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Risk factors for hepatocellular carcinoma recurrence after liver transplantation

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Abstract

Liver transplantation (LT) provides an excellent option for the long-term survival of patients with unresectable hepatocellular carcinoma (HCC) based on the Milan criteria. Despite careful selection of patients, HCC may still recur after LT, which represents the most important negative predictor of post-transplant survival. The growing demand for LT in HCC has led to the expansion of patient selection criteria, with a resultant increase in the risk of post-transplant HCC recurrence. Numerous tumor and host factors predict HCC recurrence. The morphological, histological, and serological characteristics of tumors in predicting HCC recurrence have been extensively studied. Furthermore, the type and duration of anticancer response before LT has also been considered a surrogate marker of tumor aggressiveness and is associated with the risk of recurrence. The demographic and clinical characteristics of recipients, as well as the type and duration of exposure to immunosuppressive therapy, represent the main host-related risk factors. Many studies have attempted to describe predictive models for the risk of HCC recurrence, considering evaluable parameters both before and after LT. Although many models have been proposed, relatively few have been externally validated on different patient populations. This paper aims to comprehensively summarize the available data on the predictive factors of HCC recurrence after LT, and to examine and discuss those that have been externally validated.

Keywords: Liver transplantation, hepatocellular carcinoma, tumor recurrence, risk predictive model

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer and is ranked as the sixth most common neoplasm and the third leading cause of cancer death[1]. In Western countries, the vast majority of HCCs develop in the presence of liver cirrhosis caused by chronic viral hepatitis, alcohol abuse, and - more recently - following the complications of non-alcoholic steatohepatitis[2,3].

In 1963, liver transplantation (LT) was originally introduced in clinical practice with the concept of treating unresectable liver tumors[4]. Early LT experiences were unsatisfactory since it became clear that post-transplant survival was reduced due to high rates of primary tumor recurrence[5]. The major revolution in improving the survival of HCC transplanted patients has been the introduction of more stringent selection criteria. The Milan criteria, published in 1996, highlighted that the selection of patients to be transplanted should be based on both the number (up to three) and diameter (up to 5 cm) of HCC nodules[6]. In the absence of macrovascular invasion and extrahepatic spread, LT allowed patients a 4-year survival rate of 75%[6]. Despite these excellent results, HCC recurred in 8% of patients and was the main cause of death[6]. In subsequent studies focused on LT patients fulfilling the Milan criteria, HCC recurrence were found in 10%-16% of cases[7-9]. These data demonstrated that HCC recurrence occurs despite the application of stringent selection criteria for LT, and is likely due to HCC dissemination from circulating cancer cells and micrometastases before or during total hepatectomy[10]. A recent debate on the need to expand the Milan criteria for LT poses the question of whether the price to pay for adopting this policy is increased HCC recurrence[11].

The purpose of this paper is to present a review of the scientific data available on the risk factors for HCC recurrence after LT. Risk factors related mainly to cancer and the host, as well as the impact of immunosuppressive therapy regimens adopted after transplantation, have been considered. Since many of the risk factors have been incorporated into predictive HCC recurrence risk models, this review focuses only on models that have been validated in different cohorts of LT patients.

FACTORS ASSOCIATED WITH HCC RECURRENCE AFTER LIVER TRANSPLANTATION

The development of post-LT HCC recurrence appears to be multifactorial [Table 1]. Risk factors involved in HCC recurrence may be divided into factors related to the tumor and those unrelated to the tumor. Risk factors related to the tumor are those pertaining to the morphological, histological, and serological characteristics of HCC, as well as those from the response of HCC to anticancer treatments. Risk factors unrelated to the tumor are those referring to the demographic and clinical characteristics of the recipient (age, gender, severity of underlying liver disease), of the liver graft (percentage of steatosis, cold ischemia time, living versus cadaveric donor), and the impact of immunosuppression after LT. All of these have been studied extensively to develop risk prediction models of HCC recurrence after LT.

Risk prediction models can be classified into three categories: preoperative, postoperative, and general models. Preoperative models consider the morphological, serological, and histological characteristics of HCC [Table 2]. Thus, these models may be adopted to select a candidate for LT by estimating the future risk of developing HCC recurrence. Postoperative models [Table 3] are developed from histological risk factors based on the evaluation of HCC characteristics in the explanted liver, such as tumor grade and the presence of microvascular invasion (MVI). General risk models are derived from a combination of pre- and postoperative risk factors; for this reason, they cannot be used to select candidates with HCC for LT. Conversely, general risk models can be adopted to determine optimal screening intervals for HCC recurrence in high-risk patients or to design clinical trials on neo-adjuvant therapies[12].

Table 1. Classification of risk factors implicated in hepatocellular carcinoma recurrence after liver transplantation

Risk factors
Tumor-related
Morphology
Number and size of the nodules
Macrovascular invasion
Extrahepatic spread
Histology
Grading
Microvascular invasion
Genetic signature
Expression of serum markers
AFP
DCP
CRP
NLR
PLR
Molecular markers
TP53 mutations
Gene expression signatures
FAI
HDACs and MMPs
miRNAs
CTC
Response to anticancer treatments
Bridge treatments
Downstaging treatments
Tumor unrelated
Recipient characteristics
Age, gender
Severity of underlying liver disease
Immunological status
Donor characteristics
Age, gender
LDLT *vs*. DCD
Percentage of graft steatosis
Cold and warm ischemia times
Immunosuppressive regimen after liver transplantation
CNI
mTOR inhibitors

AFP: alpha-fetoprotein; DCP: des-gamma-carboxyprothrombin; CRP: C-reactive protein; NLR: neutrophil/lymphocyte ratio; PLR: platelet/lymphocyte ratio; TP53: tumor protein p53; FAI: fractional allelic imbalance; HDACs: histone deacetylases; MMPs: matrix metalloproteinases; miRNAs: micro-RNAs; CTC: circulating tumor cells; LDLT: living donor liver transplantation; DCD: deceased donor, CNI: calcineurin inhibitors; mTOR: mammalian target of rapamycin

PRE-LIVER TRANSPLANTATION TUMOR-RELATED RISK FACTORS ASSOCIATED WITH HCC RECURRENCE

Morphological factors

The simplest morphological characteristics of HCC, such as the number of nodules and their size, were adopted to develop the Milan criteria[6]. The reason why these criteria, based exclusively on morphological features, made it possible to obtain an accurate selection of patients with HCC for LT, is linked to the size and number of HCC nodules which are considered as a surrogate marker for the presence of MVI and/or poor differentiation of the tumor[13,14]. It has been demonstrated that the presence of MVI and/or poor HCC differentiation are independent predictors for HCC recurrence[15]. Since the Milan criteria were published, several studies conducted in Western countries have reported similar survival rates of HCC liver transplanted patients using less stringent morphologic selection criteria. These results suggest that the Milan criteria might exclude some patients with HCC who may benefit from LT[16]. Thus, several studies have since explored the possibility of expanding the Milan criteria by considering only the morphologic characteristics of the tumor(s), which are assessed in the pre-LT period using radiologic techniques[17-20]. Among these studies, two were validated in different patient cohorts. The first was conducted in China

Table 2. The pre-operative models assessing the risk of hepatocellular carcinoma recurrence after liver transplantation. Only risk models that have been externally validated for use before liver transplantation are presented

Authors - model name	Factors included in the model	Outcomes after liver transplantation
Mazzaferro *et al*.[6] Milan criteria	Number (up to three identified as < 3 cm in diameter) and size (up to 5 cm if single) of nodules	4-year post-LT survival 75% 4-year RFS 83%
Fan *et al*.[21] Fudan-Shanghai criteria	Number and size of nodules (≤ 9 cm if single, no more than 3 lesions with the largest ≤ 5 cm), total tumor diameter ≤ 9 cm	3-years post-LT survival 80% 3-years RFS 88%
Yao *et al*.[23] San Francisco (UCSF) criteria	Number and size of nodules (≤ 6.5 cm if single or 2-3 lesions ≤ 4.5 cm), total tumor diameter ≤ 8 cm	5-year RFS 80.7%
DuBay *et al*.[51] Toronto criteria	Tumor confined to the liver, no poor histologic differentiation on biopsy, AFP serum levels < 400 ng/mL	5-year post-LT survival 70% 5-year RFS 66%
Toso *et al*.[55] Toso criteria	Total tumor volume ≤ 115 cm^3 and AFP serum levels ≤ 400 ng/mL	4-year post-LT survival 74.6% 4-year RFS 68%
Duvoux *et al*.[43] French model	Size and number of nodules (≤ 3 cm, between 3-6 cm or ≥ 6 cm) and AFP serum levels ≤ 100, between 100-1000, or > 1000 ng/mL	5-year post-LT survival 69.9% 5-year RFS 66.6%
Mazzaferro *et al*.[59] Metroticket 2.0	Number and size of nodules (up-to-seven criteria) and AFP serum levels	5-year post-LT survival 74.9% 5-year RFS 77.9%
Zheng *et al*.[53] Hangzhou criteria	HCC ≤ 8 cm or > 8 cm associated with AFP < 400 ng/mL and tumor histological grade I or II	5-year post LT survival 70.7% 5-years RFS 62.4%
Kaido *et al*.[68] Kyoto criteria (LDLT)	Up to 10 nodules ≤ 5 cm in diameter and DCP serum levels ≤ 400 mAU/mL	5-year post-LT survival 82% 5-year HCC recurrence rate 4.4%
Lee *et al*.[71] MoRAL model (LDLT)	DCP and AFP serum levels	5-year post-LT survival 86% 5-year RFS 66.3%

LDLT: living donor liver transplantation; AFP: alpha-fetoprotein; DCP: des-gamma-carboxyprothrombin; RFS: recurrence-free survival

Table 3. The post-operative models to assess the risk of hepatocellular carcinoma recurrence after liver transplantation. In the table are presented the only risk models that have been externally validated for use after liver transplantation

Authors - model name	Factors included in the model	Outcomes after liver transplantation
Onaca *et al*.[113]	Single lesion ≤ 6 cm or 2-4 lesions ≤ 5 cm each	5-year post-LT survival 67.8% 5-year RFS 63.9%
Mazzaferro *et al*.[11] Up-to-seven Metroticket criteria	Sum of the size of the largest tumor (in cm) and the number of tumors not exceeding 7 in the absence of MVI	5-year post-LT survival 71.2% 5-year recurrence rate 9.1%
Decaens *et al*.[114]	Number of nodules, largest tumor diameter, and tumor differentiation	5-year RFS 60.2% 5-year recurrence rate 20.8%
Halazun *et al*.[115] Combo-MoRAL score	Pre LT largest HCC nodule < 3 cm, NLR < 5 and AFP < 200 ng/mL plus post LT HCC number < 3, largest nodule < 3 cm, HCC histological grade < 4 and no MVI	5-years RFS 80%
Mehta *et al*.[116] RETREAT criteria	AFP serum levels at LT, the sum of the largest viable tumor diameter, and number of viable tumors	5-year recurrence rate 12.8%

MVI: microvascular invasion; NLR: neutrophil/lymphocyte ratio; AFP: alpha-fetoprotein; LT: liver transplantation; RFS: recurrence-free survival

in patients transplanted for HCC due mainly to chronic hepatitis B virus (HBV) infection. The authors demonstrated that expanding the indications for LT in patients with solitary HCC ≤ 9 cm in diameter, or with no more than 3 lesions (the largest ≤ 5 cm) with a total tumor diameter of ≤ 9 cm, there was no significant difference in 1- and 3-year survival and in recurrence-free survival as compared to the Milan criteria[21]. The aforementioned Fudan-Shanghai criteria were subsequently validated in seven Shanghai liver transplant centers, which included 1,078 patients[22]. The second study was conducted in the US and gave rise to the University of California San Francisco (UCSF) criteria[23]. These criteria suggested that having a single lesion ≤ 6.5 cm or 2-3 lesions ≤ 4.5 cm each, with a total tumor diameter ≤ 8 cm, resulted in 5-years post-LT recurrence-free survival in 80.7% of cases, which was not worse compared to that observed when applying the Milan criteria.

The most important limitation of morphologic criteria based exclusively on radiological imaging -performed by contrasted computed tomography (CT) scan or magnetic resonance imaging (MRI) - is the accuracy in detecting any single lesion in the liver, and, more importantly, to properly characterize it.

The American College of Radiology developed the Liver Imaging Reporting and Data System (LI-RADS) to standardize the acquisition, interpretation, reporting, and data collection of liver imaging[24]. LI-RADS is being increasingly adopted in clinical practice for patients at high risk of HCC, thereby enabling the categorization of observations from LR-1 (definitely benign) to LR-5 (definitely HCC) based on the level of suspicion for HCC. However, a recent meta-analysis[25] derived from 14 studies showed that the performance of LI-RADS for diagnosing HCC has a sensitivity of 67% and specificity of 92%. These data confirm previous reports indicating that radiologic imaging alone inaccurately stages as many as 20%-25% of patients undergoing LT for HCC[26-28].

Histological factors

To increase the prognostic accuracy of the predictive models of HCC recurrence based exclusively on morphological data, some authors explored using the histological characteristics of HCC obtained by nodule biopsy performed before LT. Cillo *et al.*[26] selected 33 patients with HCC for LT based on tumor grading obtained by liver biopsy. Patients with moderately to well-differentiated HCC had a 5-year post-LT survival of 75% despite approximately one-third of them failing to meet the Milan criteria at explanted liver examination. With respect to MVI, Shah *et al.*[28]. evaluated 155 patients with confirmed HCC after LT that satisfied the Milan criteria, then assessed the presence of MVI via pathological analysis. The presence of MVI was significantly associated with both the number and size of the nodules and, more importantly, 68% of patients who developed HCC recurrence were positive for MVI. Despite the undoubted diagnostic value of pre-LT pathological assessment of tumor grading and MVI, routine tumor biopsy is often unfeasible either due to the presence of multiple nodules or the risk of cancer cells seeding[29].

To overcome these limitations, a recent approach to non-invasively detect the presence of MVI in HCC is the application of 18F-FDG PET/CT imaging[30]. In HCC, the growth rate and activity of glycolytic enzymes are related[31]. Thus, contrary to what occurs in well-differentiated HCC, poorly differentiated HCC cells exhibit low glucose-6 phosphatase activity and high 18F-FDG uptake[32]. Recent studies appear to confirm that maximum standardized uptake values of 18F-FDG PET/CT imaging are strongly correlated with the histological characteristics of HCC, such as MVI and tumor grade[33-35]. The optimal cutoff values for the SUVmax of HCC (SUVmax T) and SUVmax of the normal liver (SUVmax L) in predicting MVI have been identified as 3.80 and 1.49, respectively[36]. Moreover, in a study that enrolled 34 HCC liver transplanted patients, none of the patients with a SUVmax L/T ratio > 1.5 had well-differentiated HCC[37]. A further improvement in the radiological detection of MVI in patients with HCC was derived from the application of gadoxetic acid-enhanced MRI and 18F-FDG PET/CT examinations. With the application of these radiological techniques, the presence of peritumoral enhancement and the ratio of SUVmax T/SUVmean L ≥ 1.2 had a statistically significant association with MVI, with an odds ratios of 10.6 and 14.2, respectively[38]. When both MRI and PET/CT imaging techniques have been applied in combination, the sensitivity and specificity for the prediction of MVI were 78.6 and 80% respectively[30]. Recent experiences have demonstrated how the combination of 18F-FDG PET/CT can represent valid help in selecting LT patients with HCC who exceed the Milan criteria. A Japanese study[39] that enrolled 182 living donor liver transplanted (LDLT) patients with HCC demonstrated that, in patients exceeding the Milan criteria with negative 18F-FDG PET/CT and alpha-fetoprotein (AFP) serum levels < 115 ng/mL, the 5-year HCC recurrence rate was not statistically different from those fulfilling the Milan criteria (19% compared to 7%, $P = 0.1$). Similar results have been obtained in a Korean study involving LDLT, wherein patients exceeding either the Milan or UCSF criteria, but with negative 18F-FDG PET/CT, had 5-year HCC-free survival rates after LT of 73.3 and 72.8%, respectively[40]. However, these encouraging results require extensive validation in Western populations, in patients with different etiologies of liver disease, and in LT performed using cadaveric donors. Undoubtedly, the combined use of 18F-FDG PET/CT could represent a new and more accurate system to non-invasively assess the morphological and histological features of HCC in the near future, which could guide the selection of patients for LT.

Biological markers

Biological markers can be divided into three categories: (1) serum markers directly related to HCC biology, such as AFP and des-gamma-carboxyprothrombin (DCP); (2) systemic inflammation markers, such as neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR)[41]; and (3) molecular biomarkers in tumor tissue and in serum, such as DNA alterations/mutations, enzymes, and micro-RNAs (miRNAs)[42].

Serum markers

AFP

AFP is a surrogate marker of HCC differentiation and vascular invasion[43,44]; thus, the measurement of AFP serum levels before LT has been proposed as a useful tool to identify patients with a high risk of HCC recurrence[45]. Although AFP has proven to be a valid and simple tool to discriminate HCC recurrence risk, opinions regarding what the discriminating plasma values of the protein should be are not unanimous. Several authors have suggested that serial measurements of AFP levels might be more accurate than a single measurement. Increasing AFP levels to more than 15 ng/mL[46,47], more than 50 ng/mL/month[48], or 0.1 ng/mL/day[49] during the LT waiting period have been proposed as strong predictors for HCC recurrence.

The most effective method of using AFP serum levels to predict HCC recurrence after LT is to associate it with tumor morphological criteria[50]. Four selection criteria for LT in HCC patients, including AFP serum levels and the morphologic characteristics of the tumor - both evaluated pre-LT - have been extensively validated. The "Toronto criteria"[51] were derived from a general assumption that acceptable survival rates in LT for HCC can be achieved for any size or number of HCC, provided that: imaging studies ruled out vascular invasion, the HCC was confined to the liver, and the HCC was not poorly differentiated on biopsy. The authors demonstrated that by applying these criteria, the only pre-transplant variable associated with 5-year disease-free survival was an AFP serum level value > 400 IU/mL at the time of transplant. In the validation cohort of the study[52], it was confirmed that AFP was the only independent predictive variable associated with post-LT survival and HCC recurrence, albeit with a cutoff value of 500 IU/mL.

The association of morphologic and histologic characteristics of HCC and AFP serum levels has also been utilized in China to develop the Hangzhou criteria[53]. These criteria were defined as no portal vein tumor invasion, HCC diameter ≤ 8 cm, or patients who have HCC larger than 8 cm would still be eligible for LT if their AFP serum levels were < 400 ng/mL, and their HCC histological grade was I or II. The 1- and 3-year survival rates of patients transplanted for HCC within the Hangzhou criteria were not significantly different from those transplanted within the Milan criteria.

The criteria proposed by Toso *et al.*[54] were derived from a large study including over 6000 patients. The authors demonstrated that a total tumor volume (TTV) of ≤ 115 cm^3 and AFP serum levels ≤ 400 ng/mL identified patients at low risk of HCC recurrence after LT more effectively than both the Milan and UCSF criteria. The TTV-AFP criteria were validated in a prospective study with patients from different European countries and Canada[55].

The Liver Transplantation French Group developed and validated a prognostic model for predicting HCC recurrence after LT, known as the AFP model[43]. This model considered the predictive variables of AFP serum level, and size and the number of nodules, with different cutoffs for each variable. The following points were assigned for tumor size: 0, 1, or 4 points when the largest tumor size was ≤ 3 cm, 3-6 cm, or > 6 cm, respectively. Concerning the number of nodules, 0 or 2 points were assigned for the presence of ≤ 3 or ≥ 4 nodules. Moreover, 0, 2, or 3 points were assigned to AFP serum levels ≤ 100, 100-1000, or > 1000 ng/mL, respectively. The maximum score obtainable from the AFP model was 9. Patients with a final score of up to

2 points were classified as having a low risk of HCC recurrence; on the contrary, patients with a final score ≥ 3 were classified as high risk. Interestingly, patients with AFP serum levels > 1000 ng/mL reached 3 points irrespective of the number or size of nodules; therefore, they could immediately be classified as patients at high risk of HCC recurrence. The validation of the AFP model in several countries[44,56-58] and LDLT confirms that this model better discriminated the risk of HCC recurrence compared to the Milan criteria. It is evident that patients within the Milan criteria but with AFP serum levels > 1000 ng/mL experienced HCC recurrence in 37.7% of cases, compared to patients exceeding the Milan criteria but with AFP < 100 ng/mL, with HCC recurrence in 14.4% of cases[41]. Due to the aforementioned characteristics, the AFP model was adopted for the liver allocation policy of France in 2013.

More recently, Mazzaferro *et al.*[59] developed a new predictive model of HCC recurrence based on AFP serum levels and the morphological characteristics of HCC in Italy, and validated this model in Asian patient cohorts. This model, known as the Metroticket 2.0 model, identified the sum of tumor number and size, and the $\log_{10}$ of AFP level as being significantly associated with HCC-specific death. For patients with HCC to have a 70% chance of HCC-specific survival 5 years after transplantation, their AFP level should be < 200 ng/mL and the sum of the number and size of tumors (in centimeters) should not exceed 7 cm; if the AFP level was 200-400 ng/mL, the sum of the number and size of tumors should be ≤ 5 cm; if their AFP level was 400-1000 ng/mL, the sum of the number and size of tumors should be ≤ 4 cm. This model, based on serum AFP level and HCC number and size, outperformed the Milan, UCSF, and AFP French model to predict which patients will survive for 5 years after LT.

The availability of recent models that combine the morphological and biological elements of the tumor has made it possible for patients with HCC, who would have been excluded by applying selection models based exclusively on morphological characteristics of the tumor, to undergo LT. More importantly, this has been achieved by keeping post-transplant survival unchanged. The relative simplicity of calculating the size and number of nodules in addition to AFP serum levels as surrogate biological markers of the tumor, make these models suitable for extensive and standardized use. An important innovation of these models is their possibility of being used "dynamically", to evaluate the evolution of the tumor in the patient before the transplant. This implies that these models could be used in addition with the response to neo-adjuvant treatments as the reference criteria for defining transplant feasibility in patients with HCC.

DCP

Increased serum DCP levels have been detected in patients with HCC[60] and they correlate with the degree of HCC malignancy, including the presence of intrahepatic metastases, capsule infiltration, and portal vein invasion[61,62]. Moreover, HCC expressing normal levels of AFP and increased levels of DCP showed a lower grade of differentiation and more frequent MVI[63,64]. For these reasons, DCP has been proposed as a stronger predictor of HCC recurrence after LT than AFP[61,65]. Two Japanese groups have developed and validated the selection criteria for LDLT by considering the size and number of nodules as well as DCP serum levels. The Kyoto criteria[66-68] were considered for LT patients with up to 10 HCC ≤ 5 cm in diameter and DCP serum levels ≤ 400 mAU/mL. Patients that exceeded the Milan criteria but met the Kyoto criteria had similar HCC recurrence rates to patients within the Milan criteria[66]. Similar results were obtained by adopting the Kyushu criteria[69], which selects for LT patients with any number of HCC < 5 cm in diameter and DCP serum levels < 300 mAU/ml. These criteria were more sensitive than the UCSF and Kyoto criteria in predicting HCC recurrence[61,70].

A further attractive strategy to construct a model to predict HCC recurrence involves combining AFP and DCP levels. The MoRAL model[71] was developed from this hypothesis in patients exceeding the Milan criteria who underwent LDLT. The authors included a total of 566 consecutive patients who underwent LDLT in Korea, 410 of which exceeded the Milan criteria. Serum levels of AFP and DCP provided good

discriminatory function with respect to time to HCC recurrence. A low MoRAL score (cutoff ≤ 314.8) was associated with significantly longer recurrence-free (*vs.* > 314.8) and overall survival in the cohort exceeding the Milan criteria. The 5-year recurrence-free and overall survival rates of patients exceeding the Milan criteria with a low MoRAL score were as high as 66.3% and 82.6%, respectively. Moreover, patients within the Milan criteria with a high MoRAL score showed a higher risk of recurrence than patients exceeding the Milan criteria with a low MoRAL score.

Only one retrospective study[72] combining AFP and DCP serum levels to predict HCC recurrence was performed outside of Asia, in the US. The authors demonstrated that AFP ≥ 250 ng/mL and DCP ≥ 7.5 ng/mL were associated with a higher risk of HCC recurrence. When AFP and DCP were combined with the Milan criteria, the hazard ratio increased from 2.6 for outside the Milan criteria to 8.6 for outside the Milan criteria and AFP ≥ 250 ng/mL, and to 7.2 for outside the Milan criteria and DCP ≥ 7.5 ng/mL.

Systemic inflammation biomarkers

The option of considering inflammatory markers as elements associated with greater HCC invasiveness or with more frequent recurrence after LT arises from certain studies conducted on C-reactive protein (CRP). CRP is a protein synthesized by hepatocytes in response to systemic inflammation and has been implicated in the prognosis of HCC[73]. Some Asian studies have demonstrated that in patients outside the Milan criteria, high serum CRP levels were significantly associated with a higher risk of HCC recurrence[74,75].

Besides CRP, recent studies have identified NLR and PLR as two inflammation markers involved in the prognosis of HCC[41]. Halazun *et al.*[76] reported that in patients within the Milan criteria, an NLR ≥ 5 was associated with worse recurrence-free survival after LT than in patients with an NLR < 5 (25% *vs.* 75%). These observations led the authors to propose a risk model for predicting HCC recurrence that includes NLR and tumor size > 3 cm in diameter. Further studies have been conducted to assess the real impact of NLR on the independent risk of HCC recurrence along with MVI and the size and number of nodules. Recent meta-analyses have highlighted that the cutoff value of NLR is rather heterogeneous among different studies, which is justified since the results obtained were not comparable. By applying an NLR cutoff value of 4, the majority of studies have indicated that a high NLR value is associated with MVI and a lower HCC recurrence-free survival after LT[77,78].

Regarding PLR, high PLR has been associated with a significant increase in HCC recurrence after LT[79]. Notably, the prognostic value of PLR in determining the risk of HCC recurrence should be evaluated with caution. Moreover, some reports indicate that the absolute platelet count seems to be as important as PLR. In patients with a platelet count ≥ 75×10^9/L on the day before transplant, the HCC recurrence rate was significantly higher than in patients with a platelet count < 75×10^9/L (28.2% *vs.* 13.2%)[80]; interestingly the former group of patients presented more frequently with poorly differentiated HCC, MVI, and bile duct invasion compared to the latter group.

Many hypotheses have been proposed in an attempt to explain the pathophysiological mechanisms involved in determining the influence of NLR and PLR in HCC recurrence after LT. Both neutrophils and platelets are implicated in promoting vascular invasion and the development of metastasis of tumors by increasing the production of vascular endothelial growth factor (VEGF)[81,82]. Furthermore, platelets may promote the establishment of HCC metastases by blocking tumor cell removal[83,84]. Since high NLR and PLR are determined by low lymphocyte numbers, it may be hypothesized that the reduction in lymphocyte numbers could result in impaired immune surveillance against HCC[41]. While existing studies conducted on inflammatory markers have provided interesting results, they also have several limitations. Specifically, the retrospective nature of the studies and the small number of cases represent the major limitations. Moreover, the different cutoffs applied to NLR and PLR make adoption of these models for predicting the risk of HCC recurrence in the clinical practice unvalidated.

Molecular biomarkers

Several studies investigated the usefulness of molecular biomarkers to predict HCC recurrence in liver transplanted patients[85]. Regarding DNA alterations/mutations evaluable in liver tissue, the presence of TP53 mutations, high fractional allelic loss, significant hypo-methylation of 8 tumor suppression genes, and the absence of CTNNB1 mutations, identified a molecular subclass of aggressive HCC. These features were predictive of reduced recurrence-free survival in a small group of 25 liver transplanted patients[86]. A further interesting approach is to evaluate the impact of gene expression signatures in liver tissue, both inside and outside of the tumor, in predicting HCC recurrence. Applying this approach in a large cohort of 132 liver transplanted patients for HCC outside Milan criteria, Miltiadous *et al.*[87] demonstrated that the S2 molecular subclass[88] and progenitor cell markers (CK19 signature[89]) were independent predictors of overall survival and of HCC recurrence after LT, respectively.

The fractional allelic imbalance rate index (FAI), determined from tissue samples, is used to compare the acquired mutational load between different tumors[90]. FAI was evaluated in a cohort of 71 liver transplanted patients with HCC, 18 of whom experienced tumor recurrence. Among the 19 microsatellites evaluated, 3 loci (D3S2303, D9S251, and D9S254) were found to be predictive of recurrence after LT[91]. If confirmed in other prospective studies, and in patients outside the Milan criteria, FAI could represent an interesting tool to identify recipients at higher risk of tumor recurrence.

Enzymes such as histone deacetylases (HDACs) and matrix metalloproteinases (MMPs) have also been studied in liver transplanted patients. HDACs regulate genes and are involved in tumor cell proliferation, differentiation, invasion, and metastasis. Liver transplanted patients carrying T alleles in HDAC1 rs1741981 and HDAC3 rs 2547547 single nucleotide polymorphisms have been found to have a low risk of HCC recurrence[92]. On the contrary, high expression of HDAC3 was related to high risk of HCC recurrence in HBV positive recipients[93].

MMPs are extracellular matrix-degrading enzymes that can be secreted by tumor cells to enhance tumor invasiveness and metastasis. Although results among different studies are conflicting[42], MMP-9 and MMP-2 positive staining in stromal tissue adjacent to tumors seems to be associated with HCC recurrence[94]. While there is a rationale for investigating the role of enzymes in predicting HCC recurrence, the results of the studies are still too heterogeneous to draw solid conclusions for use in clinical practice.

Besides DNA changes and aberrant gene expression, miRNAs have been evaluated as potential prognostic biomarkers in HCC. An interesting report proposed a prognostic score incorporating the expression in liver tissue of miR-214, miR-3187, and the Milan criteria, which improved the accuracy of predicting HCC recurrence compared with the Milan criteria alone[95]. Despite several studies reporting a potential role of miRNAs in predicting HCC recurrence[85], none of them were really prospective and adequately powered. The major limitation in introducing miRNAs in clinical practice is probably the need for a liver tissue biopsy to evaluate their expression. To overcome this important limitation, liquid biopsy has emerged as a minimally invasive alternative approach to analyze HCC components without the need of tissue samples. This method allows the analysis of DNA, RNA in extracellular vescicles such as the exosomes, or circulating tumor cells released by the tumor in the bloodstream[85]. Among the few studies that have applied this technology, Nakano *et al.*[96] demonstrated that circulating exosomal miR-92b could have clinical value for predicting HCC recurrence post-LT. The main limitation of liquid biopsy is the low sensitivity and lack of reproducibility when different technologies were applied[97].

Among the serological markers that can be measured before transplantation, AFP remains the most clinically useful to date, albeit with the major limitation that it can only be used in protein secreting HCC.

HCC response to anticancer treatments as a surrogate biological marker of tumor aggressiveness

Alongside the morphological, histological, and serological criteria used to predict the risk of HCC recurrence after LT, an innovative and more flexible approach involves considering the response of HCC to anticancer loco-regional treatments as a surrogate marker of the biological aggressiveness of the tumor - and thus, of the risk of post-LT recurrence. This approach shifts the concept of selecting HCC patients for LT from the characteristics of disease presentation to the final characteristics of the tumor after it has undergone available treatment. Regarding this point, it is important to differentiate "bridge treatments" from "downstaging treatments". Bridge treatments refer to patients already on the waiting list for LT that undergo HCC loco-regional treatments to reduce dropout rates from the waiting list. Downstaging treatments refer to those initially applied to patients beyond the accepted criteria for LT (e.g., Milan, UCSF, TTV-AFP, and others) to bring the tumor back within the accepted criteria for LT[98]. The increasing rate of applying the approach of merging HCC tumor stage and response to anticancer treatments in European countries[99] is justified by observations that post-LT survival outcomes in patients with HCC exceeding the Milan criteria with objective and sustained response to pre-LT therapies are not significantly different compared to those patients who meet conventional criteria at presentation[100]. In the US, the UCSF downstaging protocol has recently been adopted as a national policy for granting priority listing for LT[101]. This protocol implies that the initial selection criteria are single lesions ≤ 8 cm, or 2-3 lesions < 5 cm with total tumor diameter < 8 cm, or 4-5 nodules all < 3 cm with total tumor diameter < 8 cm. In a retrospective analysis of the UNOS database of 3819 patients who underwent LT from 2012 to 2015, and were classified as always within the Milan criteria or achieving UNOS downstaging criteria (UNOS-DS), 3-year post-LT survival was 83.2% for the Milan criteria and 79.1% for the UNOS-DS. The 3-year HCC recurrence probability was 6.9% for Milan and 12.8% for UNOS-DS. Interestingly, AFP ≥ 100 ng/mL was the only independent predictor of HCC recurrence in downstaging groups[102]. The application of downstaging protocols involves a minimum observation period of 3 months of disease stability from successful downstaging to LT[101,103]. This implies that liver transplant centers should adopt dynamic graft allocation protocols to assure "on time" LT in those patients who can maximize the benefit of successful downstaging[99]. Thus, both AASLD and EASL guidelines recommended loco-regional treatments in patients with HCC exceeding the Milan criteria and to consider those who underwent successful downstaging as candidates for LT, when this status is maintained for at least 3-6 months[104-106].

Although the rationale for supporting the application of downstaging strategies exists, it is much more complex to evaluate the ways in which to obtain it. There are in fact multiple loco-regional therapies applicable for the treatment of patients with HCC with both bridging and downstaging purposes. These therapies include transarterial chemoembolization (TACE) and radioembolization, percutaneous ethanol injection, radiofrequency ablation, and stereotactic body radiation. Liver resection may be a further part of a multimodal downstaging strategy[107,108]. The long list of therapeutic strategies highlights how indications to perform them can be varied between different transplant centers, thus results obtained are not always comparable[107]. Since every treatment can negatively impact upon residual liver function, it has been proposed that only patients with adequate liver function (Child Pugh class A/B) and with serum bilirubin ≤ 3 mg/dL can be candidates for downstaging procedures[102,103]. TACE is the most commonly used treatment, so it is recommended as first-line for downstaging[108].

A further critical element relates to the method used in various studies to judge the response to loco-regional treatments. In more recent studies, this has been done by applying the modified Response Evaluation Criteria in Solid Tumors (mRECIST), which assesses both the change in tumor volume and in arterial enhancement by means of contrast CT or MRI scan of the liver[109]. A very recent report demonstrated that the addition of the mRECIST criteria into the Metroticket 2.0 framework improved its predictive ability[110]. However, although sufficiently detailed, the mRECIST criteria may have reproducibility limits between different transplant centers, such that the results obtained by loco-regional therapies are not always reproducible.

Despite these important limitations, it has recently been accepted that patients with HCC listed for LT and receiving loco-regional treatments associated with objective response, improved waitlist and post-transplant outcomes. More importantly, the degree of tumor response to loco-regional treatments may help in defining LT priority in candidates with HCC[108].

POST-LIVER TRANSPLANTATION TUMOR-RELATED RISK FACTORS ASSOCIATED WITH HCC RECURRENCE

In all prognostic risk models, the number and size of tumors, as well as the presence of MVI, were found to be statistically associated with the risk of HCC recurrence after LT. As previously mentioned, it is known that pre-transplant staging methods based on radiologic imaging fail to predict the exact number and size of HCC at pathology in approximately 25%-35% of patients due to over- or understaging[28,111,112]. These observations justified the development of HCC recurrence risk models based on the accurate assessment of tumor burden in the explanted liver. In a large US database of HCC liver transplanted patients, Onaca *et al.*[113] evaluated the number and size of HCC in all explanted livers and correlated them with HCC-free survival after LT. The authors demonstrated that patients with 2-4 tumors < 5 cm or with a single lesion < 6 cm had recurrence-free survival equivalent to patients with a single tumor of 3.1-5.0 cm or 2-3 lesions all < 3 cm in diameter, which represents the Milan criteria.

As previously reported, the assessment of MVI before LT is difficult and often inaccurate[29]; on the contrary, evaluation of the presence of MVI appears very accurate on histological examination obtained in the explanted liver. On this basis, Mazzaferro *et al.*[11] developed a predictive model of the risk of mortality and recurrence of HCC after LT based on histopathological analysis performed on the explanted liver. The histological characteristics evaluated include the number and size of the nodules, the presence of MVI, and the grading of the tumor. The authors collected a sample of 1,556 patients transplanted for HCC from several US, European, and Asian centers, of which only 444 had tumor characteristics under the Milan criteria at explant. The combination of HCC characteristics exceeding the Milan criteria but resulting in an estimated 5-year overall survival of at least 70% generated a subgroup of patients that, in the absence of MVI, fulfilled the so-called "up-to-seven criteria", which involves seven being the result of the sum of the size (in cm) and the number of tumors, for any given HCC. The overall survival reported in this subgroup of patients was 71.2%, which was similar (73.3%) to that obtained in the subgroup of patients fulfilling the Milan criteria, irrespective of the presence of MVI. On the contrary, patients exceeding the up-to-seven criteria, plus patients with MVI who were beyond the Milan criteria and within the up-to-seven criteria, had a 5-year survival rate after LT of 48.1%. The presence of MVI at any size and number category of tumors was paralleled by a significant worsening of survival and the cumulative incidence of HCC recurrence.

A similar model was developed and validated in France[114], which considered the pathological characteristics of HCC assessed in the explanted liver, including the number, size, and grading of tumors. To obtain a final numeric risk score, the authors attributed point values for any of the following tumor characteristics: the number of nodules, the diameter of the largest nodule, and tumor differentiation (well, moderate and poor). For Cox regression analysis, the number of nodules, maximal diameter of the largest nodule, and tumor differentiation were independent predictors of HCC-free survival. Interestingly, in patients with a score < 4, there was no significant difference in 5-year tumor-free survival between those within and exceeding the Milan criteria. A very similar approach was followed in one of the largest single institution dataset of HCC patients undergoing LT in the US[115], to update the original MoRAL score. The evaluation of histology in the explanted liver showed that grade IV HCC, the presence of more than 3 lesions, the largest tumor size > 3 cm, and MVI were independently associated with HCC recurrence. This model, called the post-MoRAL score, was therefore combined with the original pre-MoRAL score to

develop a new model, called the combo-MoRAL score. In this new model, patients were stratified according to an increased HCC recurrence risk score ranging from 0 to 26. Those patients presenting a combo-MoRAL score of up to 6 were identified as low-intermediate risk, while those exceeding 6 points were considered to have a high-very high risk of HCC recurrence. Patients outside the Milan criteria who had a combo-MoRAL score of up to 6 experienced a risk of recurrence similar to those within the Milan criteria. A recent and particularly relevant Risk Estimation of Tumor Recurrence After Transplant (RETREAT) score[116] was developed and validated for patients with HCC that met the Milan criteria based on imaging. A total of 1061 patients were enrolled in the study, which was developed in the US and validated in Canada. In the development cohort, 9.4% of patients had MVI and 22.1% exceeded the Milan criteria on explant. Cumulative probabilities of HCC recurrence at 1 and 5 years were 5.7% and 12.8%, respectively. For multivariable Cox proportional hazards regression, three variables were independently associated with HCC recurrence: MVI, AFP value at time of LT, and the sum of the largest viable tumor diameter and the number of viable tumors on explant. The RETREAT score was created using these three variables, with scores ranging from 0 to 5 or higher being highly predictive of HCC recurrence. The RETREAT model was able to stratify 5-year post-LT recurrence risk ranging from less than 3% with a score of 0 to greater than 75% with a score of 5 or higher.

A critical point that can be applied to the majority of models that aim to predict HCC recurrence is that the time of recurrence after transplant is rarely mentioned. Although this time is variable, several studies have identified a peak of HCC recurrence within 2-3 years after transplant while after 5 years, recurrence is very infrequent[117]. Since HCC recurrence after LT impacts negatively on overall survival, the time to recurrence represents an important prognostic factor. Early (within 12 months after LT) HCC recurrence is associated with a more severe prognosis[117]. The reasons why it occurs may be related to the presence of non-detected extra-hepatic HCC metastases at the time of transplant, or as a consequence of circulating neoplastic clones of HCC able to engraft and growing in the transplanted liver or in other organs. Recurrences occurring more than 12 months following LT, particularly if associated with AFP serum levels < 100 ng/mL at the time of recurrence, are associated with a better prognosis and with 5-year survival nearing 50%[118,119]. These data strongly suggest maintaining a very stringent surveillance of the recurrence of HCC in the first 3 years after LT, prolonging up to the fifth year, since very late recurrence of the tumor is also associated, if promptly discovered and treated, with a better prognosis[10]. Regarding the best way to perform surveillance, in the absence of specific evidence-based risk stratification criteria, the majority of LT centers suggest to perform a total body contrast CT or MRI scan every 6 months for at least 3 years, that can then be extended to 5 years after LT, in addition to AFP serum measurements[117].

Taking into account the aforementioned considerations HCC recurrence risk models based on the evaluation of tumor characteristics on the explanted liver should improve post-LT HCC surveillance strategies and to help identify patients who may benefit from future adjuvant therapies. On the contrary, all of these models have the major limitation in that they cannot be used to select patients with HCC for LT.

TUMOR UNRELATED RISK FACTORS ASSOCIATED WITH HCC RECURRENCE

Transplant type

Conflicting results exist regarding the potential impact of influencing HCC recurrence following LT performed using cadaveric donors versus LDLT[120-126]. In addition to more advanced clinical characteristics of the tumor that are often recognized in LDLT[120], there are other potential explanations to support the hypothesis that HCC recurrence may be more frequent in LDLT when compared to using cadaveric donors. A potential mechanism to promote tumor progression and recurrence may be related to the release of growth factors in the course of liver regeneration involving a partial graft. Moreover, it has been demonstrated that small-sized grafts are more likely to cause acute phase graft injury, promoting cell adhesion, increased angiogenesis, and cell migration. All of these factors may contribute to increased

HCC recurrence[127-129]. A further mechanism could be linked to the short waiting time on LDLT waiting lists[130,131]. This short waiting time may not be able to highlight tumors with greater biological aggressiveness, expressed as having rapid growth over time. Finally, the LDLT technique associated with sparing of the inferior vena cava and with more extensive manipulation of the liver during transplant operations may contribute towards increasing the risk of HCC recurrence[132]. The same considerations may be applied to LT performed by the piggyback technique with cadaveric donors since it theoretically carries a higher risk of positive vena cava margins and requires greater manipulation of the diseased liver, thus leading to an increased risk of HCC spread[133]. Nevertheless, it remains important to highlight that the piggyback technique is the preferred approach in many liver transplant centers since it provides several advantages compared to conventional techniques, such as shorter operation times, anhepatic phases, warm ischemia times, and stays in intensive care units[132,134].

Graft and donor-related factors

Besides the use of partial grafts for LT, several reports have indicated that prolonged cold and warm ischemia times could be associated with an increased risk of HCC recurrence[135]. Multiple biological mechanisms have been proffered to explain how ischemia-reperfusion injury can affect the risk of HCC recurrence, based on *in vivo* and *in vitro* experiments[136-138] One of these mechanisms hypothesizes that the exposure of micrometastases to prolonged hypoxia could lead to the abnormal expression of genes and cytokines that increase angiogenesis, cell proliferation, and growth[136]. Notably, it has been hypothesized that female grafts may be more susceptible to ischemia-reperfusion injury and may have increased sensitivity to reoxygenation damage following prolonged cold storage[139,140]. Furthermore, hypoxia stabilizes and activates the transcription factor for hypoxia - inducible factor, which represents a key oxygen response regulator able to activate the transcription of genes stimulating angiogenesis such VEGF-A[141,142]. However, the relationship between prolonged graft ischemia time and the risk of HCC recurrence was most convincing in recipients who presented additional risk factors for recurrence at baseline, such as HCC beyond the Milan criteria, the presence of MVI, or high AFP serum levels[126].

The increased susceptibility to ischemia-reperfusion injury of older grafts support some observations, thus indicating that recipients transplanted with older donors experienced HCC recurrence more frequently than those transplanted with younger donors[143]. Although these observations are of interest, previous studies have not confirmed them in subsequent studies[144,145].

Given the growing number of donors with metabolic syndrome, one aspect that appears to be of particular interest is the potential impact of graft steatosis on the risk of HCC recurrence. It has been accepted that grafts with moderate to severe steatosis present low tolerability to ischemia-reperfusion injury[146]. This injury may be able to promote a release of lipid peroxides and downregulate the secretion of adipokines that can protect the steatotic grafts[147]. Thus, the induced inflammatory cascade within the graft could be responsible for the increase in angiogenesis, which is considered the key factor in promoting tumor recurrence. All of these observations have not been validated in prospective studies and a large number of patients; thus, no current evidence exists to modify the allocation criteria for steatotic grafts based on the presence of HCC in recipients.

There is emerging evidence that persistent HBV infection contributes to cancer development within the liver, increasing genetic instability of and promoting hepatocyte destruction and regeneration. Several reports indicate that in patients transplanted for HCC related to chronic HBV infection, the reappearance of active HBV replication in the graft was associated with an increased risk of HCC recurrence and with shorter overall survival[126]. These reports were more frequent in the past when prophylaxis against HBV recurrence was given only with immunoglobulins against HBV or with Lamivudine.

Recipient characteristics

Overweight or obese recipients transplanted for HCC seem to be more exposed to HCC recurrence compared to lean recipients[148]. The mechanisms proposed to support this observation are very similar to those discussed for steatotic grafts. The altered production of adipokines could occur in obese patients, and are known to be responsible for increased cell proliferation and the reduction of apoptosis in neoplastic cell lines. In certain cases, adipokines can also be responsible for the increased expression of VEGF and other mediators capable of increasing angiogenesis and tumor recurrence[149,150].

Clinical studies have identified a body mass index cutoff value of > 30 kg/m^2 or the presence of obesity as independent predictors of more frequent HCC recurrence[148,151]. Regarding recipient age, several studies have reported that elderly patients who underwent LT experienced lower survival and higher rates of HCC recurrence[126]; however, the mechanisms involved in explaining these observations are not entirely clear. The most probable hypothesis is that advanced age represents a factor associated with reduced efficiency of the immune system in reducing the development of neoplastic cell clones, which would be more evident during prolonged immunosuppressive therapy[152].

Impact of immunosuppression schemes adopted after LT

The impact of immunosuppressive therapy after LT has been extensively studied with regard to the development of metabolic complications such as diabetes, arterial hypertension, hyperlipidemia, and renal failure. This was motivated by the observation that the main cause of mortality in the medium to long term after LT is not linked to allograft dysfunction, but rather to the development of metabolic complications and *de novo* tumors[153].

Much less studied has been the impact of immunosuppressive therapy in modifying the risk of HCC recurrence after LT. The role of immunosuppression in promoting oncologic cell transformations has been extensively proven in both *in vitro* and *in vivo* studies[154]. Furthermore, research conducted in cell lines and observational clinical studies indicate that not only the type and the schemes of immunosuppressive treatments, but also the total immunosuppressive load, are likely determinants in promoting cancer recurrence[154].

Among the very few studies designed to evaluate the impact of immunosuppressive treatment on HCC recurrence after LT, a high level of heterogeneity among patients and HCC selection criteria are present. Furthermore, many immunosuppressive schemes have adopted the simultaneous use of different classes of drugs, with potential pro- or anti-oncogenic effects that may not always be synergistic. Thus, the inherent limitations in the design of these studies make it very difficult to draw solid conclusions.

A recent literature review[154] identified 21 studies, of which only one was prospective and randomized, while two were meta-analyses and evaluated the potential anticancer effect of sirolimus[155]. Other studies evaluated the impact of the type and load of calcineurin inhibitors (CNIs), as well as the impact of corticosteroids or anti-thymoglobulin antibodies. By summarizing the results of these studies, which highlighted HCC recurrence rates ranging from 12% to 54%, two key messages may be reported: (1) an increased rate of HCC recurrence risk was associated with higher exposure to both CNIs (cyclosporine or tacrolimus); and (2) the use of a mammalian target of rapamycin (mTOR) inhibitors was associated with a lower risk of HCC recurrence[156]. To confirm the potential beneficial role of the mTOR inhibitor sirolimus in decreasing the risk of HCC recurrence after LT, a prospective multicenter and randomized study was performed[157]. The results of this study were disappointing since they only showed a lower recurrence rate 3 years after LT in patients with early HCC, though this advantage was lost at 5 years. Moreover, in patients with HCC within the Milan criteria, the rate of recurrence was not statistically different from that obtained by adopting immunosuppressive schemes without mTOR inhibitors[154]. The main limitations of this study

were likely related to the high heterogeneity of the immunosuppressive drugs added to sirolimus and to having left the LT centers completely free to manage therapy with steroids.

Studies performed with the other mTOR inhibitor, everolimus[158], suggested that patients treated with this inhibitor had significantly lower HCC recurrence rates when compared to those treated with sirolimus or with CNI. However, it is important to note that everolimus-treated recipients had a shorter follow-up period and were more frequently transplanted for HCC within the Milan criteria. Overall, based on the available data from retrospective studies, meta-analyses, and post-hoc assessments of randomized trials, it seems advisable to consider mTOR inhibition-based immunosuppression after transplantation for HCC, particularly in patients who exceed the Milan criteria; however, prospective data are required to verify this claim.

If immunosuppression load appears to serve a determinant role in cancer recurrence[159,160], it seems justified to explore immunosuppressive protocols aimed at weaning exposure to immunosuppressive drugs. The liver is a solid organ that - more than other organs - is suitable for tolerating transplantation with total withdrawal of immunosuppression without developing rejection. This phenomenon is known as operational tolerance[161]. A large European prospective study on immunosuppressive treatment withdrawal, which enrolled 102 adult LT recipients, showed that 40% of them reached the status of operational tolerance for at least 1 year[162]. The clinical predictors for achieving operational tolerance were a minimal time of 3 years from LT to immunosuppression weaning, the absence of autoimmune liver disease, and recipient age > 60 years[163]. Regarding the impact of operational tolerance on HCC recurrence, 17 patients transplanted for HCC were enrolled in a protocol of immunosuppression weaning in Italy[163]. The results demonstrated that no HCC recurrence was detected in those patients achieving operational tolerance, while one patient who presented only transient tolerance experienced HCC recurrence after 3 years from LT and died within 4 years. Despite these promising results, the identification criteria for precisely selecting patients who may develop operational tolerance remain lacking. Furthermore, if the purpose of utilizing the development of operational tolerance is to reduce the risk of HCC recurrence, it would be essential to obtain the suspension of immunosuppression within the first 2 to 3 years after LT, since this is the time interval during which the recurrence of HCC is more likely[117].

CONCLUSION

LT remains an effective treatment in patients with unresectable HCC within the Milan criteria or in those who can be downstaged to being within the Milan criteria; however, HCC recurs despite careful selection of recipients. The growing demand for LT for HCC is expected, and the probable modest increase in the availability of organs to allocate for this indication - in the face of a clear decrease in transplantation for hepatitis C virus-related liver disease - makes the intention to expand the Milan criteria justifiable. The key question will now be: how can we balance the expansion of the Milan criteria with the risk of an exponential increase in HCC recurrence after LT? From a mathematical perspective, squaring the circle is a geometry problem that consists of constructing a square with the same compass and ruler with the same area as a circle. The study of the relationship between circumference and diameter of the rim has accompanied the history of humanity since the invention of the wheel. In 212 B.C., Archimedes was the first to attempt a calculation based on geometry through the method of exhaustion, which consisted of attempting to trap the circumference between a registered and a circumscribed polygon. Using 96-sided polygons, he calculated the value of π at 3.14163. Trapping that number in a finite series of digits is a feat that almost all mathematicians do, and when the mathematician Johan Lambert showed that it is irrational in 1761, he took the real challenge to calculate the highest number of decimal places to make it as precise as possible. Lambert's proof marks a turning point since it shows, that our number cannot be trapped in the relationship between two numbers in practice (one in the numerator and one in the denominator); thus, it cannot be "rationalized".

From a clinical perspective, the search for the perfect solution between expanding the Milan criteria and maintaining a sufficiently low risk of tumor recurrence to allow a patient survival rate of at least 60% at 5 years represents a major challenge for the coming years. The models used to predict the risk of HCC recurrence based on the pre-transplant evaluation of all tumor characteristics (including its response to treatments) currently represent those that combine both rigorous aspects of patient selection and organ allocation for transplant. These two factors work synergistically to increase the benefit, effectiveness, and justice of transplantation for HCC. The evaluation of the results obtained by adopting these criteria on large patient series in different geographical areas will be crucial for defining their validity and applicability.

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Authors' contributions

Wrote the paper: Toniutto P
Selected bibliography and collaborate in writing the paper: Fornasiere E, Fumolo E, Bitetto D

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