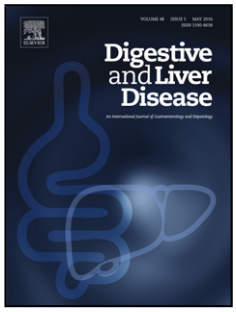

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PII: S1590-8658(18)30986-1
DOI: https://doi.org/10.1016/j.dld.2018.09.016
Reference: YDLD 3874

To appear in: *Digestive and Liver Disease*

Received date: 24-5-2018
Accepted date: 16-9-2018

Please cite this article as: { https://doi.org/

Immune inflammation indicators and ALBI score to predict liver cancer in HCV-patients treated with direct-acting antivirals

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Abstract

Background: Unexpectedly high occurrence or recurrence rate of hepatocellular carcinoma (HCC) has been observed in patients with chronic hepatitis C receiving direct-acting antivirals (DAAs) therapy.

Aims: We evaluated the predictive value of albumin-bilirubin (ALBI) score and immune-inflammation indicators to identify the risk of occurrence or recurrence of HCC in patients treated with DAAs in a real life setting.

Methods*:* In this retrospective cohort study, we analysed data from 514 patients with cirrhosis who were prospectively enrolled for treatment with DAAs. We assessed baseline neutrophil to lymphocyte ratio (NLR), systemic immune-inflammation index (SII), platelet to lymphocyte ratio (PLR), aspartate aminotransferase-lymphocyte ratio (ALRI) index and ALBI score.

Results: In patients with no history of HCC (N=416), increased AST, bilirubin, ALRI, and ALBI score, and decreased albumin and platelets were significantly associated with an increased risk of HCC development, at univariate analysis. At multivariate analysis, increase in ALBI grade (p = 0.038, HR: 2.35, 95% CI: 1.05–5.25) and decrease in

platelets (p = 0.048, HR: 0.92, 95% CI: 0.85–1.0) were independently associated with HCC development. In patients with previous HCC (N=98), adjusting for the time from HCC treatment, increased ALRI (p = 0.008, HR: 1.05, 95% CI: 1.01–1.09) was significantly associated with a risk of recurrence.

Conclusion: ALBI score, platelet count and ALRI are promising, easy to perform and inexpensive tools for identifying patients with higher risk of HCC after treatment with DAAs.

Abbreviations

HCC: hepatocellular carcinoma; DAA: direct-acting antivirals; HCV: hepatitis C virus; SVR: sustained virological response; AIFA: Italian Medicines Agency; CEUS: contrast-enhanced ultrasonography; CT: computerized tomography; MRI: magnetic resonance imaging; RFA: radiofrequency ablation; TACE: trans-arterial chemoembolization; PEI: percutaneous ethanol injection; kPa: kilo Pascal; HBsAg: hepatitis B surface antigen; BMI: body mass index; SOF: sofosbuvir; SMV: simeprevir; RBV: ribavirin; DCV: daclatasvir; LDV: ledipasvir; AFP:α-fetoprotein; BCLC: Barcelona Clinic Liver Cancer; US: ultrasound.

Keywords: albumin; bilirubin; ALRI; SII; NLR; PLR; interferon-free therapy; cirrhosis; cancer immunosurveillance.

1. Introduction

Interferon(IFN)-free regimen using new direct acting antivirals (DAAs) has represented a turning point in the treatment of patients with chronic hepatitis C [1].

The impact of DAAs-based treatment on the development of hepatocellular carcinoma (HCC) in patients with cirrhosis has grown controversial due to potential clinical implications, particularly for HCC recurrence after a successful curative treatment. Some studies have recently reported an unexpectedly high HCC recurrence rate of 27-29% among subjects treated with liver resection or ablation, who had received DAAs therapy [2-4]. However, similar results were not confirmed in other studies [5-9].

The mechanism that could explain such a high rate of tumor recurrence after DAAs treatment is one of the main topics of these studies. Microenvironment and viral-induced inflammation are supposed to play a key role in chronic liver injury and tumor initiation [10]. However, the human immune system itself has also an anti-tumor function [11]. Overall, there is a complex yet fragile balance between the pro- and anti-tumor functions of the immune system. Some studies have proposed that DAAs treatment could modify the natural killer function and the expression of IFN response genes [12,13].

Several inflammation prognostic scores, such as the systemic immune-inflammation index (SII), the neutrophil to lymphocyte ratio (NLR), the platelet to lymphocyte ratio (PLR) and the aspartate aminotransferase-lymphocyte ratio index (ALRI), have been developed to predict survival and recurrence in patients with HCC [14-23].

The albumin-bilirubin (ALBI) score was first proposed by Johnson et al. [24], with the aim to obviate the need of subjective variables, such as ascites and encephalopathy, in the Child-Pugh grading system, for liver function evaluation in HCC patients.

The objective of this study was to evaluate the predictive value of ALBI score and immune-inflammation indicators to identify the risk of HCC occurrence or recurrence in patients treated with DAAs for chronic hepatitis C.

2. Materials and Methods

2.1Patient selection and treatment

In this retrospective cohort study, we analysed data from all the consecutive HCV-infected cirrhotic patients treated with DAAs in seven hepatology centers in the area of Bologna, Italy, between January 2015 and August 2016. Follow-up ended in January 2017. Data were first retrieved from the electronic regional registry database (Piattaforma SOLE). All the additional data were obtained from the individual patient records.

Eligibility for treating hepatitis C with DAAs was assessed for each patient following the priority criteria established by the national registry of the Italian Medicines Agency (AIFA). When possible, alternative treatment options were sought at the clinician's discretion.

Diagnosis of liver cirrhosis was established if at least one of the following was present: 1) previous liver biopsy with stage 4 fibrosis with METAVIR score; 2) presence of esophageal and/or gastric varices at endoscopy; 3) liver stiffness higher than 12 kPa at transient elastography by FibroScan (Echo Sense, Paris, France) [25,26].

Patients with previous liver transplantation or with treated HCC without radiological complete response before starting DAAs were excluded.

The database included 688 patients treated with different regimens of DAAs (105 with and 563 without HCC history). Since our analysis was limited to patients with liver cirrhosis, 144 were excluded because of a F3 METAVIR score or a transient elastography result of less than 12 kPa. Further 10 patients (5 in each category) were not included in the analysis due to liver transplantation (Fig. 1).

Before starting antiviral therapy, all patients with no HCC history underwent abdomen ultrasound (US). If a potential focal lesion was detected in the liver, the diagnostic work-up was completed with contrast-enhanced ultrasonography (CEUS), and a subsequent computerized tomography (CT) scan or magnetic resonance imaging (MRI) was performed to exclude the presence of HCC. All the patients with a history of HCC underwent ultrasound and CT scan or MRI to exclude recurrent HCC.

All patients were followed-up after EOT. During follow-up, patients repeated US evaluation as recommended by the surveillance programme guidelines [27]; in case of suspicion of HCC development, CEUS/CT/MRI were carried out to determine the presence of HCC.

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

All blood values were obtained at baseline and at the end of the treatment.

2.2 Statistical Analysis

Data were summarized by mean ± standard deviation or median and minimum or maximum value for continuous variables, and by frequency and percentage for

categorical variables. Clinical characteristics between patients with and without a history of HCC were compared using the Chi-square test or the Fisher Exact test, when appropriate, for categorical variables and the Student-t test or the Wilcoxon rank-sum test, when appropriate, for continuous variables.

With regard to the time to HCC detection (occurrence or recurrence), we performed distinct analyses for patients with or without a history of HCC.

The Nelson-Aalen estimator was used to obtain cumulative hazard rates at specific follow-up time points within the two groups of patients (with and without a history of HCC).

The median censoring time was computed as the median of the time points since the start of DAA treatment until death, last patient contact or the end of follow-up (censored times), that is, considering only patients not experiencing the event of interest and included in the two groups of patients. The median time to HCC was computed as the median of the time points since the start of DAA treatment to HCC occurrence, considering only patients experiencing the event of interest and included in the two groups of patients. Results are reported as point estimate and 95% confidence intervals (CIs) in round brackets.

Kaplan-Meier curves were used to represent the time to HCC onset since the beginning of DAA treatment. Time was censored at the time of death, last patient contact or end of follow-up. Cox regression was used to evaluate the association between inflammation indices and other covariates and the time to HCC. When not specified, continuous covariates were included in the models as they were, that is, without dichotomization or categorization. For ease of interpretation, some of the reported hazard ratios (HRs) for

continuous covariates correspond to a 10-unit increase in the corresponding variable rather than a 1-unit increase.

The underlying assumption of proportional hazard was tested on the basis of Schoenfeld residuals.

Given the limited number of HCC events and thus the need for a more parsimonious model as well as the presence of correlation among variables, to specify the final Cox regression model we decided to focus our attention of the inflammation indices rather than their single components. Due to the presence of missing values for the continuous variables, multiple imputation using multivariate normal distribution with ten imputed datasets was performed [28].

NLR was computed as the ratio of the absolute neutrophil count to the absolute lymphocyte count, PLR as the ratio of absolute platelet count to the absolute lymphocyte count, SII as platelet count × neutrophil count/lymphocyte count, and ALRI as the ratio of aminotransferase to absolute lymphocyte count. ALBI was obtained through the following formula: $(\log_{10}$ bilirubin × 0.66) + (albumin × -0.085) where bilirubin is in µmol/L and albumin in g/L. PALBI was obtained using the following formula: $(2.02 \times \log_{10}$ bilirubin) + $[-0.37 \times (\log_{10}$ bilirubin$)^2]$ + (-0.04 × albumin) + $(-3.48 \times \log_{10}$ platelets) + $[1.01 \times (\log_{10}$ platelets$)^2]$. For patients with a history of HCC, the time from HCC treatment until start of DAA treatment was computed and categorized as follows: < 6 months, 6 - 12 months, 12 - 24 months, and ≥ 24 months.

Exploratory analyses were performed to test for differences in the mean values of clinical parameters at three different time points (baseline, end-of-treatment, and at 12 weeks of follow-up). An ANalysis Of VAriance (ANOVA) for repeated measurements

was used for this purpose with Bonferroni adjustment for post-hoc comparisons. The analysis was performed only on patients with data at all three time points.

To evaluate the effect of changes in specific clinical parameters from the baseline to the end-of-treatment, Cox regression models were fitted including the absolute change (i.e. lymphocytes at the end-of-treatment minus lymphocytes at baseline) as covariate of interest and adjusting for the value of the clinical parameter at the baseline. This exploratory analysis was performed only on patients with data at both time points.

A p-value less than 0.05 was considered statistically significant. Analyses were performed using STATA 14.0 (College Station, Texas, USA) and R version 3.4.0 statistical software (package "rms" for drawing the nomogram).

3. Results

3.1 Patient characteristics

We performed our analysis on the 514 consecutive patients with HCV-related liver cirrhosis treated with different DAA regimens between January 2015 and August 2016. We split the population into two groups for analysis: patients with history of HCC (98) and without (416).

The median follow-up was 18.04 months (range 0.43–26.41) from the start of the treatment. The baseline characteristics of the two groups before starting DAA therapy are shown in Table 1.

HCC was detected and confirmed by at least two independent imaging techniques, or biopsy, in 30 out of 98 patients (30.6%) with a history of HCC, and in 29 out of 416 patients (7.0%) without a history of HCC.

In patients with a history of HCC the cumulative hazards of HCC recurrence at 6, 12, and 18 months were 0.074 (95% CI: 0.035-0.156), 0.261 (95% CI: 0.172-0.079) and 0.380 (95% CI: 0.262-0.551), respectively. Fig 2a shows the Kaplan-Meier curve for HCC recurrence-free probability.

In subjects without a history of HCC the cumulative hazards of HCC occurrence at 6, 12 and 18 months were 0.010 (95% CI: 0.004-0.026), 0.050 (95% CI: 0.031-0.079) and 0.072 (95% CI: 0.048-0.108), respectively. Fig. 2b shows the Kaplan-Meier curve for HCC occurrence-free probability.

The median censoring time was 19.19 (1.12-26.41) for patients with a history of HCC and 18.23 months (1.84-24.41) for patients without a history of HCC.

The median time to HCC from start of DAA treatment was 8.5 months (0.4-20.8) for patients with and 10.8 (0.9-20.1) for patients without a history of HCC.

3.2 Predictors of HCC recurrence and occurrence

Results from univariable Cox regression models for patients without a history of HCC are reported in Table 2. Based on univariable analysis, a 10-unit increase of AST (p = 0.036, HR: 1.06, 95% CI: 1.01–1.12), a 1-unit increase of bilirubin (p = 0.035, HR: 1.46, 95% CI: 1.03–2.08), a 10-unit increase of ALRI (p =0.002, HR: 1.07, 95% CI: 1.02–1.11), and a 1-unit increase in ALBI score (p = 0.001, HR: 2.99, 95% CI: 1.45–6.15) were significantly associated with a higher hazard of HCC occurrence. In addition, a 1-unit increase of albumin (p = 0.004, HR: 0.34, 95% CI: 0.17–0.71), and a 10-unit increase of platelet count (p = 0.007, HR: 0.89, 95% CI: 0.82–0.97) were significantly associated with a decreased hazard of HCC occurrence.

At multivariable analysis, ALBI score (p = 0.038, HR: 2.35, 95% CI: 1.05–5.25) and the platelet count (p =0.048, HR: 0.92, 95% CI: 0.85–1.00) resulted independently associated with HCC occurrence.

Based on this final multivariable model, a nomogram for predicting the HCC-free probability at 1-year of start of DAA treatment was built (Fig 3).

At univariable analysis, applying the cut-off values published by Johnson et al [24] to ALBI grade, patients with ALBI grade 2 or 3 had a risk of HCC occurrence over three times higher than those with ALBI grade 1 (p = 0.01, HR: 3.22, 95% CI: 1.32-7.86). Using a cut-off value of 100 x 10^9/L for platelets, patients with platelets < 100 x10^9/L had about a two-fold higher risk of HCC occurrence than patients with platelets > 100 x10^9/L (p = 0.033, HR: 2.15, 95% CI: 1.03-4.51). When the categorized variables for ALBI grade and platelets were both included in a multivariable model, ALBI grade resulted statistically significant (ALBI grade 2-3 vs ALBI grade 1: p = 0.01, HR: 2.71, 95% CI: 1.08-6.83), while platelet count did not (platelets < 100 x10^9/L vs platelets > 100 x10^9/L: p = 0.169, HR 1.70, 95% CI: 0.80-3.64).

In patients with a history of HCC, only a 10-unit increase in ALRI index (p = 0.037 HR: 1.03, 95% CI: 1.00–1.05) resulted significantly associated with HCC recurrence at univariable analysis. Neither AST (p = 0.243), nor bilirubin (p = 0.937), nor ALBI score (p = 0.405), nor albumin (p = 0.364), nor platelet count (p = 0.562) were associated with recurrence (Tab 3). Adjusting for the time from HCC treatment, ALRI index remained independently associated with the risk of HCC recurrence (p=0.007 HR: 1.05, 95% CI: 1.01 – 1.09).

3.3 Inflammatory parameters changes after treatment

At baseline, patients without a history of HCC had a higher number of neutrophils and lymphocytes than those with a history of HCC (neutrophils: 2.96 ± 1.33 vs 2.59 ± 1.10 $x10^9$/L respectively; lymphocytes: 1.70 ± 0.85 vs 1.37 ± 0.77 $x10^9$/L, respectively). As shown in Supplementary Table 1, baseline NLR, PLR and SII significantly increased and ALRI significantly decreased at the end of DAA treatment in patients without a history of HCC. Conversely, no changes were observed in patients with a history of HCC apart from ALRI that resulted significantly decreased.

In patients without a history of HCC, a significant increase in neutrophil counts (2.96 ± 1.33 vs 3.17 ± 1.47 $x10^9$/L, p=0,003) and a significant decrease in lymphocyte counts (1.70 ± 0.85 vs 1.47 ± 0.79 $x10^9$/L, p<0.001) were observed between baseline and the end of treatment.

4. Discussion

This study evaluated ALBI score and immune-inflammation parameters as potential predictors of HCC occurrence or recurrence in patients treated with DAA for chronic hepatitis C.

The annual risk of HCC in untreated patients with HCV-related cirrhosis ranges between 3-5% [29] for those without a history of HCC, and 15-20% for patients with a history of HCC treated with surgical resection or radiofrequency ablation [30-40]. Some recent studies have shown unexpected high HCC recurrence rates after DAA therapy [2-4], but other studies contradicted these findings [5-9]. Given these contrasting data, it is pivotal to identify the patients at a higher risk and to determine the possible mechanisms associated with early occurrence or recurrence.

Our study considered a homogenous population of cirrhotic patients divided into two subgroups characterized by the presence of absence of a history of HCC. We found that in patients without a history of HCC, the risk of HCC development seemed dependent on the stage of liver disease (as evidenced by the ALBI score and platelet count). Liver function impairment and cirrhosis remain the main predictors of the risk of HCC for these patients as well as for the untreated patients with hepatitis C [41].

Interestingly, our data show that pre-treatment liver stiffness was not associated with the prediction of the risk to develop HCC at univariate analysis, probably suggesting that liver dysfunction is a better predictor than the stage of liver fibrosis.

Platelets are involved in thrombosis, inflammatory responses, liver regeneration [42-44], and the regulation of angiogenesis [45]. Low platelet count in cirrhosis is associated with a higher risk of developing HCC representing an indirect marker of advanced disease [46].

A nomogram was developed to predict the risk of HCC recurrence in this population at 1 year. This graphical representation may help computing the patient's risk of HCC within a specific timeframe and potentially planning differentiated follow-up schedules. For example, a patient without previous HCC with an ALBI score of -3 (ALBI grade 1 category) and a platelet count of 150 $x10^9$/L would have a total of 95.5 points (ALBI grade = 12.5 points and platelets = 83 points). For a patient with these characteristics, the predicted probability of HCC recurrence in the first year of start of DAA therapy is 0%. Conversely, for a patient with ALBI score of -1.40 (ALBI grade 2 category) and a platelet count of 50 $x10^9$/L would have a total of 127 points (ALBI grade = 32 points and platelets = 95 points) with a risk of recurrence HCC of about 20%. The nomogram

can be used to evaluate the HCC risk for each patient and plan a closer follow-up in high-risk patients.

Hepatic function does not seem to predict the risk of cancer recurrence in patients with a history of HCC, although ALRI index seems to be a good predictor. One explanation could be that a dysregulation of the antitumor response after the sharp decrease in HCV viral load induced by DAA therapy may lead to tumor recurrence. Recurrence could be accelerated by DAA effects, boosting the growth of undetectable HCC as a consequence of a perturbation of the immune surveillance, caused by a swift clearance of HCV.

ALRI index is an inflammation and immune-based prognostic score. Several studies have shown that ALRI index can predict therapy outcome in patients with HCC [14-16]. ALRI index can reflect the efficacy of the immune response based on lymphocytes and hepatic index aspartate aminotransferase.

We reported different results in two different populations (patients without a history of HCC and patients with a history of HCC). The greater risk factor for the development of HCC in patients without a history of HCC is the severity of liver cirrhosis, similarly to the tpopulation no treated with DAA. Conversely, we believe that the immuno-related factor was the only predictor of occurrence in patients with a history of HCC because they may had already carried an immunologic switch that had promoted cancer development.

Other interesting results of this study are the significant decrease in lymphocyte counts and the significant increase in neutrophils during DAA therapy. We think that this created a favorable microenvironment for the growth of a misinterpreted focus of cancer cells that had promoted HCC progression. Other markers tested in this study (NLR, PLR and SII) showed an increase during treatment with DAA. This imbalance reflects

an increase in the inflammatory state during treatment, allowing us to conclude that this change may underlie HCC occurrence and recurrence during DAA therapy.

With this respect, a recent study by Villani et al. [47] demonstrated that DAA therapy induces a rapid reduction in TNF-α, IL-10, and induces an increase in serum VEGF. In particular, IL-10 is a immunoregulatory molecule involved in anti-inflammatory processes in chronic HCV infection. During persistent infections the virus exploits the production of IL-10 by dentritic cells (DCs) to exhaust antiviral T cells. High IL-10 levels produced by DCs suppress their antigen presenting capacity and lead to inefficient T cell activation. Chronic antigen presence further exhausts T cells and induce IL-10 production. T cells therefore become tolerant to viral antigens and infection persists [48]**.** Moreover, IL-10 has pleiotropic and controversial functions in tumor biology, as it seems to function at the crossroads between immune stimulation and immune suppression in cancer [49]. For this reason evaluation of ALRI index allows to evaluate the patient's general inflammatory state and to predict which patients are at greater risk of relapse after DAA treatment. This hypothesis, however, needs to be supported in a specific translational trial.

Among the limitations of this study is its retrospective nature, which might have precluded the collection of data on potential confounding factors or important prognostic factors. However, all cases were consecutively selected, thus reducing any potential bias. Missing data were imputed using multiple imputation, a method proven superior to single value imputation methods (i.e. mean imputation), and the complete case approach, which generally generates problems in case of missing at random data, with the effect of reducing the sample size, and in case of missing not at random data,

with the effect of producing biased estimates [28]. Finally, another limitation is the relatively short follow-up of patients.

In conclusion, to our knowledge, this is the first study to have investigated the risk factors of HCC development after DAA treatment using immune inflammation indicators and ALBI score. Our results indicate that an impaired liver function, but not immune inflammation indicators, is an independent risk factor for the occurrence of HCC after DAA in patients without a history of HCC. On the contrary, the immune-inflammation ALRI index seems to be an independent predictor of recurrence in patients with a history of HCC. Furthermore, we demonstrated that DAA therapy leads to an increase in neutrophils and a decrease in lymphocytes, resulting in a potential imbalance and a subsequent favorable microenvironment for the growth of cancer cells. Low cost, easy determination, and reproducibility of these markers could be usefully in clinical practice. Further studies in larger cohorts of patients are needed to confirm these results.

Conflict of interest

Stefano Brillanti: Advisory Board for MSD and GILEAD Sciences;

Pietro Andreone: Advisory Board for MSD, GILEAD Sciences, ABBVIE, BMS, INTERCEPT; Research grant from MSD, ABBVIE, GILEAD Sciences, BMS.

Author contributions

Conception and design: ACG, FGF, FC, PA, SB.

Provision of study materials or patients: All authors.

Collection and assembly of data: All authors

Data analysis and interpretation: ACG, FGF, FC, PA, SB, EP.

Manuscript writing: ACG, FC, PA, SB, EP, GM.

Final approval of manuscript: All authors

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Figure legends

Fig 1. Flow chart of the study population.

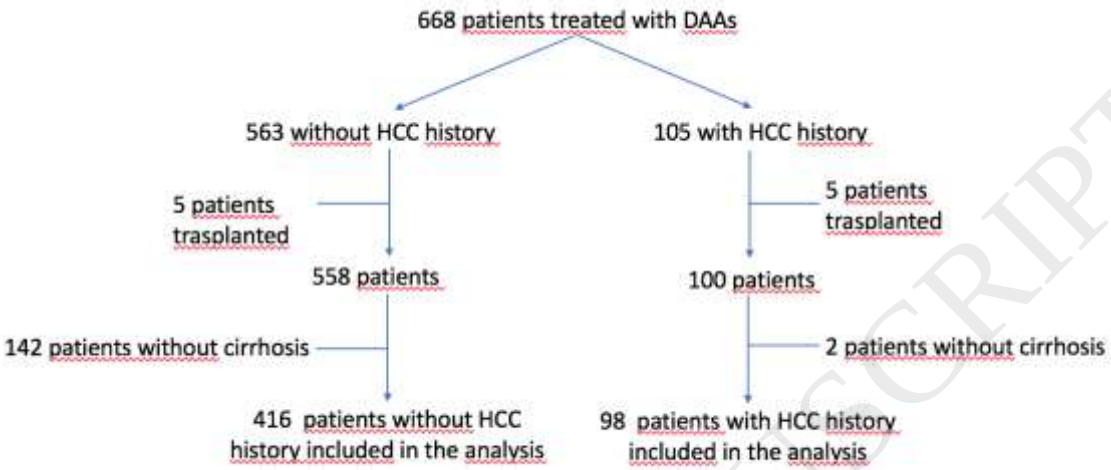

Fig 2. Kaplan-Meier curves for HCC-free probability among patients with a history of HCC (2a) and without a history of HCC (2b).

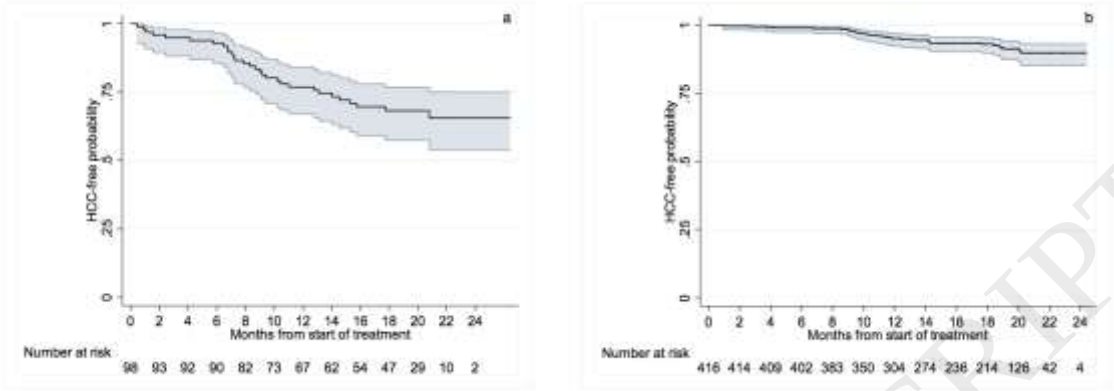

Fig 3. Nomogram for HCC-free probability at 12 months for patients without a history of HCC.

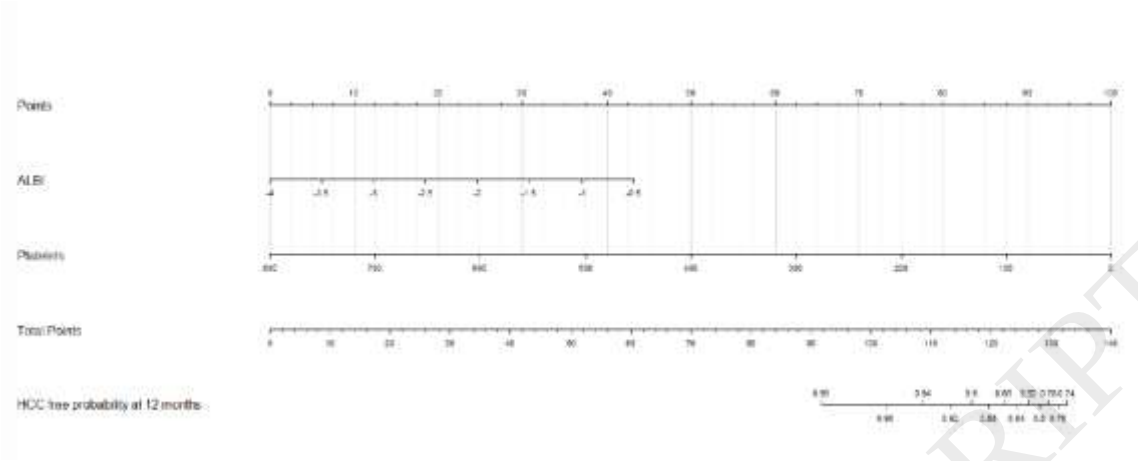

Table legends

Table 1. Baseline characteristics of patients with and without a history of HCC.

Baseline characteristics	Without history of previous HCC (N=416)	With history of previous HCC (N=98)	P-value
Male sex	242 (58.2%)	60 (61.2%)	0.581
Age at start of therapy, years	63.3 [30.9 – 89.7]	71.0 [47.3 – 86.0]	0.016
Oesophageal varices	141 (51.8%)	46 (60.5%)	0.179
Diabetes	96 (23.1%)	28 (28.6%)	0.253
Child-Pugh A B	 351 (84.4) 65 (13.1)	 72 (73.5) 26 (26.5)	 0.004
Arterial hypertension	210 (50.7%) 0,58	40 (40.8%)	0.078
HBsAg positive	3 (0.7%)	1 (1.0%)	0.569
HCV genotype: 1a 1b 2 3 4	 53 (12.7%) 240 (57.7%) 45 (10.8%) 61 (14.7%) 17 (4.1%)	 10 (10.2%) 57 (58.2%) 10 (10.2%) 12 (12.2%) 9 (9.2%)	0.303
Antiviral treatment: SOF+SMV SOF+LDV SOF+DCV SOF+RBV Omb/Par/Rit+Das Omb/Par/Rit	 107 (25.7%) 79 (19.0%) 75 (18.0%) 57 (13.7%) 84 (20.2%) 14 (3.4%)	 28 (28.6%) 12 (12.2%) 19 (19.4%) 20 (20.4%) 17 (17.4%) 2 (2.0%)	0.377
Ribavirin use	303 (73.0%)	69 (70.4%)	0.604
HCV RNA, IU/mL	961083 [504 – 23800000]	608178 [227 – 24400000]	0.172
Time from HCC treatment (months) < 6 6-12 12-24 >= 24 mesi		 34 (35.8%) 17 (17.9%) 25 (26.3%) 19 (20.0%)	
Missing	1	3	
Stiffness	19.1 [5 – 70.6]	21.3 [5 – 58]	0.393
Missing	84	32	
AST, IU/L	71 [16 – 344]	66 [11 – 206]	0.284
Missing	66	17	
ALT, IU/L	65 [14 – 428]	57 [11 – 376]	0.172
Missing	7	2	
Bilirubin, mg/dL	0.8 [0.2 – 5.9]	1.0 [0.3 – 3.8]	0.100
Missing	4	1	
INR	1.1 [0.9 – 3.0]	1.1 [1.0 – 2.1]	0.341
Missing	46	8	

Albumin, g/dL	3.9 [2.1 – 5]	3.7 [2.1 – 4.9]	0.003
Missing	22	6	
Creatinin, md/dL	0.8 [0.3 – 2.3]	0.8 [0.5 – 1.7]	0.092
Missing	4	-	
Hemoglobin, g/dL	13.8 [8.2 – 18.3]	13.1 [8.8 – 17.8]	0.043
Missing	9	3	
Neutrophils, x10^9/L	2.9 [0.6 – 10.1]	2.5 [0.3 – 5.8]	0.042
Missing	28	3	
Lymphocytes, x10^9/L	1.7 [0.3 – 8.2]	1.3 [0.1 – 4.7]	<0.001
Missing	28	3	
Platelets, x10^9/L	114 [15 – 767]	95.5 [26 – 308]	0.006
Missing	9	2	
NLR	1.8 [0.2 – 12.1]	2.1 [0.1 – 20.9]	0.064
Missing	28	3	
PLR	73.2 [10.6 – 430.9]	76.3 [25.4 – 400]	0.819
Missing	29	3	
SII	202.3 [29.0 – 1659.1]	207.3 [9.3 – 1385.2]	0.100
Missing	29	3	
ALRI	46.5 [5.8 - 418.4]	64.2 [9.4 – 700]	0.005
Missing	84	19	
ALBI	-2.5 [-3.7 - -0.8]	-2.4 [-3.3 - -0.7]	0.034
Missing	22	7	
PALBI	-2.6 [-3.5 - -1.1]	-2.5 [-3.3 - -1.5]	0.401
Missing	25	8	

Data are given as median [min-max] or as number of cases and percentage (%).

Table 2. Results from univariable and multivariable Cox regression for the time to HCC occurrence in patients without a history HCC.

	Univariate analysis			Multivariate analysis		
	HR	**(95% CI)**	**P-value**	**HR**	**(95% CI)**	**P-value**
Sex (ref. Male)	0.61	0.28 – 1.35	0.225			
Age at start of therapy, years	1.02	0.99-1.05	0.262			
Varices (ref. No)	1.60	0.75-3.41	0.221			
Child-Pugh (ref. A) B	1.72	0.69-4.23	0.242			
Diabetes (ref. No)	0.77	0.29-2.03	0.602			
Hypertension (ref. No)	0.66	0.32-1.38	0.271			
AST‡	1.06	1.01-1.12	**0.036**			
ALT‡	1.03	0.97-1.09	0.402			
Bilirubin	1.46	1.03-2.08	**0.035**			
INR	1.54	0.27-8.70	0.625			
Albumin	0.34	0.17-0.71	**0.004**			
Creatinine	0.87	0.14-5.39	0.879			
Hemoglobin	0.88	0.73-1.07	0.216			
Neutrophil	0.76	0.54-1.07	0.119			
Lymphocyte	0.58	0.33-1.01	0.056			
Platelet‡	0.89	0.82-0.97	**0.007**	**0.92**	**0.85-1.00**	**0.048**
NLR	1.05	0.81-1.37	0.689			
PLR‡	0.93	0.84-1.04	0.226			
SII‡	0.98	0.95-1.01	0.200			
Liver Stiffness	1.02	0.99-1.05	0.213			
ALRI‡	1.07	1.02-1.11	**0.002**			
ALBI	2.99	1.45-6.15	**0.003**	**2.35**	**1.05-5.25**	**0.038**
PALBI	2.06	0.79-5.42	0.142			
SVR	3.1	0.98-9.8	0.054			

‡Reported as a 10-unit increase

Table 3. Results from univariable Cox regression for the time to HCC recurrence in patients with a history of HCC.

	Univariable analysis			**Multivariate analysis**		
	HR	**(95% CI)**	**P-value**	**HR**	**(95% CI)**	**P-value**
Sex (ref. male)	1.39	0.68 – 2.85	0.370			
Age at start of therapy, years	0.99	0.96-1.03	0.764			
Esophageal varices (ref. No)	0.75	0.36-1.55	0.430			
Child-Pugh (ref. A) B	0.60	0.23-1.58	0.300			
Diabetes (ref. No)	1.17	0.54-2.56	0.691			
Ipertension (ref. No)	0.99	0.48-2.05	0.971			
Ast‡	1.00	0.91-1.11	0.243			
Alt‡	1.04	0.97-1.11	0.391			
Bilirubin	0.98	0.59-1.64	0.937			
INR	1.37	0.19-10.07	0.757			
Albumin	0.70	0.33-1.48	0.346			
Creatinine	1.33	0.26-6.89	0.730			
Hemoglobin	1.06	0.88-1.29	0.521			
Neutrophil	0.89	0.62-1.27	0.512			
Lymphocyte	0.83	0.48-1.43	0.496			
Platelet‡	0.98	0.91-1.05	0.562			
NLR	1.09	0.99-1.19	0.092			
PLR‡	1.01	0.96-1.07	0.616			
SII‡	1.01	0.99-1.02	0.405			
Stiffness	1.02	0.99-1.04	0.264			
ALRI‡	1.03	1.00-1.05	**0.037**	1.05	1.01-1.09	**0.008**
ALBI	1.35	0.66-2.77	0.405			
PALBI	1.31	0.48-3.57	0.600			
SVR	0.83	0.21-3.39	0.799			
Time from HCC (months) <6 6-12 12-24 >=24	 1 0.48 1.29 0.30	 0.14-1.67 0.58-2.87 0.07-1.33	 0.248 0.532 0.112	 1 0.40 1.42 0.17	 0.11-1.44 0.63-3.16 0.02-1.16	 0.160 0.395 0.070

‡Reported as a 10-unit increase