HCC risk stratification after cure of hepatitis C in patients with compensated advanced chronic liver disease

Graphical abstract

**Highlights**

- We studied *de novo* HCC development in patients with cACLD after SVR in a derivation cohort (n = 475) and validation cohort (n = 1,500).

- Algorithms based on post-treatment age/albunim/LSM, and optionally, AFP and alcohol consumption, accurately stratified *de novo* HCC risk.

- Approximately two-thirds of patients were identified as having an HCC risk <1%/year.

- In these patients, HCC-surveillance might not be cost-effective.

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**Lay summary**

Simple algorithms based on age, alcohol consumption, results of blood tests (albumin and α-fetoprotein), as well as liver stiffness measurement after the end of hepatitis C treatment identify a large proportion (approximately two-thirds) of patients with advanced but still asymptomatic liver disease who are at very low risk (<1%/year) of liver cancer development, and thus, might not need to undergo 6-monthly liver ultrasound.

https://doi.org/10.1016/j.jhep.2021.11.025

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HCC risk stratification after cure of hepatitis C in patients with compensated advanced chronic liver disease

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Background & Aims: Hepatocellular carcinoma (HCC) is a major cause of morbidity and mortality in patients with advanced chronic liver disease (ACLD) caused by chronic hepatitis C who have achieved sustained virologic response (SVR). We developed risk stratification algorithms for de novo HCC development after SVR and validated them in an independent cohort.

Methods: We evaluated the occurrence of de novo HCC in a derivation cohort of 527 patients with pre-treatment ACLD and SVR to interferon-free therapy, in whom alpha-fetoprotein (AFP) and non-invasive surrogates of portal hypertension including liver stiffness measurement (LSM) were assessed pre- and post-treatment. We validated our results in 1,500 patients with compensated ACLD (cACLD) from other European centers.

Results: During a median follow-up (FU) of 41 months, 22/475 patients with cACLD (4.6%, 1.45/100 patient-years) vs. 12/52 decompensated patients (23.1%, 7.00/100 patient-years, p < 0.001) developed de novo HCC. Since decompensated patients were at substantial HCC risk, we focused on cACLD for all further analyses. In cACLD, post-treatment-values showed a higher discriminative ability for patients with/without de novo HCC development during FU than pre-treatment values or absolute/relative changes.

Conclusions: Simple algorithms based on post-treatment age/alcohol consumption (optional), age, LSM, and albumin, accurately predicted de novo HCC development (bootstrapped Harrell’s C with/without considering alcohol: 0.893/0.836). Importantly, these parameters also provided independent prognostic information in competing risk analysis and accurately stratified patients into low- (−2/3 of patients) and high-risk (−1/3 of patients) groups in the derivation (algorithm with alcohol consumption; 4-year HCC-risk: 0% vs. 16.5%) and validation (3.3% vs. 17.5%) cohorts. An alternative approach based on alcohol consumption (optional), age, LSM, and albumin (i.e., without AFP) also showed a robust performance.

Lay summary: Simple algorithms based on post-treatment age/alcohol consumption, results of blood tests (albumin and α-fetoprotein), as well as liver stiffness measurement after the end of hepatitis C treatment identify a large proportion (approximately two-thirds) of patients with advanced but still asymptomatic liver disease who are at very low risk (<1%/year) of liver cancer development, and thus, might not need to undergo 6-monthly liver ultrasound.

Keywords: hepatocellular carcinoma; hepatitis C; cACLD; SVR; surveillance.

Received 3 June 2021; received in revised form 15 November 2021; accepted 21 November 2021; available online xxx

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https://doi.org/10.1016/j.jhep.2021.11.025
Introduction

Direct acting antiviral (DAA)-based interferon (IFN)-free therapies for chronic hepatitis C (CHC) are highly effective, achieving sustained virologic response (SVR; i.e., HCV cure) in almost all patients with advanced chronic liver disease (ACLD). SVR following IFN-free treatment has not only been associated with improvements in surrogates of portal hypertension such as liver stiffness measurement (LSM) or von Willebrand factor (VWF) levels, but also with amelioration of portal hypertension as assessed by hepatic venous pressure gradient (HVPG). Decreases in the severity of portal hypertension translate into reductions in hepatic decompensation and concordantly liver-related mortality. Nevertheless, a considerable proportion of patients remains at risk of developing complications of ACLD. While the incidence of hepatic decompensation seems to be comparatively low and non-invasive markers such as LSM and VWF/platelet count ratio (VITRO) facilitate risk stratification, de novo HCC development remains a major concern. Specifically, the incidence of HCC ranged from 1.5–1.8 to 3.6/100 patient-years in patients with ACLD/cirrhosis. Of note, clinically significant portal hypertension (CSPH, as defined by an HVPG ≥10 mmHg) is accompanied by a 6-fold increased risk of HCC in compensated ACLD (cACLD), suggesting that the aforementioned surrogates of portal hypertension may also indicate HCC risk. While no data on VITRO is available, the occurrence of de novo HCC has previously been associated with LSM as well as traditional HCC risk factors such as age, serum albumin, and alpha-fetoprotein (AFP). Several risk prediction models have been proposed based on these and other factors; however, all of these previously published scores have yet to undergo external validation. Thus, no recommendation regarding the identification of a low-risk subgroup of patients with ACLD in whom HCC surveillance is not cost-effective/warranted has been implemented in recent guidelines on the management and follow-up (FU) of CHC.

We investigated the incidence of de novo HCC and its prediction in a comprehensively characterized cohort of patients with ACLD from 3 tertiary centers and aimed to validate the prognostic algorithms we developed in a large, independent validation cohort comprising patients with cACLD from other European centers. In addition, we applied previously published risk prediction models to both cohorts to evaluate their prognostic accuracy.

Patients and methods

Derivation cohort

All patients achieving SVR after DAA-based IFN-free treatment at the Medical University of Vienna, Padua University Hospital, and Ordensklinikum Linz Barmherzige Schwestern with pretreatment ACLD (defined as baseline [BL]-LSM ≥10 kPa, HVPG ≥26 mmHg, or advanced fibrosis/cirrhosis on liver histology [F3/4]) were screened for eligibility for this retrospective study based on prospectively collected data. After excluding all patients with Child-Pugh stage C who were not candidates for liver transplantation (i.e., patients in whom surveillance is not recommended), a history or a current diagnosis of HCC, porto-sinusoidal vascular disease, previous orthotopic liver transplantation (OLT), or an HCC diagnosis/OLT during treatment from the dataset, 527 patients were included. Notably, subgroups of these patients have been previously investigated with regard to changes in HVPG and their prognostic value, the diagnostic/predictive ability of non-invasive markers for portal hypertension and hepatic decompensation, the predictive value of VITRO for hepatic decompensation, the influence of genetic variants on liver disease regression, as well as changes in coagulation after HCV cure. However, none of these studies focused on HCC.

Clinical and laboratory parameters and liver stiffness measurement

Clinical and laboratory parameters were evaluated by chart review. Alcohol consumption above the threshold for non-alcoholic fatty liver disease was defined as >30 g/day and >20 g/day for males and females, respectively. Plasma VWF antigen levels were measured by a latex agglutination assay (STA LIATEST VWF, Diagnostica Stago, Asnieres, France). VITRO score was calculated by dividing VWF (%) over platelet count (PLT) (G × L⁻¹), as described previously. Paired measurements of non-invasive markers were performed prior to antiviral therapy, as well as after the end of treatment (EOT). Due to the retrospective design of this study (and also for logistical reasons), the time points were not standardized. Vibration-controlled transient elastography (FibroScan; Echosens, Paris, France) was used for LSM. All measurements were performed after a minimum fasting period of 4 hours and in the absence of relevant amounts of ascites.

HCV therapy

All patients were treated with IFN-free therapies. The choice of the regimen was at the physicians’ discretion and depended on their availability, reimbursement policies, and national as well as international clinical practice guidelines at the time of treatment initiation. Treatment duration ranged from 8 to 24 weeks.

HCC surveillance

All patients underwent HCC surveillance either by ultrasound, computed tomography, or magnetic resonance imaging on a 6-monthly basis. HCC was diagnosed based on EASL clinical practice guidelines at the time.

Validation cohort

Data was collected from 1,500 patients with cACLD and without a history of HCC/OLT treated at other European centers (Hospital General Universitario Gregorio Marañón and Hospital Universitario 12 De Octubre [Madrid, Spain]; Hospital Universitari Vall d’Hebron and Hospital Clinic [Barcelona, Spain]; and Klinikum Ottakring [Vienna, Austria]). All patients achieved SVR after DAA-based IFN-free treatments. For the Spanish cohorts, details regarding inclusion and exclusion criteria as well as study design are provided in the individual publications, whereas for patients from the other Viennese hospital (Klinikum Ottakring) contributing to the derivation cohort, criteria/design were similar to the validation cohort (Table S1). Patients with missing data on FU-LSM and FU-albumin were not considered for our analyses.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 25 (SPSS Inc., USA) and R 4.0.5. (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were compared using the t-test. Categorical variables were compared using the chi-square or Fisher’s exact test. A p-value < 0.05 was considered statistically significant. All analyses were performed as a two-tailed test.
were reported as mean ± standard deviation or median (interquartile range), while categorical variables were reported as proportion of patients with/without a certain characteristic. Student’s t test was used for group comparisons of normally distributed variables and Mann-Whitney U test for non-normally distributed variables, respectively. Group comparisons of categorical variables were performed using either Pearson’s Chi-squared or Fisher’s exact test. The areas under the curve (AUC) and respective 95% CIs of receiver-operating characteristic (ROC) analyses were calculated for continuous variables using the R package ‘cutoffR’, applying Youden’s J-statistic to obtain the respective optimized cut-offs for classifying patients regarding HCC development. To increase the reliability of these cut-offs, we performed bootstrap resampling 5,000 times. Univariable and multivariable Cox regression analyses were performed using the R ‘survival’ package to investigate the association of individual (continuous and binary) parameters with HCC development. For further model development, backward elimination excluding variables with p > 0.10 was applied to identify variables that provide certain information for HCC prediction. For these analyses, the time to event was calculated from the EoT, and patients were censored at OLT, death, or end of FU. Harrell’s C-indices for the respective models were derived using the R package ‘cmprsk’ with bootstrap resampling performed 5000 times to increase the generalizability of these models. Fine and Gray competing risks regression models were calculated with the R package ‘cmprsk’ to test whether variables included in the final model were still independently associated with HCC when considering OLT and death as competing risks. Finally, a score was derived from respective adjusted subdistribution hazard ratios (aSHRs). Moreover, published prediction models were tested in our cohort using Gray’s test for subdistribution hazards. Applying Youden’s J-statistics and bootstrap resampling, the following cut-offs denoted a high risk for HCC (1.45/100 patient-years) vs. 12 patients with dACL (23.1%, p < 0.001, 7.00/100 patient-years). Since patients with dACL were at very high risk of de novo HCC development, we abstained from merging them with patients with cACL. Moreover, the limited number of patients precluded dedicated analyses on risk factors for HCC in patients with dACL. Accordingly, all other analyses focused on cACL.

**cACL subgroup of the derivation cohort**

Characteristics of patients with cACL with and without HCC during FU (median 41 [IQR 33] months) are presented in Table S3. Also, time points of FU measurements are shown in Fig. S1A which clustered around 12 weeks after EoT. Differences in patient characteristics were observed for age, presence of varices and non-invasive markers of portal hypertension (i.e., LSM, PLT, VWF, and VITRO), hepatic function (i.e., MELD and serum albumin), as well as aspartate aminotransferase, AFP, and composite scores (i.e., aspartate aminotransferase-to-platelet ratio index [APRI] and Fibrosis-4 [FIB-4]) both at BL and FU. Of note, none of the patients who developed HCC during FU had uncharacterized nodules at BL.

Following univariable ROC analyses, a similar moderate accuracy (AUC < 0.800) to identify patients with HCC was evident for several continuous variables (Table 2). Specifically, FU-albumin, FU-LSM, BL-VWF, BL-/FU-VITRO, FU-APRI, BL-/FU-FIB-4, BL-/FU-AFP showed an AUC of 0.700-0.800 with FU-AFP having the numerically highest AUC (0.796; 95% CI 0.726-0.866). Of note, FU variables tended to be more informative than BL parameters. Again, absolute and relative changes were considerably less accurate (AUC < 0.700) with relative Δ LSM showing the highest AUC (0.674; 95% CI 0.570-0.778). These analyses indicated that single parameters are incapable of accurately predicting HCC development in the post-SVR setting.

We aimed at identifying cut-offs that denote a high vs. low risk for HCC for the most promising parameters. Applying Youden’s J-statistics and bootstrap resampling, the following cut-offs were identified: Age ≥ 59.27 years, FU-albumin < 42 g·L⁻¹, FU-LSM ≥ 19.0 kPa, FU-PLT < 190 G·L⁻¹, FU-VWF ≥ 186%, FU-VITRO ≥ 1.02, FU-FIB-4 ≥ 1.93, and FU-AFP ≥ 4.6 ng·ml⁻¹ (Table 3, Fig. S2). We abstained from further analyzing APRI, as it basically contains the same information as FIB-4, but FU-FIB-4 yielded a higher AUC.

**Cox regression analyses and model estimation in the derivation cohort**

Next, we performed Cox regression analyses including dichotomized FU-values of non-invasive parameters and age as a central risk factor, since these values were superior to or equally accurate as BL values, and the utilization of data obtained at a single time point may facilitate the clinical application of the resulting risk prediction model (Table 4). We also included alcohol consumption above the threshold, while we did not include metabolic factors, since no statistically significant associations with HCC development were evident (Table S4).

Following significant univariable associations with HCC development, 7 different multivariable models were built based on combinations of these variables. All of them accurately predicted HCC, however, FU-FIB-4, FU-VWF, and FU-VITRO were not independently associated with HCC development. Following
4 According to the reverse Kaplan-Meier method. Group comparisons of categorical variables were performed using Pearson χ² test or Fisher’s exact test, as applicable. Values in bold indicate p < 0.05. *Fasting blood glucose >125 mg /dL; HbA1c ≥6.5%, or antidiabetic medication. **≥30 kg/m² and >20 g/d for males and females, respectively. †According to the reverse Kaplan-Meier method.

backward elimination, the models comprising age ≥59 years, FU-AFP ≥4.6 ng·mL⁻¹, FU-LSM ≥19 kPa, and FU-albumin <42.0 g·L⁻¹ with and without alcohol consumption above the threshold showed the highest predictive ability (Harrel’s C: 0.893 and 0.874), while the same models without FU-AFP ≥4.6 ng·mL⁻¹ also showed a high discriminative ability (Harrel’s C: 0.836 and 0.815).

**Competing risk analysis and modelling of a score**
To test whether the parameters included in the final version of models 5 and 7 (including alcohol) provided independent information for the prediction of HCC, while accounting for OLT and death as competing risks, we performed a competing risk regression analysis (Table 5). Importantly, all parameters were independently associated with HCC development during FU.

### Table 1. Comparison of patient characteristics at BL and FU in the derivation and validation cohorts.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Derivation cohort, cACLD, n = 475</th>
<th>Validation cohort, AFPI, n = 691</th>
<th>Validation cohort, non-AFPI, n = 1,300</th>
<th>p value († vs. ‡)</th>
<th>p value (‡ vs. †‡)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>57.6 ± 11.3</td>
<td>59.1 ± 12.3</td>
<td>61.4 ± 11.7</td>
<td>0.024</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>296 (62.3%)</td>
<td>435 (63.0%)</td>
<td>840 (56.0%)</td>
<td>0.825</td>
<td>0.015</td>
</tr>
<tr>
<td>Female</td>
<td>179 (37.7%)</td>
<td>256 (37.0%)</td>
<td>660 (44.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>n = 475</td>
<td>n = 689</td>
<td>n = 1,321</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>African</td>
<td>438 (92.2%)</td>
<td>654 (94.9%)</td>
<td>1,278 (96.7%)</td>
<td>0.452</td>
<td>0.343</td>
</tr>
<tr>
<td>Asian</td>
<td>30 (6.3%)</td>
<td>13 (1.9%)</td>
<td>15 (1.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latin-American</td>
<td>7 (1.5%)</td>
<td>20 (2.9%)</td>
<td>22 (1.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥30 kg/m²</td>
<td>26.9 ± 5.0 (n = 470)</td>
<td>27.2 ± 4.5 (n = 503)</td>
<td>26.9 ± 4.4 (n = 1,096)</td>
<td>0.305</td>
<td>0.942</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below the threshold¹</td>
<td>443 (93.8%)</td>
<td>605 (92.5%)</td>
<td>1,206 (93.7%)</td>
<td>0.627</td>
<td>0.736</td>
</tr>
<tr>
<td>Above the threshold¹</td>
<td>32 (6.7%)</td>
<td>47 (7.5%)</td>
<td>81 (6.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FU-albumin, g·L⁻¹</td>
<td>41.5 ± 4.2</td>
<td>41.3 ± 4.3</td>
<td>41.3 ± 4.4</td>
<td>0.452</td>
<td>0.343</td>
</tr>
<tr>
<td>FU-LSM, kPa</td>
<td>18.0 (14.3)</td>
<td>15.0 (13.1)</td>
<td>16.3 (12.5)</td>
<td>0.745</td>
<td>0.402</td>
</tr>
<tr>
<td>FU-PLT, G·L⁻¹</td>
<td>157 ± 65</td>
<td>155 ± 68</td>
<td>150 ± 66</td>
<td>0.709</td>
<td>0.048</td>
</tr>
<tr>
<td>FU-AFP, ng·mL⁻¹</td>
<td>6.5 (10.7)</td>
<td>6.7 (9.0)</td>
<td>6.7 (9.0)</td>
<td>0.413</td>
<td>0.388</td>
</tr>
<tr>
<td>Bmi, mg·L⁻¹</td>
<td>170 ± 69</td>
<td>168 ± 66</td>
<td>159 ± 68</td>
<td>0.479</td>
<td>0.003</td>
</tr>
<tr>
<td>≥ 30 mg·L⁻¹</td>
<td>42.4 (39.6–45.1)</td>
<td>44.4 (42.7–46.0)</td>
<td>40.4 (39.7–41.2)</td>
<td>-</td>
<td>0.015</td>
</tr>
<tr>
<td>HCC</td>
<td>22 (4.5%)</td>
<td>36 (5.2%)</td>
<td>65 (4.3%)</td>
<td>0.655</td>
<td>0.783</td>
</tr>
<tr>
<td>Incidence/100 patient-years</td>
<td>1.45</td>
<td>1.74</td>
<td>1.40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AUC values of pre-treatment and post-treatment parameters, as well as their absolute and relative changes, for predicting hepatocellular carcinoma development in the derivation cohort.

### Table 2. AUC values of pre-treatment and post-treatment parameters, as well as their absolute and relative changes, for predicting hepatocellular carcinoma development in the derivation cohort.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC (95% CI)</th>
<th>Parameter</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.664 (0.546–0.782)</td>
<td>Absolute Δ albumin, g·L⁻¹</td>
<td>0.541 (0.423–0.658)</td>
</tr>
<tr>
<td>FU-albumin, g·L⁻¹</td>
<td>0.691 (0.586–0.796)</td>
<td>Relative Δ albumin, %</td>
<td>0.534 (0.414–0.654)</td>
</tr>
<tr>
<td>FU-LSM, kPa</td>
<td>0.631 (0.522–0.741)</td>
<td>Absolute Δ LSM, kPa</td>
<td>0.610 (0.476–0.744)</td>
</tr>
<tr>
<td>FU-PLT, G·L⁻¹</td>
<td>0.678 (0.598–0.776)</td>
<td>Relative Δ LSM, %</td>
<td>0.674 (0.570–0.778)</td>
</tr>
<tr>
<td>FU-VITRO, %</td>
<td>0.713 (0.621–0.805)</td>
<td>Absolute Δ VITRO, %</td>
<td>0.703 (0.573–0.833)</td>
</tr>
<tr>
<td>BL-AST</td>
<td>0.567 (0.445–0.689)</td>
<td>Relative Δ VITRO, %</td>
<td>0.503 (0.373–0.633)</td>
</tr>
<tr>
<td>BL-ALT</td>
<td>0.501 (0.376–0.626)</td>
<td>Absolute Δ AST</td>
<td>0.516 (0.378–0.653)</td>
</tr>
<tr>
<td>FU-ALT</td>
<td>0.598 (0.483–0.714)</td>
<td>Relative Δ AST, %</td>
<td>0.516 (0.387–0.645)</td>
</tr>
<tr>
<td>BL-APRI</td>
<td>0.677 (0.560–0.794)</td>
<td>Absolute Δ APRI</td>
<td>0.586 (0.463–0.709)</td>
</tr>
<tr>
<td>FU-AFC</td>
<td>0.702 (0.594–0.809)</td>
<td>Relative Δ APRI, %</td>
<td>0.600 (0.463–0.738)</td>
</tr>
<tr>
<td>BL-VITRO</td>
<td>0.730 (0.648–0.812)</td>
<td>Absolute Δ VITRO, %</td>
<td>0.627 (0.471–0.773)</td>
</tr>
<tr>
<td>FU-VITRO</td>
<td>0.720 (0.627–0.813)</td>
<td>Relative Δ VITRO, %</td>
<td>0.543 (0.421–0.665)</td>
</tr>
<tr>
<td>BL-AFP</td>
<td>0.720 (0.655–0.785)</td>
<td>Absolute Δ AFP</td>
<td>0.631 (0.526–0.737)</td>
</tr>
<tr>
<td>FU-AFP</td>
<td>0.796 (0.726–0.866)</td>
<td>Relative Δ AFP, %</td>
<td>0.536 (0.421–0.652)</td>
</tr>
</tbody>
</table>
Table 3. AUC values of pre-treatment and post-treatment parameters for predicting hepatocellular carcinoma development, and the respective Youden-optimized cut-offs (both without and after bootstrapping), in the derivation cohort.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC (95% CI)</th>
<th>Youden-optimized cut-off</th>
<th>Bootstrapped AUC</th>
<th>Bootstrapped Youden-optimized cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>0.664 (0.546-0.782)</td>
<td>59.27</td>
<td>0.67</td>
<td>59.24</td>
</tr>
<tr>
<td>FU-albumin, g·L⁻¹</td>
<td>0.714 (0.594-0.835)</td>
<td>42.0</td>
<td>0.71</td>
<td>42.0</td>
</tr>
<tr>
<td>FU-LSM, kPa</td>
<td>0.713 (0.621-0.805)</td>
<td>19.0</td>
<td>0.71</td>
<td>19.0</td>
</tr>
<tr>
<td>FU-PLT, G·L⁻¹</td>
<td>0.674 (0.580-0.768)</td>
<td>198.5</td>
<td>0.67</td>
<td>190</td>
</tr>
<tr>
<td>FU-VWF, %</td>
<td>0.687 (0.577-0.798)</td>
<td>186</td>
<td>0.69</td>
<td>186</td>
</tr>
<tr>
<td>FU-VITRO</td>
<td>0.713 (0.613-0.813)</td>
<td>0.95</td>
<td>0.71</td>
<td>1.02</td>
</tr>
<tr>
<td>FU-FIB-4</td>
<td>0.720 (0.627-0.813)</td>
<td>1.70</td>
<td>0.72</td>
<td>1.83</td>
</tr>
<tr>
<td>FU-AFP, ng·ml⁻¹</td>
<td>0.796 (0.726-0.866)</td>
<td>4.6</td>
<td>0.80</td>
<td>4.6</td>
</tr>
</tbody>
</table>

AFT, alpha-fetoprotein; AUC, area under the curve; BL, baseline; FU, follow-up; FIB-4, fibrosis-4; LSM, liver stiffness measurement; PLT, platelet count; VITRO, von Willebrand factor antigen/platelet count ratio; VWF, von Willebrand factor.

1Bootstrapped AUCs and median Youden-optimized cut-offs from 5000 bootstrap samples are reported.

Table 4. Cox regression analyses of risk factors for hepatocellular carcinoma development in the derivation cohort.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hazard ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥59 years</td>
<td>5.454 (1.835-16.210)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FU-albumin ≥42 g·L⁻¹</td>
<td>5.642 (2.067-15.400)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FU-PLT ≥190 G·L⁻¹</td>
<td>9.619 (1.290-71.750)</td>
<td>0.027</td>
</tr>
<tr>
<td>FU-VWF ≥2180%</td>
<td>3.491 (1.354-9.005)</td>
<td>0.010</td>
</tr>
<tr>
<td>FU-VITRO &gt;2.0</td>
<td>3.704 (1.244-11.030)</td>
<td>0.019</td>
</tr>
<tr>
<td>FU-AFP &gt;4.6 ng·ml⁻¹</td>
<td>17.130 (3.988-73.570)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FU-LSM ≥19.0 kPa</td>
<td>4.739 (1.964-11.440)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FU-AFP ≥4.6</td>
<td>4.535 (1.334-15.420)</td>
<td>0.016</td>
</tr>
<tr>
<td>Alcohol consumption above the threshold</td>
<td>3.106 (1.044-9.244)</td>
<td>0.042</td>
</tr>
</tbody>
</table>

Values in bold indicate p <0.05. Parameters have been dichotomized according to the Youden’s index-optimized cut-offs (see Table 3). AFT, alpha-fetoprotein; FU, follow-up; FIB-4, fibrosis-4; LSM, liver stiffness measurement; PLT, platelet count; VITRO, von Willebrand factor antigen/platelet count ratio; VWF, von Willebrand factor.

1Stepwise exclusion of variables with p >0.100.

2Calculated from Cox regression analyses.

3Calculated from last step of backward elimination using bootstrapped Harrel’s C-indexes.
A simple score was derived from adjusted (subdistribution) hazard ratios assigning 3 points for FU-AFP ≥4.6 ng·mL⁻¹, 2 points for age ≥59 years, 2 points for alcohol consumption above the threshold, 1 point for FU-LSM ≥19 kPa, and 1 point for FU-albumin <42 g·L⁻¹ (0 points were assigned if the respective criterion was not met). Following this approach, the derivation cohort was stratified according to the number of assigned points (Fig. S3). The subdistribution hazard ratio (SHR) was 2.47 (95% CI 1.91-3.19, p <0.001) per point. Patients were then stratified into low-risk (0-3 points, n = 308 [65.8%]) and high-risk (4-9 points, n = 160 [34.2%]) groups. Of note, 61.4% of the low-risk group had AFP ≤4.6 ng·L⁻¹ and thus, showed evidence of cirrhosis. This dichotomization identified patients at very low and substantial risk of HCC at 4 years: 0% vs. 16.5% (Fig. 1A; HCC incidence rate per 100 patient-years: 0 vs. 4.3).

Since AFP is not routinely assessed at many centers, we also tested whether a simple score derived from the adjusted (subdistribution) hazard ratios of model 6 (i.e., without AFP) was also able to stratify HCC risk. We assigned 3 points for age ≥59 years, 2 points for alcohol consumption above the threshold, 2 points for FU-albumin <42 g·L⁻¹, and 2 points for FU-LSM ≥19 kPa and stratified patients into low-risk (0-3 points, n = 322 [68.8%]) and high-risk (4-9 points, n = 146 [31.2%]) groups. The HCC risk at 4 years was 1.3% vs. 14.8% (SHR 13.70; 95% CI 4.02-46.40; p <0.001; Fig. 1B; HCC incidence per 100 patient-years: 0.3 vs. 3.9).

Finally, both approaches also yielded a high discriminative ability without including alcohol consumption above the threshold as a variable, thereby acknowledging uncertainties regarding the quantification of alcohol consumption (AFP-based algorithm at 4 years: 0.5% vs. 16.7% [95% CI 5.10-94.00; p <0.001]; non-AFP-based algorithm: 1.8% vs. 15.0% [95% CI 3.67-32.40; p <0.001]; Fig. S4AB).

External validation of proposed risk scores
In an attempt to externally validate these findings, we tested these 4 scores in an independent validation cohort comprising patients from Madrid, Barcelona, and another Venncene center (validation cohort). Overall, 691 patients were included in the validation cohort for the FU-AFP-based algorithm, while 1,500 patients were included in the validation cohort for the algorithm without FU-AFP. Despite small differences existing for age and FU-LSM, disease severity, FU time and HCC incidence were comparable (Table 1). In the validation cohort, FU measurements clustered around 48 weeks after EoT (Fig. S1B).

As depicted in Fig. 2, both approaches including alcohol consumption as a risk factor efficiently stratified the risk of HCC during FU, with a probability of 3.3% vs. 17.5% developing HCC within 4 years according to the AFP-based algorithm (SHR 5.11; 95% CI 2.54-10.30, p <0.001; HCC incidence per 100 patient-years: 0.9 vs. 4.4) and 3.7% vs. 11.6% developing HCC within 4 years according to the algorithm without AFP (SHR 3.46; 95% CI 2.05-5.84; p <0.001; HCC incidence per 100 patient-years: 0.9 vs. 3.0). Comparable results were achieved without considering alcohol consumption as a risk factor (Table S4C,D).

Sensitivity analysis stratifying patients according to time between EoT and FU-LSM
Both scores considering alcohol maintained an adequate discriminative ability when combining the derivation and validation cohort and stratifying patients into tertiles of the time between EoT and FU-LSM (Fig. S5).
points for FU-albumin <42 g

with ACLD/advanced liver

previous attempts –

novo

FU, follow-up; LSM, liver stiffness measurement.

whom surveillance is clearly warranted (high-risk;

whom HCC surveillance may not be cost-effective (low-risk) or in

ng

(4-9 points) assignment. 3 points are assigned for age>

ative incidences are displayed according to low risk (0-3 points) and high risk

(4-9 points) assignment. 3 points are assigned for age ≥59 years, 2 points for alcohol consumption above the threshold, 2 points for FU-LSM ≥19 kPa, and 2 points for FU-albumin <42 g·L⁻¹ (0 points if the respective criterion is not met). FU, alpha-fetoprotein; cACLD, compensated advanced chronic liver disease; FU, follow-up; LSM, liver stiffness measurement.

Discussion

In the present study, we investigated predictive factors for HCC development in patients with cACLD who achieved SVR on IFN-free therapies. We focused on patients with cACLD, since HCC incidence has been reported to be significantly higher in patients with ACLD/advanced liver fibrosis or cirrhosis and (this is also reflected by current European surveillance recommendations). We provided an easily applicable score that facilitates risk stratification in clinical routine, as it identified patients in whom HCC surveillance may not be cost-effective (low-risk) or in whom surveillance is clearly warranted (high-risk; i.e., AFP ≥4.6 ng·ml⁻¹ OR age ≥59 years WITH either FU-LSM ≥19 kPa AND/OR FU-albumin <42 g·L⁻¹) due to a considerable probability of de novo HCC despite SVR. Importantly – and in contrast to most previous attempts – our proposed algorithms underwent extensive external validation, which is critical due to the profound implications of a delayed diagnosis of HCC that may result from an unwarranted termination of surveillance due to an underestimation of HCC risk. The analysis of the multicenter validation cohort confirmed that approximately two-thirds of patients (i.e., those who do not meet the aforementioned high-risk criteria) are classified as low-risk and that these patients exhibit an HCC risk <1%/year. Accordingly, the incidence of HCC in these patients clearly falls below the cost-effectiveness threshold (at 50,000 USD/quality-adjusted life year) for HCC surveillance, which has been estimated at 1.32/year. Of note, this low-risk group also included a large proportion (61.4%) of patients with evidence of cirrhosis, indicating that current recommendations to identify at-risk patients who should undergo ultrasound surveillance have very limited accuracy.

Our approach has important advantages that may promote its application in the clinic. First, a ‘one-time’ assessment after treatment (e.g., around 12 weeks after EoT or up 48 weeks after EoT) is easily applicable in clinical routine, since patients can be stratified according to their individual risk of HCC while confirming SVR. In addition, HCC risk stratification can be combined with the evaluation of the probability of hepatic decompensation by additionally assessing FU-PLT and FU-VWF to calculate the FU-VITRO score. In contrast, if approaches rely on BL values, they cannot be applied later in the series of incomplete pre-treatment work-up or unavailable information (due to changes in treatment center), leaving these patients unclassified. Similarly, consideration of absolute/relative changes seems particularly problematic, as it doubles the number of required variables, and thus, the chance of missing information.

Secondly, our approach combines indicators of liver fibrosis and portal hypertension (i.e., LSM) and hepatic dysfunction (i.e. serum albumin) with age (a strong driver of carcinogenesis in general) and AFP – a broadly available biomarker that is commonly applied for HCC surveillance in clinical practice, which is obligatory according to Asian Pacific Association for The Study of Liver, optional according to the American Association for the Study of Liver Disease, and not recommended due to concerns about cost-effectiveness by the European Association for the Study of the Liver (EASL) clinical practice guidelines. However, the latter clinical practice guidelines also emphasize that the use of AFP should be re-evaluated in patients who achieved etiological cure, as it may perform better after the amelioration of hepatic inflammation. Interestingly, AFP showed the highest individual AUC for HCC development during FU and was considered as a binary variable in our risk prediction model at a cut-off of ≥4.6 ng·ml⁻¹. Interestingly, this AFP cut-off is considerably lower than the cut-offs proposed for HCC surveillance that were either 20 or 200 ng·ml⁻¹. However, AFP usually decreases with HCV cure (e.g., -2.5 ng·ml⁻¹ or -41.3% in our study) and the proposed application of AFP (i.e., for risk stratification) differs from its common use as a biomarker for HCC surveillance (i.e., diagnosis of [very] early-stage HCC).

Considerable evidence supports the use of LSM for predicting HCC risk in patients who achieved SVR, and which was recently summarized by a meta-analysis. However, specific cut-offs for identifying patients at relevantly increased risk varied substantially according to the studied population, ranging from ≥20 kPa and ≥21.5 kPa to ≥30 kPa post-treatment. Although several studies proposed that absolute and relative changes in LSM are related to HCC development, they showed (if at all) modest prognostic value in our series of patients. Moreover, when compared to the non-invasive diagnosis of CSPH and prediction of hepatic decompensation, the predictive ability of LSM for HCC seems inferior, which argues...
for the consideration of additional variables in order to increase prognostic accuracy.

The combination of high LSM and AFP with the traditional risk factors such as old age and low serum albumin might optimize previous approaches: these were often established in studies that were based on less thoroughly characterized patient cohorts, and were therefore unable to establish synergistic effects between these variables. Importantly, we have also included alcohol consumption above the threshold as a (modifiable) risk factor. Alcohol consumption has previously been associated with the development of HCC after SVR and – according to the Baveno VII recommendations – prohibits the discharge of patients with cACLD who achieved SVR from portal hypertension surveillance. The consideration of alcohol consumption highlights the importance of this co-factor for progressive liver disease after SVR, even resulting in increased liver-related mortality, which may raise awareness both for physicians and patients. Fully acknowledging uncertainties regarding the quantification of alcohol consumption, we have confirmed that our risk scores perform appropriately and do not require any modifications, even if alcohol consumption is not considered. Although diabetes and metabolic comorbidities have been discussed as other potential risk factors for HCC in the post-SVR context, we did not observe such a significant association. Of note, our score does not include composite variables such as VITRO (which had a higher AUC than its individual components, VWF and PLT) or FIB-4, since none of these scores was predictive of HCC, when also considering other variables. Currently, the EASL (2020) clinical practice guidelines for the treatment of hepatitis C do not recommend a personalized surveillance strategy, and thus, a 6-monthly ultrasound surveillance is recommended in all patients with pre-treatment advanced liver fibrosis (F3) or cirrhosis (F4). Importantly, this approach might not be cost-effective, especially not in patients who only have advanced liver fibrosis (F3) pre-treatment. A recent analysis estimated that the number of HCC surveillance candidates with SVR will increase more than 6-fold from 2012 to 2030. Therefore, personalized surveillance strategies are urgently needed to optimize resource utilization and these surveillance strategies should be based on a comprehensive evaluation of de novo HCC risk – such as our proposed algorithms – rather than the pre-treatment liver fibrosis stage.

Since a late diagnosis of HCC has serious implications for the outcome of an individual patient, extensive external validation is mandatory, before risk stratification approaches are applied in the clinic to identify low-risk patients in whom HCC surveillance can be deferred. Therefore, we also evaluated previously proposed approaches based on the cACLD subgroup of our derivation cohort and the validation cohort. In this context, several specific aspects of individual scoring/grading systems were notable, and these are extensively discussed in the supplementary information.

Of note, the international multicenter design increases the generalizability of our findings. Since we only included specialized centers, we were able to acquire a large, comprehensively characterized derivation cohort that provided information on the vast majority of potential predictors of HCC development in patients with ACLD who achieved SVR. However, presumably the most important strength of our study is the external validation of our algorithms in up to 1,500 patients from different centers across Europe. Although there were statistically significant differences in patient characteristics, the differences between cohorts were very small (<10%) for variables considered in our prognostic models (age and FU-LSM). Moreover, slight variations between the derivation and validation dataset may even increase the generalizability of our models.

The main limitation of our study is its retrospective design, which introduced considerable variability regarding the time point for the assessment of post-treatment data. These measurements were clustered around 12 weeks after EoT in our derivation cohort and around 48 weeks after EoT in the validation cohort. These differences are mainly due to the design of the contributing studies as well as limited patient compliance and capacity restrictions. However, the heterogeneity in the assessment time point may actually improve the robustness and generalizability of our risk stratification approach, as it showed an excellent discriminative ability both in the derivation and validation cohort, despite differences in the time point of assessment. In addition, sensitivity analyses using time point-dependent stratification revealed that the discriminative ability of our risk stratification approach was maintained at all assessment time points. Accordingly, the lack of standardization may also be seen as a strength of our study, as some variation in the time point of assessment will be unavoidable in ‘real-world’ clinical practice, and thus, risk stratification systems should show a robust performance under rather unstandardized conditions to ascertain external validity.

Similar to the variability in the time point of post-treatment measurements, HCC surveillance prior to therapy and EoT was not standardized. However, all patients had at least one unsuspicious imaging after EoT. Currently, lifelong HCC surveillance is recommended since a similar HCC incidence over time has been reported after SVR to IFN-free and IFN-based therapies. However, our study cannot provide information on the long-term risk of HCC, and this is an unavoidable limitation of all studies available to date. Accordingly, long-term studies are warranted: these should also address the question of whether a re-evaluation of the laboratory/elastography parameters at a later time point may refine risk stratification regarding events occurring during long-term FU. Since the vast majority of included patients were Caucasian (>90%), it remains to be shown whether our findings can be extrapolated to other ethnicities. Data from Asia will be important to confirm the accuracy of the proposed algorithms.

In conclusion, based on our international multicenter study, we developed and externally validated simple algorithms for HCC prediction in patients with cACLD who achieved SVR to IFN-free treatments, comprising a set of broadly available parameters, which were all evaluated at a single post-treatment time point. Approximately two-thirds of patients were identified as having an HCC risk <1%/year, thereby clearly falling below the cost-effectiveness threshold for HCC surveillance.

**Abbreviations**

ACLD, advanced chronic liver disease; AFP, alpha-fetoprotein; AUC, area under the curve; BL, baseline; cACLD, compensated ACLD; CHC, chronic hepatitis C; CSPH, clinically significant portal hypertension; dACLD, decompensated ACLD; EoT, end of treatment; FIB-4, fibrosis-4; FU, follow-up; HCC, hepatocellular carcinoma; HVPG, hepatic venous pressure gradient; IFN, interferon; LSM, liver stiffness measurement; MELD, model of end-stage liver disease; OLT, liver transplantation; PLT, platelet count; ROC, receiver-operating characteristic; SVR, sustained virologic response; VITRO, von Willebrand factor antigen/platelet count ratio; VWF, von Willebrand factor
Financial support

This work was supported by a grant from the Medical Scientific Fund of the Mayor of the City of Vienna (No. 17035) as well as the Andrew K. Burroughs short-term training fellowship of the European Association for the Study of the Liver.

Conflict of interest

G.S. has nothing to disclose. E.M. received grants from Novartis. K.K. received travel support from AbbVie, Bristol-Myers Squibb, and Gilead. P.S.C. received consulting fees from PharmaIN, and travel support from Falk and Phenex Pharmaceuticals; S.H.-S. served as a speaker and/or consultant and/or advisory board member for AbbVie, Bristol-Myers Squibb, Eisai, Gilead, and Intercept and received travel support from AbbVie and Gilead; A.Z. has nothing to disclose; D.B. received travel support from AbbVie and Gilead; D.C. served as a speaker and/or consultant and/or advisory board member for AbbVie, Gilead, and MSD, and received travel support from AbbVie, MSD, ViIV Healthcare and Gilead; B.S.I. received travel support from AbbVie and Gilead. B.Sch. received travel support from AbbVie, Ipsen and Gilead. A.F.S. served as a speaker and/or consultant and/or advisory board member for Boehringer Ingelheim, Gilead, and MSD. M.Pi. served as a speaker and/or consultant and/or advisory board member for Bayer, Bristol-Myers Squibb, Ipsen, Eisai, Lilly, MSD, and Roche, and received travel support from Bayer and Bristol-Myers Squibb. R.S. has nothing to disclose. F.P.R. served as a speaker and/or consultant and/or advisory board member for AbbVie, Biotest, Gilead, and MSD, and received travel support from AbbVie, Biotest, and Gilead. H.G. has nothing to disclose. M.S. received speaking honoraria from BMS and travel support from Bristol-Myers Squibb, AbbVie, and MSD. C.S. has nothing to disclose. M.G. received grants from Gilead, and Janssen. P.Sc. received consulting fees from PharmaIN, and travel support from Falk and Phenex Pharmaceuticals; S.H.-S. served as a speaker and/or consultant and/or advisory board member for AbbVie, Bristol-Myers Squibb, Gilead, Collective Acumen, and W. L. Gore & Associates and received travel support from AbbVie, Bristol-Myers Squibb, and Gilead.

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

Authors’ contributions

Study concept and design (M.M.), acquisition of data (all authors), analysis and interpretation of data (G.S., E.M., T.R., M.M.), drafting of the manuscript (G.S., T.R., M.M.), critical revision of the manuscript for important intellectual content (all authors), language editing (H.L.).

Data availability statement

Data are available from the corresponding author (mattias.mandorfer@meduniwien.ac.at) upon reasonable request.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2021.11.025.

References

Author names in bold designate shared co-first authorship


Journal of Hepatology 2021 vol. 73:1–10 9
Research Article

Viral Hepatitis


