Short communication

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Risk of hepatocellular carcinoma after HCV eradication: Determining the role of portal hypertension by measuring spleen stiffness

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Background & Aims: Hepatitis C virus (HCV) eradication with direct-acting antivirals (DAAs) reduces but does not eliminate the risk for hepatocellular carcinoma (HCC). The development of surveillance strategies for HCC after the sustained virologic response (SVR) is therefore warranted. We aimed to evaluate the role of spleen stiffness measurement (SSM) in the prediction of HCC risk in a cohort of patients with advanced chronic liver disease (ACLD) treated with DAAs.
Methods: This is a retrospective cohort study of 140 patients with HCV-related ACLD successfully treated with DAAs in our centre between 2015 and 2017. Patients with available liver stiffness (LSM) and SSM before treatment and 6 months after (SVR24) were included. A Cox regression model investigated the association between SSM and HCC development.
Results: During a median follow-up of 41.5 (IQR 32–49) months, 20 patients presented with HCC. SSM at SVR24 predicted HCC development in univariate and adjusted multivariate analysis (hazard ratio: 1.025; 95% CI: 1.001–1.050); the best cut-off was 42 kPa. Patients with LSM-SVR24 ≤10 kPa were at the lowest risk of HCC. In patients with LSM-SVR24 >10 kPa, HCC incidence was not further influenced by LSM values (10–20 kPa *vs.* >20 kPa), but only by SSM-SVR24 values (≤42 *vs.* >42 kPa).
Conclusions: Portal hypertension, as evaluated by SSM, plays a significant role in liver carcinogenesis after DAA treatment. We proposed a new algorithm based on post-treatment values of LSM and SSM for the stratification of HCC risk after SVR achievement.
Lay summary: Spleen stiffness predicts the development of hepatocellular carcinoma after viral eradication, especially in patients with post-treatment liver stiffness values >10 kPa. An algorithm based on liver and spleen stiffness can stratify for the risk of liver cancer development and guide the surveillance strategies after treatment with direct-acting antivirals.
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Introduction

The introduction of direct-acting antivirals (DAAs) has markedly increased the rate of sustained virologic response (SVR) in patients with hepatitis C virus (HCV), even among patients with advanced chronic liver disease (ACLD).[1] It is well-established now that treatment with DAAs reduces the overall risk of hepatocellular carcinoma (HCC) by 50–70%.[2–4] However, some patients remain at considerable risk for HCC despite SVR achievement, so the development of adequate HCC surveillance strategies after HCV eradication is a very relevant topic.[5] Increasing evidence supports the role of liver stiffness measurement (LSM), both before and after DAA treatment, to predict HCC risk despite SVR.[6] Pons *et al.*[7] recently described how post-SVR LSM values (<10 kPa *vs.* 10–20 kPa *vs.* ≥ 20 kPa), combined with albumin levels, were able to predict HCC development with good accuracy (Harrell's C index = 0.73). However, none of these studies has included portal hypertension-related variables, even though portal hypertension plays a significant role in liver carcinogenesis.[8,9] In a recent paper by our study group,[10] we found that spleen stiffness measurement (SSM), a direct non-invasive surrogate of portal hypertension,[11,12] was the only independent predictor of late HCC recurrence after hepatic resection in cirrhotic patients.

In this study we aimed to evaluate the role of SSM assessed by transient elastography (TE) as a mirror of portal hypertension, in the prediction of HCC development after SVR achievement and explore whether its combination with LSM can improve the risk stratification for HCC after viral eradication.

Materials and methods

Study participants and follow-up

This is a retrospective cohort study of patients with HCV-related ACLD, defined by LSM >10 kPa at baseline,[13] who were successfully treated with DAAs in our centre between 2015 and 2017 and had available valid measurements of LSM and SSM before and 6 months (SVR24) after antiviral treatment.[14,15] These patients

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Table 1. Patient characteristics according to HCC development status after viral eradication.

Variable	All patients (n = 140)	Patients who developed HCC (n = 20)	Patients who did not develop HCC (n = 120)	*p* value
Age (years)	63 (55–74)	62 (58–71)	64 (54–74)	0.993
Male (%)	97 (69.3)	17 (85)	80 (66.7)	0.100
Diabetes mellitus (%)	41 (29.3)	5 (25)	36 (30)	0.649
Previous decompensation (%)	30 (21.4)	7 (35)	23 (19.2)	0.110
Laboratory results				
Platelets (cells×10^9/L)	111 (79–150)	93 (68–124)	117 (82–152)	0.083
ALT (U/L)	60 (40–92)	61 (51–124)	59 (39–88)	0.280
Bilirubin (mg/dl)	0.91 (0.67–1.30)	1.03 (0.76–1.66)	0.89 (0.67–1.23)	0.213
Albumin (g/dl)	3.8 (3.6–4.1)	3.8 (3.5–4)	3.8 (3.6–4.1)	0.334
Creatinine (mg/dl)	0.80 (0.70–0.98)	0.88 (0.70–1.01)	0.80 (0.70–0.98)	0.590
INR	1.1 (1.06–1.19)	1.2 (1.09–1.24)	1.1 (1.05–1.16)	**0.007**
MELD score	8 (7–10)	10 (9–119	8 (7–10)	**0.008**
Child-Pugh B (%)	21 (15)	3 (15)	18 (15)	1
Non-invasive tests				
LSM at baseline (kPa)	18.6 (14.1–26.5)	25.6 (21.7–39.4)	17.3 (14.1–25.9)	**0.0004**
LSM at SVR24 (kPa)	13.3 (9.5–21.2)	18.7 (16.9–29.5)	12 (9.1–19.7)	**0.0005**
SSM at baseline (kPa)	58.8 (42.2–75)	63.9 (48.8–75)	57.2 (38.5–75)	0.154
SSM at SVR24 (kPa)	38.2 (29.9–67.7)	57.8 (39.8–73.5)	37.4 (28.7–66.4)	**0.0185**

Qualitative data are expressed as number and percentual (%); quantitative data are expressed as median (IQR). For group comparisons the chi-square test or Mann-Whitney *U* test, and the McNemar test were used, as appropriate. *p* values in bold denote statistical significance. ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; INR, international normalised ratio; LSM: liver stiffness measurement; MELD, model for end-stage liver disease; SSM, spleen stiffness measurement; SVR, sustained virologic response.

have been included in a previous study evaluating the role of non-invasive tests in predicting decompensation after SVR achievement[15]; however, the relationship between elastography and HCC development was not previously explored.

All patients underwent a standard follow-up in agreement with international guidelines.[16,17] No patient started or changed the dose of non-selective beta-blockers in between the two SSM measurements. The incidence of ACLD-related events, such as HCC, decompensation, liver transplantation, or death, was recorded. On February 1, 2020 the follow-up ended; patients who did not develop the event during follow-up were censored at the time of death, liver transplantation, or the last visit to the study centre. This study was conducted in compliance with the Declaration of Helsinki and approved by local institutional review board.

TE examination

The LSM and SSM values were assessed by TE (FibroScan®), 'M' probe, (Echosens, Paris, France) after overnight fasting and an abdominal ultrasound examination. The LSM reliability criteria were in agreement with recent guidelines.[18] The SSM was assessed on the same day as LSM, as previously described.[19,20] The same reliability criteria as for LSM were applied.

Statistical analysis

Categorical data were expressed as n (%), and continuous variables as medians (IQR). The primary outcome was the development of HCC after SVR. The association between TE parameters and the primary outcome was evaluated in univariate and adjusted multivariate Cox regression analysis (including prespecified variables, such as the model for end-stage liver disease (MELD) at baseline, presence of diabetes, and previous HCC). The estimated hazard ratio (HR) with the 95% CI and the c-statistics were reported. Kaplan-Meier curves were used to depict the risk of HCC development during follow-up. All *p* values referred to 2-tailed tests of significance. A value of $p < 0.05$ was considered significant. The statistical analysis was carried out using Stata/SE (Version 14.0; Stata Corp, College Station, TX, USA).

Results

Patient characteristics and follow-up

Of the 183 patients with LSM >10 kPa and available SSM at baseline initially evaluated, 22 patients were lost at follow-up and did not have a second evaluation of SSM, 15 were excluded because SSM was not feasible, and 6 did not achieve SVR. Therefore, a total of 140 SVR patients with paired TE evaluations were included in the final analysis. Most of the patients were male (97, 69.3%), and the median age was 63 (55–74); median MELD at baseline was 8 (7–10). A history of previous decompensation or HCC with complete remission was present in 30 (21.4%) and 13 (9.3%) patients, respectively. The patients' characteristics are summarised in Table 1.

Median follow-up was 41.5 (32–49) months. During this period, 20 (14.3%) patients developed HCC after SVR, of whom 13 presented *de novo* HCC. Moreover, 2 patients underwent liver transplantation, and 8 patients died during follow-up.

Transient elastography and the prediction of HCC development

SSM at SVR24, but not at baseline, was significantly associated with HCC development in univariate analysis (HR 1.029, 95% CI: 1.006–1.053, $p = 0.013$). This association remained significant after adjusting for known confounders (MELD at baseline, previous HCC, and presence of diabetes), with an adjusted HR of 1.025 (95% CI: 1.001–1.050, $p = 0.049$). SSM-SVR24 values predicted HCC development with a good accuracy (c-statistic = 0.682, 95% CI: 0.577–0.802). The best SSM cut-off was 42 kPa (sensitivity 75%, specificity 61%, negative predictive value 93.6%, positive predictive value 24.2%).

LSM-SVR24 was also associated with HCC development both in univariate (HR: 1.039; 95% CI: 1.014–1.065, $p = 0.001$) and adjusted multivariate analysis (HR: 1.044; 95% CI: 1.016–1.073); the overall LSM-SVR24 accuracy was good (c-statistic =0.739, 95% CI: 0.645–0.840).

A new algorithm for the stratification of HCC risk after HCV

Firstly, we evaluated the incidence of HCC in 3 subgroups of patients stratified according to the LSM-SVR24 values (≤10 kPa

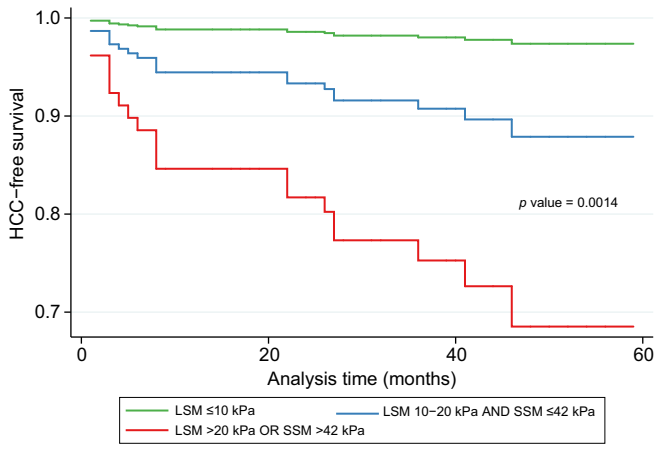

Fig. 1. HCC-free survival according to the new algorithm based on LSM and SSM values after viral eradication. Value of *p* by log-rank. HCC, hepatocellular carcinoma; LSM, liver stiffness measurement; SSM, spleen stiffness measurement.

vs. 10–20 kPa *vs.* >20 kPa). HCC developed in 2.2% (1/45; 95% CI: 0.3–15.4%) of the patients with LSM-SVR24 ≤10 kPa, 17.8% (10/56; 95% CI: 10.2–31.3%) of the patients with LSM between 10 and 20 kPa, and 23.1% (9/39; 95% CI: 13–40.9%) of patients with LSM-SVR24 >20 kPa.

We then explored whether the newly identified SSM-SVR24 cut-off (42 kPa) could improve the risk stratification within each of these subgroups (Fig. S1). The 1 patient with HCC in the first category of patients also had a low SSM (22 kPa). In patients with LSM-SVR24 between 10 and 20 kPa, HCC developed in 10% (3/30; 95% CI: 3.4–29.3%) of the patients with SSM-SVR24 ≤42 kPa and 26.9% (7/26; 95% CI: 14.3–50.7%) of the patients with SSM-SVR24 >42 kPa. In patients with LSM-SVR24 >20 kPa, HCC developed in 14.2% (1/7; 95% CI: 2.3–87.7%) of the patients with SSM-SVR24 ≤42 kPa and 25% (8/32; 95% CI: 13.7–45.6%) of the patients with SSM-SVR24 >42 kPa.

Finally, we reported the risk of HCC-free survival in 3 subgroups (Fig. 1, $p = 0.0014$); according to the new algorithm. The risk was defined as low in patients with LSM-SVR24 ≤10 kPa (incidence rate of 0.65%/year, 95% CI: 0.09–4.57%), moderate in patients with LSM-SVR24 10–20 kPa and SSM-SVR24 ≤42 kPa (incidence rate 3.14%/year, 95% CI; 1.01–9.74%), and high in patients with LSM-SVR24 >20 kPa or SSM-SVR24 >42 kPa (incidence rate 9.71%/year, 95% CI: 5.95–15.83%). Given the limited number of patients and events in the subgroup of patients with LSM-SVR24 >20 kPa and SSM-SVR ≤42 kPa, we considered these patients at high-risk for HCC.

Discussion

In the present study, we demonstrated that the severity of portal hypertension, evaluated by the SSM, is a major determinant of HCC risk after HCV eradication, especially in patients with post-treatment values of LSM >10 kPa. A speculative risk-based surveillance strategy was proposed based on a new algorithm including values of LSM (≤10 kPa or >20 kPa) and SSM (≤42 kPa) after treatment.

In the DAAs era, the risk of HCC development is significantly (50–70%) reduced but not eliminated after viral eradication.[2–4] The development of predictive models that estimate the residual risk for HCC and identify the best candidates for lifelong surveillance after SVR is highly warranted. Several attempts have been made to identify high-risk patients, mainly based on biochemical, elastosonographic, multivariate, and deep learning models.[5] In particular, different models based on LSM values have been proposed for this purpose.[6,7,21–23] However, none of the predictive models reported included variables reflecting the severity of portal hypertension.[5] In their seminal paper, Faillaci *et al.*[8] showed that large oesophageal varices and high levels of angiopoietin-2, a reflection of neoangiogenesis and extensive splanchnic collateralisation, were independent predictors of both HCC occurrence and recurrence after DAAs. This study strongly suggests that the role of DAA in liver carcinogenesis acts through the main predisposing condition: severe portal hypertension and the linked modification of hepatic and splanchnic microcirculation. Therefore, we hypothesised that SSM values could play a significant role in predicting the HCC risk after DAA treatment.

We found that SSM values at SVR24 could predict HCC development both at univariate and adjusted multivariate analysis with good accuracy; the best cut-off for this purpose was 42 kPa. We then evaluated whether SSM-SVR24 values could improve the LSM-based algorithm for HCC risk prediction after SVR. The incidence of liver cancer was lowest in patients with LSM-SVR24 ≤10 kPa (2.2%, incidence rate of 0.65%/year). In patients with more advanced liver disease (LSM-SVR24 >10 kPa), the risk of HCC was determined only by the degree of portal hypertension, as the incidence of HCC within the 2 subgroups defined by SSM-SVR24 cut-off of 42 kPa (10.8% *vs.* 25.9%) was not further influenced by LSM-SVR values (10–20 kPa *vs.* 20 kPa). These results confirm our hypothesis and the findings by Faillaci *et al.*[8] and show that portal hypertension is a major determinant of liver carcinogenesis, also in the post-SVR context.

We finally proposed a new algorithm and a risk-based speculative surveillance strategy based on LSM and SSM after DAA treatment. According to our proposed model, biannual screening could be avoided in low-risk patients (annual incidence <1%/year).[17] In patients at moderate risk for HCC, standard surveillance by ultrasound and biochemical markers every 6 months should be performed. High-risk patients for HCC could benefit from a more intensive follow-up by magnetic resonance imaging or computer tomography spaced out with ultrasound.[5,24,25]

In conclusion, prediction models estimating the HCC risk after HCV eradication should include parameters that mirror the presence of portal hypertension, such as spleen stiffness. The combination of LSM and SSM offers a unique possibility of capturing together liver fibrosis, portal hypertension, and their dynamic changes after specific treatment. Thus, it would be a useful prognostic tool for the risk stratification of complications in patients with liver disease.

Abbreviations

ACLD, advanced chronic liver disease; ALT, alanine aminotransferase; DAA, direct-acting antiviral; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INR, international normalised ratio; LSM, liver stiffness measurement; MELD, model for end-stage liver disease; SSM, spleen stiffness measurement; SVR, sustained virologic response; TE, transient elastography.

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Conflicts of interest
The authors declare that they have no conflicts of interest that pertain to this work.

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

Authors' contributions
Collected data: E.D., G.M., F.R. Analysed data: E.D., G.M., F.R., L.C., A.F. Provided overall oversight of the study: A.C., D.F. Wrote the manuscript: E.D., G.M., F.R. Contributed to the drafting and final approval of the manuscript: E.D., G.M., F.R., L.C., A.F., A.C., D.F. Approved the final manuscript: E.D., G.M., F.R.

Data availability statement
Data are available from the corresponding author upon reasonable request.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2021.100289.

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Author names in bold designate shared co-first authorship

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