Digestive and Liver Disease 55 (2023) 907–917

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Liver, Pancreas and Biliary Tract

Profiling the risk of hepatocellular carcinoma after long-term HCV eradication in patients with liver cirrhosis in the PITER cohort

Loreta A. Kondili[a,b,#], Maria Giovanna Quaranta[a], Luisa Cavalletto[c], Vincenza Calvaruso[d], Luigina Ferrigno[a], Roberta D'Ambrosio[e], Ilaria Simonelli[f], Giuseppina Brancaccio[g], Giovanni Raimondo[h], Maurizia R. Brunetto[i], Anna Linda Zignego[j], Carmine Coppola[k], Andrea Iannone[l], Elisa Biliotti[m], Gabriella Verucchi[n], Marco Massari[o], Anna Licata[p], Francesco Barbaro[q], Marcello Persico[r], Francesco Paolo Russo[s], Filomena Morisco[t], Maurizio Pompili[u], Mauro Viganò[v], Massimo Puoti[w,x], Teresa Santantonio[y], Erica Villa[z], Antonio Craxì[d], Liliana Chemello[c,##], on behalf of PITER Collaborating Investigators[*]

[a] *Center for Global Health, Istituto Superiore Di Sanità (ISS), Rome, Italy*
[b] *UniCamillus-Saint Camillus International University of Health Sciences, Rome, Italy*
[c] *Department of Medicine-DIMED, Padua University, University Hospital, Clinica Medica 5, Refering Regional Center for Liver Diseases, Padova, Italy*
[d] *Gastroenterology and Hepatology Unit, PROMISE, University of Palermo, Palermo, Italy*
[e] *Division of Gastroenterology and Hepatology, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
[f] *L'altrastatistica srl, Consultancy & Training, Biostatistics Office, Rome, Italy*
[g] *Department of Molecular Medicine, Infectious Diseases Unit, University of Padua, Padua, Italy*
[h] *Department of Internal Medicine, University Hospital of Messina, Messina, Italy*
[i] *Department of Clinical and Experimental Medicine, University Hospital of Pisa, Pisa, Italy*
[j] *Department of Experimental and Clinical Medicine, Interdepartmental Centre MASVE, University of Florence, Florence, Italy*
[k] *Department of Hepatology, Gragnano Hospital, Gragnano, NA, Italy*
[l] *Section of Gastroenterology, Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy*
[m] *Infectious and Tropical Medicine Unit, Department of Public Health and Infectious Diseases, "Policlinico Umberto I" Hospital, Sapienza University of Rome, Rome, Italy*
[n] *Clinic of Infectious Diseases and Microbiology Unit, Alma Mater Studiorum, Bologna University, Bologna, Italy*
[o] *Malattie Infettive, Azienda Unità Sanitaria Locale, IRCCS di Reggio Emilia, Reggio Emilia, Italy*
[p] *Infectious Diseases Unit, DIBIMIS, University of Palermo, Palermo, Italy*
[q] *Department of Medicine, Infectious Diseases Unit, University of Padua, Padua, Italy*
[r] *Internal Medicine and Hepatology Division, Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno, Baronissi, Italy*
[s] *Department of Surgery, Oncology and Gastroenterology, Gastroenterology Unit, University of Padua, Padua, Italy*
[t] *Gastroenterology Unit, Federico II University, Naples, Italy*
[u] *Internal Medicine and Gastroenterology, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Largo Agostino Gemelli, Rome, Italy*
[v] *Hepatology Unit, San Giuseppe Hospital, Milan, Italy*
[w] *Infectious Disease Unit, Niguarda Hospital, Milan, Italy*
[x] *Università degli Studi di Milano-Bicocca, School of Medicine, Milan, Italy*
[y] *Infectious Diseases Unit, Department of Clinical and Surgical Sciences, University of Foggia, Foggia, Italy*
[z] *Gastroenterology Unit, Department of Medical Specialties, University of Modena & Reggio Emilia and Modena University-Hospital, Modena, Italy*

[#] Correspondence: Dr. L.A. Kondili, Center for Global Health, Istituto Superiore di Sanità, Viale Regina Elena 299 - 00161 Rome, Italy.
[##] Prof. L. Chemello, Department of Medicine-DIMED, Padua University, University Hospital, Clinica Medica 5, Refering Regional Center for Liver Diseases, Via Giustiniani, 2 - 35128 Padova, Italy.
E-mail addresses: loreta.kondili@iss.it (L.A. Kondili), liliana.chemello@unipd.it (L. Chemello).
[*] **PITER Collaborating Investigators:** names and affiliations of each member of this study group are listed in Appendix.

https://doi.org/10.1016/j.dld.2023.01.153

ARTICLE INFO

Article history:
Received 20 October 2022
Accepted 16 January 2023
Available online 10 February 2023

Ms. Ref. No.: DLD-22-1433R1

Keywords:
Direct-acting antiviral
Long term outcomes
Predictive factors
Real-life cohort
HCC

ABSTRACT

Background and aims: Severe liver disease markers assessed before HCV eradication are acknowledged to usually improve after the SVR. We prospectively evaluated, in the PITER cohort, the long-term HCC risk profile based on predictors monitored after HCV eradication by direct-acting antivirals in patients with cirrhosis.

Methods: HCC occurrence was evaluated by Kaplan-Meier analysis. Cox regression analysis identified the post-treatment variables associated with *de-novo* HCC; their predictive power was presented in a nomogram.

Results: After the end of therapy (median follow-up:28.47 months), among 2064 SVR patients, 119 (5.8%) developed *de-novo* HCC. The HCC incidence was 1.90%, 4.21%, 6.47% at 12-, 24- and 36-months from end-of-therapy, respectively (incidence rate 2.45/100 person-years). Age, genotype 3, diabetes, platelets (PLT)≤120,000/μl and albumin ≤3.5g/dl levels were identified as pre-treatment HCC independent predictors. Adjusting for age, the post-treatment PLT≤120,000/μl (AdjHR 1.92; 95%CI:1.06-3.45) and albumin≤3.5g/dl (AdjHR 4.38; 95%CI 2.48-7.75) values were independently associated with HCC occurrence. Two different risk profiles were identified by combining long-term post-therapy evaluation of PLT ≤ vs. >120,000/μl and albumin ≤ vs. >3.5g/dl showing a significant different HCC incidence rate of 1.35 vs. 3.77/100 p-y, respectively.

Conclusions: The nomogram score based on age, PLT and albumin levels after SVR showed an accurate prediction capability and may support the customizing management for early HCC detection.

1. Introduction

Hepatocellular carcinoma (HCC) remains a global health challenge, with an estimated incidence of more than 1 million cases by 2025 [1]. It has been the leading cause of death in patients with cirrhosis, showing an annual incidence rate (IR) of 1–6% [2]. The risk attributable to hepatitis C virus (HCV) infection is decreased by the achievement of a sustained virological response (SVR) after interferon (IFN) and DAA treatments. Still, clearance of HCV infection does not eliminate the risk of HCC occurrence when advanced fibrosis or cirrhosis was present before treatment [3–5]. The likelihood of developing HCC after SVR ranges from 2 to 2.5% per year among different studies [6,7]. Factors involved in these differences have mostly been analyzed retrospectively (i.e., length of pre- or post-treatment follow-up (FU), mean age, liver disease stage or biochemical surrogates, and characteristics of undefined nodules). Albeit factors relating to the stage of liver disease have often been linked to the higher risk of developing HCC, no apparent risk profile widely applicable in the clinical practice has emerged [4,8,9]. This may be partly explained because the above items were assessed before SVR, while it is now acknowledged that the disease severity parameters usually improve following SVR [10]. Since patients with cirrhosis remain susceptible to developing HCC after the SVR attainment, the identification of risk profiles applicable at the individual patient's level will help customize management policies.

Some studies have investigated the improvements in surrogate features of liver fibrosis and portal hypertension after HCV eradication, documenting different categories of HCC incidence and some predictive factors associated with an unfavorable outcome [11–13]. However, real-life data focusing on these potential predictors, when evaluated prospectively at long-term after achieving the SVR in real-life practice, are scarce.

Drawing from the experience of the PITER platform [14], which collected a prospective multicenter real-life cohort representative of HCV patients in care in Italy, we prospectively assessed potential predictive factors associated with HCC occurrence after achieving the SVR by DAA therapy in patients with cirrhosis. The final goal was to make available to clinicians a simple nomogram plotting the likelihood of an individual patient with advanced liver disease being HCC-free at specific time points during FU after SVR.

2. Patients and methods

PITER is an ongoing platform that enrolled and prospectively evaluated a representative cohort of chronic hepatitis C patients, since June 2014, from 60 specialized centers of liver and infectious diseases distributed throughout Italy [14].

Data were collected in dedicated electronic Case Report Forms (eCRFs), at different time points: pre-DAA treatment (at baseline) and during the long-term FU, according to each clinical center real-life practice.

Liver cirrhosis was defined either by liver biopsy (Metavir or Ishak score) or by Transient Elastometry using a liver stiffness measurement (LSM) cut-off >12.5 kPa [15]. In the lack of these procedures, it was agreed among clinical centers by the attendance of biochemical and/or clinical and instrumental features of liver cirrhosis, or portal hypertension. The Child-Pugh-Turcotte (CPT) class and the Fibrosis-4 (FIB-4) index, as non-invasive estimation tools of liver fibrosis, were generated automatically in the eCRF of each patient enrolled.

The presence of liver steatosis by abdominal ultrasound associated with hypertension or cardiovascular disease, type 2 diabetes, and BMI >25 kg/m^2 were considered surrogate markers of the metabolic syndrome in patients with no alcohol consumption.

2.1. Inclusion and exclusion criteria

All consecutively enrolled patients diagnosed with liver cirrhosis who achieved the SVR by DAA therapy were evaluated. Patients with a compensated CPT-A or B class or with a previous decompensation history, but showing a stable clinical condition with compensation within the last 12-month period were included.

Patients with a previous diagnosis of HCC or pre-treatment unclassified nodular lesions, CPT-C class, orthotopic liver transplantation (OLT) and those on the OLT waiting list were excluded. The FU period of the study started from the EOT evaluation.

2.2. Hepatocellular carcinoma occurrence

HCC surveillance was performed in all patients by ultrasound (US) examination every six months. In addition, HCC was diagnosed by needle biopsy or by non-invasive criteria according to international guidelines [16]. Only HCC occurring after the EOT was considered as *de-novo* HCC.

2.3. Statistical analysis

Patients' main characteristics were reported as the median and interquartile range (IQR) for continuous or as proportions for categorical variables. Albumin level, PLT count and LSM were evaluated using clinically relevant threshold cut-offs. The Mann-Whitney U test, the Chi-squared test and the Wilcoxon matched-pairs signed-rank test were used as appropriate.

HCC occurrence was evaluated using Kaplan–Meier survival analysis. The log-rank test was used to identify differences in survival among groups.

Cox regression analyses were used to identify variables independently associated with HCC incidence adopting a stepwise forward selection. Gender variable was forced to be included in the model. PLT count ≤120.000/ µl was used, as a cut-off for HCC risk discrimination based on better performance associated with albumin level ≤3.5 g/dl in our study population, as also previously reported [3].

A further model was then constructed to explore the association of HCC incidence and variables related to the severity of liver damage individuated by the previous model and subjected to changes after achieving SVR. This predictive model was presented as a nomogram by a graphical representation of the probability of HCC free-survival for each patient at 1-, 3- and 5-years of FU, according to calculated points associated with each risk factor evaluated. The predictive accuracy of the models was measured through the concordance index (Harrell's c-index), quantifying the level of agreement between the predicted probabilities and the actual possibility of having the event of interest. The bootstrap resampling method was chosen to internally validate the predictive models' selecting a 500 repetition. The calibration curve was used to assess the goodness of the model fit. A p-value <0.05 was considered statistically significant. Statistical analyses were performed with STATA version 16.1 (StataCorp, College Station, TX, USA) and R software (version 4.1.2, Regression Modelling Strategies package. The R Foundation for Statistical Computing, Vienna, Austria).

2.4. Ethics

The study was conducted following the Declaration of Helsinki guidelines and the Good Clinical Practice principles. The study protocol was approved by the Ethics Committee of the Istituto Superiore di Sanità and of each participating center. Patients' data were evaluated anonymously, adopting generated codes. All patients gave their written informed consent to participate in the study.

3. Results

3.1. Pre-treatment characteristics and HCC incidence rate in SVR patients by DAA therapy

The study population consisted of 2064 patients with liver cirrhosis who achieved the SVR following the DAA treatment of whom 1368 (66%) were diagnosed by liver stiffness >12.5kPa and/or liver biopsy and 696 (34%) by biochemical and/or clinical or instrumental findings of cirrhosis or portal hypertension. Pre-treatment characteristics of patients, who achieved the SVR, followed up for a median time of 28.47 months (IQR 13.33-41.61 months) are shown in Table 1. One-hundred and nineteen patients (5.7%), who developed HCC, were older and reported more frequently clinical features of severe liver disease (lower PLT or albumin levels, LSM ≥20 kPa, FIB-4 >3.25 or showed a CPT class B or previous episode of decompensation), diabetes or a SOF-based treatment schedule use.

The overall HCC-IR in patients who achieved SVR was 2.45/100 person-years (p-y). The 12-, 24- and 36-month cumulative HCC-IR was 1.90%, 4.21%, and 6.47%, respectively (Fig. 1A). The 12, 24- and 36-month HCC-IR in class CPT-A and CPT-B cases was 1.63%, 3.70%, and 5.97% and 3.41%, 7.13%, and 9.33%, respectively (log-rank test p=0.0227) (Fig. 1B).

3.2. Pre-treatment predictive factors of HCC development

Pre-treatment variables independently associated with HCC development in patients who achieved the SVR are shown in Table 2. After adjusting for the confounding effect of each variable by stepwise forward selection: age (Adjusted Hazard Ratio (AdjHR) 1.06, 95%CI 1.04-1.09), HCV genotype 3 (AdjHR 4.27, 95%CI 2.22-8.23), PLT ≤120,000/µl (AdjHR 1.67, 95%CI 1.05-2.65), albumin ≤3.5 g/dl (AdjHR 2.51, 95%CI 1.62-3.88) and diabetes (AdjHR 1.75, 95%CI 1.10-2.76) were variables independently associated with HCC occurrence.

The cumulative HCC-IR according to pretreatment evaluation of PLT and albumin levels and by their combination are reported in Supplementary Fig. 1 (Panels A, B and C).

3.3. Prospective evaluation of HCC-risk predictors

Among patients who achieved SVR following the DAA therapy, those who have available paired pre- treatment and post-treatment data were evaluated prospectively. Pre-treatment characteristics by HCC occurrence are shown in Supplementary Table 1. Pre- and post-treatment LSM were available in 420 patients, 22 of whom developed HCC. As shown in Fig. 2A, the median pre-treatment LSM was similar in both groups of patients with or without the development of HCC (p=0.551), and a significant drop in LSM was observed after viral eradication both in cases with (p=0.024) or without (p<0.001) HCC occurrence. Patients who developed HCC during the FU showed significantly higher median post-treatment LSM compared with those who did not (18.5 vs. 12.0 kPa; p=0.016).

Following HCV eradication, the PLT count increased significantly compared with the pre-therapy values in patients without HCC (p<0.001) but not in those who developed HCC (p=0.889) (Fig. 2B). Albumin levels increased in both groups of patients with or without the development of HCC during pre-and post-therapy evaluation (both, p<0.001) (Fig. 2C).

3.4. Post-treatment predictors of different HCC-risk groups

The cumulative HCC-IR at 12-, 24-, and 36-month in patients with post-treatment PLT count ≤120,000/µl was 2.81%, 6.40%, and 8.96%, respectively, which appeared significantly higher (log-rank test p<0.001) compared to patients with PLT count >120,000/µl (IR: 1.48%, 2.78% and 4.36%, at 12-, 24-, and 36-month, respectively) (Fig. 3A). The same profile was observed between groups with albumin levels <3.5 or >3.5 g/dl (IR at 12-, 24- and 36-month: 7.52%, 14.12%, and 21.49% vs. 1.30%, 3.16 %, and 4.72%, respectively; log-rank test p<0.001) (Fig. 3B).

By combining the two surrogate markers of liver disease severity (i.e., PLT count and albumin level) (Fig. 3C), the highest HCC-IR following HCV eradication was found in the group of patients with PLT count ≤120,000/µl and/or albumin level ≤3.5 g/dl (IR: 3.90%,

Table 1
Pre-treatment characteristics of DAA successfully treated patients by HCC occurrence.

		No HCC (N=1945)		HCC occurrence (N=119)			TOTAL (N=2064)	
Epidemiological features								
Age (years)		*Median (IQR)* 64 (54 - 72)		*Median (IQR)* 68 (62 - 72)		*p*** **<0.001**	*Median (IQR)* 64 (55 - 72)	
		N.	*%*	*N.*	*%*	*p****	*N.*	*%*
Gender	Male	1099	56.5	73	61.3	0.301	1172	56.8
	Female	846	43.5	46	38.7		892	43.2
BMI*	Underweight-Normal	860	44.3	57	47.9	0.625	917	44.5
	Overweight	783	40.3	47	39.5		830	40.3
	Obese	300	15.4	15	12.6		315	15.3
Alcohol use*	Never	1299	68.8	74	63.3	0.373	1373	68.4
	Current	205	10.9	13	11.1		218	10.9
	Past	385	20.4	30	25.6		415	20.7
HCV- genotype	1a	248	12.8	7	5.9	0.207	255	12.4
	1b	1107	56.9	72	60.5		1179	57.1
	2	289	14.9	17	14.3		306	14.8
	3	181	9.3	15	12.6		196	9.5
	Other	120	6.2	8	6.7		128	6.2
HBV Infection	Anti-HBc+/HBsAg+	22	1.1	0	0.0	0.386	22	1.1
	Anti-HBc+/HBsAg-	383	19.7	27	22.7		410	19.9
Potential metabolic syndrome		253	13.0	15	12.6	0.899	268	13.0
Diabetes		415	21.3	35	29.4	**0.038**	450	21.8
Previous Interferon use		905	46.5	64	53.8	0.124	969	46.9
Clinical features							.	
Platelets count (n/μL)*	≤ 120,000	979	51.9	82	70.7	**<0.001**	1061	53.0
	> 120,000	908	48.1	34	29.3		942	47.0
Albumin level (g/dL)*	≤ 3.5	400	22.9	54	47.8	**<0.001**	454	24.5
	> 3.5	1343	77.1	59	52.2		1402	75.5
Liver Stiffness (kPa)*	≥ 20	714	46.8	54	60.7	**0.011**	768	47.5
	< 20	813	53.2	35	39.3		848	52.5
FIB-4*	≤ 3.25	629	33.6	22	19.1	**0.001**	651	32.8
	> 3.25	1243	66.4	93	80.9		1336	67.2
Child-Pugh Class	A	1663	85.5	93	78.2	**0.029**	1756	85.1
	B	282	14.5	26	21.8		308	14.9
Past decompensation§		204	10.5	21	17.6	**0.015**	225	10.9
Treatment regimen		.						
Ribavirin use		1119	57.5	76	63.9	0.174	1195	57.9
SOF-based treatment		1499	77.1	102	85.7	**0.028**	1601	77.6

Abbreviations: BMI, Body Mass Index; DAA, Direct Acting Antiviral; HBV, FIB-4, Fibrosis-4; Hepatitis B Virus; IQR, Interquartile Range; SOF, Sofosbuvir.
* Inconsistencies are due to missing values.
** p value Mann–Whitney rank-sum test.
*** p value Chi-square test.
§ All patients did not have sign of liver decompensation at treatment start.

Table 2
Pre-treatment predictive factors associated with HCC occurrence in DAA successfully treated patients at baseline. Univariate and multivariate analysis.

Pre-treatment variables evaluated	Crude HR	95% CI	p value	Adjusted HR*	95% CI	p value
Age (increasing years)	**1.04**	**1.02 - 1.06**	**<0.001**	**1.06**	**1.04 - 1.09**	**<0.001**
Gender (ref. female)	1.32	0.91 - 1.91	0.139	1.39	0.89 - 2.17	0.143
BMI: overweight (ref. under-normalweight)	0.91	0.62 - 1.33	0.615			
obese (ref. under-normalweight)	0.86	0.49 - 1.51	0.596			
Alcohol use: current (ref. never)	1.38	0.76 - 2.48	0.290			
past (ref. never)	1.36	0.89 - 2.08	0.157			
HCV-genotype (3 vs others)	1.49	0.87 - 2.57	0.146	**4.27**	**2.22 - 8.23**	**<0.001**
Anti-HBc+	1.14	0.74 - 1.76	0.542	1.58	0.98 - 2.55	0.058
Previous Interferon treatment	1.11	0.78 - 1.60	0.563			
Platelets count (ref. >120,000/μL)	**1.95**	**1.30 - 2.90**	**0.001**	**1.67**	**1.05 - 2.65**	**0.029**
Albumin level (ref. > 3.5 g/dL)	**3.08**	**2.13 - 4.46**	**<0.001**	**2.51**	**1.62 - 3.88**	**<0.001**
LSM (ref. < 20 kPa)	1.50	0.98 - 2.30	0.063			
Past decompensation	**1.74**	**1.09 - 2.80**	**0.021**			
Diabetes	**1.56**	**1.05 - 2.31**	**0.027**	**1.75**	**1.10 - 2.76**	**0.017**
Ribavirin use	0.82	0.56 - 1.20	0.300			
SOF-based treatment	**1.72**	**1.03 - 2.88**	**0.038**			

Abbreviations: BMI, Body Mass Index; CI, Confidence Interval; DAA, Direct Acting Antiviral; HCC, Hepatocellular Carcinoma; HCV, Hepatitis C Virus; HR, Hazard Ratio; LSM, Liver Stiffness Measurement; SOF, Sofosbuvir.
* Cox forward stepwise selection. Gender variable was forced to be included in the model.Statistically significant hazard ratios and related 95% confidence intervals are reported in bold.

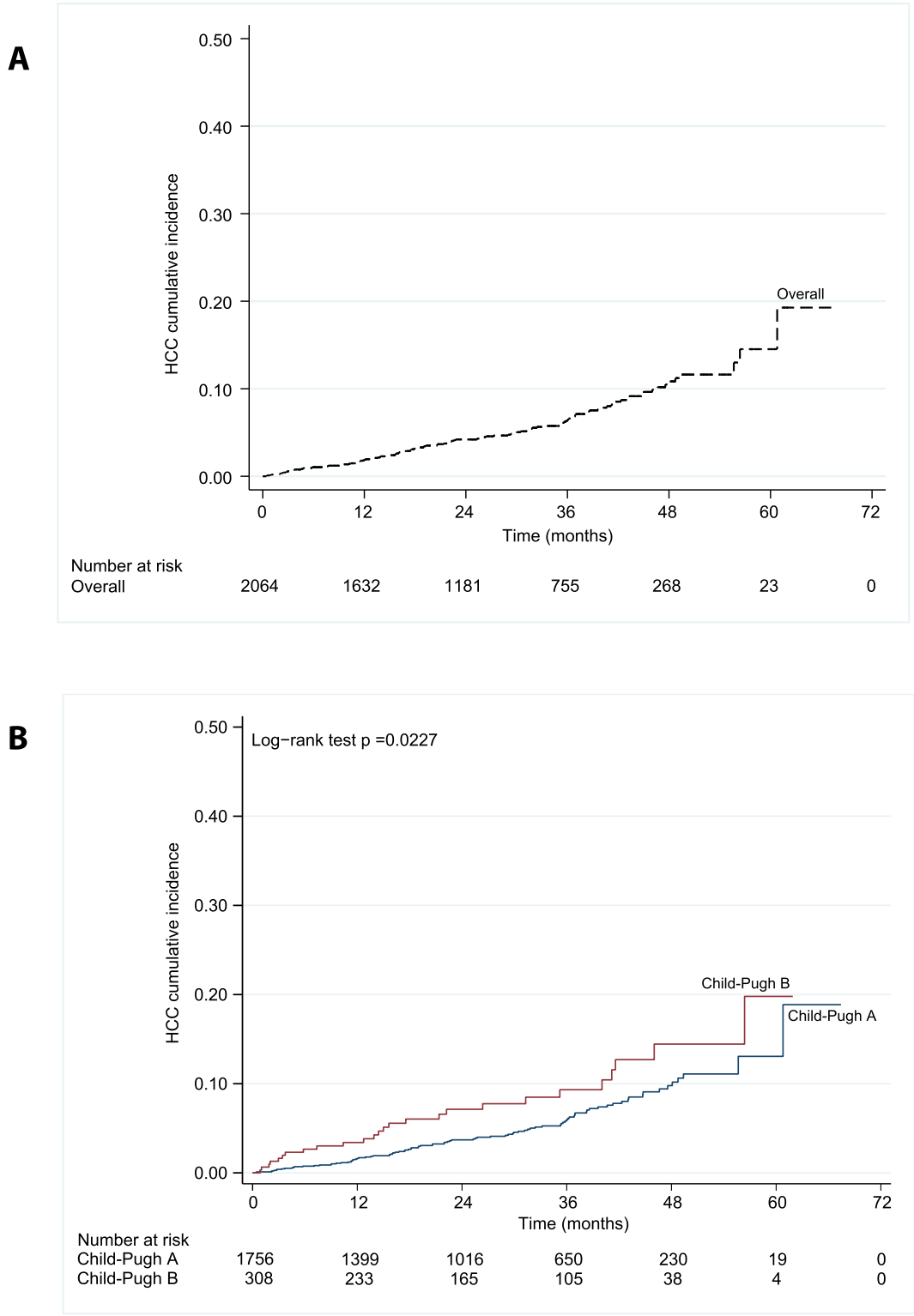


Fig. 1. Cumulative incidence of HCC development in patients with SVR after DAA therapy. (A) Cumulative incidence curve in patients with SVR. (B) Cumulative incidence curves in patients with SVR stratified for CPT-A and CPT-B class. Abbreviations: CPT, Child-Pugh-Turcotte; DAA, Direct Acting Antiviral; HCC, Hepatocellular Carcinoma; SVR, Sustained Virologic Response.

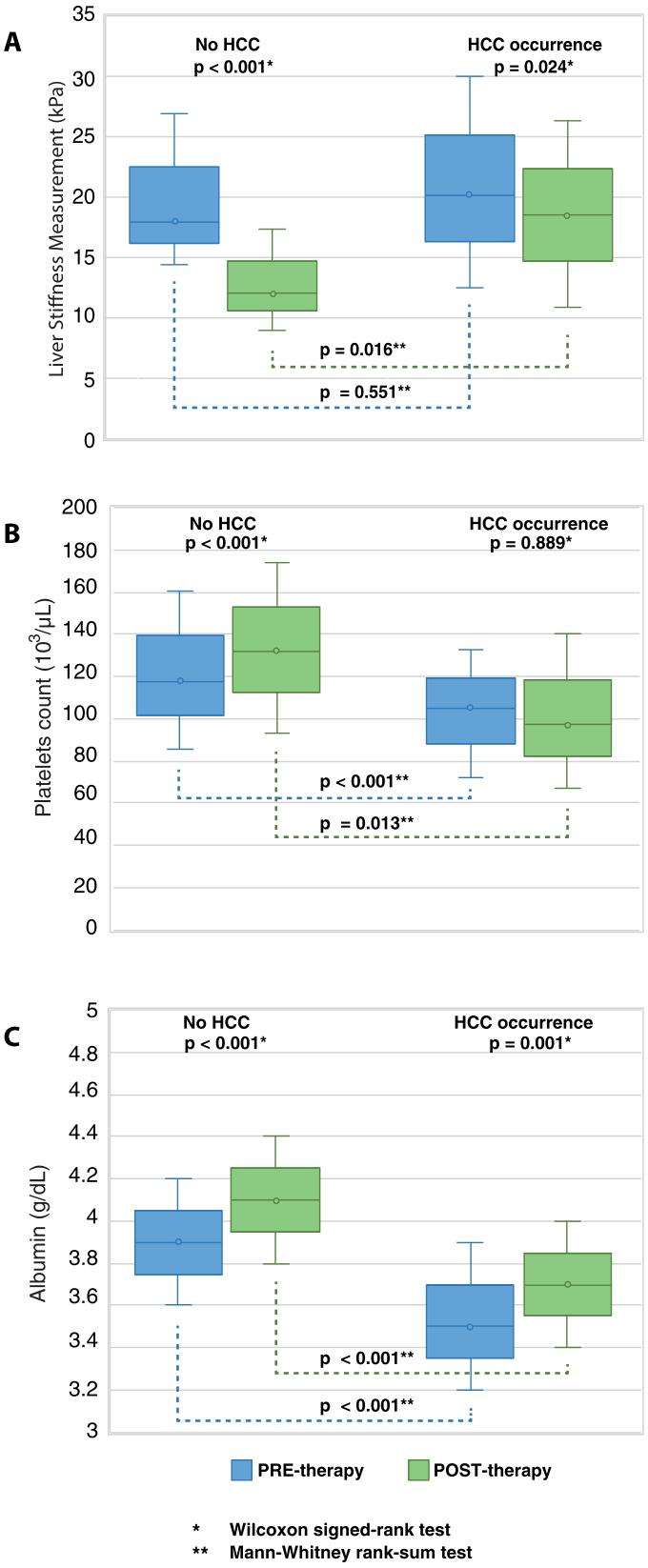


Fig. 2. Pre- and post-treatment evaluation of liver disease severity factors in patients with SVR. Pre- and post-treatment LSM (A), platelets count (B) and albumin level (C) in patients with and without HCC occurrence. Values were reported as the median and interquartile range. Abbreviations: HCC, Hepatocellular Carcinoma; LSM, Liver Stiffness Measurement; SVR, Sustained Virologic Response.

6.37%, 9.38%, at 12-, 24- and 36-month, respectively) compared to the group of patients categorized by both PLT >120,000/µl and albumin >3.5 g/dl (IR: 0.78%, 1.30%, and 2.04%, at 12-, 24- and 36-month, respectively; log-rank test p<0.001), who maintained the lowest risk of HCC occurrence during the FU compared to other combinations.

3.5. Prediction model for HCC free-survival probability after SVR

The nomogram was developed based on the multivariable Cox regression model. It included the variables evaluated post-therapy that were independently associated with HCC development such as: age (AdjHR 1.03; 95%CI 1.00-1.06), PLT count (AdjHR 1.92; 95%CI 1.06-3.45) and albumin level (AdjHR 4.38; 95%CI 2.48-7.75). It was assigned a score to each variable, and thus by summing up each point to obtain a total score and, drawing a vertical line from the total score (Total Point) up to the probability scale, at 1-, 3- and 5-year of overall survival at the bottom of the nomogram, it was possible to determine the probability of being HCC-free (overall survival probability) (Fig. 4). For example, a patient aged 65 years (55 points), with a PLT count ≤120,000/µl (37 points), and albumin level ≤3.5 g/dl (83 points) has a total scoring of 175 points; corresponding to a probability of being HCC-free survival at 1-year of about 90%, at 3-years ranging 80-75% and at the 5-year FU between 60-50%.

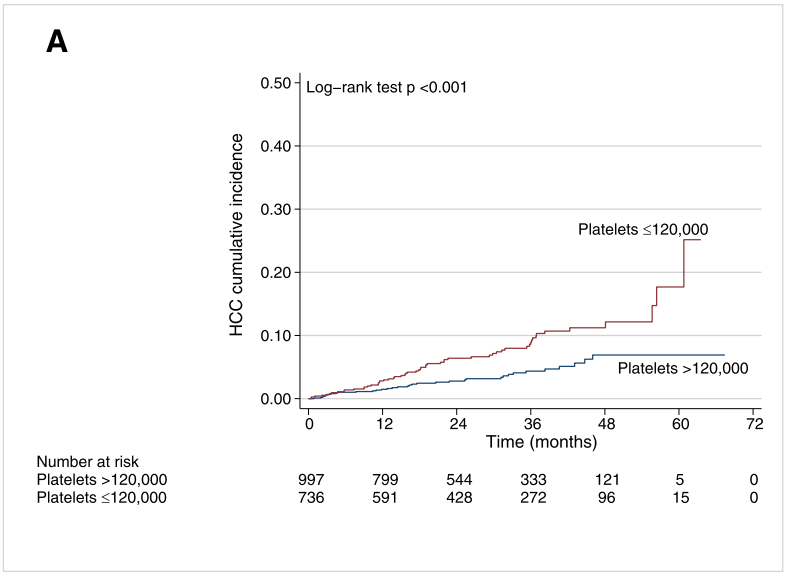
The model showed a satisfactory prediction capability with a Harrell's c-index of 0.74 (95%CI 0.67-0.82). The Calibration Plot is reported in the Supplementary Material (Supplementary Fig. 2).

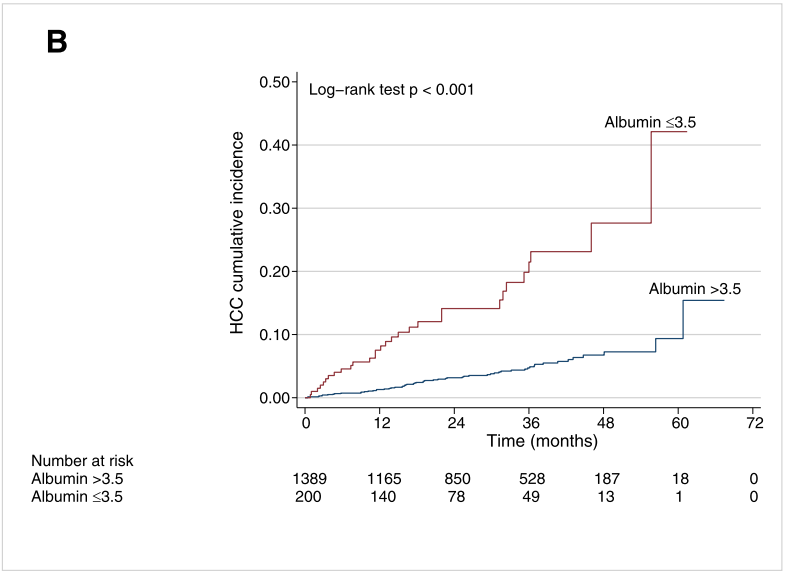
4. Discussion

To our knowledge, this is the first study focusing on the post-treatment predictive parameters of HCC occurrence at long-term FU in successfully DAA-treated patients with liver cirrhosis from a large ongoing prospective cohort.

In this analysis, we observed a cumulative HCC-IR of 6.47% at the 36-month FU after HCV eradication (2.45/100 p-y). This rate is somewhat higher than previously reported ones, ranging from 2 to 2.5% [3,4,17–20]. The HCC risk is reported to persist at least 10 years following viral eradication [4], and the different rates reported by several cohorts, compared to our data, may be explained by a different FU duration and, in particular, by different degrees of liver disease severity [21] among studies. In the PITER cohort, we reported a higher number of patients with features of severe liver disease (i.e., assessed by FIB-4 >3.25 in 67.2%; PLT ≤120,000/µl in 53.0%; LMS ≥20kPa in 47.5%; CPT-B in 14.9% and cases with previous liver decompensation in 10.9%), compared with some of the other cohorts described in literature [3,17,18,22]. The peculiarity of our cohort was possibly related to the fact that in Italy between 2015 to 2017, the DAAs could only be prescribed for patients with progressive liver disease and previous resistance to IFN, but being in stable compensation at pretreatment evaluation and within the previous 12 months.

The pre-treatment factors independently associated with HCC occurrence by multivariate analysis were: older age, genotype 3, presence of diabetes, decreased PLT count and albumin level confirming previously reported data [12,17,23,24]. The role of genotype needs further clarification. Some studies of literature found an increased risk of HCC occurrence among HCV-1 cases [7,18]. In Italy, genotype 3 is a prevalent genotype in younger patients, who had acquired HCV infection by drug use, less in older cohorts, that have acquired the infection by different transmission routes mainly infected by HCV genotype-1b. These last cases are the most representative group of patients with liver cirrhosis in Italy, and this could be one of the reasons for the lack of association between genotype 3 and HCC risk in other cohorts described pre-





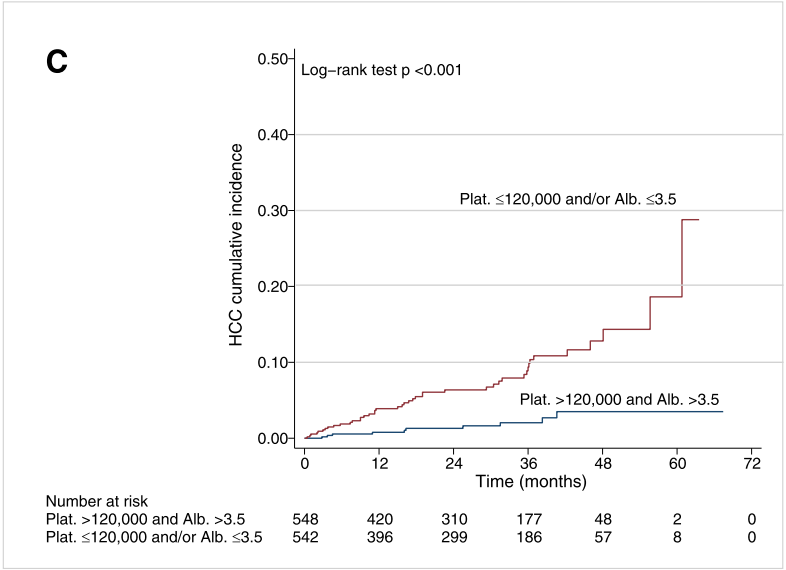


Fig. 3. Cumulative incidence of HCC according to different post-treatment characteristics. (A) Post-treatment platelets count (Platelet count ≤120,000/μl vs. Platelet count >120,000/μl). (B) Post-treatment albumin level (albumin level ≤3.5 g/dl vs. albumin level >3.5 g/dl). (C) Combination of post-treatment platelets and albumin values (Platelet count ≤120,000/μl and/or albumin level ≤3.5 g/dl vs. Platelet count >120,000/μl and albumin level >3.5 g/dl). Abbreviations: Alb, Albumin; HCC, Hepatocellular Carcinoma; Plat, Platelet.

Variables	Crude HR	95% CI	p value	Adjusted HR	95% CI	p value
Age (increasing years)	1.04	1.02 - 1.06	<0.001	1.03	1.00 - 1.06	0.021
Platelets (ref. >120,000/μL)	2.48	1.41 - 4.35	0.002	1.92	1.06 - 3.45	0.030
Albumin (ref. > 3.5 g/dL)	4.94	2.83 - 8.63	<0.001	4.38	2.48 - 7.75	<0.001

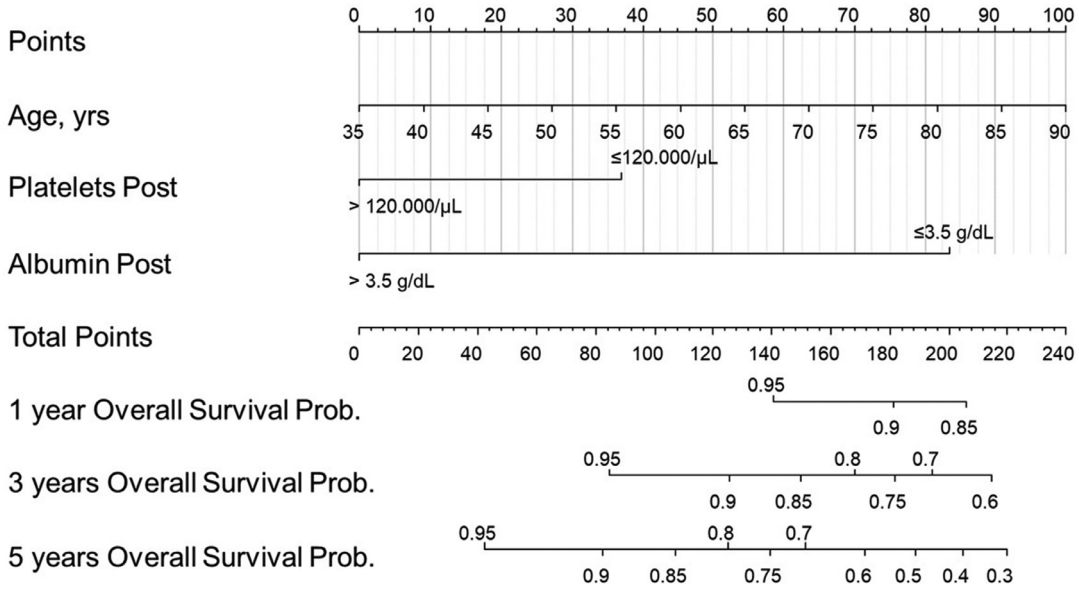


Fig. 4. Nomogram for HCC prediction in patients with SVR. Multivariable Cox regression model (including age, post-treatment platelets count and albumin levels) and graphical representation as nomogram. For each risk factor draw a vertical line upwards and note down the corresponding points. Each score should be summarized ending up with a total score that corresponds to a predicted probability of 1-, 3- and 5- years of overall survival at the bottom of the nomogram.
Abbreviations: CI, Confidence Interval; HCC, Hepatocellular Carcinoma; HR, Hazard Ratio; SVR, Sustained Virologic Response.

viously. Age by itself is one of the main confounding factors and after age-adjusted Cox regression analysis in our study, genotype 3 became independently associated with HCC risk after viral eradication. Alcohol consumption, differently from other results [12], was not associated with HCC development after SVR, potentially explained by prevalent social alcohol use, rather than the presence of heavy drinkers in this relatively old-age patient cohort.

The comparison of albumin levels in available paired data collected pre- and post-treatment in both cases with or without HCC occurrence improved. However, it could be emphasized, that the median albumin level was fairly normal in patients who did not develop HCC, and in contrast, it appeared frankly pathological in patients who developed HCC. Furthermore, some features associated with portal hypertension, such as: thrombocytopenia, deterioration of sinthetic liver function, as reflected by low albumin levels and increased liver stiffness tend to persist in patients who developed HCC. Thus, the PITER cohort tends to depict two different categories of cases, those with main characteristics that are just apparently similar at pre-treatment data, but whose real picture can be unmasked after viral eradication, being associated to a higher HCC risk.

Our findings confirm that even at a 36-month FU period after SVR, a substantial residual risk of HCC development remained, particularly in those with pre-treatment severe liver damage and the presence of portal hypertension (i.e., in cases with albumin level <3.5 g/dl and/or PLT count <120,0000/μl) [7,11,17,20,21,23]. In these patients, the severity of liver disease was identified among the most critical predictors of *de-novo* HCC.

Considerable evidence and a meta-analysis support the use of LSM for predicting HCC-risk in patients who achieved SVR, and different cut-offs have been proposed, ranging from >20 or >30 kPa at pre-treatment, and >10 kPa at post-treatment evaluations, in relation to higher incidence of HCC [8,17,25,26]. We did not have sufficient post-treatment LSM data to include the stiffness values in the risk evaluation modelling. However, the pre-treatment LSM ≥ 20 in the PITER cohort, was significantly associated with HCC development, as confirmed also by a recent HCC modeling risk [8]. Specifically, 60.7% vs. 46.8% of cases who developed HCC had pre-treatment LSM ≥20 kPa, respect to cases who did not (p=0.011). These cases showed a higher median stiffness value at 1-year FU compared to those who did not develop HCC (18.5 vs. 12.0 kPa; p=0.016), while a significant drop of LSM was observed after viral eradication both in cases without (p<0.001) or with HCC development (p=0.024). A similar pattern has been observed also in other studies [17,25]. However, unlike previously reported [27], we could not find any change in LSM between pre-treatment and post-treatment as a predictor of HCC development, probably due to a low sample size with available paired data. However, pointing out the significantly lower drop in LSM among patients with HCC (-18.0% vs. -28.9% p = 0.005), the relation between LSM and switch-off of liver inflammation after viral eradication must be also considered, independently of HCC development [8,27]. In this cohort, HCC developed mainly among cases with LSM ≥ 20 kPa, when obtained within one year from EOT; however, in accordance with data of Pons et al [8], at post-treatment long-term FU after SVR, the LSM did not show an independent HCC predictive value.

The best predictor of HCC occurrence after SVR in our study was given by the association of two easy and accurate markers, undoubtedly associated with portal hypertension and liver function, as PLT count and albumin level, analyzed prospectively at long-term FU after SVR.

The highest HCC incidence in SVR cases was found in patients with PLT count ≤120,000/μl and/or albumin level ≤3.5 g/dl (IR 3.90%; 6.37% and 9.38%, at 12-, 24- and 36-months, respectively) compared with patients categorized by both PLT >120,000/μl and albumin levels >3.5 g/dl (IR 0.78%, 1.30% and 2.04%, at 12-, 24- and 36- months, respectively). The latest group maintained the lowest HCC risk during the FU compared to other combinations of these two parameters.

We followed the analysis based on the aforementioned markers, which combine indicators of hepatic function (i.e., serum albumin) and severe stage of liver disease (assessed with PLT count), evaluated during the FU after viral eradication, with the patient's age (a strong driver of carcinogenesis) [7,11]. The nomogram constructed in our study, using PLT and albumin values measured at long-term FU after EOT in patients who achieved the SVR, allows the estimation of the probability of being HCC-free over time (at 1-, 3- and 5-years of FU) in the real-life practice. Combining variables independently associated with HCC occurrence (age and PLT count ≤ vs. >120,000/μl and albumin level ≤ vs. >3.5 g/dl), we stratified two risk groups post-therapy, with an HCC-IR significantly different of 1.35 vs. 3.77/100 p-y. This developed model, calculated by nomogram maps, based on age, PLT, and albumin obtained post-therapy, showed an accurate prediction of overall survival probability to be HCC-free at 1, 3, and 5-years of FU in the individual patient.

Our data, as others recently reported, fails to identify a group with no risk in whom the HCC surveillance may be stopped, perhaps because we evaluated "*as predictors*" the biochemical tools strongly related to the progression of liver damage. Instead, we reinforce the need for very tight HCC surveillance in the risk group of patients with advanced liver disease, based, in addittion to the liver ultrasound, also in a periodical evaluation of PLT count and albumin level combination, which should be considered to drive the risk of HCC occurrence. Genetic determinants or algorithms based on other predictive variables, possibly identified by machine learning approaches, are warranted to refine the individualized prediction of HCC-risk ([28]).

4.1. Strengths and limitations

Major strengths of our study are the large patient cohort, the consecutive enrolment, the long-term prospectively evaluation , and the complete clinical characterization with specific essential real-life data obtained in tertiary health care centers not explicitly dedicated to HCC cure.

This multicenter ongoing life-practice patient enrolment could suffer from possible limitations, as the potential inclusion of cases with very early and undetectable liver nodules that cannot have been ruled out before the DAA therapy by the six-month US or other imaging surveillance. To limit this potential bias and to be more conservative in the analysis only HCC occurring after the EOT was considered *de-novo* HCC.

The potential heterogeneity among the network of clinical centers in terms of competence in diagnosing HCC could be another limitation of the study. However, we believe this limitation was overcome by the strict application of guidelines to diagnose HCC with adequate monitoring of cases by the participating centers.

The accurate monitoring of LSM during FU was lacking in some patients, making this tool not thoroughly evaluated in our analysis.

The real-world clinical practice of HCC surveillance, focusing on the risk stratification approach based only on two easy-accessible and accurate surrogate markers of liver function over time could be helpful. Further dedicated studies will be useful to fully confirm its utility in real-life clinical practice.

4.2. Conclusions

A scoring system, based on combined PLT and albumin values evaluated post-treatment, may be helpful to identify patients free of HCC risk after SVR. Using the produced nomogram, based on: patient age, albumin level ≤3.5 g/dl and PLT count ≤120,000/μl, performed at 1-, 2- and 3-year after SVR, could accurately identify the population with the highest HCC risk deserving of a careful long-term FU. Further dedicated studies are useful to fully confirm its utility in real-life clinical practice.

Conflict of interest

None declared.

Data availability statement

This study was approved by the Ethical Committee of Istituto Superiore di Sanità and the local Ethical Committees of the participating centers. By protocol, the property of the data is of participating clinical centers while Istituto Superiore di Sanità acts as a coordinating center for data management and analysis. Cumulative data are reported within the paper whereas each patient's data are not fully available and without restrictions for ethical reasons. Dr. LA Kondili (loreta.kondili@iss.it) is in charge data management and the readers may contact her for specific data requests. She will provide the necessary ethical clearances for access to data. Interested readers may contact Carlo Petrini (carlo.petrini@iss.it) from the Ethical Committee of Istituto Superiore di Sanità to request access to patient level data.

Acknowledgements

The authors wish to thank all PITER collaborating group and all participating centers, investigators and research staff (available in www progettopiter.it) who are involved in the study on a voluntary basis, for their time and effort. We also thank and Giampaolo La Terza (Medisoft Informatic Services) for Database maintenance and implementation. We additionally acknowledge Federica Magnani, Rosangela Duranti, Erika Olivieri, Alessandra Mattei for secretarial and administrative assistance.

This study was supported by Italian Ministry of Health (Grant number RF-2016-02364053 and Ricerca Corrente Annual Program 2023).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2023.01.153.

Appendix

PITER collaborating investigators:

Valentina Panetta (L'altrastatistica srl, Consultancy & Training, Biostatistics Office, Rome, Italy)

Giovanni Battista Gaeta (Infectious Diseases, University of Campania "Luigi Vanvitelli", Naples, Italy)

Roberto Filomia (Department of Internal Medicine, University Hospital of Messina, Messina, Italy)

Barbara Coco (Department of Clinical and Experimental Medicine, University Hospital of Pisa, Pisa, Italy)

Monica Monti (Department of Experimental and Clinical Medicine, Interdepartmental Centre MASVE, University of Florence, Florence, Italy)

Daniela Caterina Amoruso (Department of Hepatology, Gragnano Hospital, Gragnano (NA), Italy)

Salvatore Madonia (Department of Internal Medicine, Villa Sofia-Cervello Hospital, Palermo, Italy)

Donatella Ieluzzi (Liver Unit, University Hospital of Verona, Verona, Italy)

Gloria Taliani (Infectious and Tropical Medicine Unit, Department of Public Health and Infectious Diseases, "Policlinico Umberto I" Hospital, Sapienza University of Rome, Rome, Italy)

Lorenzo Badia (Clinic of Infectious Diseases and Microbiology Unit, Alma Mater Studiorum Bologna University)

Guglielmo Marco Migliorino (Department of Infectious Diseases, San Gerardo Hospital - ASST Monza, Monza, Italy)

Alessia Giorgini (Gastroenterology and Hepatology Unit, San Paolo Hospital, University of Milan, Italy)

Mario Masarone (Internal Medicine and Hepatology Unit, University of Salerno, Salerno)

Pierluigi Blanc (Infectious Disease Unit, Santa Maria Annunziata Hospital, Florence, Italy)

Valentina Cossiga (Gastroenterology Unit, Federico II University, Naples, Italy)

Martina DE Siena (Internal Medicine and Gastroenterology, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy)

Xhimi Tata (Center for Global Health, Istituto Superiore Di Sanità, Rome, Italy)

Maria Grazia Rumi (Hepatology Unit, San Giuseppe Hospital, Milan, Italy)

Luchino Chessa (Liver Unit, University Hospital, Monserrato, Cagliari, Italy)

Pietro Lampertico (Division of Gastroenterology and Hepatology, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy)

Carlo Ferrari (Department of Medicine and Surgery, University of Parma, Unit of Hematology, Azienda Ospedaliero-Universitaria di Parma, Italy)

Ivan Gentile (Department of Clinical Medicine and Surgery, University of Naples Federico II, Napoli, Italy)

Giustino Parruti (Infectious Diseases Unit, Spirito Santo General Hospital, Pescara, Italy)

Leonardo Baiocchi (Hepatology Unit, University of Tor Vergata, Rome, Italy)

Alessia Ciancio (Gastroenterology Unit, Città della Salute e della Scienza of Turin, University Hospital, Turin, Italy)

Pietro Invernizzi (Division of Gastroenterology and Center for Autoimmune Liver Diseases, San Gerardo Hospital, Monza, Italy)

Alessandro Federico (Department of Hepato-Gastroenterology, University of Campania Luigi Vanvitelli, Naples, Italy)

Carlo Torti (Department of Medical and Surgical Sciences, Infectious and Tropical Diseases Unit, "Magna Graecia" University, Catanzaro, Italy)

Giulia Morsica (Department of Infectious Diseases, San Raffaele Hospital, Milan Italy)

Pietro Andreone (Department of Internal Medicine, University of Modena and Reggio Emilia, Modena, Italy)

Alessio Aghemo (Department of Gastroenterology, IRCCS Humanitas Research Hospital IRCCS, Rozzano, Italy)

Patrizia Popoli (National Center for Drug Research and Evaluation, Istituto Superiore Di Sanità, Rome, Italy)

Stefano Vella (Global Health, Catholic University, Rome, Italy)

References

[1] Cancer today n.d. http://gco.iarc.fr/today/home (accessed May 11, 2022).
[2] Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. Nat Rev Dis Primers 2021;7:6. doi:10.1038/s41572-020-00240-3.
[3] Calvaruso V, Cabibbo G, Cacciola I, Petta S, Madonia S, Bellia A, et al. Incidence of hepatocellular carcinoma in patients with HCV-associated cirrhosis treated with direct-acting antiviral agents. Gastroenterology 2018;155:411–21 e4. doi:10.1053/j.gastro.2018.04.008.
[4] Ioannou GN, Beste LA, Green PK, Singal AG, Tapper EB, Waljee AK, et al. Increased risk for hepatocellular carcinoma persists up to 10 years after HCV eradication in patients with baseline cirrhosis or high FIB-4 scores. Gastroenterology 2019;157:1264–78 e4. doi:10.1053/j.gastro.2019.07.033.
[5] Carrat F, Fontaine H, Dorival C, Simony M, Diallo A, Hezode C, et al. Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. Lancet 2019;393:1453–64. doi:10.1016/S0140-6736(18)32111-1.
[6] Ioannou GN, Feld JJ. What are the benefits of a sustained virologic response to direct-acting antiviral therapy for hepatitis C virus infection? Gastroenterology 2019;156:446–60 e2. doi:10.1053/j.gastro.2018.10.033.
[7] D'Ambrosio R, Degasperi E, Anolli MP, Fanetti I, Borghi M, Soffredini R, et al. Incidence of liver- and non-liver-related outcomes in patients with HCV-cirrhosis after SVR. J Hepatol 2022;76:302–10. doi:10.1016/j.jhep.2021.09.013.
[8] Pons M, Rodríguez-Tajes S, Esteban JI, Mariño Z, Vargas V, Lens S, et al. Non-invasive prediction of liver-related events in patients with HCV-associated compensated advanced chronic liver disease after oral antivirals. J Hepatol 2020;72:472–80. doi:10.1016/j.jhep.2019.10.005.
[9] Alonso López S, Manzano ML, Gea F, Gutiérrez ML, Ahumada AM, Devesa MJ, et al. A model based on noninvasive markers predicts very low hepatocellular carcinoma risk after viral response in hepatitis C virus–advanced fibrosis. Hepatology 2020;72:1924–34. doi:10.1002/hep.31588.
[10] van der Meer AJ, Feld JJ, Hofer H, Almasio PL, Calvaruso V, Fernández-Rodríguez CM, et al. Risk of cirrhosis-related complications in patients with advanced fibrosis following hepatitis C virus eradication. J Hepatol 2017;66:485–93. doi:10.1016/j.jhep.2016.10.017.
[11] Semmler G, Meyer EL, Kozbial K, Schwabl P, Hametner-Schreil S, Zanetto A, et al. HCC risk stratification after cure of hepatitis C in patients with compensated advanced chronic liver disease. J Hepatol 2022;76:812–21. doi:10.1016/j.jhep.2021.11.025.
[12] Kanwal F, Kramer JR, Asch SM, Cao Y, Li L, El-Serag HB. Long-term risk of hepatocellular carcinoma in HCV patients treated with direct acting antiviral agents. Hepatology 2020;71:44–55. doi:10.1002/hep.30823.
[13] Ioannou GN, Green PK, Beste LA, Mun EJ, Kerr KF, Berry K. Development of models estimating the risk of hepatocellular carcinoma after antiviral treatment for hepatitis C. J Hepatol 2018;69:1088–98. doi:10.1016/j.jhep.2018.07.024.
[14] Kondili LA, Vella S. PITER: an ongoing nationwide study on the real-life impact of direct acting antiviral based treatment for chronic hepatitis C in Italy. Dig Liver Dis 2015;47:741–3. doi:10.1016/j.dld.2015.05.022.
[15] Castéra L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, et al. Prospective comparison of transient elastography, fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128:343–50. doi:10.1053/j.gastro.2004.11.018.
[16] Galle PR, Forner A, Llovet JM, Mazzaferro V, Piscaglia F, Raoul J-L, et al. EASL clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 2018;69:182–236. doi:10.1016/j.jhep.2018.03.019.
[17] Degasperi E, D'Ambrosio R, Iavarone M, Sangiovanni A, Aghemo A, Soffredini R, et al. Factors associated with increased risk of de novo or recurrent hepatocellular carcinoma in patients with cirrhosis treated with direct-acting antivirals for HCV infection. Clin Gastroenterol Hepatol 2019;17:1183–91 e7. doi:10.1016/j.cgh.2018.10.038.
[18] Nahon P, Layese R, Bourcier V, Cagnot C, Marcellin P, Guyader D, et al. Incidence of hepatocellular carcinoma after direct antiviral therapy for HCV in patients with cirrhosis included in surveillance programs. Gastroenterology 2018;155:1436–50 e6. doi:10.1053/j.gastro.2018.07.015.
[19] Waziry R, Hajarizadeh B, Grebely J, Amin J, Law M, Danta M, et al. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: a systematic review, meta-analyses, and meta-regression. J Hepatol 2017;67:1204–12. doi:10.1016/j.jhep.2017.07.025.
[20] Romano A, Angeli P, Piovesan S, Noventa F, Anastassopoulos G, Chemello L, et al. Newly diagnosed hepatocellular carcinoma in patients with advanced hepatitis C treated with DAAs: a prospective population study. J Hepatol 2018;69:345–52. doi:10.1016/j.jhep.2018.03.009.
[21] Krassenburg LAP, Maan R, Ramji A, Manns MP, Cornberg M, Wedemeyer H, et al. Clinical outcomes following DAA therapy in patients with HCV-related cirrhosis depend on disease severity. J Hepatol 2021;74:1053–63. doi:10.1016/j.jhep.2020.11.021.
[22] ANRS collaborative study group on hepatocellular carcinoma (ANRS CO22 HEPATHER, CO12 CirVir and CO23 CUPILT cohorts)Electronic address: stanislas.pol@aphp.fr. Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: data from three ANRS cohorts. J Hepatol 2016;65:734–40. doi:10.1016/j.jhep.2016.05.045.
[23] Rinaldi L, Perrella A, Guarino M, De Luca M, Piai G, Coppola N, et al. Incidence and risk factors of early HCC occurrence in HCV patients treated with direct

acting antivirals: a prospective multicentre study. J Transl Med 2019;17:292. doi:10.1186/s12967-019-2033-x.
[24] Hedenstierna M, Nangarhari A, Weiland O, Aleman S. Diabetes and cirrhosis are risk factors for hepatocellular carcinoma after successful treatment of chronic hepatitis C. Clin Infect Dis 2016;63:723–9. doi:10.1093/cid/ciw362.
[25] Rinaldi L, Guarino M, Perrella A, Pafundi PC, Valente G, Fontanella L, et al. Role of liver stiffness measurement in predicting HCC occurrence in direct-acting antivirals setting: a real-life experience. Dig Dis Sci 2019;64:3013–19. doi:10.1007/s10620-019-05604-8.
[26] You M-W, Kim KW, Shim J-J, Pyo J. Impact of liver-stiffness measurement on hepatocellular carcinoma development in chronic hepatitis C patients treated with direct-acting antivirals: a systematic review and time-to-event meta-analysis. J Gastroenterol Hepatol 2021;36:601–8. doi:10.1111/jgh.15243.
[27] Ravaioli F, Conti F, Brillanti S, Andreone P, Mazzella G, Buonfiglioli F, et al. Hepatocellular carcinoma risk assessment by the measurement of liver stiffness variations in HCV cirrhotics treated with direct acting antivirals. Dig Liver Dis 2018;50:573–9. doi:10.1016/j.dld.2018.02.010.
[28] Audureau E, Carrat F, Layese R, Cagnot C, Asselah T, Guyader D, et al. Personalized surveillance for hepatocellular carcinoma in cirrhosis - using machine learning adapted to HCV status. J Hepatol 2020;73:1434–45. doi:10.1016/j.jhep.2020.05.052.