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Predicting recurrence of hepatocellular carcinoma after liver transplantation using a novel model that incorporates tumor and donor-related factors

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I, the corresponding author, confirm that I had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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LAO, GO, and CT designed the study, analyzed the data, and wrote the manuscript.

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CC and MF did statistical analysis, designed the figures and tables, and provided critical intellectual input in constructing the manuscript.
PC, AA and TB designed the study, wrote the first draft of the manuscript and provided critical intellectual input in constructing the manuscript.

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Abstract

Evidence suggests that liver graft quality impacts on post-transplant recurrence of hepatocellular carcinoma (HCC). As of today, selection criteria only use variables related to tumor characteristics.

Within the Scientific Registry of Transplant Recipients, we identified patients with HCC who underwent liver transplantation between 2004 and 2016 (development cohort, n=10,887). Based on tumor recurrence rates, we fitted a competing-risk regression incorporating tumor- and donor-related factors, and we developed a prognostic score. Results were validated both internally, and externally in the Australia and New Zealand Liver Transplant Registry

Total tumor diameter (sub-hazard ratio [sub-HR] 1.52 [1.28-1.81]), alpha-feto protein (sub-HR 1.27 [1.23-1.32], recipient male gender (sub-HR 1.43 [1.18-1.74]), elevated donor body mass index (sub-HR 1.26 [1.01-1.58] and shared graft allocation policy (sub-HR 1.20 [1.01-1.43] were independently associated with tumor recurrence. We next developed the Darlica score (sub-HR 2.72 [2.41-3.08] p<0.001), that allows identifying risky combinations between a given donor and a given recipient. Results were validated internally (n=3,629) and externally in the Australia and New Zealand Liver Transplant Registry (n=370).

The current score is based on variables that are readily available at the time of graft offer. It allows identifying hazardous donor-recipient combinations in terms of risk of tumor recurrence and overall survival.

Introduction

Liver transplantation offers the best chance of cure for patients with early-stage hepatocellular carcinoma (HCC), and the proportion of patients with HCC as an indication for liver transplantation is rising [1]. Post-transplant tumor recurrence is the main drawback of this strategy, with 8-20% of patients experiencing tumor relapse five years after deceased-donor liver transplantation [2].

Careful candidate selection is crucial when inscribing an HCC-bearing patient on the waiting-list, especially when considering global organ shortage. Since their publication in 1996, the Milan criteria [3] have become an international standard in this matter, and most US centers still use these criteria nowadays. However, the Milan criteria have been criticized for their restrictiveness, and several groups have proposed alternative approaches [4-6] achieving comparable or even better outcomes, amid some debate [7]. Further improvements in patient selection have been achieved by taking tumor biology into account, for instance by using surrogate markers such as alpha-feto protein (AFP) and protein induced by vitamin K absence-II [8-10].

It is noteworthy that, as of today, routinely used selection criteria are solely based on the patients' tumor characteristics. But over the last decade, going one step forward, our group and others have reported clinical [11-13] and experimental [14] evidence supporting that, in addition to tumor characteristics, liver graft quality and donor-related factors may have an impact on tumor recurrence after liver transplantation. While donor marginality can be defined in several manners, it most commonly reflects a continuum of risk based on characteristics impacting liver graft quality, such as donor age, body mass index (BMI), mechanism of death, presence of underlying conditions, graft steatosis, and duration of cold and warm ischemia [15].

Building on this evidence, the objective of this study was to develop and validate a prognostic score combining tumor and donor characteristics, to predict post-transplant HCC recurrence.

Materials and Methods

Data Source, Study Population and Variables of Interest

We used data from the Scientific Registry of Transplant Recipients (SRTR) to derive and internally validate the score. The SRTR includes data on all donors, wait-listed candidates, and transplant recipients in the US, submitted by members of the Organ Procurement and Transplantation Network. The study population consisted of adult patients with HCC as a primary or secondary diagnosis, undergoing a first liver transplantation between January 2004 and December 2016. Patients with cholangiocarcinoma, or mixed HCC-cholangiocarcinoma were excluded. We excluded partial grafts (living donation and split livers) to avoid the bias induced by the association of small-for-size livers with tumor cell proliferation [16]. For external validation, we used data from the Australia and New Zealand Liver and Intestinal Transplant Registry (ANZLITR), which contains data on liver and intestinal transplants performed in Australia and New Zealand since establishment of first liver transplant unit in 1985.

Given the design of the current study, local ethical approval was not required. But access to both registries was subject to local institutional review by the SRTR and ANZLITR (Protocol n.9293, and HREC/58592/Austin-2019, respectively) with written agreements on data use and safety management. Patient-related variables are anonymized in both registries, and information on the geographical location of the transplant centers is not available in any of the standard analysis files.

In the SRTR dataset, using computer-generated random sequence, we split the study population into two cohorts. The development cohort comprised three quarters of the population (n=10,887), and the remaining patients were allocated to the internal validation cohort (n=3,629). We collected information on patient age, gender, body mass index (BMI), underlying liver disease, date of inscription on the waiting-list, date of transplantation, date of tumor recurrence, and date of death.

To evaluate tumor morphology, we retrieved the most recent pre-transplant data, describing the number of tumor nodules and their diameter (cm). Total tumor diameter was calculated by summing the diameters of individual nodules. Of note, when looking at the whole SRTR-derived population, the most recent radiological assessments of HCC nodules were made via magnetic resonance imaging, computed tomography, or ultrasound in 7,554 (52%), 6,673 (46%) and 289 (2%) of cases, respectively. Because candidates for liver transplantation undergo repeated oncological assessments while on the waiting-list, we used the most recent AFP value. This approach, which has been used by other internationally validated scores [17], is supported by previous evidence from the SRTR, where only the last AFP values (as opposed to the value at listing, or AFP dynamic changes) independently predicts post-transplant survival rates [18].

Before doing any statistical modelling, we selected donor-related predictor variables that were considered to be plausibly related, both biologically and clinically, to the recurrence of HCC. In addition, the selection of candidate predictors was restricted to those that would be readily available at the time of graft offer and allocation. For instance, in our main analysis, we did not evaluate factors that may not be anticipated before organ procurement surgery (e.g. cold ischemia time, or warm ischemia time during graft implantation). Such variables would not be of use in the real-world setting, our score being designed to inform clinical decision-making at the time of graft offer. Donor-related variables of interest were donor age, BMI, gender, cause of death, blood group, diabetes, hypertension, smoking, cocaine use, need for inotropic support, region in which organ procurement took place, and whether the liver graft was shared among different organ procurement organizations. We calculated the donor risk index (DRI) according to Feng et al. [19], and assessed whether donors were considered marginal according to the Organ Procurement and Transplantation Network (OPTN) expanded donor criterion. Briefly these criteria describe donors over the age of 60 years without comorbidities or donors over the age of 50 years with two comorbidities among hypertension, death from cerebrovascular accident, or serum creatinine levels >1.5 mg/dL [20].

Statistical Analysis and Design of the Prediction Score

We identified patients with post-transplant HCC recurrence according to the methodology by Samoylova [21], where post-transplant HCC recurrence is identified at the time a diagnosis of recurrence is made, or when a patient dies with the cause of death being recurrent HCC. A step-by-step guidance on how to use this approach can be found in the supplementary materials. Because patients receiving a liver transplant in the presence of HCC are at risk of mutually-exclusive events, we used a competing-risk model to calculate adjusted post-transplant HCC recurrence rates, with death as the event competing with tumor recurrence. For tumor characteristics, we used blood level of AFP and total tumor diameter, because these variables are easy to assess in the clinical context, and they respected the proportional hazard assumption in the current model.

In the development cohort, a multivariable competing-risk model was fitted to assess the impact of putative predictors on the risk of tumor recurrence. Next, based on the weights derived from the coefficient of each independent variable, we constructed a prognostic score predicting the five-year rate of post-transplant recurrence. This score was called the **D**onor **A**nd **R**ecipient score for **Li**ver **Ca**ncer (Darlica). We assessed the score's discrimination capacity by calculating the Wolbers's c-statistic for the primary outcome (tumor recurrence) [22] and we further calculated Harrell's c-statistic with regards to overall survival analyses. In brief, a value of 0.5 indicates no discrimination and a value of 1 indicates perfect discrimination. To validate the model (both internally, in the SRTR-based validation cohort, and externally, using data from the ANZLITR), we calculated the predicted recurrence rates for each patient in the validation cohorts (n=3,629 and n=370 in the internal and external validation sets, respectively) using the coefficients from the model obtained in the development cohort. As a post-hoc analysis (supplementary Table 4-6, supplementary Figure 6), and for the ease of use in case of external validation by other groups, we also calculated an alternative version of our score where donor characteristics are pooled through the DRI [19], rather than by individual predictors. Statistical analyses were performed using *cmprsk* for R version 3.0.1 (R-Foundation for Statistical Computing, Vienna, Austria), and Stata® 15 (StataCorp, College Station, Texas, USA).

Results

Patient Characteristics in the American Scientific Registry of Transplant Recipients.

The SRTR study population comprised 14,516 patients, divided in a development (n=10,887) and an internal validation set (n=3,629, supplementary Figure 1). In the whole SRTR dataset, there were 77% of males, and the median (interquartile range [IQR]) recipient age was 59 years (54-63). The median total tumor diameter, AFP level, and DRI were 2.5cm (1.1-3.6), 10 ng/ml (5-36) and 1.82 (1.56-2.18), respectively. Note that as expected, shared livers were associated with prolonged cold ischemia (mean ± standard deviation: 7.65 h ± 2.97 versus 6.37 h ± 2.86, $p<0.001$). The median (IQR) length of follow-up was 48.1 months (24.4-82.7). Baseline characteristics were similar in patients allocated to the development and internal validation sets (Table 1). Overall survival rates (95% CI) at one, three and five years were respectively 92.6% (92.1-93), 82% (81.3-82.6), and 74.1% (73.2-74.9). Corresponding graft survival rates were 92.4% (91.9-92.8), 81.1% (80.5-81.8), and 72.7% (71.9-73.5). At the same time points, tumor recurrence was present in 2.2% (1.9-2.4), 5.6% (5.2-6.1), and 7.7% (7.2-8.3) of the population (supplementary Figure 2).

Derivation and Internal Validation of the Predictive Score

To rule out the potential bias that marginal grafts may be selected for patients with more advanced tumors, we looked for an association between the distribution of categories of donor characteristics through strata of patients with distinct tumor characteristics (supplementary Table 1). We found no evidence that the approach of using suboptimal grafts in recipients with more advanced tumors was reflected in the present dataset.

As a preliminary assessment of the impact of donor characteristics on post-transplant HCC recurrence, we used two composite variables (the DRI and the OPTN criterion for expanded donor) that aggregate multiple data on donor quality. Using this approach, we found that patients receiving a liver graft displaying an elevated DRI, or that was procured from a donor meeting the OPTN criterion for being qualified as expanded, had a significantly increased risk of tumor recurrence compared to patients receiving leaner livers (supplementary Figure 3). To further dissect this result, we evaluated the impact of single donor characteristics on the outcome of tumor recurrence. Univariable analysis indicated that donor age, donor BMI, donor cause of death, history of donor diabetes and graft sharing were associated with tumor recurrence (Table 2). Other variables such as donation after cardiac death, donor smoking history, hypertension, history of cocaine use, blood group and need for inotropic support were not associated with the outcome.

For HCC characteristics, by categorizing total tumor diameter at two cut-off values ([<3cm] *vs*. [≥3cm to <4cm] *vs*. [≥4cm]), we identified three groups of patients with statistically different 5-year tumor recurrence and overall survival rates (supplementary Figure 4a). Similarly, for blood level of AFP, when we used the validated cutoff value of 400 ng/ml [9], patients displayed significantly different post-transplant recurrence rates (supplementary Figure 4b). Note that in the current dataset, the model for end-stage liver disease (MELD) score (sub-HR=0.99 [0.99-1.01], p=0.976), recipient immunosuppression type (sub-HR=1.38 [0.60-3.5], p=0.392) and waitlist time (sub-HR 0.99 [0.99-1.016, p=0.275) were not associated with the outcome.

Next, we ran a multivariable, competing-risk regression and we retained the predictors that were significantly and independently associated with tumor recurrence: $\log_{10}$ AFP, total tumor diameter, recipient gender, donor BMI and remote organ procurement (Table 3). The Darlica score was derived from this model by calculating the natural logarithm of the estimated coefficient and by summing points attributed to each clinical condition, according to the following formula:

$$\textbf{\textit{DARLICA score}} =$$

$$\begin{aligned} & \textbf{0.361} \textit{ if Recipient is a Male} \\ & + \textbf{0.209} \textit{ if Total Tumor Diameter} \geq 3 \ \& < 4 \textit{ cm} \\ & + \textbf{0.418} \textit{ if Total Tumor Diameter} \geq 4 \textit{ cm} \\ & + \textbf{0.241} \textit{ x } Log_e \textit{ AFP (ng/ml)} \\ & \\ & + \textbf{0.198} \textit{ if Liver Graft is Shared} \\ & + \textbf{0.081} \textit{ if Donor Body Mass Index} \geq 30 \ \& < 35 \textit{ kg/m}^2 \\ & + \textbf{0.234} \textit{ if Donor Body Mass Index} \geq 35 \textit{ kg/m}^2 \end{aligned}$$

The hazard ratio for tumor recurrence associated with unit increments in score values was sub-HR 2.72 [2.41-3.08] p<0.001. Supplementary Figure 5 shows the frequency distribution of the score values within the development cohort. As estimated by the score, the 5-year recurrence rate was 4.1% (3.3 to 5.0) in patients scoring less than 0.8, versus 12.1% (10.9-13.4) in patients scoring 1.4 or more (p<0.001, supplementary Table 2). The 5-year overall survival rates in the same risk categories were respectively 81.4% (79.7 to 83.1) and 64.5% (62.7 to 66.5), p<0.001. The Wolbers's c-statistic for the 5-year prediction of tumor recurrence was 0.64. Harrell's c-statistic for overall survival was 0.66 (0.64-0.69). Figure 1 illustrates the rates of HCC recurrence and overall survival after stratifying the population in quartiles of the score.

To internally validate the present results, we re-calculated the score for each patient in SRTR sub-cohort (n=3,629), and we computed tumor recurrence and overall survival rates, as indicated by the attributed values. Results were similar to the development cohort (Figure 1, supplementary Table 2), both in terms of the impact of the score on the outcome (sub-HR 2.49, 1.99-3.12, p<0.001), and of the c-statistics of the model (tumor recurrence: Wolbers's c=0.63, overall survival: Harrell's c=0.67, 0.62-0.72). Supplementary Table 3 exemplifies variations in the risk of tumor recurrence according to selected donor-recipient combinations (from the less hazardous to the most hazardous donor-recipient pair). Supplementary Table 7 indicates the c-statistics of other scores that are currently in circulation, these were recalculated in the current dataset. Finally, to further translate our results in a user-friendly format, a visual scorecard was designed, where navigation through relevant donor-recipient combinations allows estimating the corresponding 5-year tumor recurrence rates (Figure 2).

Characteristics of Patients in the Australia and New Zealand Liver and Intestinal Transplant Registry and External Validation of the Darlica Score.

We used data of 370 patients undergoing liver transplantation for HCC in Australia and New Zealand between 2004 and 2015, and for whom the ANZLITR possessed complete information, including in terms of tumor recurrence. The median age was 56 years (52-59), and 87% of patients were males. Patients in the ANZLITR displayed n=2 (1-3) nodules on average, with a median total tumor diameter of 3 cm (1.7-4.1), and an AFP level of 9.2ng/ml (4-28) (Table 1). As compared to the SRTR, there were more patients with chronic hepatitis B in the ANZLITR, predominantly in the Asian immigrant population and in the Maori population in New Zealand. Of note, the ANZLITR stopped using the Milan criteria and adopted UCSF criteria in 2007, which may explain the higher number of nodules and greater tumor diameter in this cohort. Median donor BMI was 25.8 kg/m^2 (23.5-30), and 45 (12.2%) liver grafts were shared. Overall survival rates in the ANZLITR at 1, 3 and 5 years were 92.7% (89.5-94.9), 84.3% (80.2-87.6), and 78.9% (74.5-82.8). Corresponding tumor recurrence rates were 4.3% (2.6-7.1), 9.4% (6.8-13.1) and 12.3% (9.2-16.4), respectively. Using the coefficients obtained from the competing-risk regression in the SRTR, we calculated values of the score in each observation in the ANZLITR. When transposing our risk-scoring tool to this cohort, the sub-hazard ratio for tumor recurrence associated with the score (continuous scale) was 1.90 (1.06 to 3.42), p=0.032. Wolbers's c-statistic was 0.57. When looking at overall survival, Harrell's c-statistic was 0.59 (0.58-0.62). Because of limited sample size, we assigned classification of risk according to the score into two categories of patients: those at low- and high-risk of tumor recurrence (supplementary Figure 8). The 5-year tumor recurrence rate in these two risk groups were respectively 8.5% (5.1-13.9) and 16.3% (11.5-22.9) (p=0.036), corresponding to a 52% lower probability of tumor relapse in the low-risk group compared to the high-risk group.

Discussion

Here, we have derived and validated a risk-scoring tool that informs decision-making when allocating liver grafts for patients with HCC. This score combines, for the first-time, tumor- and donor-related characteristics, and uses five variables (donor BMI, remote graft procurement, total tumor diameter, AFP and patient gender) that are readily available at the time of an organ offer. The score allows distinguishing between categories of graft-recipient combinations carrying an incremental risk of post-transplant tumor recurrence.

Among the variables that compose the current score, it is noteworthy that AFP had the strongest impact. This finding is consistent with many studies published over the last decade, that showed AFP to be a important factor to account for when evaluating the risk of post-liver transplant HCC recurrence [8, 9, 23-29]. By dynamically assessing AFP while on the waitlist, Vibert et al. reported that a 15 ng/ml increase in AFP per month was a strong determinant of poor overall- and disease-free survival after liver transplantation for HCC [27]. A simpler approach of assessing AFP consists of looking at this marker in a static manner. Yet, finding an optimal cut-off remains a matter of debate. Evidence gathered from an international, multicenter, prospective study indicated that, in centers with at least 8-month waiting time, patients with a tumor burden beyond the Milan criteria but matching the total tumor volume/AFP criterion (TTV; ≤115 cm^3) / alpha-fetoprotein (AFP; ≤400 ng/ml) achieved satisfactory outcomes in terms of tumor recurrence and overall survival [9]. Furthermore, the French-AFP study indicated that for patients beyond the Milan criteria, a static AFP-value of 100 ng/ml or less was associated with low risk of recurrence and 5-year survival rates of nearly 70% [8]. In contrast, patients within the Milan criteria but with an AFP value greater than 1000 ng/ml displayed high risk of recurrence and markedly reduced survival. More recently, the Metroticket 2.0 study [17] further improved risk estimation by proposing a model that takes into account not only tumor burden and alpha-fetoprotein value, but also pre-liver transplant downstaging treatments. Using this approach, Mazzaferro and colleagues constructed a prediction tool with the greatest discriminatory capacity ever achieved as of today.

The score presented in the current study may contribute to a change in clinical reasoning in the practice of allocating liver grafts, which currently consists of interpreting the risk of tumor recurrence by looking only at the recipients' tumor characteristics. Indeed, beyond tumor size and AFP level, we show that marginal grafts portend an additional negative impact on the probability of tumor relapse. In the situation where a high-risk combination of donor- and recipient characteristics is identified, clinicians may consider declining the organ offer, and let the graft being allocated to another (potentially non-HCC bearing) candidate on the waiting-list. Pending further prospective validations, including in patients from other continents than North America and Oceania, the current approach could theoretically contribute in optimizing graft allocation, by minimizing the "waste" of liver grafts that portend an increased risk of tumor recurrence. This is of particular relevance to the context of liver transplantation, where the use of marginal grafts has been shown to yield satisfactory outcomes in selected end-stage liver disease patients [30]. Of note, in the current study, the MELD score (and the variables which it is based on) did not have an impact on the risk of tumor recurrence, a finding that contrasts with other recent evidence [31, 32].

Several mechanisms may explain how donor/liver graft characteristics may impact on post-transplant tumor recurrence. First, marginal livers, which are prone to surgical stress and ischemia-reperfusion, display upregulated expression of genes associated with vasculogenesis and cell proliferation, thereby enhancing the implantation and proliferation of circulating tumor cells [33-35]. Second, evidence indicates that the formation of neutrophil extracellular traps in the injured liver promotes the expansion of circulating cancer cells in the liver sinusoid, thereby facilitating the growth of tumor foci [36]. Third, the shear stress prevailing in the liver sinusoid during the reperfusion phase provokes mechanical damage to the capillary barrier, favoring the implantation of circulating cancer cells [37]. In this regard, recent advances in the field of ex-vivo organ preservation (either in the short- or the long-term) [38, 39] provide an appealing opportunity to rescue damaged liver grafts. Such an approach could prove beneficial not only to recover organs that would otherwise be discarded, but also notably in the current oncological context.

Our study has several strengths. First, its conceptual novelty, our score being the first HCC-prediction tool that incorporates both tumor- and donor-related characteristics. Second, the length of follow up (median 48.1 months (IQR 24.4-82.7)), allowing us to identify late tumor recurrences, which are not uncommon [40]. Third, our study is based on a very large sample size, data were gathered from prospectively acquired continental databases, and results were externally validated. Fourth, our statistical modelling was constructed using a multivariable competing-risk regression. This approach allowed overcoming methodological pitfalls that commonly hamper the interpretation of survival analyses in the field of oncology [41]. Finally, our interest in designing this score relies both on biological and epidemiological evidence linking graft characteristics and tumor recurrence [11, 42-45].

Rather than attempting at making obsolete other selection criteria that are routinely used, we consider the current score as an attempt at elaborating on the concept of incorporating graft quality in our understanding of the risk of post-transplant recurrence. In this regard, our model is mostly relevant to those situations where the candidate's prognosis is dictated by the tumor itself rather than the underlying liver disease. For instance, a patient with a single tumor nodule but with decompensated liver cirrhosis should not be restricted from receiving liver transplantation, even with a liver graft procured from a marginal donor because in this case, short-term mortality is portended by liver failure and not by the tumor. In contrast, one could speculate that a patient with multiple small nodules that remain stable while on the waiting-list may be wrongfully put at risk of tumor recurrence if he or she is offered a liver graft of poor quality. Such a patient could benefit more from spending additional time on the waiting-list and being eventually offered a lower risk liver graft. With these observations in mind, the consideration of giving better quality organs to patients with more advanced HCC should be carefully weighed, notably in light of the principles of equity and equality.

Our study also has several shortcomings. First, the retrospective nature of the analysis makes our results prone to some selection bias and therefore the current score should not be recommended for clinical use. For instance, graft allocation policy in North America is a dynamic process, and a registry-based analysis may tend to analyze data in a fixed manner, missing some trends in how liver graft are allocated, and how exception points are distributed to patients with HCC. Second, for the 5-year prediction of tumor recurrence, the Wolbers's c-statistic of our model was 0.64 (Harrell's c for survival=0.66 [0.64-0.69]), a value that may be considered a drawback by itself. The c-statistics in the ANZLITR cohort was even lower, further hindering practical application of our score. A list of previously published scores and their respective discriminatory statistics can be found in supplementary Table 7. Values of these scores were recalculated in the current cohort. The Metroticket 2.0 study reported a moderately higher discrimination capacity of 0.72 (0.65-0.79) [17]. But when we transposed the Metroticket 2.0 the present SRTR-based population, we found a c-statistic of 0.63 (0.61-0.65). Another famous score, the French AFP-model [8] reported a c-statistic 0.67 (0.61-0.73). Upon application of this model to the current dataset, the c-statistic was of 0.61 (0.59-0.63). The recalculated c-statistics corresponding to the Hazard Associated with Liver Transplantation for HCC (HALT) score [46], were of 0.63 (0.61-0.66). Finally, the predictive power of the Milan and the TTV/AFP [9] criteria were markedly lower (supplementary Table 7).

Another limitation to the current study is that we could not evaluate downstaging therapies [17, 47] that patients may have received while on the waiting-list, due to the limited granularity of these variables in the SRTR dataset. Along this line, we cannot exclude that the calculation of tumor size may have ignored non-vital areas of previously ablated nodules. Fourth, we did not assess dynamic changes of AFP and this approach may have missed some clinically relevant changes in the patient's oncological status (such as successful or failing bridging therapy). However, evidence does not uniformly support this assumption [17] and a previous SRTR-based study has shown that using the last pre-transplant AFP value was the best alternative [18]. Finally, we categorized total tumor diameter and donor BMI. Although this strategy was applied to make the score more clinically-relevant and user-friendly, the use of continuous variables may provide more complete information in multivariable statistical modelling [48].

In conclusion, we have designed the first score that aggregates graft- and tumor-related factors and that predicts the risk of tumor recurrence after liver transplantation for patients with hepatocellular carcinoma. This score offers a practical and intuitive tool combining a limited number of variables that are readily available at the time of organ offer. While the use of this tool could be of interest to identify high risk situations, the clinical relevance of the current approach needs to be further addressed. For instance, by evaluating whether avoiding risky donor-recipient pairs truly contributes to an improvement in graft allocation policy, including in terms of the ethical principles that form the basis of organ transplantation.

Table Legends

Table 1. Characteristics of the Study Populations.

Data show median ± interquartile range, unless specified. * mean ± standard deviation. Body mass index (BMI), hepatitis C virus (HCV), hepatitis B virus (HBV), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alpha-feto protein (AFP), Scientific Registry of Transplant Recipients (SRTR), Australia and New Zealand Liver and Intestinal Transplant Registry (ANZLITR).

Table 2. Univariable analysis of the impact of donor-related characteristics on the risk of post-transplant tumor recurrence.

Body mass index (BMI), donation after cardiac death (DCD).

Table 3. Multivariable competing-risk regression analysis.

Calculated sub-hazard ratios go along with 95 percent confidence intervals. Alpha-feto protein (AFP), body mass index (BMI).

Figure Legends

Figure 1. Cumulative risk of post-transplant tumor recurrence (left panels) and overall survival (right panels), stratified by risk group (quartile of the calculated risk score).

(A) Development cohort (n=10,887). (B) Internal validation cohort (n=3,629).

Data are derived from competing-risk regression analysis for the analysis of tumor recurrence, and Kaplan-Meier for overall survival.

Score category 1 (**blue**)=Darlica score <0.8

Score category 2 (**orange**)=Darlica score ≥ 0.8 & <1.1

Score category 3 (**red**)=Darlica score ≥ 1.1 & <1.4

Score category 4 (**black**)=Darlica score ≥ 1.4

Figure 2.

User-friendly scorecard of the Darlica Score, allowing a quick assessment of the 5 year risk of post-transplant tumor recurrence. Women (left panels), men (right panels). The risk of tumor recurrence is summarized by a color heatmap, with corresponding rates depicted in the upper-right insert. Risk-categories were derived from combination of each of the relevant variables (recipient sex, recipient alpa-feto protein level, total tumor diameter, use of a shared liver graft, and donor body mass index), according to the score formula*. 5-year probabilities of tumor recurrence were then calculated for each patient based on calculated score values. Body mass index (BMI), hepatocellular carcinoma (HCC). Data are derived from competing-risk regression analysis.

* Calculation: DARLICA score = [0.361 if recipient is a male] + [0.209 if total tumor diameter ≥ 3 & <4 cm] + [0.418 if total tumor diameter ≥ 4 cm] + [0.241 x $\log_{10}$ afp (ng/ml)] + [0.198 if liver graft is shared] + [0.081 if donor body mass index ≥ 30 & <35 kg/m^2] + [0.234 if donor body mass index ≥ 35 kg/m^2]

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Supplementary material.

Supplementary Figure 1. Study flow chart. Hepatocellular carcinoma (HCC), Scientific Registry of Transplant Recipients (SRTR), Australia and New Zealand Liver and Intestinal Transplant Registry (ANZLITR).

Supplementary Figure 2. Cumulative risk of post-transplant tumor recurrence (left panels) and overall survival (right panels), in the whole cohort. 95 percent confidence interval are depicted by the light blue area.

Supplementary Figure 3. Cumulative risk of post-transplant tumor recurrence according to composite variables informing on donor quality. (A) Donor Risk Index. (B) OPTN expanded donor criteria. Data are derived from competing-risk regression analysis. Organ Procurement Transplant Network (OPTN).

Supplementary Figure 4. Cumulative risk of post-transplant tumor recurrence stratified by categories of the assessed tumor-related variable. (A) Total Tumor Diameter. (B) Blood level of alpha-feto protein. Data are derived from competing-risk regression analysis.

Supplementary Figure 5. Frequency distribution of the Darlica score in the development cohort.

Supplementary Figure 6. Alternative version of the score, based on the Donor Risk Index (DRI). Cumulative risk of post-transplant tumor recurrence (left panels) and overall survival (right panels), stratified by risk group (quartile of the calculated risk score).

(A) Development cohort (n=10,887). (B) Validation cohort (n=3,629). Data are derived from competing-risk regression analysis for the analysis of tumor recurrence, and Kaplan-Meier for overall survival. The sub-hazard ratio (95 percent confidence interval) for tumor recurrence associated with point increments of the DRI-adapted score was 2.70 (2.39-3.05). Wolbers's c-statistic for tumor recurrence: 0.66. Harrell's c-statistic for overall survival: 0.67 (0.65-0.69).

Score category 1 (**blue**)=DRI-adapted score <1.29
Score category 2 (**orange**)=DRI-adapted score ≥ 1.29 & <1.55
Score category 3 (**red**)=DRI-adapted score ≥ 1.55 & ≤1.88

Score category 4 (**black**)=DRI-adapted score > 1.88

Supplementary Figure 7. Graft survival, stratifying the population by risk categories (quartile of the calculated Darlica score).

(A) Development cohort (n=10,887). (B) Validation cohort (n=3,629).

Score category 1 (**blue**)=Darlica score <0.8

Score category 2 (**orange**)=Darlica score ≥ 0.8 & <1.1

Score category 3 (**red**)=Darlica score ≥ 1.1 & <1.4

Score category 4 (**black**)=Darlica score ≥ 1.4

Supplementary Figure 8. External validation in the Australia and New Zealand Liver and Intestinal Transplant Registry (ANZLITR, n=370).

(A) Cumulative risk of post-transplant tumor recurrence, stratified by Darlica Score risk group (high risk vs. low risk). (B) Overall survival, stratified by Darlica Score risk group (high risk vs. low risk).

Data are derived from competing-risk regression analysis for the analysis of tumor recurrence, and Kaplan-Meier for overall survival.

Low risk (**green**)=Darlica score <1.32

High risk (**black**)=Darlica score ≥ 1.32

Supplementary Table 1. Donor characteristics (as assessed by history of donor diabetes, donor age and donor body mass index) are evenly distributed through strata of patients with increasingly elevated tumor burden (as assessed by α-fetoprotein and total tumor diameter).

Supplementary Table 2. Cumulative risk of post-transplant tumor recurrence stratified by risk group (quartile of the calculated risk score). Upper panel: SRTR development cohort (n=10,887). Lower panel: SRTR internal validation cohort (n=3,629). Data are derived from competing-risk regression analysis.

Supplementary Table 3. Representative situations where the Darlica Score may help identifying hazardous donor-recipient combinations. Examples 1-2, 3-4 and 5-6 should be examined pairwise, looking at the increased risk of tumor recurrence upon using a liver graft procured from a more marginal donor. 5-year rates of recurrence are derived from competing-risk regression analysis.

Supplementary Table 4. Multivariable competing-risk regression, using the donor risk index (DRI) as a surrogate for donor quality.

Supplementary Table 5. **Five-year recurrence rates, as calculated by the alternative version of the score, using the donor risk index (DRI) as a surrogate for donor quality.**

Supplementary Table 6. Five-year overall survival rates, as calculated by the alternative version of the score, using the donor risk index (DRI) as a surrogate for donor quality.

Supplementary Table 7. C-statistics of other commonly used scores and models predicting post-transplant outcomes of patients receiving liver transplantation for hepatocellular carcinoma.

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Table 1

Characteristics of the Study Population

	SRTR Development cohort	SRTR internal validation cohort	ANZLITR, external validation cohort
Recipient Age, years	59 (54-63)	59 (54-63)	56 (52-59)
Recipient Gender (M:F)	8439 : 2448	2807 : 822	323 : 47
Recipient BMI, kg/m^2	27.8 (24.8-31.5)	27.8 (24.7-31.5)	26.7 (24.7-29.5)
Underlying liver disease, %			
HCV	6506 (59%)	2173 (60%)	219 (59%)
Alcohol	985 (9%)	316 (9%)	40 (11%)
NAFLD/NASH	642 (6%)	220 (6%)	14 (4%)
HBV infection	657 (6%)	220 (6%)	65(18%)
Cryptogenic cirrhosis	292 (3%)	111 (3%)	8 (2%)
Primary Biliary Cirrhosis	110 (1%)	31 (1%)	3 (1%)
Autoimmune	109 (1%)	40 (1%)	4 (1%)
Other	1586 (15%)	518 (14%)	16 (4%)
Tumour characteristics			
AFP blood level, ng/ml	10 (5-35)	10 (5-37)	9.2 (4-28)
No. of tumour nodules	1.31 (± 0.61)	1.33 (±0.63)	2.35 (±1.62)
Total tumour diameter, cm	2.5 (1.1-3.6)	2.5 (1.2-3.7)	3 (1.7-4.1)
Donor characteristics			
Age, years	44 (28-55)	44 (28-55)	48.5 (34-60)
BMI, kg/m^2	26.5 (23.2-30.6)	26.5 (23.2-30.6)	25.8 (23.5-30)
Graft cold ischemia time, hours	6.2 (5-8)	6.2 (5-8)	6.6 (5.2-8.7)
Organ was shared, %	2298 (21.1)	790 (21.8)	45 (12.2)

Data show median ± interquartile range, unless specified. * mean ± standard deviation. Body mass index (BMI), hepatitis C virus (HCV), hepatitis B virus (HBV), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alpha-feto protein (AFP)

Table 2

Univariable analysis of the impact of donor-related characteristics on the risk of post-transplant tumor recurrence

	Sub-Hazard Ratio (95% CI)	p value
Donor Age ≥ 60y	1.26 (1.04-1.53)	**0.040**
Donor BMI (kg/m^2)		
<30	Ref	
30-34.99	1.11 (0.96-1.28)	0.174
≥35	1.37 (1.02-1.84)	**0.035**
Donor cause of death (stroke vs. other)	1.14 (1.00-1.29)	**0.043**
History of donor diabetes	1.21 (1.01-1.45)	**0.036**
Liver graft was shared	1.29 (1.12-1.49)	**0.001**
Donor meets criteria to be an expanded donor	1.17 (1.02-1.35)	**0.021**
Donor Risk Index	1.18 (1.03-1.36)	**0.021**
DCD	0.97 (0.74-1.27)	0.825
Donor tobacco smoking ≥20 pack-year	1.05 (0.91-1.21)	0.479
Donor history of hypertension	1.06 (0.93-1.20)	0.403
Donor history of cocaine use	1.06 (0.84-1.35)	0.616
Donor inotropic support	0.99 (0.88-1.13)	0.987
Donor blood group		
A	Ref	
AB	1.26 (0.82-1.94)	0.290
B	0.88 (0.72-1.08)	0.215
O	0.93 (0.82-1.07)	0.324

Body mass index (BMI), donation after cardiac death (DCD)

Donor meets criteria to be an expanded donor	1.17 (1.02-1.35)	**0.021**
Donor Risk Index	1.18 (1.03-1.36)	**0.021**
DCD	0.97 (0.74-1.27)	0.825
Donor tobacco smoking ≥20 pack-year	1.05 (0.91-1.21)	0.479
Donor history of hypertension	1.06 (0.93-1.20)	0.403
Donor history of cocaine use	1.06 (0.84-1.35)	0.616
Donor inotropic support	0.99 (0.88-1.13)	0.987
Donor blood group		
A	Ref	
AB	1.26 (0.82-1.94)	0.290
B	0.88 (0.72-1.08)	0.215
O	0.93 (0.82-1.07)	0.324

Body mass index (BMI), donation after cardiac death (DCD)

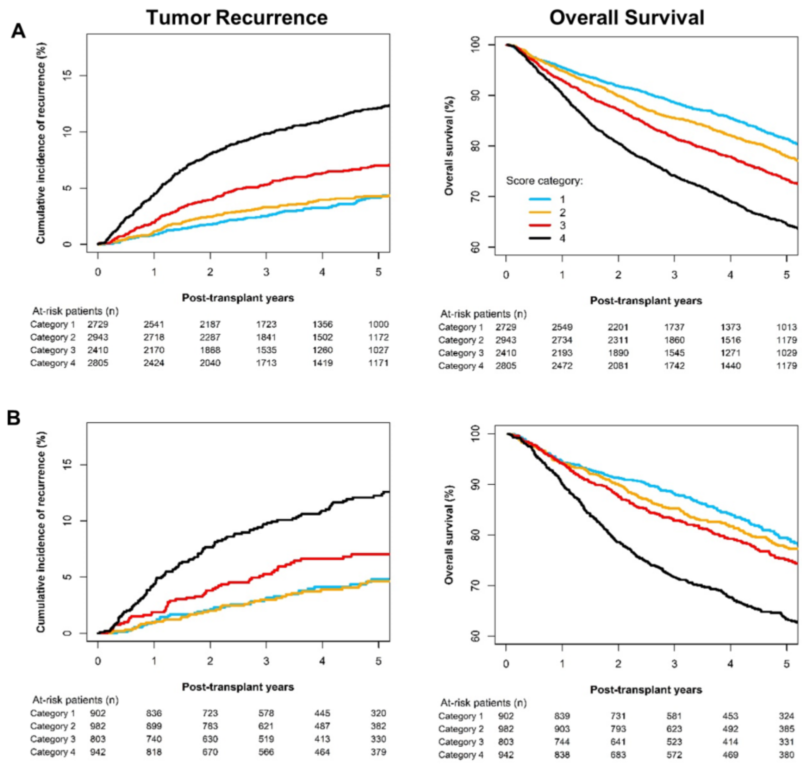
Table 3

Multivariable competing-risk regression analysis

	Sub-Hazard ratio (95%CI))	p value
Total Tumor Diameter		
<3 cm	ref	
3-4 cm	1.23 (1.02-1.49)	**0.028**
≥ 4 cm	1.52 (1.28-1.81)	**<0.001**
Log AFP (ng/ml, per unit increase)	1.27 (1.23-1.32)	**<0.001**
Recipient Gender (male)	1.43 (1.18-1.74)	**<0.001**
Donor Age (years)		
≥60	1.19 (0.97-1.56)	0.124
Donor BMI (kg/m^2)		
<30	ref	
30-34.99	1.08 (0.90-1.31)	0.410
≥35	1.26 (1.01-1.58)	**0.037**
Donor cause of death (stroke vs. other)	1.04 (0.89-1.21)	0.620
History of donor diabetes	1.16 (0.93-1.45)	0.190
Liver graft was shared	1.20 (1.01-1.43)	**0.020**

Calculated sub-hazard ratios go along with 95 percent confidence intervals. Alpha-feto protein (AFP), body mass index (BMI)

Figure 1



Cumulative risk of post-transplant tumor recurrence (left panels) and overall survival (right panels), stratified by risk group (quartile of the calculated risk score).
(A) Development cohort (n=10,887)· (B) Validation cohort (n=3,629).
Data are derived from competing-risk regression analysis.

Score category 1 (**blue**)=Darlica score <0·8
Score category 2 (**orange**)=Darlica score ≥ 0·8 & <1·1
Score category 3 (**red**)=Darlica score ≥ 1·1 & <1·4
Score category 4 (**black**)=Darlica score ≥ 1·4

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Darlica Score

Prediction of Post-Transplant HCC recurrence

LOCAL LIVER GRAFT

WOMEN

Alpha-feto protein (ng/ml)

Total Tumor Diameter	Donor BMI (kg/m²)	<5	5-10	10-100	100-200	200-400
< 3 cm	< 30	0.4	0.5	0.8	1.2	1.4
	30-34.9	0.4	0.5	0.9	1.2	1.4
	≥35	0.6	0.7	1	1.4	1
3 cm – 3.9 cm	< 30	0.6	0.7	1	1.4	1.6
	30-34.9	0.7	0.7	1	1.5	1.7
	≥35	0.8	0.9	1.3	1.7	1.7
≥ 4 cm	< 30	0.8	0.9	1.2	1.6	1.8
	30-34.9	0.9	1	1.2	1.7	1.8
	≥35	1.1	1.1	1.4	1.8	2

MEN

Alpha-feto protein (ng/ml)

Total Tumor Diameter	Donor BMI (kg/m²)	<5	5-10	10-100	100-200	400
< 3 cm	< 30	0.6	0.8	1.1	1.6	1.7
	30-34.9	0.7	0.9	1.2	1.6	1.8
	≥35	0.8	1	1.4	1.8	1.9
3 cm – 3.9 cm	< 30	0.8	1	1.3	1.8	1.9
	30-34.9	0.9	1.1	1.4	1.9	2
	≥35	1.1	1.2	1.6	2	2.2
≥ 4 cm	< 30	1	1.2	1.6	2	2.1
	30-34.9	1.1	1.3	1.6	2	2.2
	≥35	1.3	1.5	1.8	2.2	2.4

SHARED LIVER GRAFT

WOMEN

Alpha-feto protein (ng/ml)

Total Tumor Diameter	Donor BMI (kg/m²)	<5	5-10	10-100	100-200	400
< 3 cm	< 30	0.5	0.7	1	1.4	1.6
	30-34.9	0.5	0.7	1.1	1.5	1.6
	≥35	0.7	0.9	1.2	1.5	1.8
3 cm – 3.9 cm	< 30	0.6	0.9	1.2	1.6	1.9
	30-34.9	0.6	0.9	1.3	1.7	1.9
	≥35	0.9	1.1	1.5	1.8	2
≥ 4 cm	< 30	0.9	1.1	1.4	1.8	2
	30-34.9	0.9	1.2	1.4	1.8	2
	≥35	1.1	1.2	1.6	2	2.2

MEN

Alpha-feto protein (ng/ml)

Total Tumor Diameter	Donor BMI (kg/m²)	<5	5-10	10-100	100-200	400
< 3 cm	< 30	0.81	1.01	1.32	1.7	1.9
	30-34.9	0.90	1.09	1.41	1.8	2
	≥35	1.05	1.24	1.52	2	2
3 cm – 3.9 cm	< 30	1.02	1.23	1.57	2	2
	30-34.9	1.1	1.3	1.6	2.1	2.2
	≥35	1.22	1.48	1.8	2.2	2.4
≥ 4 cm	< 30	1.23	1.42	1.7	2.2	2.3
	30-34.9	1.31	1.52	1.9	2.3	2.4
	≥35	1.48	1.68	2	2.4	2.5

Darlica Score

5 year prediction of HCC recurrence

- 20% and higher
- 14%
- 10%
- 6.5%
- Less than 5%