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ORIGINAL ARTICLE

External validation of the French alpha-fetoprotein model for hepatocellular carcinoma liver transplantation in a recent unicentric cohort – a retrospective study

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SUMMARY

Prognostic models of liver transplantation (LT) for hepatocellular carcinoma (HCC) mainly derive from LT cohorts with numerous hepatitis C virus (HCV) patients. The AFP model, which is currently used in France to select LT candidates, was derived from a cohort of LT performed between 1988 and 2001, including a majority of HCV-positive recipients. The emergence of new direct-acting antiviral therapies and subsequent decrease of HCV incidence may change the generalizability of such models. We performed an external validation of the AFP model in a cohort of recipients transplanted between 2005 and 2018. Although multivariable analysis identified all three model's factors (AFP level, largest tumor size, number of nodules) as predictors of tumor recurrence, the AFP model showed poor discrimination and calibration in the present cohort. This poor performance could be related to significant differences between the derivation and the present cohort in terms of etiology, severity of underlying liver disease, tumor burden and differentiation, and use of neoadjuvant treatments. The present findings suggest that the decline of HCV-induced HCC among LT candidates may compromise the generalizability of the AFP model in more recent LT cohorts. Further studies are required for updating or building more robust prognostic models.

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Introduction

The majority of hepatocellular carcinomas (HCCs) develop in patients with a background of underlying liver disease [1]. For decades, hepatitis C virus (HCV) represented the main cause of hepatic disease in Western countries, and thus, patients with HCV-induced HCC represented a large proportion of patients undergoing liver transplantation (LT), since the latter represents the optimal treatment modality for HCC. However, due to organ shortage, access to LT remains limited to very selected patients with the best possible outcome [2].

Several prognostic models have been developed over the past 2 decades in order to allow appropriate selection

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of LT candidates. The Milan criteria, which were designed more than 20 years ago, are still the most admitted criteria for LT eligibility [3]. These criteria are based on 2 main factors, namely number of tumors and size of the largest tumor, which are both strongly correlated with oncological outcome. Recently, several reports from various origins suggested that adding alpha-fetoprotein (AFP) level to the two previous factors could significantly increase the prognostic values of the various models proposed, and therefore, the most recent prognostic models nowadays are based on tumor size, tumor number, and AFP [4–7]. As such, recipient selection for LT in France is based on the AFP model since 2013.

Meanwhile, the incidence of HCV has been decreasing, as a result of the emergence of new direct antiviral drugs, which result in sustained virological response in up to 95% of HCV patients [8]. As a consequence, the proportion of HCV-induced HCC among LT candidates is decreasing, while other underlying liver disease such as metabolic-associated fatty liver disease is rapidly emerging. Yet, the majority of the prognostic models that are currently used, such as the AFP model, derive from LT cohorts with a large proportion of HCV-positive recipients. This may compromise the validity of existing prognostic models such as the AFP model, owing to the specific biology of HCV-induced HCC. The purpose of this study was to perform an external validation of the AFP model in a more recent cohort of LT recipients who benefitted from more effective anti-HCV therapies.

Patients and methods

Patients

The study population consisted of consecutive adult patients who underwent LT at a single center from January 2005 to July 2018 for HCC. Patients undergoing LT for an etiology other than HCC and those with a substantial amount (>5%) of missing data were excluded from analysis. The retrospective study cohort was divided into two groups: a group of recipients with HCV-induced HCC (HCV group) defined by a positive HCV serology and a group with HCC not induced by HCV (non-HCV group). This study was approved by the institutional review board and conformed to the precepts of the 1975 Helsinki declaration.

AFP model

The AFP model was developed and validated in 2012, and it is applied in France since 2013 [4] for liver allograft allocation. It consists of a Cox proportional hazard model based on 3 factors (AFP, number of nodules, and size of largest nodule), which predicts the risk of tumor recurrence after LT (Table 1). It was developed on a cohort of 537 LT recipients transplanted between 1988 and 2001, and it was validated on a cohort of 435 recipients transplanted between 2003 and 2004. A simplified user-friendly risk score was derived from the Cox model, by transforming the β-coefficients of each variable (multiplied by 3 and rounded in integers).

Organ allocation and transplantation

Eligibility criteria for LT at our institution were based on Milan criteria before 2013 and on the AFP model since 2013, in accordance with French national guidelines [4]. As such, LT is considered for patients with an AFP score ≤2, with some exceptions. Patients with a score beyond 2 are considered for locoregional therapies such as local ablation or trans arterial chemoembolization and may be further listed in the event of successful and sustainable downstaging. After careful selection, some patients with a score beyond 2 may also be considered for LT using rescue allocation allografts [9]. The LT procedure has been described elsewhere [10,11].

Study design and endpoint

The aim of this study was to test the generalizability of the AFP model in a more recent cohort of LT recipients at the era of direct antiviral drugs, which supposedly may change the long-term prognosis of HCC patients, owing to a decreased incidence and a better disease control of HCV recipients. Firstly, we compared the baseline characteristics and outcomes between the HCV and non-HCV groups. Secondly, we performed a comparative analysis between the original cohort on which the AFP model was derived and the present cohort, for all recipient and tumor-related variables deemed to possibly have an influence on outcome. Finally, we performed an external validation of the AFP model on the present cohort, following the recommendations made by Steyerberg [12].

Statistical analysis

Continuous variables were expressed in median with interquartile range (IQR) or mean ± standard deviations (SD) and compared using the Mann–Whitney test or Student's *t*-test, as appropriate. Categorical variables

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Table 1. Original AFP Cox model and user-friendly risk score.

Variables	Original Cox model		User-friendly risk score
	β-Coefficient	Hazard ratio (95% CI)	Points
Tumor size			
≤3 cm	0	1 (reference)	0
>3 and ≤6 cm	0.272	1.31 (0.84–2.04)	1
>6 cm	1.347	3.84 (2.23–6.62)	4
Number of nodules			
≤3 nodules	0	1 (reference)	0
>3 nodules	0.696	2.01 (1.15–3.50)	2
AFP level (ng/mL)			
≤100	0	1 (reference)	0
>100 and ≤1000	0.668	1.95 (1.21–3.15)	2
>1000	0.945	2.57 (1.55–4.28)	3

AFP, alpha-fetoprotein.

were expressed in counts and percentages and compared using the chi-square test or Fisher's exact test, as appropriate. Survival curves were built using the Kaplan–-Meier method and compared using the Mantel–Cox log-rank test. Risk factors for time to recurrence were identified using univariable and multivariable Cox proportional hazard models.

External validation of the AFP model was done by testing both discrimination and calibration alternately on both the original Cox model and the user-friendly risk score, using the following steps:

Calculation of the calibration slope

By definition, the calibration slope of a model is equal to 1 in the original dataset from which it was derived. Calculating the calibration slope of an existing model in an external cohort allows to assess its discrimination: if the slope is not significantly different from 1, the discrimination of the model is considered to be equivalent to the one it had on the original dataset, and therefore, it is considered satisfactory. Conversely, if the slope is significantly lower than 1, the discrimination is considered poor.

The calibration slope was calculated by performing a Cox regression in the external dataset, using the prognostic index of the model as the only variable. The prognostic index, also known as the linear predictor, was calculated for each individual in the same manner than in the original study, as the sum of the product of the covariables by their regression coefficient from the original model $\beta_i X_i$. The obtained overall regression coefficient ($\beta_{overall}$) is the calibration slope. The Cox model that we used could thus be defined as:

$$\ln h(t) = \ln h_0(t) + \beta_{overall} * \beta_i X_i \qquad \text{(model 1)}$$

where ln $h(t)$ designates the natural log of the hazard function, ln $h_0(t)$ designates the natural log of the newly estimated baseline hazard function, $\beta_i X_i$ designates the prognostic index, and $\beta_{overall}$ designates the calibration slope.

To test whether the slope was significantly lower than 1, a likelihood ratio test (which has a chi-square distribution) was performed, testing the null hypothesis $\beta_{overall} = 1$. To do so, the likelihood ratio test is performed between the calibration model (model 1) and an alternative model (model 2) with the regression coefficient set at the previously calculated calibration slope minus 1:

$$\ln h(t) = \ln h_0(t) + (\beta_{overall} - 1) * \beta_i X_i \qquad \text{(model 2)}$$

Search for misspecification of the model

Misspecification of a Cox model could be related to the fact that one or more variables may no longer validate the proportional hazard assumption or may have a different regression coefficient than in the original dataset. Therefore, we tested the proportional hazard assumption for each of the three variables included in the original model, using the scaled Schoenfeld residuals both statistically and graphically. To test whether variables in the new dataset had a different estimated regression coefficient (β) than in the original dataset ($\beta_{original}$), we built three alternative models (model 3), each including

the prognostic index as an offset variable (meaning its regression coefficient is constrained at 1), and adding one of the three variables of the original AFP model (AFP, size or number of nodules), in the following manner:

$$\ln h(t) = \ln h_0(t) + \beta_i X_i + \beta' * X \quad \text{with} \beta' = \beta_{\text{original}} - \hat{\beta} \qquad \text{(model 3)}$$

To determine whether β′ is different than 0 (meaning that $\hat{\beta}$ is different than β_{original}), a likelihood ratio test was performed between model 1 and each of the three newly developed models.

Concordance measures

Concordance statistics, such as the area under the receiver operating characteristic curve (AUROC), are widely used to assess the discrimination of a prediction model in an independent cohort. Due to the time-to-event setting of the outcome analyzed herein (post-transplant tumor recurrence), standard AUROC could not be used. Several alternative discrimination measures have been proposed in the literature for testing the discrimination in survival models. Here, we tested three different discrimination measures: Harrell's *c*-index, Somers *D*, and Gönen and Heller *K* statistic. For c-index and *K*, values above 0.80 are considered very good, and values between 0.80 and 0.70 are considered good, while values below 0.70 are considered poor. For Somers D, values above 0.60 are considered very good, values between 0.60 and 0.40 are considered good, while values below 0.40 are considered poor.

Kaplan–Meier estimates and hazard ratios for risk groups

We further assessed calibration of the model by calculating Kaplan–Meier estimates according to risk groups using the user-friendly AFP risk score. As recommended by Steyerberg [12], calibration of a prediction model is preferably performed by dividing cohorts into four risk groups. Therefore, we estimated the rate of recurrence across four groups according to the AFP score (0, 1, 2, and ≥3). Since the original study determined the cutoff value of two in order to define a low-risk and a high-risk group, we also compared Kaplan-Meier estimates between these two risk groups.

In addition, a table displaying hazard ratios and their confidence intervals per risk group was constructed in an attempt to check for discrimination between groups, as suggested by Royston and Altman [13].

All tests were two-tailed and statistical significance was established for $P < 0.05$. All calculations were performed with SPSS software version 23.0 (IBM Corp., Armonk, NY, USA) and R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/).

Results

Between January 2005 and July 2018, a total of 822 LTs were performed at the institution. After exclusion of 516 patients transplanted for etiologies other than HCC and 26 patients with incomplete data, 280 recipients transplanted for HCC were included in the present study. Of these, 90 were HCV-positive and 190 were HCV negative. Comparison between recipients according to HCV status is reported in Table 2, showing significant differences between the two groups in terms of recipient age, background of hypertension and dyslipidemia, and for several tumor characteristics at listing such as size of largest nodule and AFP level, use of pre-transplant locoregional therapies, while there were no differences in terms of tumor characteristics on explant.

In the non-HCV group, the main indication was alcoholic cirrhosis in 130 (68.4%), followed by nonalcoholic fatty liver disease in 52 recipients (27.4%). Among patients in the HCV group, there were 41 (45.6%) recipients who also presented with alcohol-related liver disease, 6 (6.7%) who presented with nonalcoholic fatty liver disease, and 4 (4.4%) who had a co-infection with hepatitis B virus. A specific anti-HCV treatment had been administered to 72 (80.0%) recipients before LT, including 38 (42.2%) recipients with a sustained virological response at the time of LT, whereas 15 (16.7%) recipients received a treatment after LT. The type of treatment was a combination of ribavirin and interferon in 46 (51.1%) recipients, combination of a direct-acting antiviral drug with interferon (with or without ribavirin) in 7 (7.8%) recipients, and combinations of various direct-acting antiviral drugs in 34 (37.8%) recipients, with corresponding sustained virological response rates of 21.7% ($n = 10$), 85.7% ($n = 6$), and 94.1% ($n = 32$), respectively (Table 2). The remaining 3 (3.3%) patients did not receive any anti-HCV treatment.

Perioperative results

Median estimated blood loss was 1225 ml (IQR: 750–2000) in the HVC group vs 900 ml (IQR: 600–1500) in the non-HCV group ($P = 0.065$), and

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Table 2. Comparison of baseline tumor and recipient characteristics between the HCV and non-HCV groups.

	HCV group (n = 90)	Non-HCV group (n = 190)	P
Age (years, mean ± SD)	55.3 ± 6.3	58.6 ± 7.3	<0.001
Sex M/F	78/12	168/22	0.675
Medical background			
Hypertension	26 (28.9)	82 (43.2)	0.022
Diabetes mellitus	26 (28.9)	70 (36.8)	0.190
Dyslipidemia	8 (8.9)	36 (18.9)	0.031
Cardiac disease	11 (12.2)	39 (20.5)	0.090
Chronic kidney disease	2 (2.2)	15 (7.9)	0.063
COPD	17 (18.9)	28 (14.7)	0.377
Smoker	60 (66.7)	105 (55.3)	0.070
HBV infection	4 (4.4)	27 (14.2)	0.015
Alcoholic cirrhosis	41 (45.6)	130 (68.4)	<0.001
NASH	6 (6.7)	52 (27.4)	<0.001
MELD	11 (7–16)	11 (8–17)	0.806
Child–Pugh			
A	43 (47.8)	85 (44.7)	0.472
B	24 (26.7)	64 (33.7)	
C	23 (25.6)	41 (21.6)	
Sustained virological response after anti-HCV therapy			
Overall	48 (53.3)	–	
At the time of transplant	38 (42.2)	–	
According to the treatment received			
Interferon + ribavirin	10 of 46	–	
DAA + interferon ± ribavirin	6 of 7	–	
Combination of DAA	32 of 34	–	
No therapy	0 of 3	–	
Initial tumor characteristics			
Size of largest nodule (mm, mean ± SD)	25.5 ± 14.3	33.0 ± 22.5	0.001
No. of nodules (median, IQR)	2 (1–3)	2 (1–3)	0.497
AFP level (μg/l, median, IQR)	14 (6–33)	8 (4–21)	0.018
Score alpha ≤2	83 (92.2)	156 (82.1)	0.025
Milan status in	67 (74.4)	127 (67.2)	0.219
Pretransplant neoadjuvant therapy	66 (73.3)	141 (74.2)	0.876
TACE/ablation	57 (63.3)	99 (52.1)	0.043
Surgery/combination	9 (10.0)	42 (22.1)	
None	24 (26.7)	49 (25.8)	
Tumor morphology on explant			
Number of tumors (median, IQR)	2 (1–4)	2 (1–4)	0.684
Size of largest nodule, mm (median, IQR)	17 (9–26)	18 (8–30)	0.661
Microvascular invasion	15 (18.8)	40 (22.6)	0.486
Tumor differentiation			
Well	35 (52.2)	82 (51.9)	0.888
Moderate	31 (46.3)	71 (44.9)	
Poor	1 (1.5)	5 (3.2)	
No active tumor	23	32	

AFP, alpha-fetoprotein; COPD, chronic obstructive pulmonary disease; DAA, direct-acting antiviral drug; HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range; MELD, model for end-stage liver disease; NASH, nonalcoholic steatohepatitis; SD, standard deviation; TACE, transarterial chemoembolization.

median operative time was 375 min (IQR: 300–453) in the HCV group vs. 409 min (IQR: 355–485) in the non-HCV group (P = 0.033). The two groups had similar postoperative outcomes: median hospital stay was 25 days (IQR: 19–39) in the HCV group vs. 24 (IQR: 18–36) in the non-HCV group (P = 0.492), whereas the rates of 90-day mortality and severe morbidity (Clavien–Dindo grade ≥3) were 5.6% (n = 12) and

35.0% ($n = 63$) in the HCV group vs. 5.6% ($n = 5$) and 29.5% ($n = 26$) in the non-HCV group, respectively ($P = 0.804$ and $P = 0.373$).

Oncological outcome

Median follow-up was 33 (IQR: 13–65) months in HCV group vs. 30 (IQR: 10–66) months in non-HCV group $P = 0.660$. The 1-, 3-, and 5-year recurrence rates were 9.9%, 14.7%, and 19.8% in the HCV group and 7.7%, 13.3%, and 16.9% in the non-HCV group, respectively ($P = 0.792$). The 1-, 3-, and 5-year disease-free survival was 81.4%, 71.6%, and 58.6% in the HCV group and 83.3%, 73.4%, and 69.3% in the non-HCV group, respectively ($P = 0.514$). The 1-, 3-, and 5-year overall survival was 86.1%, 75.6%, and 62.3% in the HCV group and 87.3%, 78.5%, and 75.3% in the non-HCV group ($P = 0.288$).

Univariable Cox regression identified all three variables of the AFP model as independent predictors of tumor recurrence (Table 3). In addition, three tumor characteristics on explant (number of nodule, microvascular invasion, and tumor differentiation) were also found to be significantly associated with recurrence. On multivariable analysis, the three variables of the AFP model were still retained in the model.

Comparison between the original and the present cohort

Baseline recipient and tumor characteristics of this cohort were compared to those of the original derivation cohort of the AFP model (Table 4), showing several significant differences between the two cohorts in terms recipient age, underlying liver disease, Child–-Pugh class, tumor size, use of pretransplant therapies, and tumor differentiation of explant pathology.

External validation

Calibration slope

The calibration slope of the prognostic index of the original Cox model was 0.76 (SE: 0.25), which was significantly lower than 1 ($\chi^2 = 11.94$, $P < 0.001$). Therefore, the discrimination of the model in the present cohort was considered poor (see Appendix S1).

Model misspecification

All three variables of the original Cox model (largest tumor size, number of nodules, log10 AFP) met the proportional hazard assumptions both statistically ($P = 0.761$, $P = 0.166$, and $P = 0.280$, respectively) and graphically (see Fig. S1). All three attempts for re-estimation of the regression coefficient of the three variables failed to improve the original Cox model (all $3\hat{\beta}$ were close to 0, see supplementary material).

Concordance statistics

For the original Cox model, Harrell's *c*-index was 0.609 (SE: 0.048), Somers' *D* was 0.219 (SE: 0.096), and Gönen and Heller's *K* was 0.584 (SE: 0.026). For the user-friendly risk score, Harrell' *c*-index was 0.613 (SE: 0.048), Somers' *D* was 0.226 (SE: 0.097), and Gönen and Heller's *K* was 0.580 (SE: 0.027). Subsequently, all three concordance measures suggest that the discrimination of the model in the present cohort is weak.

Kaplan–Meier estimates and hazard ratios per risk groups

The Kaplan–Meier estimates of tumor recurrence at 1, 3, and 5 years, and corresponding hazard ratios according to risk groups are reported in Table 5, showing poor calibration: recipients with a score of 2 had the highest risk of recurrence, and they even had a significantly higher recurrence rate than recipients with a score ≥3. There was no difference between recipients with a score of 0 and those with a score of 1. Of note, the cutoff value of 2, which is currently used for selecting LT candidates, did not allow to discriminate between low- and high-risk candidates [HR = 1.53 (95% CI: 0.70–3.35), $P = 0.287$].

Discussion

In the present study, we performed an external validation of the AFP model, a clinical prediction tool currently used in France to predict the risk of recurrence among patients undergoing LT for HCC. While it confirmed that the 3 factors included in the model were significantly associated with tumor recurrence, the model itself showed poor discrimination and calibration, thus raising concern regarding its generalizability among recipients in more recent LT cohorts.

In an attempt to carefully select the best HCC patients for LT, several prognostic models have been developed. The Milan criteria proposed by Mazzaferro et al. in 1996 have long been the most admitted criteria for LT eligibility [3]. This prognostic model was based on two factors—tumor size and number of nodules—and was built upon a cohort of 48 LT recipients, of

Table 3. Cox univariable and multivariable regression analysis for time to recurrence.

	Univariable analysis			Multivariable analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Male sex	1.71	0.53–5.58	0.371			
Age (per year)	0.99	0.94–1.03	0.611			
MELD	0.97	0.93–1.02	0.224			
Etiology of cirrhosis						
HCV infection	1.09	0.56–2.15	0.794			
HBV infection	1.71	0.75–3.89	0.203			
Alcoholic cirrhosis	0.62	0.32–1.18	0.143			
NASH	1.12	0.49–2.56	0.785			
Comorbidities						
Dyslipidemia	1.55	0.71–3.39	0.272			
Arterial hypertension	0.81	0.41–1.59	0.536			
Diabetes mellitus	0.75	0.36–1.55	0.433			
Cardiac diseases	0.89	0.37–2.13	0.792			
Chronic kidney disease	0.78	0.10–5.72	0.803			
COPD	1.09	0.56–2.15	0.794			
Smoking	1.23	0.62–2.41	0.555			
Tumor morphology at listing						
Size of largest nodule (per cm)	1.16	1.04–1.31	0.010	1.13	0.99–1.29	0.065
No. of nodules	1.22	1.03–1.44	0.018	1.19	1.00–1.40	0.046
Log_{10} AFP level (ng/ml)	1.73	1.28–2.36	<0.001	1.53	1.11–2.11	0.009
Neoadjuvant locoregional therapy	1.98	0.86–4.55	0.109			
Tumor morphology on the explant*						
Size of largest nodule (per cm)	3.28	0.71–20.76	0.120			
No. of nodules	1.08	1.04–1.12	<0.001			
Tumor differentiation						
Well	1					
Moderate	2.46	1.09–5.54	0.029			
Poor	7.61	2.05–28.16	0.002			
Microvascular invasion	1.78	3.05–11.65	<0.001			

AFP, alpha-fetoprotein; COPD, chronic obstructive pulmonary disease; HCV, hepatitis C virus; HBV, hepatitis B virus; MELD, model for end-stage liver disease; NASH, nonalcoholic steatohepatitis.

*Only variables that are available preoperatively were included in the multivariable analysis since the aim of the model is to predict tumor recurrence before transplantation.

whom 32 (66%) had an HCV-related underlying liver disease. Since then, several alternative models such as the University of California and San Francisco criteria [14] or the up-to-seven model [15] have been developed in an attempt to expand the LT eligibility criteria without compromising the oncological outcome. All these models were based on the same two factors (namely size and number of nodules) and were again built upon cohorts including a majority of recipients transplanted for HCV-induced HCC.

In 2012, Duvoux et al. proposed a new prognostic model incorporating AFP level along with size and number of nodules in what is currently known as the French AFP model [4]. This model showed a significant improvement in predicting HCC recurrence after LT when compared to the Milan criteria. Following this report, several new models including AFP level have been developed by other teams in Europe [5], the United States [6]' and Asia [7]. Similar to previous models using only size and number of nodules, the AFP model was built upon a training cohort and successfully tested upon a validation cohort including 61% and 44% of LT recipients with HCV-induced HCC, respectively.

Meanwhile, the use of new direct-acting antiviral therapy in the management of HCV has allowed to achieve a sustained virological response in more than 95% of patients, with a subsequent drop of the annual incidence of HCC in HCV patients from 7% to 1% per year [8,13]. As a consequence, a substantial decline in the proportion of HCV patients among waiting lists for LT has been observed worldwide. According to the

Table 4. Comparison of recipients' and tumor characteristics in the original derivation cohort and in the present cohort.

	Original derivation cohort	Present cohort	*P* or SMD*
n	*537*	*280*	
Study period	1988–2001	2005–2018	
Age (years, mean ± SD)	53 ± 9	58 ± 7	0.600*
Sex M/F	465/72	246/34	0.610
Underlying liver disease			
Alcohol	149 (30.0)	130 (46.4)	<0.001
Viral hepatitis	302 (60.8)	114 (40.7)	
Others	46 (9.2)	36 (12.9)	
Missing data	40	0	
Child–Pugh class			
A	271 (53.0)	128 (45.7)	0.018
B	163 (31.9)	88 (31.4)	
C	77 (15.1)	64 (22.9)	
Missing data	26	0	
MELD score at listing	NA	11 (8–17)	–
Tumor characteristics at listing			
AFP level (ng/ml, median, IQR)	22 (7–158)	9 (4–26)	–
Number of tumors (median, IQR)	1 (1–2)	2 (1–3)	–
Size of largest nodule (mm, mean ± SD)	37 ± 25	31 ± 20	0.256*
Milan criteria			
In	362 (68.4)	194 (69.5)	0.748
Out	167 (31.6)	85 (30.5)	
Missing data	8	1	
AFP score above 2			
≤2	366 (74.2)	239 (85.4)	<0.001
>2	127 (25.8)	41 (14.6)	
Missing data	44	0	
Pretransplant treatment			
TACE/ablation	299 (56.3)	156 (55.7)	0.002
Surgery/combination	54 (10.2)	51 (18.2)	
None	178 (33.5)	73 (26.1)	
Missing data	6	0	
Pathologic features on explant			
Number of tumors (median, IQR)	2 (1–4)	2 (1–4)	–
Size of largest nodule, mm (median, IQR)	30 (20–50)	18 (9–30)	–
Microvascular invasion			
Presence	122 (23.0)	55 (21.4)	
Absence	408 (77.0)	202 (78.6)	
Missing data	7	23	
Tumor differentiation			
Well	313 (69.1)	117 (41.8)	<0.001
Moderate	122 (26.9)	102 (36.4)	
Poor	18 (4.0)	6 (2.1)	
Not evaluable/lack of active tumor	84	55	

Categorical variables are expressed in counts (percentages). Continuous variables are expressed in median values (interquartile range) or in mean ± standard deviations. *P*-values could be calculated only for categorical variables, using chi-square or Fisher's exact test.

*Continuous variables expressed in means in the original cohort were compared using the standardized difference of means, whereas continuous variables expressed in median values were not compared statistically.

European Liver Transplantation Registry, HCV patients have dropped from 22% to 17% in the period between 2007 and 2017 [16], and a similar decline was noted in the United Network for Organ Sharing in the United States from 37% to 24% in the period between 2012 and 2016 [8,17].

Table 5. Kaplan–Meier estimates of 1-, 3-, and 5-year tumor recurrence rates and hazard ratios per risk groups.

AFP score	Number of patients (%)	Recurrence rate (%, standard error)			Hazard ratio (95% CI; vs. a score of 0)
		At 1 year	At 3 years	At 5 years	
Score 0	142 (50.7)	4.9 (2.0)	10.1 (2.9)	13.4 (3.6)	
Score 1	62 (22.1)	5.9 (3.3)	10.6 (4.5)	10.6 (4.5)	1.00 (0.38–2.63)
Score 2	36 (12.9)	20.9 (7.7)	35.0 (9.7)	42.2 (11.0)	4.18 (1.82–9.57)
Score ≥3	40 (14.3)	14.5 (6.0)	14.5 (6.0)	25.3 (8.9)	2.16 (0.90–5.22)

Following the decline of the proportion of LT candidates with HCV-induced HCC among LT waiting lists, concerns could be raised regarding the generalizability of current prognostic models in a near future. The AFP model was built on a derivation cohort of patients transplanted between 1988 and 2001, and it was further validated on a cohort of transplants performed between 2003 and 2004 [4]. None of the direct-acting antiviral therapies were available at that time. In France, the first drugs became available under temporal special authorization in 2007 and obtained full marketing authorization in 2013 [18]. In the present cohort, a significant proportion of our HCV recipients had thus access to direct-acting antiviral drugs, contrary to those in the cohorts that allowed building the AFP model.

Several reports in the past have suggested that HCV-induced HCC had a substantially different oncological outcome when compared to HCC induced by other etiologies. In a recent study by our team assessing the outcome of intermediate size solitary HCC treated by resection or local ablation, we demonstrated that HCV-induced HCC was an independent factor associated with the risk of local recurrence [19]. These findings were consistent with the study by Viganò *et al.* [20] who reported better oncological outcome in metabolic syndrome-induced HCC when compared to HCV-induced HCC. Similar findings were reported in the setting of LT by studies comparing the outcome of LT for HCV- versus non-HCV-related HCC [21] or comparing the outcome of LT for NASH-associated versus HCV-associated HCC [22].

There is a well-established difference in the pathogenesis of HCC according to the etiology of underlying liver cirrhosis [23]. In the setting of chronic HCV infection, the risk of developing HCC is correlated with the degree of liver fibrosis, and HCC usually develops in advanced cirrhotic stage [24]. This is attributed to the inability of HCV to integrate its genetic material into the host genome in the liver cell to induce malignancy, the latter of which is mostly induced by cirrhosis-mediated carcinogens [25]. Conversely, HBV-related HCC tends to develop at all stages of HBV chronic infection regardless of the severity of liver fibrosis cirrhosis owing to the ability of HBV genome to integrate into the host cells [26,27]. Also, alcohol has the capability to induce carcinogenesis directly in addition to the cirrhosis-mediated carcinogens, through oxidative processes and endotoxemia [28,29]. Finally, there is controversy over whether HCC can develop in early stage of nonalcoholic steatohepatitis (NASH) cirrhosis, but former reports have suggested that the incidence of HCC development in patients with early Child A cirrhosis is significantly higher in patients with NASH cirrhosis when compared to those with chronic HCV infection [30,31].

In the present cohort, we found different operative time and blood loss between HCV and non-HCV recipients, suggesting differences between the two groups in terms of operative difficulties. These differences may reflect a higher grade of cirrhosis more severe liver fibrosis in non-HCV recipients, resulting in higher grades portal hypertension and hepatocellular insufficiency, both of which may be associated with previous episodes of spontaneous bacterial peritonitis with subsequent adhesions and a more difficult hepatectomy. Knowing that a more severe liver fibrosis more severe cirrhosis usually results in higher rates of intrahepatic metastatic noduli, these findings could be an indirect argument regarding the different tumor biology in HCV recipients compared to non-HCV recipients.

With these thoughts in mind, it could be hypothesized that the current prognostic models could fail to select the best possible LT candidates for HCC once HCV patients will disappear from LT waiting lists. For instance, in France, recipients with an AFP score above 2 are precluded from being listed for LT; however, in the present cohort, recipients with a score equal to or above 3 had a significantly lower rate of tumor recurrence when compared to those with a score of 2, suggesting poor calibration of the model in its present

form. These findings underline the need to revise the selection criteria for HCC patients eligible for LT in a future waiting list dominated by alcoholic and metabolic cirrhosis-induced HCC.

The present study had several limitations. Albeit more recent than both the derivation and the validation set that allowed to build and validate the AFP model, the present cohort was quite heterogeneous and performed at a single center. Moreover, due to a limited sample size, it was not considered appropriate to perform any attempt of model updating or even model re-estimation. However, the present study has the merit to underscore the possible drawbacks of the AFP model as well as all HCC prognostic models used worldwide that have mostly been validated upon cohorts including numerous HCV-positive recipients and the need for new prognostic models to be established in large cohorts of patients.

In conclusion, our study suggests that the decline of HCV-induced HCC among LT candidates along with the very specific biology of HCV-induced HCC may question the validity of current HCC prognostic models. Further prospective studies are warranted to test and develop new prognostic models to allow appropriate selection of patients with HCC for LT.

Authorship

MM: analyzed data and wrote the paper. FL: analyzed data and wrote the paper. PM: analyzed data and wrote the paper. FZ: interpreted data and critically revised the manuscript. TA: participated in study design and critically revised the manuscript. ML: participated in study design and critically revised the manuscript. CD: participated in study design and critically revised the manuscript. ML: collected and analyzed data. JYM: designed the study and analyzed data. KM: designed the study and wrote the paper.

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Conflict of interest

All authors have no conflict of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Schoenfeld residuals for testing the proportional hazard assumption for the 3 variables of the AFP model.

Appendix S1. Summary of statistical tests for the various models used.

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