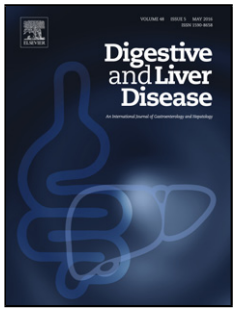
Hepatocellular carcinoma risk assessment by the measurement of liver stiffness variations in HCV cirrhotics treated with direct acting antivirals



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Authors: Federico Ravaioli, Fabio Conti, Stefano Brillanti, Pietro Andreone, Giuseppe Mazzella, Federica Buonfiglioli, Ilaria Serio, Gabriella Verrucchi, Maria Letizia Bacchi Reggiani, Agostino Colli, Giovanni Marasco, Antonio Colecchia, Davide Festi

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HEPATOCELLULAR CARCINOMA RISK ASSESSMENT BY MEASUREMENT OF LIVER STIFFNESS VARIATIONS IN HCV CIRRHOTICS TREATED WITH DIRECT ACTING ANTIVIRALS

Federico Ravaioli[1], Fabio Conti[1], Stefano Brillanti[1], Pietro Andreone[1], Giuseppe Mazzella[1], Federica Buonfiglioli[1], Ilaria Serio[1], Gabriella Verrucchi[1], Maria Letizia Bacchi Reggiani[2], Agostino Colli[3], Giovanni Marasco[1], Antonio Colecchia[1] and Davide Festi[1*]

[1] Department of Medical and Surgical Sciences, University of Bologna, Bologna

[2] Department of Experimental, diagnostic and specialty Medicine, University of Bologna, Bologna

[3] Department of Internal Medicine, General Hospital "A. Manzoni", Lecco, Italy

[4] UOC. Gastroenterology Unit, Borgo Trento University Hospital, Verona, Italy;

Corresponding author*: Prof Davide Festi, Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy; e-mail: davide.festi@unibo.it

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List of abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; DAA, direct-acting antiviral agents; IFN, interferon; SVR, sustained virological response; LSM, liver stiffness measurement; TE, transient elastography; CTP, Child-Turcotte-Pugh score; BL, baseline; EOT, end of treatment; MELD, Model for End stage Liver Disease; APRI, aspartate aminotransferase to platelet ratio index; FIB4, fibrosis-4 score; US, ultrasound; CEUS, contrast-enhanced ultrasound; CT, computerized tomography; MRI, magnetic resonance imaging; kPa, kilopascal; ΔLS, delta liver stiffness; IQR, interquartile range; HR, hazard ratio; CI, confident interval; IRB, Institutional Review Board; HBV, hepatitis B virus; NUC, nucleoside/nucleotide analogue; HVPG, hepatic venous portal gradient.

Authors contributions to manuscript: FR, FC: collected data, analysed data, wrote the manuscript, approved final manuscript. FB, IS, MLBR, AC, GM, ACO: analysed data and contributed to the drafting and final approval of the manuscript. PA, SB, GM, GV, DF: provided overall oversight of the study, analysed data and contributed to the drafting and final approval of the manuscript.

Summary (max 250)

Background: Direct-acting antivirals (DAA) are an effective treatment for hepatitis C virus infection. However, sustained virologic response (SVR) after DAA treatment does not seem to reduce the risk of hepatocellular carcinoma (HCC) development in these patients. Liver stiffness measurement (LSM) may predict the risk of developing HCC in liver cirrhosis patients.

Aims: the aim of our study was to evaluate the role of LSM variation as predictor of HCC development in patients treated with DAA.

Methods: In 139 HCV-related cirrhotic patients, LSM and laboratory tests were carried out at baseline (BL) and at the end of DAA treatment (EOT). Patients were followed for at least 6 months after the EOT. LSM reduction was expressed as Delta LS (ΔLS). Cox regression analysis was used to identify prognostic factors for HCC development after DAA.

Results: Median LSM values were significantly reduced from BL to EOT (from 18.6 to 13.8 kPa; $p<0.001$). The median ΔLS was -26.7% (IQR: -38.4% -13.6%). During a median follow-up of 15 months after DAA treatment, 20 (14.4%) patients developed HCC. Significant LSM reduction was observed both in patients who developed HCC and in those who did not, but this was significantly lower in the patients who developed HCC (-18.0% vs -28.9% $p=0.005$). At multivariate analysis, ΔLS lower than -30%, Child-Turcotte-Pugh-B and history of HCC were independently associated with HCC development.

Conclusion: Our results indicate that ΔLS is a useful non-invasive marker for predicting HCC development after DAA treatment.

Keywords: Delta Liver Stiffness, HCV, Direct-acting Antiviral Agents, Hepatocellular Carcinoma.

Introduction

Hepatitis C Virus (HCV) is the most prevalent cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) in Europe (1).

In December 2014, direct-acting antiviral agents (DAAs) were introduced into Italian clinical practice for the treatment of chronic HCV infection, replacing interferon (IFN)-based regimens (2). These drugs, directly targeting HCV replication, have been shown to achieve sustained virological response (SVR) rates in more than 90% of treated patients, regardless of the viral genotype, liver fibrosis stage and cirrhosis compensation (3).

HCV eradication with DAAs treatment in cirrhotic patients also reduces extrahepatic liver manifestations (4), liver-related morbidity and overall mortality (5), confirming previous studies carried out in HCV patients treated with IFN-based regimens (6).

Of all the complications of liver cirrhosis, HCC development is one of the most important factors affecting liver-related mortality (7), achieving SVR after IFN, reduces HCC development , however the risk remains (8).

Liver Stiffness Measurement (LSM), using transient elastography (TE), is a non-invasive marker for assessing liver fibrosis stage (9) and correlates with portal hypertension (10). LSM has also been proposed in order to evaluate the effect of antiviral treatments on liver inflammation and fibrosis, representing a possible alternative method to liver histology (11). Studies have also found a correlation between LSM values and the risk of clinically relevant outcomes and HCC development in patients with chronic liver diseases(12,13).

To date, the rate of HCC development after DAA treatment in cirrhotic patients appears to vary considerably, some authors have shown unexpectedly high rates of HCC recurrence and occurrence (14–17), while others have indicated that patients treated with DAAs have similar HCC development rates as those obtained after IFN-based regimens (18–20).

We thus decided to evaluate the role of LSM, in conjunction with other clinical liver function scores, in predicting HCC development in HCV cirrhotic patients treated with DAA-based regimens.

Materials and Methods

Patients

This retrospective analysis included all patients with HCV-cirrhosis treated with DAAs between January 2015 and June 2016 at our center, with a valid measurement of LSM by TE at baseline (BL) and at the end of DAA treatment (EOT). We excluded patients without a complete response to surgical resection or loco-regional ablation of previous HCC (i), who developed HCC during antiviral treatment (ii) and/or with follow-up duration less than six months after EOT (iii).

Antiviral treatment

Eligibility for treatment of HCV patients with DAAs was assessed following the priority criteria established in the protocol approved by the Italian Medicines Agency committee. The choice of DAA and treatment duration (12 or 24 weeks) was based on viral genotype and stage of disease, according to the guidelines available at the time of enrollment (21).

Diagnosis of cirrhosis was assessed by TE (stiffness values ≥12.5 kPa according to Castera et al. (22)), abdominal ultrasound and/or histology, if available. At baseline, anthropometric measure included body weight (kg) and height (m). In addition, Model for End stage Liver Disease (MELD) and Child-Turcotte-Pugh (CTP) scores were also calculated for each patient. Aspartate aminotransferase to platelet ratio index (APRI) and fibrosis-4 score (FIB4)(23) were calculated from BL laboratory values. SVR was defined as undetectable HCV-RNA, using real-time PCR, with a detection limit of 15 IU/mL, at 12-week post-treatment follow-up visit.

Surveillance and HCC assessment

Before starting antiviral treatment, all patients underwent abdomen ultrasound (US). 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. All patients with a history of HCC underwent CT scan or MRI, in addition to US, to exclude recurrent HCC.

All patients were followed up for a period of at least six months after EOT or until HCC development. During the follow-up period, patients repeated US evaluation as recommended by the surveillance programme guidelines (24). In the case of suspected HCC development, CEUS/CT/MRI were carried out to confirm or exclude the presence of HCC.

Liver Stiffness Measurements

LSMs were performed using TE (FibroScan, Echosens, Paris France) with a 3.5-MHz transducer (M-probe). The details of the technical background and examination procedure have been previously defined(25). LSMs were performed by one experienced clinician (who had performed at least 500 examinations) after at least six hours of fasting and after a complete abdominal US examination. Results were recorded in kilopascal (kPa) ranging from 3 kPa to a maximum value of 75 kPa. Briefly, for each patient, the examination was considered complete when at least 10 valid measurements or a maximum of 20 attempts at measurement were carried out. LS values were accepted if the success rate was >60% and the interquartile range (IQR) was <30% of the median value(26). LSM was performed at BL and EOT. The Delta LS (ΔLS) was calculated as the difference between LSM at BL and EOT and divided by LSM at BL. The predefined limit to consider ΔLS significant was ≥30% decrease from baseline, since the 30% threshold of LS change corresponds to the accepted variability of the TE assay.

Statistical analysis

Categorical data were expressed as numbers (percentages) and continuous variables as medians (IQR or range). For group comparison, the Mann-Whitney U test was used for continuous variables and the Chi^2 test for categorical variables. The ΔLS was also evaluated as a binary variable according to the median value of the entire population. Group comparisons among LSM at BL and EOT were evaluated with Friedman's non-parametric test, and Bonferroni-corrected alphas were used for post-hoc pairwise comparison. The Kaplan-Meier method was used to estimate HCC development after DAA treatment. A log-rank test was used to assess the differences in term of survival. An overall event-free survival plot for HCC development after DAA treatment was constructed for descriptive purposes.

Demographic, clinical, functional and elastometric variables were evaluated with univariate and multivariate Cox regression models in order to assess the risk factors associated with the primary outcome. After evaluation of multicollinearity, variables with a p-value of <0.10 at univariate analysis were included in several multivariate Cox regression models with stepwise backward procedures. The estimated hazard ratios (HRs) with their 95% confidence intervals (CIs), LR chi^2 and Harrell's C were presented. Only p-values of less than 0.05 were considered statistically significant. The statistical analysis was conducted using Stata/SE (Version 14.0; Stata Corp, Texas, U.S.A.).

Ethics:

The DAA-treatment protocol was approved by the National Institutional Review Board (IRB) of the Italian Medicines Agency committee. Local IRB [Institutional Ethics Committee of Sant'Orsola-Malpighi University Hospital (Bologna, Italy)] approval for subsequent anonymous retrospective analyses was authorized.

Results

A total of 139 (36.6%) patients undergoing DAA treatment met inclusion / exclusion criteria and were enrolled in the study **(Figure 1).**

The major clinical and biochemical parameters of patients included in the study are listed in Table 1. A total of 65% of the patients were males, median age was 62 (IQR: 38-83) years, with prevalent HCV genotype 1 (51%). Most patients had a functional status CTP class A (88%) and a median MELD score of 8 (IQR: 7-9). Nineteen (13.7%) patients had a history of previous HCC with a median follow-up interval of 13 (IQR: 5-62) months between the previous HCC treatment and the beginning of antiviral therapy with DAA.

In our cohort 3 (2.1%) patients had a hepatitis B (HBV) infection (all with long-term suppressive therapy with antiviral nucleoside/nucleotide analogue (NUC)) and no patient was co-infected by HIV.

Most patients (84.1%) were treated with SOF-based schedules. DAA-treatment duration was 12 weeks in 82 patients (59%), and 24 weeks in 57 patients 41%). An SVR was achieved in 131 (94.2%) patients. Patients were followed up for a median time of 15 (IQR: 12-19) months after EOT.

HCC development

During the follow-up period, 20 out of 139 (14.4%) patients developed HCC.

Patients who developed HCC after DAA treatment had a significantly higher Child Pugh Score, the majority of whom had a history of previous HCC. **(Table 1)**. Three patients had a virological relapse after antiviral treatment. The median interval between EOT and HCC diagnosis was 7 (IQR: 4 -10) months. Thirteen out of 120 (10.8%) patients developed de novo HCC, with a median time from EOT of 6 (IQR: 4-8) months. HCC recurrence was reported in 7 out of 19 patients (36.8%) with a median time of 10 (IQR: 6-15) months between EOT and HCC diagnosis. In this subgroup, the median interval between previous HCC treatment and neoplastic recurrence was 24 (IQR: 22-49) months.

Changes in Liver Stiffness Measurement in patients treated with DAA

As shown in **Figure 2A**, a significant reduction in LSM after DAA treatment was observed in the majority of patients. Overall, median LSM values decreased from 18.6 kPa (15-26.3) at BL to 13.8 kPa (10.4-20.4) at EOT (p<0.001). A similar trend was observed (**Figure 2B**), both in patients who developed HCC after DAA [from 20.1 kPa (15.7-27.9) at BL to 15.7 kPa (12.8-29.1) at EOT; and in those who did not [from 18.5 kPa (14.8-26) at BL to 13.6 kPa (10.2-20.0) at EOT; p<0.00001]. The same trend was observed comparing patients with de novo occurrence and recurrence of HCC after DAA. In 122 (87.8%) of the patients, ΔLS confirmed an LS reduction from BL to EOT. ΔLS did not correlate with ΔAST/ALT ratio (R=0.188 p= ns).

The median ΔLS was -26.7% (IQR: -38.4% - -13.6%). Patients who developed HCC after DAA had a significantly lower ΔLS than those who did not (-18.0% and -28.9%, respectively; p=0.005) (**Figure 3**). An LSM reduction of >30% was observed in 62 (44.6%) patients, of whom 59 (95.1%) did not develop HCC during follow-up **(Supplemental Material Figure 1)**.

Examining only patients without a history of HCC, the median ΔLS was -26.7% (IQR: -40.6% - -14.5%). Also in this cohort, patients who developed de novo HCC after DAA had a significantly lower ΔLS than those who did not (-18.7% and -28.9%, respectively; p=0.022). Similarly, in patients with a history of HCC, the median ΔLS was -21.8% (IQR: -34.1% - -10.5%). No statistically significant difference in ΔLS was observed in patients who developed HCC recurrence in comparison with those who did not (-13.6% and -29.6%, respectively; p=0.205).

Risk factors for HCC development after DAA treatment

At univariate Cox regression analysis, ΔLS of -30%, MELD score, CTP score, history of previous HCC, LSM at EOT were significantly associated with HCC development after DAA treatment (**Table 2**).

Table 2 shows the results of Cox multivariate models, ΔLS less than -30% (HR: 5.360, 95%CI: 1.561-18.405; p=0.008), history of previous HCC (HR: 2.758, 95%CI: 1.350-5.635; p=0.005) and CTP-B (HR: 4.046, 95%CI: 1.542 -10.618; p=0.005) were independent predictors of HCC development after DAA treatment with good predictive power (LR chi2=21.78, Harrell's C=0.7716).

Figure 4 shows Kaplan-Meier estimated curves of survival from HCC development as a function of the ΔLS value. Patients whose ΔLS was lower than -30% had shorter HCC-free survival than those with ΔLS higher than -30% (p<0.001).

A multivariate sub-analysis in patients without history of HCC confirmed ΔLS less than -30% (HR: 11.623, 95%CI: 1.508-89.606; p=0.019) and CTP-B (HR: 4.200, 95%CI: 1.289 -13.678; p=0.017) as an independent predictor of de novo occurrence of HCC development after DAA treatment (**Supplemental Material Table 1 and Supplemental Material Figure 2**). The above analysis was not performed in patients with a history of HCC due to the small sample size.

Discussion

The development of DAA-based antiviral treatment has offered the possibility to cure HCV infection even in patients with liver cirrhosis, thus to obtaining an improvement in CPT and MELD scores after HCV-eradication in patients with advanced liver disease (27,28). Similarly, LS reduction measured by TE has been observed in patients undergoing DAA treatment (29–32). However, despite clinical improvement and achievement of SVR, patients seem to maintain the risk of HCC development, especially in the short term (14,15), although the role of DAA is still an open issue (33).

In this study, we evaluated the LSM variation and its possible role in patient surveillance during a long-term follow-up after treatment with DAA-based regimens. Firstly, our study confirmed a significant improvement in LSM immediately after the EOT in cirrhotic patients, finding a median reduction of LSM equal to about a quarter of its value at BL (ΔLS=-26.7%), both in patients with and without a previous history of HCC. Our data confirm recent studies which showed a regression of LSM and biochemical fibrosis markers (FIB4 and APRI index) after DAA treatment (32,34). These findings have also been observed in patients treated with IFN-based regimens (35,36). While LSM has been considered an accurate, although surrogate, marker for fibrosis staging in different chronic liver diseases, there are preliminary and conflicting results when LSM is used to identify histologically documented fibrosis regression (37–39). In addition, a significant decrease in portal hypertension as recorded by repeated hepatic venous pressure gradient (HVPG) measurements has been reported after IFN-free therapy (40), correlated with LSM variations (41).

It is thus possible that the improvement in LSM after DAA treatment observed in our study could be the result of two time-related events: a reduction in necro-inflammatory and in fibrosis activities, the latter possibly being influenced by the reduction in portal pressure.

Our key result of the present study was finding that LSM variation could be a tool to indicate the most at-risk patients for HCC development after viral clearance with DAAs. This is particularly important since it is known that the achievement of an SVR in cirrhotic patients reduces, but does not completely abolish, the risk of HCC occurrence/recurrence. In fact, in patients treated with IFN, HCV-clearance has been associated with an improvement in the natural history of patients, but the

risk of HCC did not disappear (6,42). In addition, in cirrhotic patients treated with DAAs, the risk of HCC recurrence and occurrence is not eliminated and might even increase (33,43,44).

LSM may also be a reliable prognostic tool for HCC development (13,45,46). A retrospective study using an LSM-based score reported the risk of HCC development in a cohort of SVR HCV patients treated with IFN (47). Similar results were obtained in HBV patients, showing that early LSM changes predicted liver-related events and HCC development in patients chronically treated with NUC (48).

However, no data have been reported on the role of LSM variation in predicting HCC development/recurrence in patient treated with DAAs. We believe that is the first study to show that patients with HCC development after antiviral treatment had a significantly lower LSM reduction compared to those without HCC occurrence, although non-invasive markers of fibrosis (LSM, APRI and FIB4 scores) were not significantly different in the two groups ($p>0.05$) before the antiviral treatment. In our study ΔLS was more representative of this trend (-18% in HCC patients vs 28.9% in non-HCC patients). This finding was additionally confirmed by a Kaplan-Meir estimated curve of event-free survival which showed significantly better disease-free survival in the population of patients who had a reduction percentage > -30%. In addition, only a reduction of LSM lower than -30%, as well as the CTP B and history of previous HCC, were confirmed as independent factors associated with HCC development at multivariate analysis. Due to the different risk of HCC development in patients previously treated for HCC, we conducted a separate analysis that confirmed the same results in patients without a history of HCC which was then in turn confirmed by a Kaplan-Meir estimated curve of event-free survival in patients without a history of HCC. Due to the small number of patients with HCC in the subgroup analysis, the significance of the estimate of LSM reduction of -30% could be a limiting factor, although the clinical impact of lower delta LS reduction in terms of HCC development remains relevant.

A possible explanation of our results could be the persistence of greater fibrosis and portal hypertension after DAA treatment in patients who developed HCC compared to those who did not, in whom the reduction of LS might mainly be due to an improvement in inflammation.

The main limitations of our study include its retrospective nature and the small sample size of patients who developed HCC after DAA. However, no other concomitant causes of liver disease or HIV-coinfection were observed in our cohort as possible confounding factors for LSM variations and/or HCC development. A key strength of this study is the homogeneity of the two groups at baseline (those with and without HCC development after DAA), in particular regarding the non-invasive marker values of fibrosis (p-value>0.05). Another strength is that HCC development after DAA-treatment was assessed during a long-term follow-up (median 15 months).

In conclusion, our results confirm a significant improvement in LSM in patients with HCV-related

advanced liver disease after DAA treatment, probably due to reduced necro-inflammatory activity or improvement in portal hypertension. LSM variation, expressed as ΔLS, was correlated with HCC development after antiviral treatment. Monitoring HCV patients with LSM at EOT and subsequently calculating the ΔLS may improve the ultrasound-based screening for HCC identification after DAA treatment. However, even in patients with high decrease in LSM, this does not remove the need for US screening in patients with baseline cirrhosis of F3 fibrosis. These conclusions need to be confirmed by external validation and longitudinal studies. What is clear, however, is that the persistent risk of HCC development after HCV-eradication justifies the need for additional tools for surveillance in patients with HCV-related cirrhosis, and LSM variation could be useful in this context.

Conflict of interest statement:

FR, FC, BF, IS, MLBR, AC, GM, AC, DF declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript. PA: Consultant: Roche, MSD, Janssen Cilag, AbbVie, Boehringer, Ingelheim, Gilead Sciences, Intercept, BMS; SB: Grant: Novartis, Gilead, Other: Gilead, Janssen, MSD; GV: Consultant: AbbVie, Gilead Sciences, BMS., Sponsored Lectures (National or International): AbbVie, Gilead Sciences, BMS, MSD.

References

1. Westbrook RH, Dusheiko G. Natural history of hepatitis C. *J. Hepatol.* 2014;61:S58–S68
2. EASL Recommendations on Treatment of Hepatitis C 2016. J Hepatol. 2017; 66:153-194
3. Götte M, Feld JJ. Direct-acting antiviral agents for hepatitis C: structural and mechanistic insights. *Nat. Rev. Gastroenterol. Hepatol.* 2016; 13:338–351
4. van der Meer AJ, Berenguer M. Reversion of disease manifestations after HCV eradication. *J. Hepatol.* 2016; 65(1 Suppl):S95–S108
5. Janjua NZ, Chong M, Kuo M, et al. Long-term effect of sustained virological response on hepatocellular carcinoma in patients with hepatitis C in Canada; *J Hepatol. 2017*; 66:504-513
6. van der Meer AJ, Veldt BJ, Feld JJ, et al. Association Between Sustained Virological Response and All-Cause Mortality Among Patients With Chronic Hepatitis C and Advanced Hepatic Fibrosis. *JAMA*. 2012; 308:2584
7. de Martel C, Maucort-Boulch D, Plummer M, Franceschi S. World-wide relative contribution of hepatitis B and C viruses in hepatocellular carcinoma. *Hepatology*. 2015; 62:1190–1200
8. van der Meer AJ, Feld JJ, Hofer H, et al. Risk of cirrhosis-related complications in patients with advanced fibrosis following hepatitis C virus eradication. *J Hepatol. 2017;* 66:485-493
9. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. *J. Hepatol.* 2015; 63:237–264
10. Colecchia A, Marasco G, Taddia M, et al. Liver and spleen stiffness and other noninvasive methods to assess portal hypertension in cirrhotic patients: a review of the literature. *Eur. J. Gastroenterol. Hepatol.* 2015; 27:992–1001
11. Vergniol J, Foucher J, Terrebonne E, et al. Noninvasive tests for fibrosis and liver stiffness predict 5-year outcomes of patients with chronic hepatitis C. *Gastroenterology*. 2011; 140:1979–1973
12. Pang JXQ, Zimmer S, Niu S, et al. Liver Stiffness by Transient Elastography Predicts Liver-Related Complications and Mortality in Patients with Chronic Liver Disease. *PLoS One*. 2014; 9:e95776
13. Masuzaki R, Tateishi R, Yoshida H, et al. Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. *Hepatology*. 2009; 49:1954–1961
14. Conti F, Buonfiglioli F, Scuteri A, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J. Hepatol.* 2016; 65:727–733
15. Reig M, Mariño Z, Perelló C, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J. Hepatol.* 2016; 65:719–726
16. Kozbial K, Moser S, Schwarzer R, et al. Unexpected high incidence of hepatocellular carcinoma in cirrhotic patients with sustained virologic response following interferon-free direct-acting antiviral treatment. *J. Hepatol.* 2016; 65:856–858
17. Yang JD, Aqel BA, Pungpapong S, et al. Direct acting antiviral therapy and tumor recurrence after liver transplantation for hepatitis C-associated hepatocellular carcinoma. *J. Hepatol.* 2016; 65:859–860
18. ANRS collaborative study group on hepatocellular carcinoma (ANRS CO22 HEPATHER CC and CC cohorts). Lack of evidence of an effect of direct-acting antivirals on the

recurrence of hepatocellular carcinoma: Data from three ANRS cohorts. *J. Hepatol.* 2016; 65:734–740

19. Torres HA, Vauthey J-N, Economides MP, Mahale P, Kaseb A. Hepatocellular carcinoma recurrence after treatment with direct-acting antivirals: First, do no harm by withdrawing treatment. *J. Hepatol.* 2016; 65:862–864
20. Cammà C, Cabibbo G, Craxì A. Direct antiviral agents and risk for HCC early recurrence: Much ado about nothing. *J. Hepatol.* 2016; 65:861–862
21. Castéra L, Vergniol J, Foucher J, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology*. 2005;128:343–350
22. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J. Hepatol.* 2014; 60:392–420
23. Adler M, Gulbis B, Moreno C, et al. The predictive value of FIB-4 versus FibroTest, APRI, FibroIndex and Forns index to noninvasively estimate fibrosis in hepatitis C and nonhepatitis C liver diseases. *Hepatology*. 2008; 47:762–763
24. Dufour JF, Greten TF, Raymond E, et al. Clinical Practice Guidelines EASL – EORTC Clinical Practice Guidelines : Management of hepatocellular carcinoma European Organisation for Research and Treatment of Cancer. *J. Hepatol.* 2012; 56:908–943
25. Colecchia A, Montrone L, Scaioli E, et al. Measurement of spleen stiffness to evaluate portal hypertension and the presence of esophageal varices in patients with HCV-related cirrhosis. *Gastroenterology*. 2012; 143:646–654
26. Bonino F, Arena U, Brunetto MR, et al. Liver stiffness, a non-invasive marker of liver disease: a core study group report. *Antivir. Ther.* 2010;15(Suppl 3):69–78
27. Conti F, Brillanti S, Buonfiglioli F, et al. Safety and efficacy of direct-acting antivirals for the treatment of chronic hepatitis C in a real-world population aged 65 years and older. *J Viral Hepat. 2017;* 1365-2893
28. Fernández Carrillo C, Lens S, Llop E, et al. Treatment of hepatitis C virus infection in patients with cirrhosis and predictive value of MELD: Analysis of data from the Hepa-C registry. *Hepatology*. 2017; 1527-3350
29. Tada T, Kumada T, Toyoda H, et al. Improvement of liver stiffness in patients with hepatitis C virus infection who received direct-acting antiviral therapy and achieved sustained virological response. *J. Gastroenterol. Hepatol.* 2017; 1440-1746
30. Elsharkawy A, Abdel Alem S, Fouad R, et al. Changes in Liver stiffness measurements and Fibrosis scores following Sofosbuvir based treatment regimens without Interferon. *J. Gastroenterol. Hepatol.* 2017; 1440-1746
31. Bachofner JA, Valli P V., Kröger A, et al. Direct antiviral agent treatment of chronic hepatitis C results in rapid regression of transient elastography and fibrosis markers fibrosis-4 score and aspartate aminotransferase-platelet ratio index. *Liver Int.* 2017; 37:369–376.
32. Knop V, Hoppe D, Welzel T, et al. Regression of fibrosis and portal hypertension in HCV-associated cirrhosis and sustained virologic response after interferon-free antiviral therapy. *J. Viral Hepat.* 2016; 23:994-1002
33. Reig M, Boix L, Bruix J. The impact of direct antiviral agents on the development and recurrence of hepatocellular carcinoma. *Liver Int.* 2017; 37 Suppl 1:136–139
34. Bachofner JA, Valli P V., Kröger A, et al. Direct antiviral agent treatment of chronic hepatitis C results in rapid regression of transient elastography and fibrosis markers fibrosis-4 score and aspartate aminotransferase-platelet ratio index. *Liver Int.* 2016; 37:369–376

35. Hézode C, Castéra L, Roudot-Thoraval F, et al. Liver stiffness diminishes with antiviral response in chronic hepatitis C. *Aliment. Pharmacol. Ther.* 2011; 34:656–663
36. Stasi C, Arena U, Zignego AL, et al. Longitudinal assessment of liver stiffness in patients undergoing antiviral treatment for hepatitis C. *Dig. Liver Dis.* 2013; 45:840–843
37. Castera L. Non-invasive tests for liver fibrosis progression and regression. *J. Hepatol.* 2016; 64:232–233
38. D'Ambrosio R, Aghemo A, Fraquelli M, et al. The diagnostic accuracy of Fibroscan for cirrhosis is influenced by liver morphometry in HCV patients with a sustained virological response. *J. Hepatol.* 2013; 59:251–256
39. Maria F. Donato, Cristina Rigamonti, Federica Invernizzi, Giuseppe Colucci, Mirella Fraquelli, Marco Maggioni, Barbara B. Antonelli, Sara Monico, Giorgio Rossi MC. Paired Liver biopsy, Fibrotest and FibroScan® before and after treatment with DAA in liver transplanted recipients with recurrent hepatitis C: diagnostic accuracy and concordance. *Hepatology 2016*; 64:134A–135A
40. Schwabl P, Mandorfer M, Steiner S, et al. Interferon-free regimens improve portal hypertension and histological necroinflammation in HIV/HCV patients with advanced liver disease. *Aliment. Pharmacol. Ther.* 2017; 45:139–149
41. Mandorfer M, Kozbial K, Freissmuth C, et al. Interferon-free regimens for chronic hepatitis C overcome the effects of portal hypertension on virological responses. *Aliment. Pharmacol. Ther.* 2015; 42:707–718
42. Manthravadi S, Paleti S, Pandya P. Impact of sustained viral response postcurative therapy of hepatitis C-related hepatocellular carcinoma: a systematic review and meta-analysis. *Int. J. Cancer*. 2016; 140:1042–1049
43. Nault J-C, Colombo M. Hepatocellular carcinoma and direct acting antiviral treatments: Controversy after the revolution. *J. Hepatol.* 2016; 65:663–665
44. D'Ambrosio R, Colombo M. Should surveillance for liver cancer be modified in hepatitis C patients after treatment-related cirrhosis regression? *Liver Int.* 2016; 36:783–790
45. Narita Y, Genda T, Tsuzura H, et al. Prediction of liver stiffness hepatocellular carcinoma in chronic hepatitis C patients on interferon-based anti-viral therapy. *J. Gastroenterol. Hepatol.* 2014; 29:137–143
46. Jung KS, Kim JH, Kim SU, et al. Liver Stiffness Value-Based Risk Estimation of Late Recurrence after Curative Resection of Hepatocellular Carcinoma : Development and Validation of a Predictive Model. 2014; 9:1–7
47. Wang J-H, Yen Y-H, Yao C-C, et al. Liver stiffness-based score in hepatoma risk assessment for chronic hepatitis C patients after successful antiviral therapy. *Liver Int.* 2016; 36:1793–1799
48. Kim BK, Han K-H, Park JY, et al. A liver stiffness measurement-based, noninvasive prediction model for high-risk esophageal varices in B-viral liver cirrhosis. *Am. J. Gastroenterol.* 2010;105:1382–1390

Figure 1: The study design flow chart of the patients studied and the study time points.

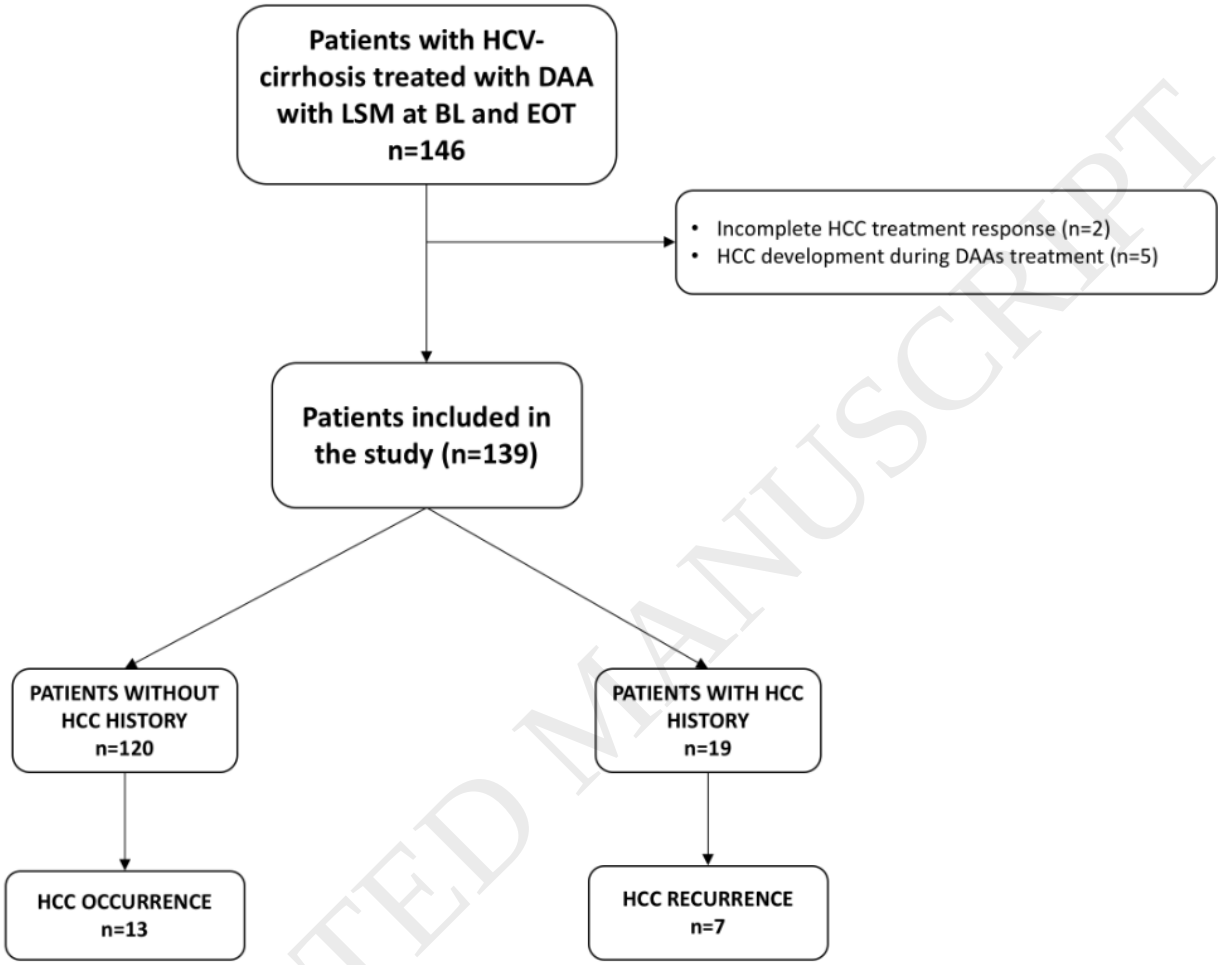

Figure 2: (A) Whole population assessment of dynamic changes of LSM by TE between BL and EOT. (B) Dynamic changes of LSM by TE in group with or without HCC development after DAA-treatment.

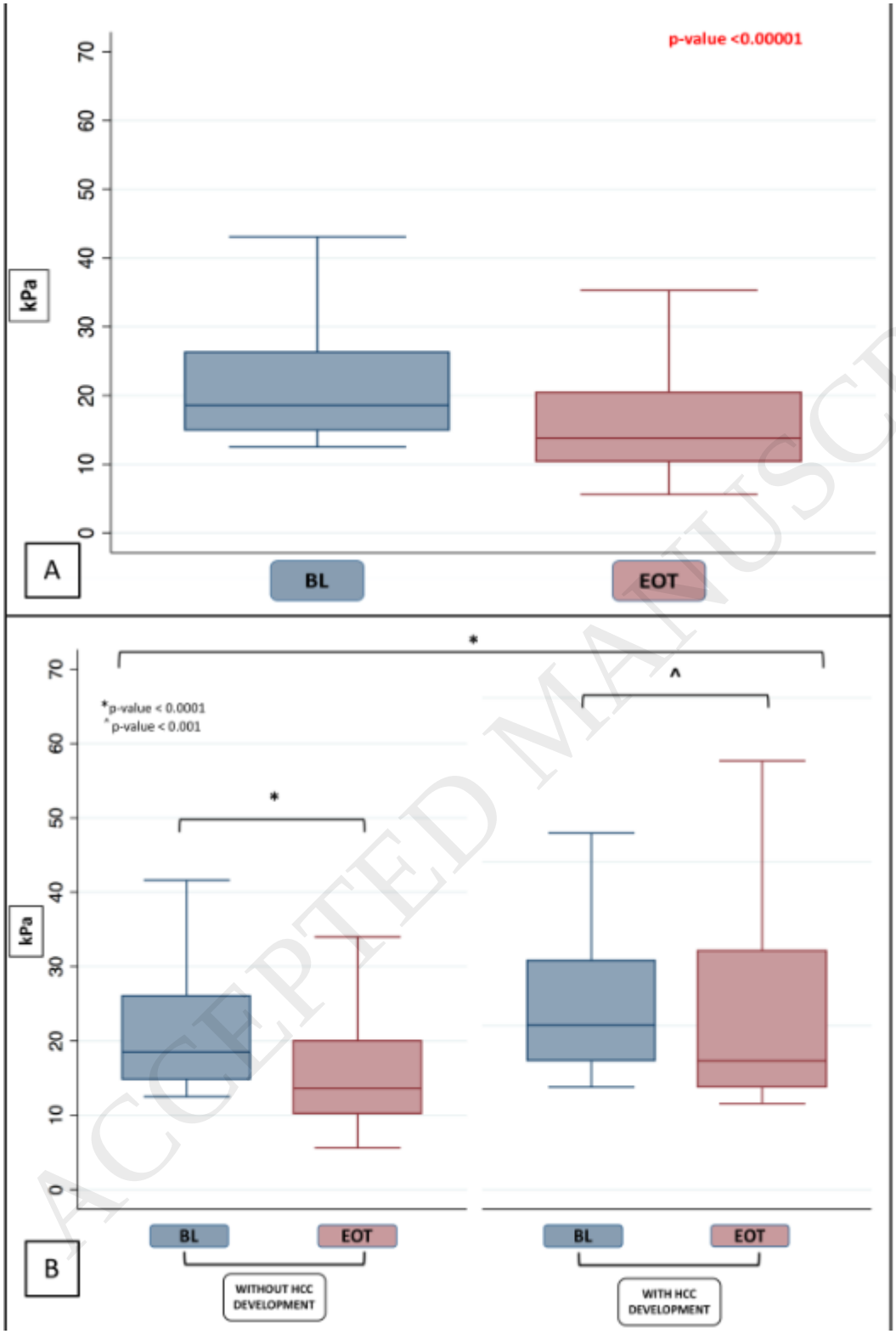

Figure 3: Delta Liver Stiffness (ΔLS) between LSM at baseline (BL) and end of treatment (EOT) according HCC development groups.

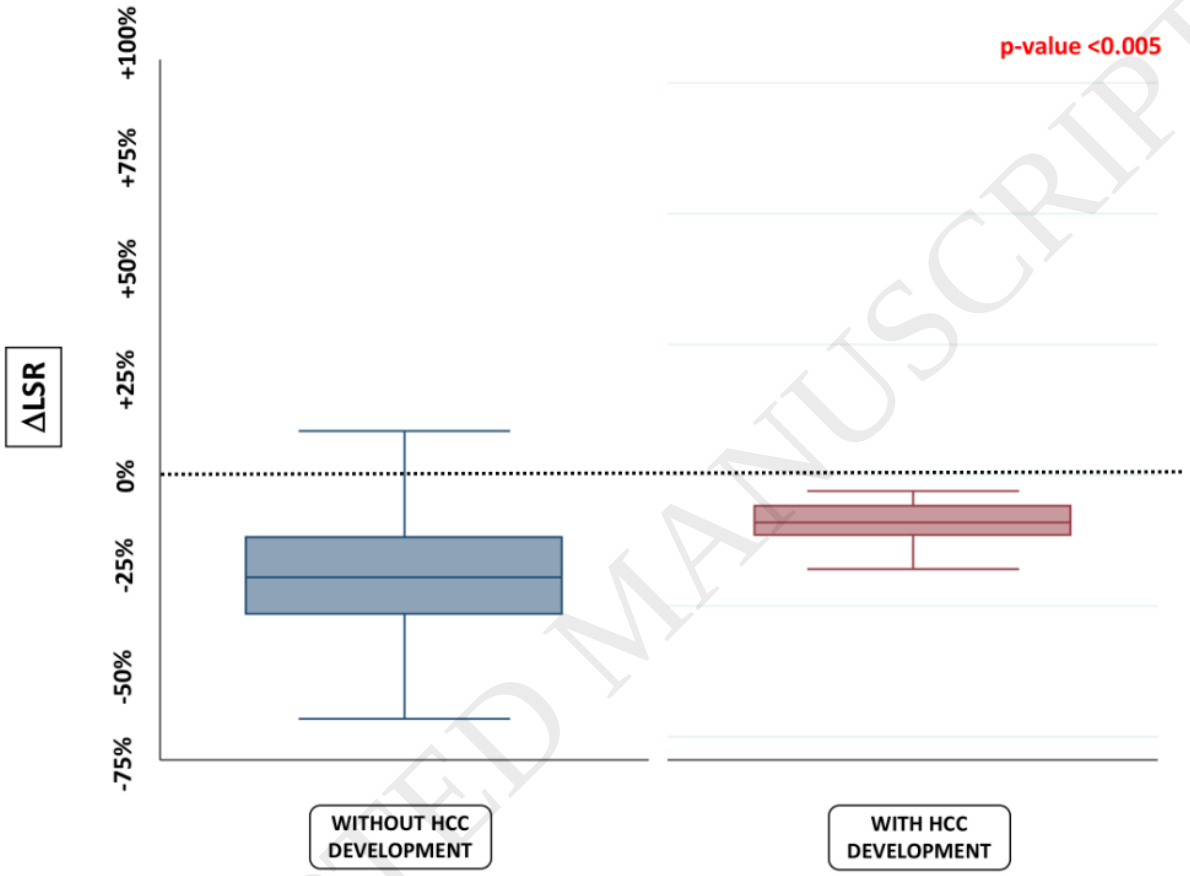

Figure 4: Kaplan-Meier estimated curve of HCC-free survival for HCC development after DAA treatment using ΔLS.

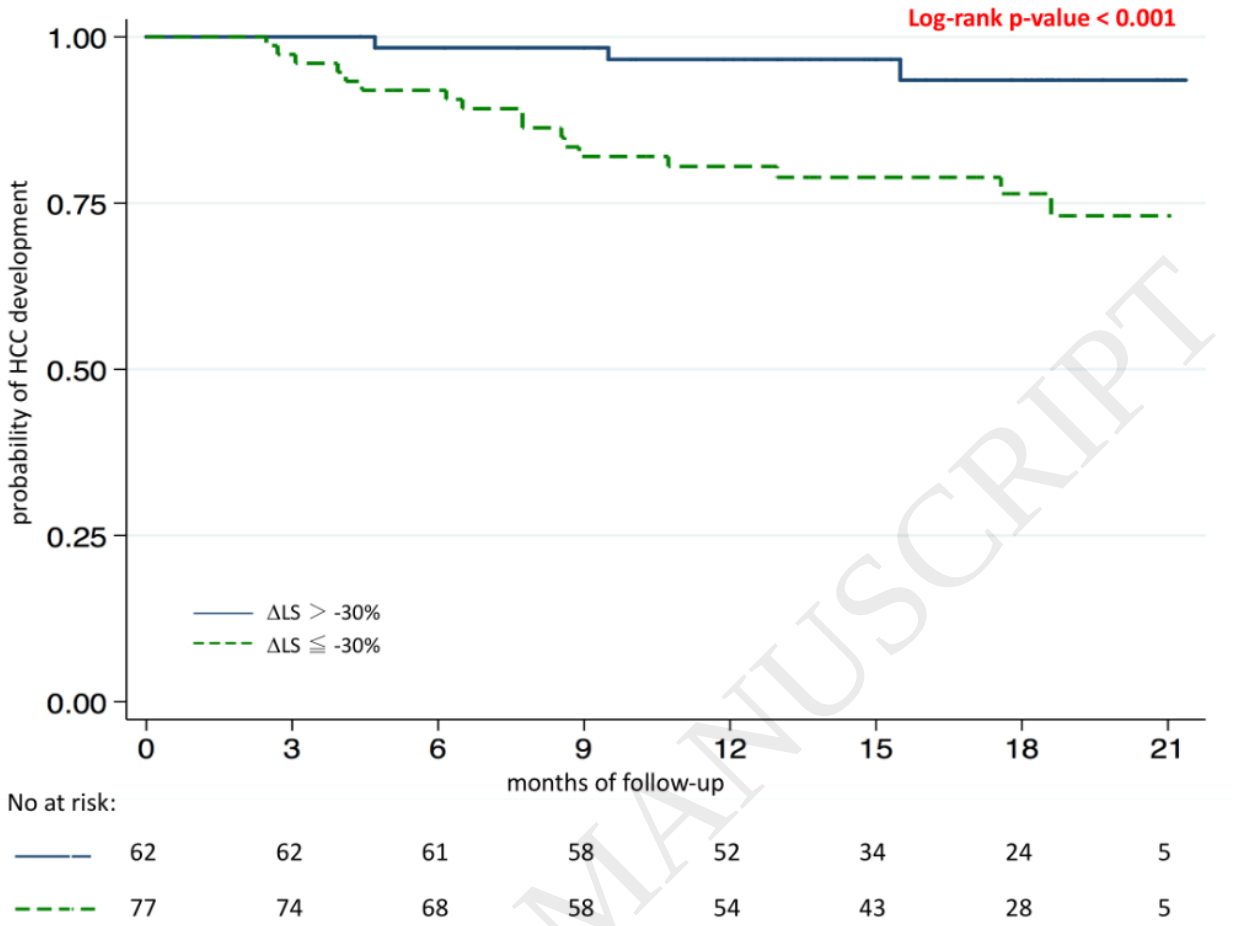

Table 1: Baseline features of 139 patients without HCC development (n=119) or with HCC development (n=20) after DAA treatment.

	Entire cohort *(n=139)*	**Without HCC after DAA *(n=119)***	**With HCC after DAA *(n=20)***	**p-value***
Male, n (%)	91 (65.5%)	79 (66.4%)	12 (60%)	0.578
Age (years), median (IQR)	63 (52 - 73)	62 (52-73)	66 (58-77)	0.229
HCV Genotype, n (%): ▪ 1a ▪ 1b ▪ 2 ▪ 3 ▪ 4	 21 (15.1%) 81 (58.3%) 16 (11.5%) 13 (9.4%) 8 (5.7%)	 19 (16%) 69 (58%) 14 (11.8%) 10 (8.4%) 7 (5.8%)	 2 (10%) 12 (60%) 2 (10%) 3 (15%) 1 (5%)	0.810
CTP score, median (IQR)	5 (5-6)	5 (5-6)	6 (5-7)	**0.022**
CTP class, n (%): ▪ A ▪ B	 123 (88.5%) 16 (11.5%)	 108 (90.8%) 11 (9.2%)	 14 (70%) 6 (30%)	**0.009**
MELD score, median (IQR)	8 (7-9)	8 (7-9)	10 (8-11)	0.060
HBsAg positive, n (%)	3 (2.2%)	2 (1.7%)	1 (5%)	0.345
DAA schedule, n (%): ▪ SOF ▪ SOF+DCV ▪ SOF+SMV ▪ SOF+LDV	 17 (12.2%) 28 (20.1%) 54 (38.8%) 18 (13%)	 15 (12.6%) 23 (19.3%) 46 (38.7%) 16 (13.4%)	 2 (10%) 5 (25%) 8 (40%) 2 (10%)	0.925

▪ PAR/r/OBV+DAS ▪ PAR/r/OBV	19 (13.7%) 3 (2.2)	17 (4.3%) 2 (1.7%)	2 (10%) 1 (5%)	
Treatment duration, n (%): ▪ 12 weeks ▪ 24 weeks	82 (59%) 57 (41%)	71 (59.7%) 48 (40.3%)	11 (55%) 9 (45%)	0.696
History of previous HCC, n. (%)	19 (13.7%)	12 (10.1%)	7 (35%)	**0.007**
PLT (cells x 10^9/L), median (IQR)	119 (82-165)	118 (82-169)	120 (102-143)	0.680
INR, median (IQR)	1.12 (1.04-1.18)	1.12 (1.03-1.2)	1.1 (1.07-1.13)	0.583
ALT (U/L), median (IQR)	56 (35-101)	55 (34-101)	58 (37-78)	1
AST (U/L), median (IQR)	59 (35-85)	65 (35-8)	51 (39-80)	0.807
Bilirubin (mg/dL), median (IQR)	0.90 (0.67-1.23)	0.85 (0.65-1.21)	0.98 (0.68-1.23)	0.523
APRI at BL	1.667 (0.865 – 2.630)	1.587 (0.859 – 2.537)	2.064 (1.047 – 3.871)	0.124
FIB4 at BL	4.743 (3.000 – 6.826)	4.688 (2.905 – 6.774)	5.848 (3.869 – 12.485)	0.056
LSM at BL	18.6 (15 - 26)	18.5 (14.8 - 26)	20.1 (15.7 – 27.9)	0.540

Table 2: Cox proportional hazard analyses for hepatocellular carcinoma development after DAA treatment.

Variables	**Univariate model**			**Multivariate model LR chi2= 21.78 AIC=171.61 Harrells'c=0.7716**		
	HR	**95%CI**	**p-value**	**HR**	**95%CI**	**p-value**
Age	1.233	0.983-1.065	0.259			
Gender (M vs F)	1.211	0.495-2.964	0.675			
MELD Score	1.138	1.001-1.294	**0.049**			
Child-Pugh (B vs A)	3.974	1.521-10.381	**0.005**	4.046	1.542 - 10.618	**0.005**
HBsAg (positive vs negative)	3.850	0.509-29.172	0.192			
APRI at BL	1.147	0.995-1.322	0.06			
APRI at EOT	2.935	1.275-6.757	**0.011**			
FIB4 score at BL	1.077	1.018-1.139	**0.010**			
FIB4 score at EOT	1.143	1.033-1.264	**0.009**			
History of previous HCC (yes vs no)	3.299	1.207-4.378	**0.011**	**2.758**	**1.350 - 5.635**	**0.005**
LSM at BL	1.019	0.975-1.064	0.400			
LSM at EOT	1.043	1.005-1.082	**0.027**			
ΔLSM (<30% vs ≥30%)	5.034	1.475 -17.189	**0.010**	5.360	1.561 - 18.405	**0.008**