysis, except the ones which had multicollinearity, were included into a logistic multivariable regression. To confirm the predictive ability of the variables resulted statistically significant at univariate analysis, receiver operating characteristic (ROC) curves were calculated, and optimal cut-offs were identified with the Youden method. Time-dependent ROC curves were constructed using STATA [18].

Individual HCC risk scores were calculated according to the hazard ratio (HR) of the predictive variables. HCC cumulative incidence among groups of patients with different risk scores was evaluated by Kaplan-Meier curves and compared by the log-rank test. Data were censored when liver events, death, or loss of follow-up occurred. All variables with a p <0.05 at univariate analysis, except ones which had multicollinearity, were included into Cox-Regression model. A Cox-Regression Nomogram was constructed based on the Cox-Regression model. All tests were two-sided, and p values <0.05 were considered statistically significant.

3. Results

3.1. Study population

Between January 2015 and October 2018, 295 patients with HCV-related cirrhosis who started DAA therapy were evaluated for eligibility in this prospective study. Sixty-one patients were excluded due to history of decompensated disease (31 with previous or current presence of ascites, 5 with encephalopathy) or HCC and 5 patients were lost to follow-up after the end of the DAA treatment. Therefore, 229 patients completed the study. Demographic and clinical data of the study population are provided in Table 1. The mean age of patients included was 67.3 years and 59.4% were male. All patients achieved SVR after treatment with DAAs.

3.2. Dynamic of LS after DAA therapy

At baseline, mean (± SD) LS was 18.1 (± 6.6) kPa and decreased to 13.6 (± 6.1) kPa at T1 and to 12.5 (± 6.1) kPa at T2 with statistically significant differences among time points (p <0.0001) (Fig. 1) (Table 2). During follow up 46 patients (20.1%) achieved a LS value <10 kPa, that is the cut-off for liver cirrhosis with Aixplorer based 2D-SWE [19].

3.3. Follow-up and liver-related events

The median follow-up was 3.25 years (range 0.5 – 4.7). During the study period, 8 patients were lost to follow-up (3.5%), 9 patients died (3.9%, 4 from liver-related causes) and 5 were transplanted (2.2%).

In our population, HCC was the most frequent liver-related event (30 patients, 13.1%). Moreover, 13 patients (5.7%) developed

Fig. 1. Liver stiffness values at different time points. * *p* <0.0001; ** <0.0001; *** 0.17. T0: baseline; T1: 6 months after end of treatment; T2: 12 months after end of treatment; kPa: kilopascal.

Table 2
Laboratory data, Liver Stiffness and Clinical Scores at different time points. Data are presented as mean±SD.

Parameter	T0	T1	*p* value	T2	*p* value	ANOVA*p* value
ALT, U/l	67 ± 62	22 ± 13	**<0.0001**	19 ± 9	**<0.0001**	**<0.0001**
AST, U/l	69 ± 54	28 ± 14	**<0.0001**	24 ± 10	**<0.0001**	**<0.0001**
INR	1.14 ± 0.24	1.14±0.25	NS	1.13 ± 0.28	NS	0.98
Creatinine, mg/dl	0.89 ± 0.76	0.92 ± 0.74	NS	0.94 ± 0.69	NS	0.79
Albumin, g/dl	4.3 ± 3.6	4.5 ± 3.9	NS	4.4 ± 3.5	NS	0.74
Bilirubin, mg/dl	1.13 ± 0.60	1.04 ± 0.56	NS	1.02 ± 0.62	NS	0.08
Platelets, 10^9/l	149 ± 184	142 ± 82	NS	140 ± 67	NS	0.73
Child-Pugh Score (A/B)	218/10	223/5	NS	219/9	NS	0.75
MELD	8.9 ± 2.7	8.7 ± 2.7	NS	8.2 ± 3.1	NS	0.68
APRI	0.84 ± 0.42	0.64 ± 0.67	NS	0.56±0.62	NS	0.30
FIB-4	5.77 ± 5.36	3.99±3.35	**<0.0001**	3.81±3.41	**<0.0001**	**<0.0001**
LS, kPa	18.1 ± 6.6	13.6 ± 6.1	**<0.0001**	12.5 ± 6.1	**<0.0001**	**<0.0001**

Significant p values are in bold.
n, number of patients; SD standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, International Normalized Ratio; MELD, Model for End-stage Liver Disease; APRI, AST to Platelet Ratio Index; FIB-4, Fibrosis-4 Score; LS, Liver Stiffness.

ascites, 11 (4.8%) PVT, 8 (3.5%) HE, 8 (3.5%) infections and 5 (2.2%) variceal bleeding. No patient developed HRS.

3.4. Predictors of HCC occurrence

Among baseline parameters, male gender, bilirubin, INR, MELD score, prior history of harmful alcohol intake, LS and portal velocity were associated with HCC occurrence by univariate analysis. The decrease in LS during follow up was inversely associated with HCC occurrence. Based on ROC curve analysis we identified a decrease in LS inferior to 20% at one year follow-up (1-year Delta LS <20%) as the best cut off to predict HCC development (AUC: 0.690, sensitivity: 74%, specificity: 65%).

By multivariate analysis using Cox regression, only MELD score (HR 1.16; CI 95% 1.01 - 1.33; $p = 0.026$) and 1-year Delta LS < 20% (HR 2.98; CI 95% 1.01 - 8.1; $p = 0.03$) were independently associated with HCC risk (Table 3). Time-dependent ROC curves analysis demonstrated that these variables maintained their predictive capacity at 24, 48, 36 and 60 months (Supplementary Table 1).

A prior history of harmful alcohol intake (HR 2.63; CI 95% 0.98 - 7.05; $p = 0.05$) and male gender (HR for female 0.23; CI 95% 0.05 - 1.08; $p = 0.06$) showed a trend towards statistical significance as a risk factor for HCC. Neither AST to platelet ratio (APRI) and Fibrosis-4 (FIB-4) nor their variation at one year after EOT were able to predict the occurrence of HCC during follow-up (Table 3).

Finally, when we considered only patients with prior history of HCC (25 patients), no variable was associated with tumor recurrence both at univariate and multivariate analysis (Supplementary Table 2).

3.5. Predictive model of HCC risk

As shown in Table 3, the risk of HCC was significantly different in patients according to baseline MELD score and 1-year Delta LS < 20%.

In order to sharpen the predictive accuracy of the variables independently associated with HCC occurrence and favor their clinical application, we constructed a nomogram. Each variable was assigned with a score equal to its HR, according to Cox regression. Since the variables maintained their predictive value over time (Fig. 2), we combined their HR in a simple model to estimate the probability of HCC occurrence at 24, 36, 48 and 60 months after

Table 3
Factors associated with the development of hepatocellular carcinoma: univariate and multivariate analysis.

Parameter	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p	HR (95% CI)	p
BMI	1.04 (0.95–1.13)	0.40		
HCV infection years pre-therapy	0.99 (0.94–1.06)	0.90		
MELD T0	1.21 (1.09–1.34)	**<0.0001**	1.16 (1.01–1.33)	**0.026**
1-y Delta MELD	1.09 (0.86–1.37)	0.45		
Platelets T0	0.98 (0.97–0.99)	0.037		
ALT T0	0.99 (0.98–1.01)	0.61		
AST T0	1.00 (0.99–1.02)	0.83		
INR T0	3.37 (1.44–7.88)	**0.005**		
Creatinine T0	1.23 80.92–1.65)	0.16		
Albumin T0	1.04 (0.98–1.10)	0.16		
Bilirubin T0	2.00 (1.31–3.03)	**0.001**		
APRI T0	1.01 (0.84–1.25)	0.85		
1-y Delta APRI	1.01 (0.99–1.02)	0.20		
FIB4 T0	1.02 (0.96–1.09)	0.58		
1-y Delta FIB4	0.99 (0.97–1.06)	0.91		
LS T0	1.06 (1.02–1.10)	**0.005**	1.03 (0.97–1.08)	0.26
1-y Delta LS <20%	4.11 (1.68–10.02)	**0.002**	2.98 (1.01–8.11)	**0.03**
HCV-RNA T0	0.97 (0.95–1.03)	0.31		
Portal Caliber T0	1.13 (0.92–1.40)	0.23		
Portal Velocity T0	0.91 (0.83–0.98)	**0.026**	0.93 (0.86–1.02)	0.12
1-y Delta Portal Velocity (%)	1.01 (0.99–1.03)	0.28		
Female	0.12 (0.03–0.53)	**0.005**	0.23 (0.05–1.08)	0.06
Hypertension	0.87 (0.38–1.98)	0.75		
Diabetes	1.16 (0.46–2.97)	0.74		
Dyslipidemia	1.27 (0.47–3.45)	0.49		
History of alcohol abuse	5.17 (2.04–13.06)	**0.001**	2.63 (0.98–7.05)	0.05

Significant p values are in bold.
HR, hazard ratio; CI, confidence interval; BMI, body mass index; HCV, hepatitis C virus; MELD, Model for End-stage Liver Disease; T0, baseline; 1-y, 1 year; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, International Normalized Ratio; APRI, AST to Platelet Ratio Index; FIB-4, Fibrosis-4 Score; LS, Liver Stiffness.

EOT (Supplementary Fig. 1). In particular, a patient with a baseline MELD score of 12 who does not achieve a 20% reduction in LS 1 year after the EOT has a HR of 9 with a probability of HCC of 6%, 15%, and 21% at 24, 36, and 48 months, respectively.

3.6. Predictors of other liver-related events occurrence

We analyzed the association of non-invasive parameters with the occurrence of other liver related events, such as the development of ascites, HE, PVT, increase in the dimension of esophageal varices and variceal bleeding.

Baseline platelet count, INR, LS and 1-year Delta LS < 20% were associated to the development of ascites by univariate analysis. However, only 1-year Delta LS < 20% was independently associated with this outcome with significant hazard ratio (HR 5.08; CI 95% 1.03 - 25.14; $p = 0.04$) (Table 4).

By Cox regression multivariate analysis, albumin (HR 1.09; CI 95% 1.03 - 1.18; $p = 0.003$) and variation of APRI score at 1-year follow-up (1-year Delta APRI) (HR 1.01; CI 95% 1.00 - 1.02; $p = 0.007$) were independently associated with the development of HE whereas MELD score (HR 1.20; CI 95% 0.98 - 1.4; $p = 0.06$) and LS (HR 1.07; CI 95% 0.98 - 1.15; $p = 0.07$) at baseline showed a trend towards statistical significance (Supplementary Table 3).

Portal vein caliber, portal vein velocity, MELD score and platelet count were associated with PVT development. However, only portal vein caliber (HR 1.39; CI 95% 1.03 - 1.87; $p = 0.03$) was independently associated with PVT by multivariate analysis (Supplementary Table 4).

Similarly, portal vein caliber was independently associated (HR 1.47; CI 95% 1.09 - 2.29; $p = 0.02$) with an increase in the staging of esophageal varices in patient who underwent follow-up esophagogastroduodenoscopy after EOT (Supplementary Table 5).

As for variceal bleeding, 4 out of 5 patients did not experience a reduction of at least 20% in LS. All patients had a platelet count below 100 × 10^9/L at baseline.

When we considered the occurrence of any portal hypertension related event, baseline LS (HR 1.07; CI 95% 1.00 – 1.14; $p = 0.03$), portal velocity (HR 0.90; CI 95% 0.81 – 0.98; $p = 0.04$) and 1-year Delta LS < 20% (HR 1.07; CI 95% 1.00 – 1.14; $p = 0.03$),were independently associated with the composite outcome of “other liver related events” with significant hazard ratio (HR 1.07; CI 95% 1.03 – 1.10; $p < 0.0001$) (Supplementary Table 6). Time-dependent ROC curves analysis demonstrated that these variables maintained their predictive capacity at 24, 48, 36 and 60 months (Supplementary Table 7).

4. Discussion

The non-invasive estimation of residual risk of HCC and other liver-related events is crucial for an adequate planning of follow-up in patients with hepatitis C and cirrhosis after eradication with DAA treatment. Although the risk of complications significantly decreases after SVR [20,21], there is an urgent need for non-invasive markers for risk stratification in this population. The identification of adequate tools to predict complications may be useful to individualize patients’ care in the perspective of personalized medicine.

The main findings of the present study were that HCC is the most frequent complication (13.1% of patients) in patients with hepatitis C and cirrhosis after eradication with DAA treatment and patients who did not achieve a significant improvement (>20%) in SWE values presented a remarkably higher risk of HCC.

In our study, the improvement of LS, rather than the baseline value, was independently associated with the occurrence of HCC. Other studies evaluated the dynamic change in LS as a risk factor for HCC after SVR. Indeed, Ravaioli et al. evidenced that a decrease

Table 4
Factors associated with the development of ascites: univariate and multivariate analysis.

Parameter	Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P
BMI	0.86 (0.69–1.05)	0.15		
HCV infection years pre-therapy	1.01 (0.94–1.09)	0.69		
MELD T0	1.15 (0.98–1.33)	0.07		
1-y Delta MELD	1.01 (0.98–1.049	0.28		
Platelets T0	0.98 (0.97–0.99)	**0.008**	0.99 (0.98–0.99)	0.11
ALT T0	0.99 (0.98–1.01)	0.55		
AST T0	1.00 (0.99–1.03)	0.69		
INR T0	3.99 (1.11–14.38)	**0.03**	5.65 (0.72–21.09)	0.09
Creatinine T0	0.56 (0.05–5.91)	0.63		
Albumin T0	0.35 (0.11–1.07)	0.06		
Bilirubin T0	1.40 (0.71–2.74)	0.32		
APRI T0	1.10 (0.91–1.33)	0.33		
1-y Delta APRI	1.00 (0.98–1.01)	0.12		
FIB4 T0	1.05 (0.99–1.21)	0.07		
1-y Delta FIB4	0.99 (0.98–1.05)	0.95		
LS T0	1.07 (1.02–1.13)	**0.006**	1.03 (0.96–1.09)	0.34
1-y Delta LS <20%	1.08 (1.03–1.15)	**0.008**	5.08 (1.03–25.14)	**0.04**
HCV RNA T0	0.99 (0.98–1.04)	0.63		
Portal Caliber T0	1.26 (0.96–1.64)	0.08		
Portal velocity T0	0.97 (0.86–1.09)	0.65		
1-y Delta Portal Velocity (%)	0.99 (0.95–1.039	0.81		
Female	2.11 (0.69–6.48)	0.18		
Hypertension	1.48 (0.48–4.54)	0.49		
Diabetes	0.28 (0.03–2.21)	0.23		
Dyslipidemia	0.75 (0.16–3.4)	0.71		
History of alcohol abuse	3.08 (0.84–11.32)	0.08		

Significant p values are in bold.
HR, hazard ratio; CI, confidence interval; BMI, body mass index; HCV, hepatitis C virus; MELD, Model for End-stage Liver Disease; T0, baseline; 1-y, 1 year; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, International Normalized Ratio; APRI, AST to Platelet Ratio Index; FIB-4, Fibrosis-4 Score; LS, Liver Stiffness.

>30% in LS was associated with a significant reduction in HCC risk [15]. Also Alonso Lopez and colleagues demonstrated that the variation of LS after the DAA therapy is a significant factor in the development of HCC after HCV eradication [12].

In patients with viral hepatitis, fibrosis and necroinflammation have been considered the main determinant of LS. Since liver fibrosis regresses very slowly, most of the decrease in LS during the early period after the SVR may be attributable to an improvement in liver inflammation [22]. Indeed, a histopathology study demonstrated that necroinflammatory changes reverted after SVR in the majority of patients, but persisted in about 13% of patients [23]. Therefore, a persistent inflammatory environment after the achievement of SVR may favor hepatocarcinogenesis [24,25].

Several studies reported that LS decreases after EOT in patients who were treated with DAAs and achieved SVR [26]. Our study confirmed these observations, using 2D-SWE to determine LS. In particular, we observed that the higher magnitude of LS decrease occurs already after 6 months from EOT and a further slight reduction occurs 12 months after the EOT. Moreover, 20% of patients achieved an improvement in LS with levels <10 kPa. This result raises the need for an appropriate redefinition of LS cut-offs to estimate liver fibrosis after the eradication of HCV.

In accordance with other recent studies [11,12], we excluded patient with prior history of decompensation, whose risk for PH-related complications was higher. However, compared with other studies, the population of our study was older, had a longer history of HCV infection and included patients with Child-Pugh class B without history of previous decompensation. Consequently, we observed a higher incidence of *de novo* HCC and other liver-related complications.

Owing to the low incidence of PH-related events, no other study analyzed the predictors of such complications. Ascites was the second most common liver-related event in our population (5.7% of patients). Interestingly, the achievement of a significant decrease in LS (Delta LS >20%) 1 year after the EOT was also independently associated with the development this complication. In patients with advanced liver fibrosis or cirrhosis, LS has showed a good correlation with hepatic venous pressure gradient (HVPG), which is considered the gold standard for the assessment of portal pressure [27]. Indeed, LS represents a measurable surrogate marker of liver fibrosis and necroinflammation, which are the first pathogenic step in the development of PH and PH-related events. Interestingly, in a study by Lens *et al.* the subgroup of patients who developed clinically significant PH had a significantly lower reduction in LS after antiviral treatment and higher MELD, HVPG and LS at baseline [28]. Our results are consistent with these observations and highlight that the dynamic of LS after DAA treatment can predict the occurrence of ascites.

Furthermore, to the best of our knowledge, this is the first study to investigate the use of 2D-SWE elastography for the prediction of liver-related events in a population of patients with HCV-related cirrhosis treated with DAAs. Compared with TE, 2D-SWE has the advantage to be inbuilt in a standard US machine, it measures LS in real time and the ROI can be adjusted in size and location. These benefits may favor the use of LS in the estimation of residual risk of liver complications in clinical practice.

In order to sharpen the predictive value for HCC and favor the application of LS in the clinical management of patients, we developed a model including parameters that were independently associated with HCC occurrence. Indeed, the risk for HCC was linear with MELD score over the time of the study. Similarly, a stability in LS 1 years after EOT represented a significant risk for the development of HCC during the follow-up period. The extreme ease of use, combining both clinical and US parameters that are commonly monitored in clinical practice, and the predictive accuracy of our model over a reasonably long period of follow-up makes it a potentially applicable tool in the management of HCV-related cirrhotic patients after SVR.

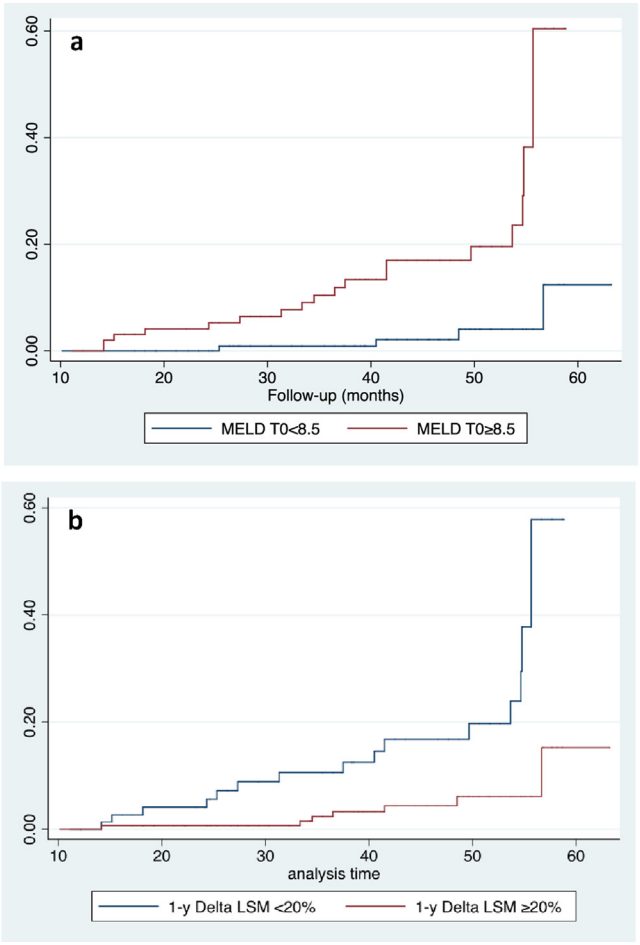

Fig. 2. Cumulative incidence of HCC according to MELD score (cut-off: 8.5, $p = 0.001$) (**A**) and LS decrease (cut-off: 20%, $p = 0.001$) (**B**). *MELD T0: Baseline Model for End-stage Liver Disease score; 1-y Delta LS<20%: decrease in liver stiffness inferior to 20% at one year follow up.*

Our study has some limitations. Compared with other studies, the design of our study was monocentric and the population was smaller. However, our cohort was selected to be homogeneous in terms of risk for liver-related events. In particular we included only patients with compensated liver cirrhosis without prior history of liver decompensation.

Moreover, a single experienced sonographer with a single US machine performed the LS in all patients.

Another limitation is that the predictive value of our model has not been validated in an external cohort.

Finally, we did not perform a direct comparison between LS measured with TE and 2D-SWE in our population. However, most recent international guidelines consider 2D-SWE an alternative option to TE in the quantification of LS [9].

In conclusion, alone and in association with clinical and biochemical variables, LS measured using 2D-SWE may be a useful tool to identify patients who are at higher risk of development of HCC and other liver related complications.

Statement of Ethics

The protocol was approved by the Institutional Review Board of Fondazione Policlinico Universitario "A. Gemelli" IRCCS. All patients were informed of the study protocol and provided written informed consent.

Conflict of interest

All authors confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

Author contributions

MAZ and AG designed the study. AN reviewed the literature, prepared the manuscript and produced tables. MEA performed US examinations. FRP and FS performed clinical follow-up after recruitment. MC analyzed data. MEA, MG, LR, BF, SP, and MDS collected data. MAZ, MP, and AG revised the manuscript critically for intellectual content. All authors approved the final version.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2023.04.018.

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