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Risk profiling of hepatocellular carcinoma occurrence after eradication of hepatitis C virus with direct-acting antiviral agents

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Abstract

Background: Treatment with direct-acting antiviral agents (DAAs) have significantly improved the outcomes of patients with HCV-related cirrhosis. However, the risk of developing hepatocellular carcinoma (HCC) still persists in these patients and predictive models are lacking. The aim of this study is to develop a predictive model including clinical and genetic features for individual risk profiling of HCC occurrence after DAA-induced sustained virologic response (SVR) in patients with HCV cirrhosis.

Methods Consecutive patients with HCV cirrhosis achieving SVR after DAA treatment without previous history of HCC were prospectively enrolled and followed-up for HCC occurrence. Clinical and biochemical features were evaluated at the time of DAA start and at the time of SVR. MERTK single-nucleotide polymorphism (SNP) rs6726639 was assessed in all patients. Cox regression model was used to identify risk factors for HCC occurrence. Accuracy was assessed by time-dependent area under the receiver operating characteristic curves (AUROCs) and net benefit was measured by decision curve analysis.

Results Four-hundred-seven patients (mean age 67 years, 57% male) were included and followed-up for a mean time of 70 months. HCC occurred in 58 (14.2%) patients. At the time of DAA start, PLT count lower than $120x10^9$/L (HR 2.12, 95%CI 1.15-3.93, p=0.017), albumin levels lower than 3.5 g/dL (HR 2.37, 95%CI 1.38-4.08, p=0.002) and MERTK AA genotype (HR 1.98, 95%CI 1.09-3.62, p=0.026) were independent risk factors for HCC occurrence, by multivariate analysis. At the time of SVR, PLT count lower than $130x10^9$/L (HR 2.14, 95%CI 1.10-4.34, p=0.036), albumin levels lower than 3.8 g/dL (HR 2.21, 95%CI 1.07-4.58, p=0.032) and MERTK AA genotype (HR 2.18, 95%CI 1.01-4.70, p=0.046) were confirmed as independent risk factors for HCC occurrence, by multivariate analysis. Time-dependent AUROCs showed good accuracy and net benefit of this model was confirmed by DCA

Conclusions The risk of HCC after DAA-induced SVR persists, although low, also after a long-term follow-up. A risk profiling based on PLT, albumin and MERTK genotype is useful to predict the risk of HCC occurrence after SVR and it could identify a subgroup of patients in which the surveillance schedule for HCC can be personalized and extended.

Summary

The outcomes of patients with hepatitis C virus (HCV)-related cirrhosis have been revolutionized by the introduction during the last decade of direct-acting antiviral agents (DAAs), that completely substituted interferon-based antiviral regimens, significantly increasing the likelihood of achieving sustained virologic response (SVR), as well as the treatment tolerability. DAAs significantly reduce the risk for hepatic decompensation, but the impact on the risk of developing HCC is substantially lower. This risk persists despite the improvement of liver function, being related to modifiable (i.e. severity of liver dysfunction, severity of liver fibrosis and severity of portal hypertension) or unmodifiable (age, sex, genetic predisposition) risk factors. Therefore, the identification of a risk profiling able to accurately predict the risk of HCC occurrence after SVR still remains an unmet medical need. Our prospective study on about 400 patients with HCV-related cirrhosis without history of previous HCC and with a mean follow-up of about 6 years substantiates the usefulness of a biochemical- and genetic-based risk profiling for predicting HCC occurrence during follow-up. Particularly, we developed a predictive model including platelet count (that is a surrogate marker of portal hypertension), albumin levels (that are a worldwide accepted biomarker of liver function) and a single nucleotide polymorphism (SNP) of MERTK gene, that is involved in hepatic fibrogenesis and carcinogenesis. We found that this model is able to accurately predict the risk of HCC occurrence both when evaluated before DAA treatment and at the time of SVR, with a slight change of cut-off levels. We believe that our results could be useful to identify a subgroup of patients with a favorable biochemical and genetic risk profile for whom a delay in the schedule of ultrasound surveillance (annual rather than semestral) after SVR can be safely proposed. Waiting for an external validation of our model, further prospective studies using a multiomic approach are needed to improve the accuracy of the risk profiling for the prediction of HCC occurrence after DAA-induced SVR.

Background, Rationale and Objectives

Hepatocellular carcinoma (HCC) represents the most common primary liver cancer, being among the top three causes of cancer-related death worldwide and it is expected that both the number of new cases and deaths will increase in the future, rising over 55% by 2040 [1]. The development of HCC is strictly linked to the presence of chronic liver disease, representing the leading cause of death in patients with compensated cirrhosis, although it could rarely occurs in patients without advanced liver fibrosis. The severity of underlying chronic liver disease and portal hypertension has a relevant clinical impact on the feasibility, the efficacy and the safety of treatments for HCC.

HCV infection is an independent risk factor for the development of HCC, both in Western countries and in Japan [2]. The prognosis of patients with HCV cirrhosis is driven by the development of hepatic decompensation and HCC, that represent competing risks for death [3]. In the past, HCV eradication obtained with interferon (IFN)-based therapies has been associated with a decrease in HCC risk in comparison with patients who did not obtain sustained virological response (SVR) [4]. During the last decade, direct-acting antiviral agents (DAAs) have deeply changed the outcomes of HCV patients, with high SVR rates (> 90%) also in those with more advanced liver disease stages. DAA-induced SVR is associated with a stabilization or improvement of liver function[5−7] and with a low rate of adverse events or contraindications [5]. This has led to a reduction in the risk of HCC occurrence after DAA-induced SVR, as showed in large prospective studies with long-term follow-up [8,9]. Following DAA-induced SVR, reported rates of HCC occurrence are estimated nearly 2–2.5% per year [8,9], with a risk that has been demonstrated to persist up to 10 years from antiviral treatment [10].

Risk factors for HCC development after SVR can be classified as

modifiable and non-modifiable. Among modifiable risk factors, the stage of liver disease has been demonstrated as one of the most relevant determinant of the risk for developing HCC, with patients with cirrhosis and clinically significant portal hypertension having the higher risk for HCC occurrence [8,9]. This finding has been demonstrated both evaluating fibrosis by invasive (i.e. liver biopsy) and non-invasive tools (i.e. FIB-4 or liver stiffness measurement [LSM]) [11]. Since it has been showed that DAA treatment could improve the severity of liver disease, it is expected that improvement in liver function after DAA-induced SVR could reduce the risk for HCC occurrence, especially in the subgroup of patients achieving a significant improvement in liver fibrosis and portal hypertension after DAA-induced SVR. On the other side, other risk factors are only partially modifiable (i.e. metabolic syndrome) or completely non-modifiable. Among these latter, older age and male sex are well-demonstrated risk factors for HCC occurrence [11], while the role of single-nucleotide polymorphisms [SNPs] have not yet been fully explained. Particularly, MERTK is a tyrosine kinase receptor that belongs to the TAM (Tyro3/Axl/Mer) receptor family. It is involved in different processes including cellular proliferation/survival, cellular adhesion/migration, and release of the inflammatory/anti-inflammatory cytokines [12,13]. Genome Wide Association Study (GWAS) reported that a SNP of MERTK (rs4374383 A>G) is associated with the risk of developing fibrosis in patients with Chronic Hepatitis C and non-alcoholic steatohepatitis [14,15]. It has been suggested that MERTK gene SNPs may play a key role not only in apoptosis, immune response and epithelial-mesenchymal transition of hepatic stellate cells (HSCs), but also in the mechanisms involved in fibrosis progression and carcinogenesis [16]. More recently, our group evaluated the liver expression of genes involved in fibrogenesis and hepatocarcinogenesis in patient with chronic hepatitis C, by using a microarray approach, showing that rs 4374383 AA homozygosity is associated with lower MERTK expression and that, according to MERTK genotype, matrix metallopeptidases and WNT gene family have a different expression in patients with or without HCC [17].

Since the number of patients that achieved DAA-induced SVR is increasing, an approach to HCC risk profiling based on the combination of clinical and genetic features could be useful to personalize clinical management, resulting in the improvement of the cost-effectiveness of surveillance, in the identification of possible targeted therapies and finally in the improvement of survival. The aim of this study is to develop a predictive model including clinical and genetic features for individual risk profiling of HCC occurrence after DAA-induced SVR in patients with HCV cirrhosis.

Materials and Methods

Patients

This prospective study enrolled all consecutive patients with HCV-related cirrhosis starting DAA treatment between March 2015 and December 2016, seen at Gastroenterology and Hepatology Unit of University Hospital "Paolo Giaccone" of Palermo.

Patients were included if they had HCV-related liver cirrhosis and if they were treated with DAA, obtaining SVR. We excluded patients with history of decompensation events before the start of DAA treatment (porto-systemic encephalopathy, ascites, variceal upper gastrointestinal bleeding, hepatocellular carcinoma) or with history of HCC before the start of DAA treatment. We also excluded patients who developed HCC occurrence during the first three months of follow-up and patients with a follow-up shorter than 24 months.

Cirrhosis was defined by liver biopsy (METAVIR F4) [18] , or by non-invasive methods (liver stiffness measurement [LSM] >11.9 kPa, irregular/nodular liver surface at abdominal ultrasound scan, splenomegaly with platelet count below 150 x 10^3/ml) [19].

All included patients underwent MERTK genotyping before DAA treatment, by using Real Time PCR by means of an allelic discrimination assay with allele-specific Taqman probes containing FAM or VIC fluorochromes as markers, which allow to highlight the two variants of the nucleic acid sequence.

This study was reviewed and approved by Institutional Review Board of University Hospital of Palermo and all patients gave their written informed consent.

Follow-up and outcomes

The inception point was defined as the start of DAA treatment (baseline). Patients underwent visit and biochemical evaluation at the start of DAA

treatment and at 12 weeks after the end of treatment (SVR) and every 6 months thereafter.

The following variables were assessed and recorded at the start of DAA treatment and during the follow-up: age, sex, body mass index (BMI), presence of comorbidities (type 2 diabetes, arterial hypertension), alanine aminotransferase (ALT) levels, platelet (PLT) count, bilirubin, albumin, international normalised ratio (INR), liver stiffness by FibroScan, Child-Pugh class, presence and size of oesophageal varices (OV).

HCC surveillance was based on 6-month ultrasound according to EASL guidelines [20]. When a new focal lesion was identified on ultrasound examination, the diagnosis of HCC had to be confirmed by multiphasic computed tomography (CT) or magnetic resonance imaging (MRI) or by a histological examination according to EASL guidelines [20].

The primary outcome of the study was the occurrence of HCC. Secondary outcomes were the occurrence of hepatic decompensation (defined as the occurrence of ascites, hepatic encephalopathy or variceal bleeding) and death.

Statistical analysis

Continuous variables were expressed as mean±standard deviation (SD), while categorical variables as frequency and percentage. Categorical variables were compared using the Chi-square, and continuous variables by the Student's t test.

HCC occurrence rates were calculated by Kaplan-Meier method, and differences among groups of patients were tested using log-rank test.

A Cox regression analysis was performed to identify risk factors for HCC occurrence. The following variables were screened at univariate analysis: age, sex, body mass index (BMI), type 2 diabetes mellitus, arterial hypertension, ALT, bilirubin, INR, albumin, PLT count, MerTK SNPs. Among these covariates, ALT, bilirubin, INR, albumin, PLT count and liver

stiffness were evaluated at baseline and at the time of SVR. Covariates screened at univariate analysis were included in a multivariate model if p-value was lower than 0.05 both at baseline and at the time of SVR. Covariates with a p-value less than .05 in the multivariate model were considered statistically significant. Results are expressed as adjusted hazard ratio (HR) with 95% confidence interval (CI).

Risks factors for HCC occurrence identified by multivariate analysis were used to generate a prediction model. The predicted probability of HCC occurrence after DAA treatment was computed for hypothetical patients identified by a combination of prognostic factors.

Discrimination of the models was assessed by time-dependent area under the receiver operating characteristic (ROC) curve, evaluated at 1, 3 and 5 years of follow-up.

We also performed a decision curve analysis (DCA) for identifying threshold probabilities at which the use of our model will translate into maximum net benefit of ruling out HCC [21,22]. DCA evaluate prediction models in comparison with default strategies of performing 6-month ultrasound in all patients or none, allowing an assessment of overall yield of prediction rules. DCA estimates a "net benefit" for a prediction rule, defined

net benefit = sensitivity X prevalence - (1 - specificity) X (1 - prevalence) X w

where w is the odds of true diagnosis (i.e., HCC in this case) across different threshold probabilities. In this setting, the net benefit represents a composite of the benefit gained by performing 6-month ultrasound for true HCC (true positive) and the cost of performing 6-month ultrasound in those without HCC (false positive). The threshold probability represents a theoretical risk level where the expected benefit of treatment is equal to the expected benefit of avoiding treatment (e.g., benefit of 6-month ultrasound equals the risk of not performing it). Thus, net benefit for ruling out HCC could be expressed

as the number of ultrasound assessment correctly spared during the follow-up.

All data were analyzed using RStudio. DCA was implemented in R using the code derived from Zhang et al. [23]. In addition to the base packages in R, tidyverse, survival, survminer, boot, reshape2, and readxl packages were used.

Results

Between March 2015 and December 2016, 410 patients with HCV-related cirrhosis were treated with DAAs. We excluded 3 patients who developed HCC during the first three months of follow-up. Finally, we included 407 patients. Baseline demographic, clinical, biochemical and genetic characteristics are reported in **Table 1**. Mean age was 72 years and 57% of patients were male. Most of patients (86%) were in Child-Pugh class A and OV were present in 75% of patients. MERTK deSNP rs6726639 AA genotype was present in about 15% of patients.

When evaluating biochemical features and liver stiffness at the time of SVR after DAA treatment, a significant improvement of ALT levels, PLT count and albumin levels was found (**Table 2**).

After a mean follow-up duration of 70 months, 58 patients (14.2%) developed HCC. Cumulative incidence rates of HCC occurrence are showed in **Figure 1.** The median time to HCC occurrence was not reached and actuarial rates of HCC occurrence were 3% at 1 year, 8.5% at 3 years, 12.9% at 5 years and 15.9% at 7 years.

At baseline, before DAA start, patients who developed HCC showed significantly lower ALT levels, PLT count, albumin levels, significantly higher INR levels and liver stiffness and a significantly higher prevalence of Child-Pugh class B and higher prevalence of MERTK AA genotype, compared with patients who did not develop HCC during follow-up (**Table 1**). When evaluated at the time of SVR, patients with HCC occurrence showed significantly lower PLT count and lower albumin levels, compared to patients without HCC (**Table 3**).

Risk factors for HCC occurrence by univariate analysis are reported in **Table 4.** PLT count and albumin levels were the only covariates that were significantly associated with HCC occurrence both when evaluated at

baseline and at the time of SVR and they were entered into multivariate model together with MERTK SNP genotype.

At baseline, PLT count lower than $120x10^9$/L (HR 2.12, 95%CI 1.15-3.93, p=0.017), albumin levels lower than 3.5 g/dL (HR 2.37, 95%CI 1.38-4.08, p=0.002) and MERTK AA genotype (HR 1.98, 95%CI 1.09-3.62, p=0.026) were independent risk factors for HCC occurrence, by multivariate analysis (**Table 5**). Time-dependent AUROCs of this model, including PLT, Albumin and MERTK (so-called PAM model) was 0.76 at 1 year, 0.76 at 3 years and 0.67 at 5 years, that were significantly better than those of MERTK alone (1-year AUROC: 0.64. 3-year AUROC: 0.57. 5-year AUROC 0.57) (**Figure 2, left panel**).

The association between MERTK AA genotype and HCC occurrence remained significantly independent also when including in the model Child-Pugh class or Child-Pugh class with PLT count or isolated albumin levels (**Table 6**). It is noteworthy that time-dependent AUROCs of these models were all lower than those of the PAM model (Child-Pugh and MERTK: 1-year AUROC 0.69; 3-year AUROC 0.67; 5-year AUROC 0.64. Child-Pugh, PLT count and MERTK: 1-year AUROC 0.73; 3-year AUROC 0.74; 5-year AUROC 0.67. Albumin levels and MERTK: 1-year AUROC 0.74; 3-year AUROC 0.71; 5-year AUROC 0.65).

Predicted probabilities of HCC occurrence according to different risk profiles (best profile: PLT count=150x109/L; albumin=3.8 g/dL; MERTK rs6726639 AC/CC. Intermediate profile: PLT count=120x109/L; albumin=3.5 g/dL; MERTK rs6726639 AA. Worst profile: PLT count=90x109/L; albumin=3.0 g/dL; MERTK rs6726639 AA) are showed in **Figure 3 (left panel)**.

At the time of SVR, PLT count lower than $130x10^9$/L (HR 2.14, 95%CI 1.10-4.34, p=0.036), albumin levels lower than 3.8 g/dL (HR 2.21, 95%CI 1.07-4.58, p=0.032) and MERTK AA genotype (HR 2.18, 95%CI 1.01-4.70, p=0.046) were confirmed as independent risk factors for HCC occurrence, by multivariate analysis (**Table 5**). Time-dependent AUROCs of this model,

was 0.71 at 1 year, 0.77 at 3 years and 0.65 at 5 years, that were significantly better than those of MERTK alone (**Figure 2, right panel**). Predicted probabilities of HCC occurrence according to different risk profiles (best profile: PLT count=150x109/L; albumin=3.8 g/dL; MERTK rs6726639 AC/CC. Intermediate profile: PLT count=120x109/L; albumin=3.5 g/dL; MERTK rs6726639 AA. Worst profile: PLT count=90x109/L; albumin=3.0 g/dL; MERTK rs6726639 AA) are showed in **Figure 3 (right panel)**.

When we categorized patients according to PAM model as low risk (PLT count>$130x10^9$/L AND albumin levels > 3.8 g/dL AND MERTK rs6726639 AC/CC) versus high risk (PLT count<$130x10^9$/L AND/OR albumin levels < 3.8 g/dL AND/OR MERTK rs6726639 AA) according to significant predictors of HCC occurrence at the time of SVR, the PAM model showed an overall higher net benefit for ruling out HCC, when compared with the strategy of performing ultrasound in all patients, as showed in **Figure 4**. It is noteworthy that the low-risk profile according to PAM model at the time of SVR was observed in 82 out of 407 patients (20.1%).

When evaluating secondary outcomes, 37 patients (9.1%) developed hepatic decompensation events during the follow-up (25 patients developed ascites, 7 developed hepatic encephalopathy and 7 developed oesophageal variceal bleeding. Fifty patients (12.3%) died and among them, 22 patients (5.4%) died for liver-related causes while 28 (6.9%) died for non-liver related causes. Median overall survival was not reached and actuarial survival rates were 98.1% at 1 year, 91.6% at 3 years, 88.5% at 5 years and 86.4% at 7 years.

Discussion

In this single-centre, prospective study, including patients with compensated HCV cirrhosis treated with DAAs, we showed that the risk of HCC occurrence after DAA-induced SVR was low, but it persisted also after a long-term follow-up. A risk profiling based on the combination of biochemical (platelet count and albumin levels) and genetic (MERTK SNP) features (PAM model) is useful to accurately predict HCC occurrence during follow-up after DAA-induced SVR. The clinical benefit of this strategy was also confirmed by DCA.

DAA treatment has revolutionised the management of patients with HCV infection, through a significant and clinical relevant improvement of short- and long-term outcomes also in patients with cirrhosis [24]. However, DAA treatment results partially effective in completely eliminating the risk of HCC and our data confirm that this risk persists also after a long-term follow-up after SVR. The risk for HCC occurrence is strictly linked to the severity of underlying liver disease, that could be partially improved by DAA treatment, and by unmodifiable genetic characteristics of patients. Our study showed that PLT count, as a surrogate marker of the severity of portal hypertension, and albumin levels, that is a well-known biomarker of liver function, are independent risk factors for HCC occurrence. These results confirm those of previously published studies. Particularly, the presence of portal hypertension was evaluated as risk factor for HCC occurrence after SVR in several studies [11], although its definition was heterogeneous. Particularly, studies that evaluated portal hypertension through invasive methods, such as the measurement of hepatic vein pressure gradient (HVPG) or the presence of OV, showed that they were both independent risk factors for HCC occurrence [25,26]. In our study, PLT count, that is a worldwide accepted non-invasive and easily measurable surrogate marker of the severity of portal hypertension, was independently associated with the

risk of HCC occurrence, confirming the findings of previous studies [8,27]. Albumin levels are a surrogate biomarker of advanced liver disease and liver function and they were previously showed as independent predictors of HCC occurrence, both when evaluated as a single biomarker or when included in composite predictive scores, such as Child-Pugh score or albumin-bilirubin (ALBI) grade [8,28,29]. All in all, our study, as well as previously published studies, confirmed that patients with more advanced liver disease in terms of severity of portal hypertension and liver synthetic dysfunction before DAA treatment have a higher risk to develop HCC after SVR.

DAA treatment is highly effective in improving both portal hypertension and liver function, also in patients with more advanced liver disease and our study confirmed that PLT count and albumin levels significantly improved after SVR, compared to the start of DAA treatment. Nevertheless, they remained independent risk factors for HCC occurrence also when they were evaluated at the time of SVR, by using slightly different cut-off levels than those evaluated before DAA treatment. These results are particularly relevant considering that SVR represents a change status for patients with HCV cirrhosis, underlining the need of dynamically evaluating biomarkers of portal hypertension and liver function before DAA treatment and during follow-up after SVR. Notably, the positive impact of DAA treatment on liver function was also confirmed by the relatively low rate of hepatic decompensation after SVR, that was about 9%, in line with the results of previous studies [9]. The maintenance of a preserved liver function is a main determinant of the outcomes of patients with HCV-related cirrhosis and HCC, since it is essential for the feasibility, the efficacy and the safety of active treatments for HCC and finally resulting in a significant improvement of the overall survival, also in patients with more advanced liver disease and previous history of HCC [30,31].

Differently from previously published study, our study also evaluated the impact of unmodifiable genetic risk factors for HCC, such as MERTK SNP. Particularly, we showed that MERTK SNP AA genotype was a significant risk factor for HCC occurrence, independently from the improvement of PLT count and albumin levels related to DAA treatment. MERTK is expressed in human HSCs and it directly acts on these cells through the promotion of their activation, supporting their survival, increasing migration, and inducing the expression of profibrogenic genes, such as procollagen type I. Particularly, the A allele is able to differentially bind to transcription factors, conditioning MERTK expression and affecting downstream pathways [32]. In this line, the AA genotype negatively affects the transcription of genes involved in tumor suppression. A previous study from our group showed that AA genotype was associated with an upregulation of matrix metallopeptidases (MMPs) 9 and 7, enhancing epithelial-mesenchymal transition and fibroproliferative processes and finally stimulating hepatocarcinogenesis. Moreover, MERTK polymorphic status is also associated with downregulation of WNT gene family 11 (WNT11), that promotes hepatocytes differentiation, acting as an oncosuppressor [17]. It is noteworthy that the discriminating ability of MERTK SNP, evaluated by time-dependent AUROCs, was significantly improved by the combination with PLT and albumin, suggesting a synergistic role between the severity of liver disease and genetic predisposition in determining the risk of HCC occurrence after DAA-induced SVR. Moreover, the association between MERTK and HCC occurrence was independent regardless of Child-Pugh class and the prognostic accuracy of the model including PLT, albumin and MERTK was higher of those of models including Child-Pugh. These results confirmed MERTK as a significant predictor of HCC occurrence independently from functional class of cirrhosis, with the best discriminating ability achieved when combining MERTK with PLT count and albumin levels.

Among the strengthens of our study, the long-term follow-up should be underlined, with a mean follow-up time after the start of DAA treatment of about 6 years that is higher compared with other previous studies conducted in this setting [11]. Moreover, HCC surveillance was conducted in a tertiary referral centre with high expertise in the management of patients with cirrhosis and focal liver lesions, therefore we are confident that the outcome of interest (i.e. HCC occurrence) was accurately and carefully evaluated according to international clinical guidelines with a proper follow-up schedule and with proper radiological diagnostic techniques. Furthermore, we included a well-defined homogeneous cohort of patients with accurate clinical, biochemical and radiological assessment of liver disease, both at the time of DAA start and at the time of SVR. Last but not least, we used an alternative methodology (i.e. DCA) to substantiate the net benefit of our biochemical and genetic risk profiling. This is particularly relevant because, according to the last recommendations from Baveno VII consensus [33], patients with HCV cirrhosis who show consistent post-treatment improvements (namely, liver stiffness values of <12 kPa and PLT $>150x10^9$/L) after DAA-induced SVR can be discharged from portal hypertension, but they should continue HCC surveillance. Although our study was not able to identify a subgroup of patients with a low enough risk of HCC to avoid surveillance, our predictive model, based on PLT count and albumin levels evaluated after SVR and MERTK genotype is useful to correctly identify a subgroup of patients with lower risk of HCC after SVR, for whom a modification of the ultrasound surveillance schedule (annual rather than semestral) may be proposed.

Our study suffers from some limitation. First, it was a single center study and an external validation of our model is needed to confirm its accuracy in predicting HCC occurrence in other settings. Second, the discriminating ability of our model was good but not excellent, potentially limiting its clinical usefulness at individual patient-level. The integration in our model of other potentially explanatory covariates related to metabolic co-factors,

HCC biological aggressiveness, other SNPs involved in genetic predisposition to hepatocarcinogenesis or the use of a multiomics approach should be explored in order to improve the discriminating ability of our model. Particularly, the evaluation of the role of other SNPs, such as PNPLA3, MBOAT7 and TM6SF2 will be evaluated in the next step of this project. Third, we were not able to evaluate the association between HCC occurrence and other clinically relevant covariates, such as gamma-glutamil transferase (GGT), a well-known biomarker of fatty liver and a surrogate of insulin-resistance, or alpha-fetoprotein (AFP). Unfortunately, these covariates were not available and their role should be further explored. Finally, although PLT and albumin are easily evaluable biochemical features, our model also included the genotyping of MERTK SNP, that could not to be universally available outside tertiary referral centers.

In conclusion, in patients with HCV cirrhosis achieving SVR after DAA treatment, we showed that: 1) The risk of HCC persists, although low, also after a long-term follow-up; 2) a biochemical- and genetic-based predictive model, including PLT, albumin and MERTK genotype (PAM model) is useful to predict the risk of HCC occurrence after SVR; 3) the risk profiling according to this model could identify a subgroup of patients in which the surveillance schedule for HCC can be personalized and extended. Further prospective studies with long-term follow-up are needed to externally validate our predictive model and to substantiate its impact on the cost-effectiveness of surveillance programs and on patients overall survival.

Tables and Figures

Table 1. Demographic, clinical, biochemical, and genetic characteristics of 401 patients with HCV-related cirrhosis at the time of the start of the treatment with direct-acting antiviral agents (DAAs), stratified according to the development (or not) of HCC occurrence.

	Overall cohort (N=407)	Patients without HCC occurrence (n=349, 85.7%)	Patients with HCC occurrence (n=58, 14.2%)	*p*-value
Age (years)	67.35±10.05	67.08±10.16	69.02±9.26	0.174
Male sex (n,%)	231 (56.76%)	196 (56.16%)	35 (60.03%)	0.651
BMI(Kg/m2)	26.176±3.66	26.08±3.61	26.77±3.94	0.182
Type 2 diabetes	126 (30.95%)	111 (31.8%)	15 (26.86%)	0.451
Arterial hypertension: NO	154 (37.84%)	128 (36.67%)	26 (44.82%)	0.299
ALT (IU/L)	85±53	87±55	69±35	0.014
PLT (10^9/L)	119±65	122±66	100±52	0.013
Bilirubin (mg/dL)	1.09±0.59	1.07±0.58	1.21±0.66	0.111
Albumin (g/dL)	3.78±0.48	3.81±0.47	3.58±0.55	0.001
INR	1.13±0.30	1.13±0.31	1.16±0.28	0.458
Child-Pugh score A B	 350 (86%) 57 (14%)	 307 (87.97 %) 42 (12.03%)	 43 (74.14 %) 15 (25.86 %)	0.009
Liver stiffness (kPa) (mean, range)	23.9±12.8	23.3±12.7	27.6±13.2	0.019

Oesophageal varices				0.161
Absent	100(24.69 %)	86 (24.78%)	14 (24.14 %)	
F1	234(57.78%)	205 (59.08%)	29 (50 %)	
F2	59 (14.57 %)	48 (13.83 %)	11 (18.97 %)	
F3	12 (2.96%)	8 (2.31%)	4 (6.90%)	
MERTK rs6726639, deSNP				0.079
AA	59 (14.5%)	45 (12.9%)	14 (24.14%)	
AC	175 (43%)	153 (43.8%)	22 (37.93%)	
CC	173 (42.5%)	151 (43.3%)	22 (37.93%)	

Continuous variables are reported as mean±standard deviation. Categorical variables are reported as absolute number and percentage)

Table 2. Comparison of clinical and biochemical characteristics of 401 patients with HCV-related cirrhosis at the time of the start of the treatment with direct-acting antiviral agents (DAAs) and at the time of sustained virologic response (SVR)

	Start of DAA treatment	SVR	*p*-value
ALT (IU/L)	85.19 ± 53.13	23.03 ± 12.77	<0.001
PLT (10^9/L)	119.71 ± 65.56	133.98 ± 59.82	0.003
Bilirubin (mg/dL)	1.09 ± 0.59	0.96 ± 0.7	0.006
Albumin (g/dL)	3.78 ± 0.49	4.04 ± 0.49	<0.001
INR	1.13 ± 0.3	1.17 ± 0.3	0.089
Liver stiffness (kPa) (mean, range)	23.91 ± 12.81	22.12 ± 16.41	0.203

Table 3. Clinical and biochemical characteristics of 401 patients with HCV-related cirrhosis at the time of sustained virologic response (SVR) after treatment with direct-acting antiviral agents (DAAs), stratified according to the development (or not) of HCC occurrence.

	Overall cohort (N=407)	Patients without HCC occurrence (n=349, 85.7%)	Patients with HCC occurrence (n=58, 14.3%)	*p*-value
ALT (IU/L)	23.03 ± 12.77	22.57±12.48	25.94±14.31	0.173
PLT (10^9/L)	133.98 ± 59.82	137.22±61.23	114.94±47.19	0.047
Bilirubin (mg/dL)	0.96 ± 0.7	0.95±0.73	1.05±0.54	0.452
Albumin (g/dL)	4.04 ± 0.49	4.06±0.48	3.89±0.48	0.061
INR	1.17 ± 0.3	1.15±0.28	1.24±0.41	0.134

Liver stiffness (kPa) (mean, range)	22.12 ± 16.41	20.67±16.57	28.1±15.26	0.255

Table 4. Risk factors for HCC occurrence by univariate Cox regression analysis

	Hazard ratio	95% Confidence Interval	*p*-value
Age (years)	1.02	(0.99 – 1.05)	0.162
Female sex (vs male)	0.85	(0.5 – 1.44)	0.554
BMI (Kg/m2)	1.04	(0.97 – 1.11)	0.301
Type 2 diabetes (yes vs no)	0.73	(0.4 – 1.32)	0.295
Arterial hypertension (yes vs no)	1.29	(0.77 – 2.17)	0.338
ALT (IU/L) (DAA start)	0.99	(0.98 – 1)	0.009
ALT (IU/L) (SVR)	1.02	(0.99 – 1.04)	0.138
PLT ($x10^9$/L)	0.99	(0.99 – 1)	0.003

(DAA start)			
PLT (x10^9/L) (SVR)	0.99	(0.99 – 1)	0.035
Bilirubin (mg/dL) (DAA start)	1.75	(1.16 – 2.63)	0.007
Bilirubin (mg/dL) (SVR)	1.48	(0.95 – 2.31)	0.085
Albumin (g/dL) (DAA start)	0.32	(0.19 – 0.53)	<0.001
Albumin (g/dL) (SVR)	0.42	(0.21 – 0.86)	0.017
INR (DAA start)	1.63	(0.82 – 3.26)	0.164
INR (SVR)	2.45	(1.1 – 5.47)	0.029
Liver stiffness (kPa) (DAA start)	1.02	(1.01 – 1.04)	0.010
Liver stiffness (kPa) (SVR)	1.02	(0.98 – 1.06)	0.314
MERTK rs6726639, deSNP AA vs AC/CC	1.97	(1.08 – 3.6)	0.027

Table 5. Risk factors for HCC occurrence by multivariate Cox regression analysis

	Model 1 (DAA start)		
	Hazard ratio	95% Confidence Interval	*p*-value
PLT < 120 $x10^9$/L	2.12	(1.15 – 3.93)	0.017
Albumin < 3.5 g/dL	2.37	(1.38 – 4.08)	0.002
MERTK rs6726639, deSNP AA vs AC/CC	1.98	(1.09 – 3.62)	0.026
	Model 2 (SVR)		
PLT < 130 $x10^9$/L	2.14	(1.1 – 4.34)	0.036
Albumin < 3.8 g/dL	2.21	(1.07 – 4.58)	0.032

MERTK rs6726639, deSNP AA vs AC/CC	2.18	(1.01 – 4.7)	0.046

Table 6. Multivariate Cox regression analysis of risk factors for HCC occurrence including MERTK and Child-Pugh class (with or without platelet count) or albumin levels.

	Model 1 (including Child-Pugh class)		
	Hazard ratio	95% Confidence Interval	*p*-value
Child-Pugh class A vs B	0.32	(0.18 – 0.59)	<0.001
MERTK rs6726639, deSNP AA vs AC/CC	1.87	(1.03 – 3.43)	0.041
	Model 2 (including Child-Pugh and platelet count)		
PLT < 120 x10^9/L	2.23	(1.22 – 4.08)	0.009

Child-Pugh class A vs B	0.34	(0.19 – 0.62)	<0.001
MERTK rs6726639, deSNP AA vs AC/CC	1.85	(1.01 – 3.38)	0.047
	Model 3 (including albumin levels)		
Albumin < 3.5 g/dL	2.72	(1.60 – 4.64)	<0.001
MERTK rs6726639, deSNP AA vs AC/CC	1.94	(1.06 – 3.54)	0.031

Figure 1. Cumulative rate of hepatocellular carcinoma (HCC) occurrence after sustained virologic response (SVR) by direct-acting antiviral agents (DAAs)

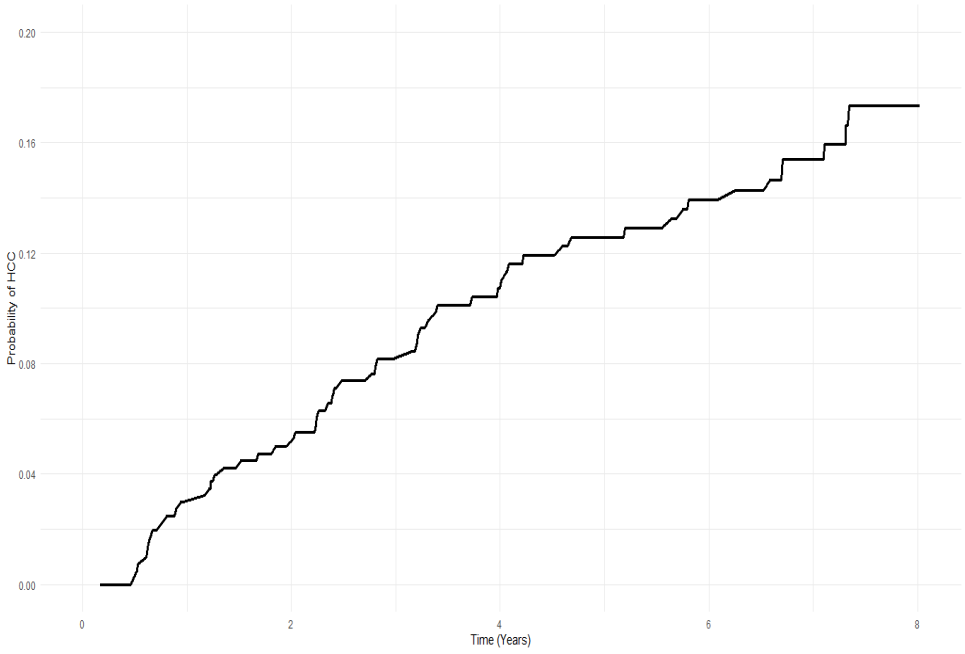


Figure 2. Discriminating ability of MERTK alone and PAM (Platelet, Albumin, MERTK) model for HCC occurrence by time-dependent area under the receiver operating characteristic curves (AUROCs) (left panel: start of direct-acting antiviral treatment; right panel: sustained virologic response)

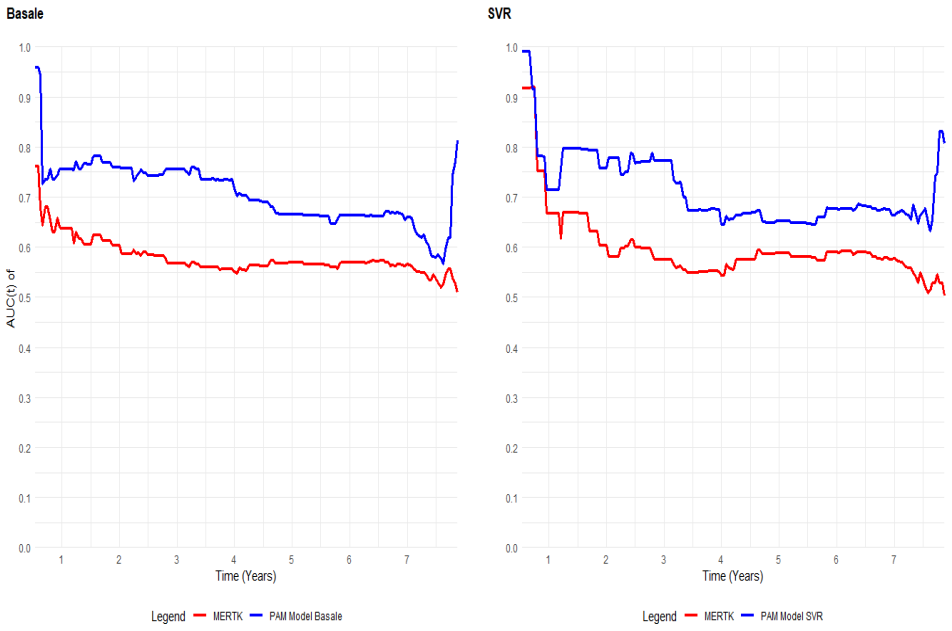


Figure 3. Predicted probabilities of HCC occurrence according to independent predictors at multivariate analysis (PAM model: platelet count, albumin levels, MERTK single nucleotide polymorphism) in three different patient profiles (Green curve: platelet count=150x10^9/L; albumin=3.8 g/dL; MERTK rs6726639 AC/CC. Blue curve: platelet count=120x10^9/L; albumin=3.5 g/dL; MERTK rs6726639 AA. Red curve: platelet count=90x10^9/L; albumin=3.0 g/dL; MERTK rs6726639 AA) at the time of the start of direct-acting antiviral agents (left panel) and the time of sustained virologic response (right panel)

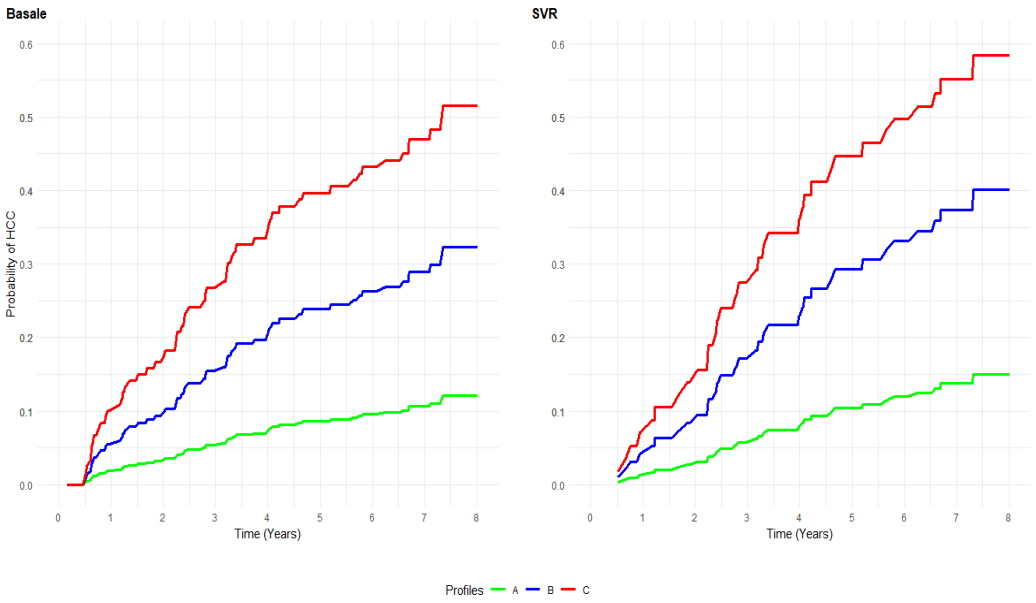
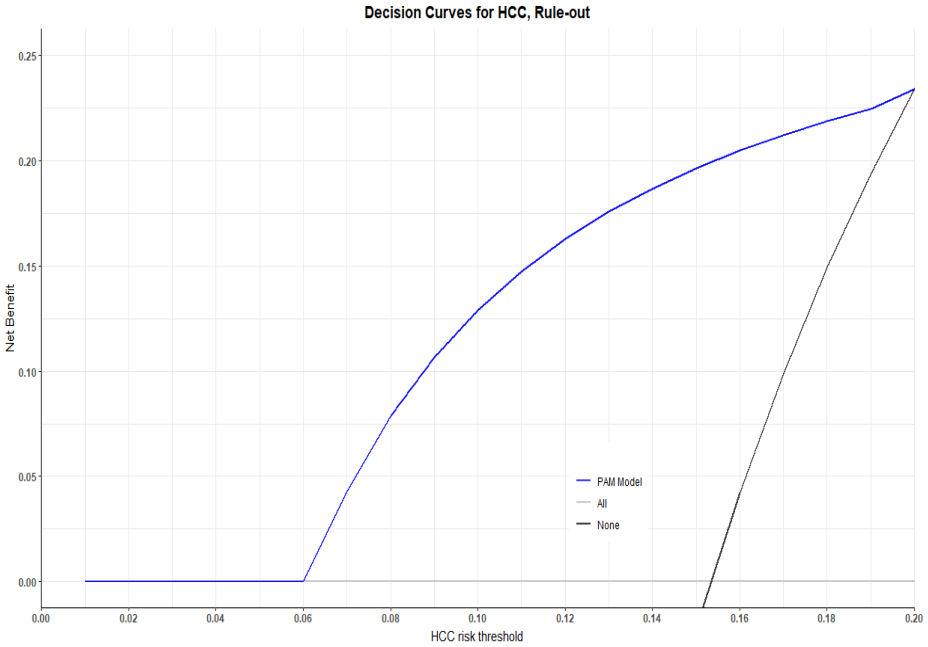


Figure 4. Decision curve analysis evaluating the net benefit of RESIST-HCC model evaluated after sustained virological response (SVR) by direct-acting antiviral agents (DAAs) for ruling out HCC occurrence


Decision Curves for HCC, Rule-out
Net Benefit
0.25
0.20
0.15
0.10
0.05
0.00
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
HCC risk threshold
PAM Model
All
None

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