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IMPACT OF REMNANT VITAL TISSUE AFTER LOCOREGIONAL TREATMENT AND LIVER TRANSPLANT IN HEPATOCELLULAR CANCER PATIENTS, A MULTI CENTRE COHORT STUDY

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Keywords: Hepatocellular Carcinoma, Liver Transplantation, Recurrence, Milan Criteria, loco-regional treatment.

Abbreviations used in the text:

AIC,	Akaike information criterion
AUC,	area under the curve
CI,	confidence intervals
CNI,	calcineurin inhibitor
CT,	computed tomography
DOR,	diagnostic odds ratio
EASL,	European Association for the Study of the Liver
HCC,	hepatocellular Carcinoma
HR,	hazard ratios
IQR,	interquartile ranges
LRT,	loco-regional treatments
LT,	liver transplantation
MC,	Milan Criteria
MDT,	multi-disciplinary teams
MELD,	model for end-stage liver disease
mRECIST,	modified Response Evaluation Criteria in Solid Tumors
mTORi,	mammalian target of rapamycin inhibitor
PEI,	percutaneous ethanol injection
RETREAT,	Risk Estimation of Tumor Recurrence After Transplant
RFA,	radio-frequency ablation
ROC,	receiver operation curve
RVT,	remnant vital tissue
TACE,	trans-arterial chemo-embolization
US,	ultrasounds

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ABSTRACT

The role of pathological findings after loco-regional treatments as predictors of hepatocellular cancer recurrence after liver transplantation has been poorly addressed. The aim of the study was to identify the role of remnant vital tissue (RVT) of the target lesion in predicting hepatocellular cancer recurrence. Two hundred seventy six patients firstly undergoing loco regional treatment and then transplanted between Jan2010-Dec2015 in four European Transplant Centres (i.e., Rome Tor Vergata, Birmingham, Brussels and Ancona) were enrolled in the study in order to investigate the role of pathological response at upfront loco regional treatment. At multivariable Cox regression analysis, RVT≥2cm was a strong independent risk factor for post-LT recurrence (HR=5.6; p<0.0001). Five-year disease-free survival rates were 60.8, 80.9 and 95.0% in patients presenting a RVT≥2cm vs. 0.1-1.9 vs. no RVT, respectively. When only Milan Criteria-IN patients were analysed, similar results were reported, with 5-year disease-free survival rates of 58.1, 79.0 and 94.0% in patients presenting a RVT≥2cm vs. 0.1-1.9 vs. no RVT, respectively. RVT is an important determinant of tumour recurrence after liver transplantation performed for hepatocellular cancer. Its discriminative power looks to be evident also in a Milan-IN setting, suggesting to more liberally use loco-regional treatments also in these patients.

INTRODUCTION

Hepatocellular Carcinoma (HCC) represents the fifth most common cancer worldwide [1]. According to current therapeutic recommendations, based on the European Association for the Study of the Liver (EASL) (www.easl.eu) and American association Study of the Liver (AASLD) guidelines (www.aasld.org), liver transplantation (LT) represents the treatment of choice in HCC patients, especially in Milan Criteria (MC)-IN patients, in which five-year disease-free survivals reach 80% [2]. The role of neo-adjuvant bridging or down-staging

therapies before LT has not yet been fully established in HCC patients waiting for LT. Although heavily controversial, current guidelines recommend the use of neo-adjuvant loco-regional treatments (LRT) as a bridge strategy only in MC-IN patients with an expected waiting time exceeding six months [3].

Similarly, the role of down-staging procedures remains a matter of debate. Nevertheless, there is evidence of their beneficial impact [1,4]. This explains why LRT are used in most liver transplant centres [5-7].

The effectiveness of LRT has so far been investigated on the basis of radiological findings obtained after the procedures and by quantification of the extent of necrosis in the histological specimens of both partial or total hepatectomy specimen [8, 9]. Only a few number of studies explored the role of pathological response after LRT as a risk factor for HCC recurrence in the setting of LT [10], no one investigating in detail the specific magnitude of the pathological remnant vital tissue (RVT) in the target nodule.

Thus, the present study aim was to quantify the actual RVT of the target HCC lesion after LRT, looking at its role in the prediction of HCC recurrence after LT.

MATERIALS AND METHODS

Study Population

The study included a population of consecutive HCC-on-cirrhosis patients first receiving LRT before or during their registration in the LT waiting list and then undergoing LT during the period January 2010 – December 2015 in four different European Centres (Tor Vergata University, Rome, Italy; Queen Elizabeth Hospital, Birmingham, United Kingdom; University Hospitals Saint Luc Brussels, Belgium; Ospedali Riuniti, Ancona, Italy). The prospectively collected data were retrospectively analysed.

Inclusion criteria for the study were: a) adult age (≥18 years); b) pre-LT

radiological/histological diagnosis of HCC; c) preoperative treatment using different methods of any LRT such as trans-arterial chemo-embolization (TACE), percutaneous ethanol injection (PEI), and radio-frequency ablation (RFA). Initial population obtained from the databases of the four collaborative Centers was composed by 408 cases: after removing patients directly transplanted without any LRT (n=82) and subjects undergoing radio-embolization, external radiotherapy or hepatic resection (n=15), the identified population consisted of 311 patients (Brussels: n=99; Birmingham: n=94; Tor Vergata Rome: n=65; Ancona: n=53). Patients with a final histological diagnosis of mixed tumours or cholangiocellular carcinoma and those in which data were missing were excluded from the analysis. Thus, the population finally used for the study included 276 patients, with a median follow-up period after LT of 2.2 years (interquartile ranges [IQR]=1.2-3.5). The median age at LT was 58.9 years (IQR: 53.0-64.3); 226 (81.9%) patients were males. The underlying liver cirrhosis was due to HCV, alcohol, HBV, and non-alcoholic steato-hepatitis in 101 (45.3%), 88 (31.8%), 30 (10.8%), and 26 (9.4%) patients, respectively. A multifactorial condition was observed in 27 (9.7%) cases; other causes of hepatopathy were reported in 4 (1.4%) cases. Median laboratory model for end-stage liver disease (MELD) at listing was 10 (IQR: 8-14). At referral, 219 (79.3%) patients were radiologically classified as MC-IN and 57 (20.7%) were MC-OUT but up-to-seven-IN. This multicentre study was approved by the Ethical Committee Boards of all the participating Institutions.

Loco-Regional Treatments

All MC-OUT patients at referral were down-staged by LRT. Out of 57 initially MC-OUT patients, 43 (75.4%) were successfully down-staged to a MC-IN status at radiological assessment. The remaining 14 (24.6%) cases all met radiological Up-to-seven criteria after down-staging. Initially MC-IN patients at referral all underwent bridging treatments.

The method of LRT was based on the clinical assessment of the patients, the number, size and localization of tumors on preoperative imaging and the vicinity of neighboring viscera, biliary and vascular structures [11]. Local multi-disciplinary teams (MDT) decided about the most appropriate interventional radiology treatment to be given [12]. All LRT were performed by senior interventional radiologists in each Center. The total number of procedures in the entire population was 330, with a median of 2 procedures per patient (IQR=1-2). The median time between last LRT and LT was 4.0 (IQR=2.0-7.9) months. The median waiting time on the transplant list was 4 (IQR=1.7-7.5) months.

TACE was applied in 221 (80.1%) of 276 patients, with a median number of 2 (IQR=1-2) procedures per patient. The procedure, planned to be as selective as possible, was performed using 50 mg of doxorubicin mixed with 10 ml of lipiodol, followed by embolization with a gelatin sponge or with degradable starch microspheres (DSM-TACE). Thirty (10.9%) patients received real-time ultrasound (US) guided PEI, with a median number of 1 (IQR=1-2) procedure per patient. The procedure was performed using a 22-gauge 20-cm-length needle and 95% sterile ethanol injection. Seventy-nine (28.6%) patients received RFA treatment, with a median of 1 (IQR=1-1) procedure per patient. Computed tomography(CT)- or US-guided radiofrequency energy was applied for 10-15 minutes at a maximum of 2000 mA using a well-defined pulsing algorithm, either through a single or a cluster electrode [13]. Fifty-three (19.2%) patients had a multi-modal treatment consisting of TACE and RFA in 33 (11.9%) cases, TACE+PEI in 17 (6.1%) cases, and PEI+RFA in 3 (1.1%) cases.

Imaging

At the first radiological evaluation, HCC was solitary in 188 (68.1%) cases. In 257 (93.1%) cases, the target lesion was ≤5 cm, with a median target lesion size of 2.6 (IQR: 1.8-3.6) cm; after 4-6 weeks of any LRT the tumor response according to modified Response Evaluation

Criteria in Solid Tumors (mRECIST) criteria was evaluated [14]. All listed patients were followed every three months by abdominal imaging procedures (i.e., CT or magnetic resonance): in case of any change in the size of the HCC target lesion, or appearance of new nodule(s), additional radiological evaluation was performed by a specialized radiologist followed by re-discussion at the local MDT. The following features were reviewed either before and after LRT: maximum unidimensional-enhanced diameter of the target lesion on arterial phase images, number of nodules, macrovascular invasion, MC-IN and up-to-seven-IN criteria [2,15,16].

Evaluation of the mRECIST criteria was performed using one dimensional axial image of the viable portion of each nodule, defined as the enhanced portion on the arterial CT phase [14]. The last imaging available before LT showed a complete response in 147 (53.3%) patients, a partial response in 65 (23.6%) cases, a stable disease in 28 (10.1%) cases and disease progression in 34 (12.3%) patients.

Pathological Examination

Each explanted liver was examined by experienced pathologists at each Center. All livers were serially sectioned and the number, size and micro/macro-vascular invasion of the nodules were recorded. The target nodule was defined as the tumor with the greatest dimension previously treated by LRT. The viable target HCC was graded according to Edmonson and Steiner Criteria [17]. In case of multiple lesions presenting different gradings, the highest grading was considered in the final report. The necrosis percentage was assessed at the dimension of the tumor section and then confirmed by the microscopic evaluation [18]. Residual RVT at the level of the target nodule was calculated as follows:

(maximum size of the lesion in cm) – (maximum size of the necrotic area in cm)

Statistical Analysis

Continuous variables were reported as medians and IQR. Dummy variables were reported as numbers and percentages. Continuous variables were compared using the Kruskal-Wallis test; dummy variables were compared using the chi-square test or the exact Fisher test when appropriate.

Receiver operation curve (ROC) analysis was used with the intent to investigate the prognostic ability of different risk factors for post-LT recurrence. Area under the curve (AUC) and 95% confidence intervals (95%CI) were reported. The diagnostic odds ratio (DOR) was estimated for different cut-off values of the remaining vital tissue in the target lesion according to the equation:

$$DOR = (sensitivity * specificity) / [(1 – sensitivity) * (1 – specificity)]$$

Two multivariable Cox regression analyses were performed for the evaluation of the risk factors for post-LT recurrence: only pre-LT available covariates or only pathological variables were initially selected for constructing the two models. Backward conditional methods were used with the intent to identify only significative covariates. Beta-coefficients, standard errors, hazard ratios (HR) and 95%CI were reported.

Linear regression analyses were done comparing the vital tissue in the target lesion with the target lesion diameter at referral and with the time elapsed between the last LRT and LT. R^2 and F test were reported. Post-LT recurrence rates were carried out on the entire population and in the sub-group of MC-IN patients at referral using Kaplan–Meier statistics and Log-rank test.

Pathological variables were tested with the intent to identify the parameters presenting the best discriminative role in terms of recurrence risk: a univariate Cox regression analysis was performed, estimating the Akaike Information Criterion (AIC) for each variable. The smallest AIC corresponded to the best discriminative power.

Variables with a two sided $p<0.05$ were considered statistically significant. SPSS statistical package version 23.0 (SPSS Inc., Chicago, IL, USA) was used.

RESULTS

Patient survival and recurrence rate

The 1-, 3- and 5-year overall recurrence-free survival rates were 96.5%, 86.2% and 80.0%, respectively; 5-year overall patient and disease specific survival rates were 68.0% and 88.0% respectively (**Figure 1**). Thirty (10.9%) patients experienced HCC recurrence after a median time from LT of 19 (IQR=11-29) months. No recurrences were observed in the first post-LT 6 months neither in patients exceeding 5 years of follow-up.

Pathological findings in the explanted liver

At final histology, the maximum median size of the target nodule, considering both vital and necrotic tissue, was 2.5 cm (IQR: 1.8-3.6), with a median number of 2 (IQR: 1-3) lesions. Seventy-nine (28.6%) patients presented a bi-lobar involvement. HCC with an unfavorable grading (G3-G4) was present in 51 (18.5%) cases, and 85 (30.8%) patients presented microvascular invasion. Ninety-eight (35.5%) patients were histologically MC-OUT.

The median necrosis of the target nodule based on diameter size was 70% (IQR: 5-100). In 102/276 (36.9%) patients the extent of necrosis in the target nodule ranged between 50% and 90%. The median RVT at the level of the target nodule was 0.7 (IQR: 0-1.8) cm. In case of multiple nodules (n=152), only 26/276 (9.4%) patients had complete necrosis and 33/276 (21.7%) reached 50-90% of necrosis rate. Among 75 patients whom achieved complete necrosis of the target nodule, 40 had multiple nodules, among which 32 (80.0%) with viable tissue.

Predictors of post-LT HCC recurrence

At ROC analysis, the presence of microvascular invasion (AUC=0.720, 95%CI=0.620-0.820; p-value<0.0001) and the RVT of the target lesion in the explanted liver tissue (AUC=0.672, 95%CI=0.562-0.781; p-value=0.002) were found to be the strongest predictors of HCC recurrence. Although the reported AUCs of these variables were not optimal, however microvascular invasion and RVT performed better than a histological MC-OUT status (evaluated considering only the residual vital tissue of each lesion), the presence of unfavorable tumor grading (namely grade 3 or 4) and serum alpha-fetoprotein (AFP) level.

As a RVT value of 2.0 cm was identified as the best predictor of HCC recurrence (DOR value=4.6) (**Table 1**), the study population was stratified in three groups according to the response to LRT: no-RVT (n=75, 27.2%), RVT from 0.1 to 1.9 cm (n=136, 49.2%), and RVT≥2cm (n=65, 23.5%). No statistical differences were observed among these 3 subgroups in terms of demographics, underlying liver disease and baseline MELD score. Wait list time was slightly (but not statistically different) longer in the RVT≥2cm group (4.9 months) vs. the other groups (p-value=0.1). The median size of the target lesion at time of initial referral ranged between 2.5 and 2.7 in the three groups (p-value=0.9). Similarly, the number of patients with an initial MC-OUT status was similar, with a negligibly higher prevalence in the RVT≥2cm group (23.1 vs. 18.7 and 20.6%; p-value=0.8). Also the total number of LRT performed was not significantly different among the groups, nor differed the time elapsed from the last LRT received and LT (5.8 vs. 3.7 and 3.8 months; p-value=0.07).

At time of LT, the median AFP value was markedly higher in the RVT≥2cm group (12.6 ng/mL; p-value=0.004). Interestingly, no cases of patients with AFP value >400 ng/mL were observed in the no-RVT group vs. six (9.2%) cases in the RVT≥2cm group (p-value<0.0001).

The radiological response evaluated following mRECIST criteria showed important differences among the 3 groups. As expected, the no-RVT group showed a higher percentage

of patients with a complete radiological response compared to the RVT≥2cm group (66.7 vs. 30.8%; p-value<0.0001). Conversely, the RVT≥2cm group had a higher percentage of cases with disease progression (12.3 vs. 5.3% in the no-RVT group; p-value=0.005).

At final pathological examination, more aggressive tumor features were observed in the RVT≥2cm group, as indicated by the presence of an unfavorable HCC grading (29.2% of patients with grade 3 or 4 vs. 8.0% in the no-RVT; p-value=0.005) and incidence of microvascular invasion (43.1 vs. 16.0% in the no-RVT; p-value=0.002). The median size of the target nodule measured at liver histology was greater in the RVT≥2cm group compared to the other groups (3.0 vs. 2.1 and 2.5 cm; p-value<0.0001). The RVT≥2cm group showed a median vital tissue diameter of 2.9 cm vs. 0.0 cm in the no-RVT and 0.8 in the RVT 0.1-1.9 cm group, respectively (p-value<0.0001). MC-OUT histological status (considering only vital tissue) was observed in 46.2% of patients in the RVT≥ 2cm group and only in 8.0% of those in the no-RVT group; p-value<0.0001) (**Table 2**).

Risk factors for HCC recurrence and survivals

Two different multivariable Cox regression analyses were performed. Using pre-LT available variables, only mRECIST progression disease resulted as an independent risk factor for post-transplant HCC recurrence (HR=2.9; p-value=0.008).

When only pathological aspects were investigated, RVT≥2cm was a highly significant independent risk factor for post-LT recurrence (HR=3.9; p-value<0.0001), together with the number of vital lesions (HR=1.1; p-value<0.0001) and microvascular invasion (HR=3.9; p-value=0.001) (**Table 3**).

Five-year disease-free survival rate was 60.8% in the RVT≥2cm group vs. 80.9% (log-rank p-value=0.006) and 95.0% (log-rank p-value<0.0001) in the RVT 0.1-1.9 cm group and no-RVT group, respectively (**Figure 2A**). When limiting the analysis to those patients who were

classified MC-IN on pre-LT radiological imaging, the 5-year disease-free survival rate was 58.1% in the RVT≥2 cm group compared to 79.0% (log-rank p-value=0.02) and 94.0% in the RVT 0.1-1.9 cm and no-RVT group, (log-rank p-value=0.002) respectively (**Figure 2B**).

As expected, the target nodule RVT correlated with pre-LT radiological lesion size: the greater the initial target lesion, the greater was the residual tissue (R^2=0.06, F=16.8; p-value<0.0001) (**Figure 3A**). An inverse correlation was observed between RVT and time elapsed between the last LRT and LT: The longer the lapse, the lower was the RVT at final histology (R^2=0.02, F=4.4; p-value=0.04) (**Figure 3B**).

Discriminative power for the risk of HCC recurrence

After testing different pathological variables in terms of discriminative power at risk for HCC recurrence after transplantation, microvascular invasion was the best variable, with an AIC=280.6. Interestingly, RVT combined with the presence of residual vital lesions presented the second best value (AIC=284.5). RVT alone had an AIC=287.1; RVT alone or in combination with the number of vital lesions both best discriminate in terms of post-LT recurrence respect to well recognized pathological risk factors for recurrence, like complete necrosis of the target lesion (AIC=294.7), poor grading (AIC=296.4) and pathological MC-OUT status (AIC=297.7) (**Table 4**).

DISCUSSION

Although twenty years have passed away from their first proposal, MC still remain the most commonly used allocation tool in HCC patients waiting for LT [19]. These criteria only take into account tumour morphology. It is now clear that biological features such as radiological progression, AFP and explant tumour burden should also become part of the allocation and post-LT management processes [20,21].

Neo-adjuvant LRT is nowadays a standard part of care of HCC patients considered for LT both as a bridge or down-staging approach and even as a real cancer-curative therapy [10,22]. Conflicting results have been reported in this context. A recent meta-analysis suggested that LRT does not affect post-LT recurrence and survival rates [23]. On the contrary, a large mono-centre experience from the US reported very low HCC recurrence rates (<3% at 5 years) in patients with a complete pathology-proven response after LRT [10], compared to 10-15% of tumour recurrence observed in patients meeting MC at explant pathology[24,25].

In the present pluri-centre study, a population of patients receiving neo-adjuvant LRT prior to LT, mostly performed within a MC-IN setting (approximately 80% of cases) was analysed. The key message in this study is that the size of the RVT in the target HCC lesion (namely, the response to LRT at the pathologic examination) is a strong independent determinant of HCC recurrence: in fact, only 5% of patients (either within or beyond MC) with no RVT showed a 5-year HCC recurrence compared to 40% of recurrences observed in patients having a RVT≥2 cm.

Moreover, RVT had a greater ability in terms of recurrence risk discrimination when compared with very well known pathological risk factor for recurrence, like complete necrosis of the target lesion, poor grading or histological MC-OUT status.

Our findings are in accordance with those of the UCLA group, who reported that patients reaching complete tumor necrosis after LRT showed excellent disease-free survivals [10]. Another recent study from the US validated a new prognostic score called Risk Estimation of Tumor Recurrence After Transplant (RETREAT), in which the size of viable tumor tissue, AFP value at time of LT and presence of microvascular invasion were all identified as predictors of tumor recurrence [26]. The RETREAT score allowed to stratify the 5-year post-LT recurrence risk from <3% (score=0) to >75% (score≥5). The present study is in full accordance with the RETREAT one, since presenting very similar recurrence and complete

pathological response rates [26].

Of great interest is the fact that the role of RVT is valid also in the specific subgroup of MC-IN patients, with 5-year disease-free survivals of only 60% in case of RVT $\geq$2 cm; this incidence improved to 80% when the RVT was limited to 0.1-1.9 cm and even to 95% in case of no RVT. These findings indicate that post-LT recurrence risk stratification using the response to LRT is of interest not only in the overall HCC-LT setting, but also in case of MC-IN patients. Moreover, it should be also postulated that pre-LT use of LRT may have some additive role also in reducing the effective risk of tumour recurrence [27].

All these data suggest that the decision to use preoperative LRT only if the waiting period is estimated to be longer than 6 months [1] should be taken with caution, even in case of MC-IN status, mainly in the presence of concomitant risk factors (i.e., high serum AFP) [28].

The main problem of RVT is its exclusive post-LT availability. For this reason, the role of radiological response as its possible surrogate was investigated in the present study. One should note that a significant discrepancy may exist between radiological findings and the final pathology report, with radiological findings under- or over-estimating the final pathological tumor burden [29]. Also in the present series, such a phenomenon was observed, with 65% of pathological MC-IN HCC patients vs. 80% of cases estimated by preoperative imaging. However, despite this discrepancy, complete radiological response was a reliable index of complete tumour necrosis at final explant pathology. Accordingly, the no-RVT group included the highest percentage of patients showing a complete radiological response (67% of cases). This observation is clinically relevant due to the need to pre-operatively predict as close as possible the pathological finding (i.e., RVT) in the explanted liver. Moreover, when multivariable models were created, it was interesting to observe that only mRECIST progression disease was an independent risk factor for recurrence among the pre-LT available covariates, while RVT was significant among the pathological ones. As a matter

of fact, radiological evidence of residual tissue was confirmed at pathology, thus suggesting a concordance between progression disease and RVT.

The potential clinical implications of the present study are manifold. This study suggests that, as postulated by Mazzaferro [29], response to LRT plays a role in the LT setting. In specific cases, in which multiple risk factors (i.e., AFP or poor radiological response) are present, a mandatory observation period after LRT should be taken into account prior to LT, as "time" can be possibly used as a useful surrogate of tumour aggressiveness in the selection process [30-32]. After LT, once the extent of RVT is known to the clinician, a more strict biochemical and imaging surveillance protocol in high-risk patients (identified by a RVT≥2 cm) at the least in the initial (i.e., 2 years) post-LT follow-up period as well as an adapted immunosuppressive protocol should be implemented [33,34], maybe also considering protocols including sorafenib [35,36].

Unfortunately, despite our intention to minimize the presence of possible statistical biases, this study presents some limitations. Indeed, the retrospective analysis of a medium-sized population of cases (n=276) minimizes the ability to obtain solid statistical conclusions in relation to the role of LRT in predicting post-LT HCC recurrence. Unfortunately, randomized controlled trials focused on this aim are difficult to set up, hampering us to construct a study aimed at minimizing possible selection biases. Furthermore, the pluri-centre design of the study might have introduced potential weaknesses, with different experiences in treating HCC in the different centers, and without any central reading neither of radiological nor histological findings. Finally, RVT does not be used as a guide to optimize the HCC allocation process, due to the fact that it is obtainable only after pathological specimen evaluation. Indeed, even if a mRECIST-based response to LRT may adequately predict the extent of RVT, the possible overestimation suggests to be cautious in implementing a prioritization model based only on this concept.

In conclusion, this study convincingly showed the importance of upfront LRT in LT patients, even in those fulfilling MC at listing. The magnitude of viable tissue after LRT, as defined by a RVT≥2 cm in the target nodule in the explanted liver, improves the capability to predict tumor recurrence after LT. Our findings underline the need to carefully measure the viable portion of the target lesion in the transplant practice, suggesting that a strict post-LT surveillance could be advisable in patients with a RVT ≥2 cm.

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FIGURES LEGENDS

Fig 1. Kaplan Meier overall survival, recurrence free survival and specific disease survival of 276 HCC recipients undergoing upfront loco regional treatment and liver transplantation. *Overall Survival was defined as the percentage of recipients who were alive after the 1st, 2nd and 5th year of LT. Recurrence free survival and disease specific survival were defined as the percentage of recipients who not experienced HCC recurrence or HCC related-death within 5 year of follow up.*

Fig 2A. Disease-free survival curves observed in the different groups stratified according to the residual vital tissue in the target lesion at pathological specimen examination: overall population.

Fig 2B. disease-free survival curves observed in the different groups stratified according to the residual vital tissue in the target lesion at pathological specimen examination: Milan Criteria-IN cases at referral.

Fig 3A. Linear regression analysis comparing the vital tissue in the target lesion with the target lesion diameter at referral.

Fig 3B. Linear regression analysis comparing the vital tissue in the target lesion with the time elapsed between the last LRT and LT.

TABLES

Table 1. ROC analysis for the predictors of post-LT recurrence.

Variables	AUC	SE	95%CI		p value
			Lower	Upper	
Micro-vascular invasion	0.720	0.05	0.620	0.820	<0.0001
Target lesion RVT	0.672	0.06	0.562	0.781	0.002
Target lesion RVT + vital lesions	0.665	0.06	0.555	0.776	0.003
MC-OUT (only vital tissue)	0.586	0.06	0.471	0.700	0.1
Poor grading (G3-G4)	0.565	0.06	0.450	0.679	0.2
Last pre-LT AFP value	0.535	0.06	0.421	0.649	0.5

Target lesion RVT cut-off	Sensitivity (%)	Specificity (%)	DOR
1.0 cm	56.7	58.1	1.8
2.0 cm	53.3	80.1	4.6
3.0 cm	26.7	91.5	3.9

Abbreviations: AUC, area under the curve; SE, standard error; CI, confidence intervals; RVT, residual vital tissue; MC, Milan criteria; DOR, diagnostic odds ratio; LT, liver transplantation; AFP, alpha-fetoprotein.

Table 2. comparison between the groups according to the residual vital tissue in the target lesion at pathological specimen examination.

Variables	**No RVT (n=75)**	**RVT 0.1-1.9 cm (n=136)**	**RVT ≥2.0 cm (n=65)**	**p value**
	Median (IQR) or n (%)			
Age at LT (years)	57.7 (51.0-62.5)	60.1 (55.0-65.0)	58.0 (52.5-64.1)	0.05
Male gender	60 (80.0)	114 (83.8)	52 (80.0)	0.7
Waiting time (months)	4.6 (1.6-9.1)	3.5 (1.7-6.5)	4.9 (2.0-8.6)	0.1
<4 months	23 (30.7)	58 (42.6)	27 (41.5)	0.2
Underlying liver disease*				
HCV-related cirrhosis	34 (45.3)	63 (46.3)	28 (43.1)	0.9
HBV-related cirrhosis	8 (10.7)	15 (11.0)	10 (15.4)	0.6
Post-alcoholic cirrhosis	28 (37.3)	55 (40.4)	29 (44.6)	0.7
NASH-related cirrhosis	7 (9.3)	11 (8.1)	8 (12.3)	0.6
Other	8 (10.7)	15 (11.0)	5 (7.7)	0.8
Lab-MELD	11 (8-15)	10 (8-14)	10 (8-13)	0.6
Last pre-LT AFP (ng/mL)	5.0 (3.6-13.6)	9.1 (3.9-36.3)	12.6 (5.0-78.9)	0.004
>400 ng/mL	0 (-)	1 (0.7)	6 (9.2)	<0.0001
Radiological findings at referral				
Target lesion diameter (cm)	2.7 (1.8-3.3)	2.6 (1.7-3.6)	2.5 (2.0-3.6)	0.9
>5 cm	3 (4.0)	12 (8.8)	4 (6.2)	0.4
Number of nodules	1 (1-2)	1 (1-2)	1 (1-2)	0.9
> 3 nodules	6 (8.0)	11 (8.1)	9 (13.8)	0.4
MC-OUT status	14 (18.7)	28 (20.6)	15 (23.1)	0.8
mRECIST radiological response				
Complete response	50 (66.7)	77 (56.6)	20 (30.8)	<0.0001
Partial response	19 (25.3)	31 (22.8)	15 (23.1)	0.9

Stable disease	1 (1.3)	12 (8.8)	15 (23.1)	<0.0001
Progression disease	4 (5.3)	15 (11.0)	34 (12.3)	0.005
Pathological findings (necrosis + vital tissue)				
Target lesion diameter (cm)				
>5 cm	2.5 (1.8-4.0)	2.1 (1.6-3.5)	3.0 (2.5-4.5)	<0.0001
Number of nodules	5 (6.7)	3 (2.2)	8 (12.3)	0.02
> 3 nodules	2 (1-3)	2 (1-3)	3 (1-4)	0.03
MC-OUT status	13 (17.3)	28 (20.6)	18 (27.7)	0.3
Pathological findings (only vital tissue)	20 (26.7)	42 (30.9)	36 (55.4)	0.001
Target lesion diameter (cm)				
>5 cm	0 (-)	0.8 (0.4-1.3)	2.9 (2.3-3.8)	<0.0001
Necrosis on target lesion (%)	0 (-)	0 (-)	5 (7.7)	<0.0001
Number of nodules	100 (100-100)	70 (20-85)	0 (0-20)	<0.0001
> 3 nodules	0 (0-1)	1 (1-3)	2 (1-4)	<0.0001
MC-OUT status	6 (8.0)	23 (16.9)	16 (24.6)	0.03
Poor grading (G3-4)	6 (8.0)	23 (16.9)	30 (46.2)	<0.0001
Microvascular invasion	6 (8.0)	26 (19.1)	19 (29.2)	0.005
	12 (16.0)	45 (33.1)	28 (43.1)	0.002
Total number of LRT	1 (1-2)	2 (1-3)	2 (1-3)	0.07
Time lapse last LRT-LT (months)	5.8 (2.1-12.3)	3.7 (1.9-7.2)	3.8 (2.0-7.0)	0.07

* More patients having multiple liver diseases.

Abbreviations: RVT, residual vital tissue; n, number; IQR, interquartile ranges; LT, liver transplantation; HCV, hepatitis C virus; HBV, hepatitis B virus; NASH, non-alcoholic steato-hepatitis; MELD, model for end-stage liver disease; AFP, alpha-fetoprotein; MC, Milan criteria; mRECIST, modified Response Evaluation Criteria In Solid Tumors; LRT, loco-regional treatments.

Table 3. Multivariable Cox regression analyses for the risk factors of post-LT recurrence: first model based only on pre-LT available variables; second model based only on pathological variables.

Variables	Beta-coefficient	SE	HR	95%CI		p value
				Lower	Upper	
First model: only pre-LT available variables						
mRECIST progression disease	1.066	0.4	2.9	1.3	6.3	0.008
Second model: only pathological variables						
RVT $\geq$ 2.0 cm	1.359	0.4	3.9	1.8	8.3	<0.0001
Number of lesions (only vital tissue)	0.120	0.03	1.1	1.1	1.2	<0.0001
Micro-vascular invasion	1.370	0.4	3.9	1.8	8.7	0.001

First model: -2 Log Likelihood=291.4
Variables initially introduced in the model and then elided using a backward conditional method: male gender, age at LT (per year), waiting time <120 days, HCV-related cirrhosis, HBV-related cirrhosis, MELD > 15 at LT, radiological dimension of the target lesion > 5 cm at referral, radiological number of nodules >3 at referral, radiological MC-OUT status at referral, AFP value > 400 ng/mL at LT, total number of LRT, time lapse last LRT-LT (months), mRECIST complete response .

Second model (only pathological variables): -2 Log Likelihood=261.2
Variables initially introduced in the model and then elided using a backward conditional method: diameter of the target lesion (only vital tissue), MC-OUT status (only vital tissue), necrosis percentage of the target lesion, complete necrosis of the target lesion, multifocal tumor, bilobar tumor, poor grading (G3-4).

Abbreviations: SE, standard error; HR, hazard ratio; CI, confidence intervals; LT, liver transplantation; mRECIST, modified Response Evaluation Criteria In Solid Tumors; RVT, residual vital tissue; HCV, hepatitis C virus; HBV, hepatitis B virus; MELD, model for end-stage liver disease; MC, Milan Criteria; AFP, alpha-fetoprotein; LRT, locoregional therapies.

Table 4. Discriminatory ability of different pathological variables in terms of post-LT HCC recurrence risk.

Variables	AIC	Beta	SE	HR	95%CI		p value
					Lower	Upper	
Microvascular invasion (Y/N)	280.6	1.748	0.4	5.7	2.6	12.5	<0.0001
Target lesion RVT cm + number of vital lesions	284.5	0.134	0.02	1.1	1.1	1.2	<0.0001
Target lesion diameter cm + number lesions (vital + necrotic)	284.9	0.133	0.02	1.1	1.1	1.2	<0.0001
Target lesion RVT cm	287.1	0.395	0.09	1.5	1.3	1.8	<0.0001
Target lesion RVT > 2.0 cm	287.8	1.448	0.4	4.3	2.1	8.7	<0.0001
Target lesion diameter cm (vital + necrotic)	290.8	0.258	0.06	1.3	1.1	1.5	<0.0001
Number of vital lesions	292.1	0.116	0.03	1.1	1.1	1.2	<0.0001
Number of lesions (vital + necrotic)	293.0	0.114	0.03	1.1	1.1	1.2	<0.0001
Target lesion complete necrosis (Y/N)	294.7	-1.400	0.6	0.2	0.1	0.8	0.02
Poor grading (G3-4)	296.4	1.051	0.4	2.9	1.3	6.3	0.009
MC-OUT (necrotic + vital tissue) (Y/N)	297.7	0.792	0.4	2.2	1.1	4.5	0.03
MC-OUT (only vital tissue) (Y/N)	298.2	0.806	0.4	2.2	1.1	4.7	0.03
Target lesion necrosis (%)	300.8	-0005	0.004	1.0	1.0	1.0	0.2

Abbreviations: AIC, Akaike information criterion; SE, standard error; HR, hazard ratio; CI, confidence intervals; Y, yes; N, no; RVT, residual vital tissue; MC, Milan Criteria.

Figure 1

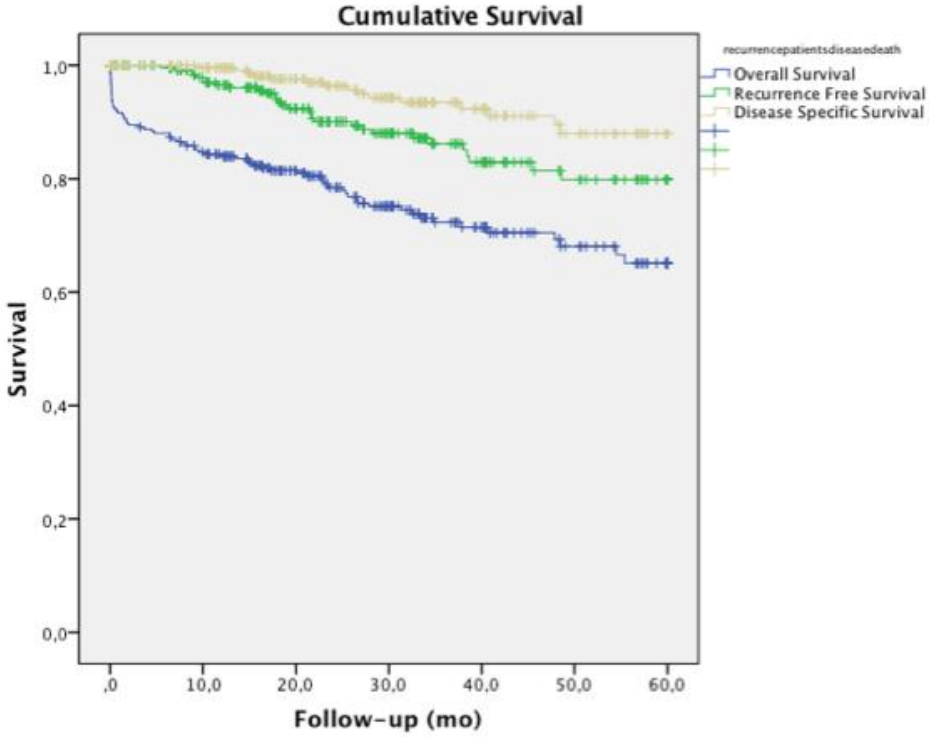

Figure 2

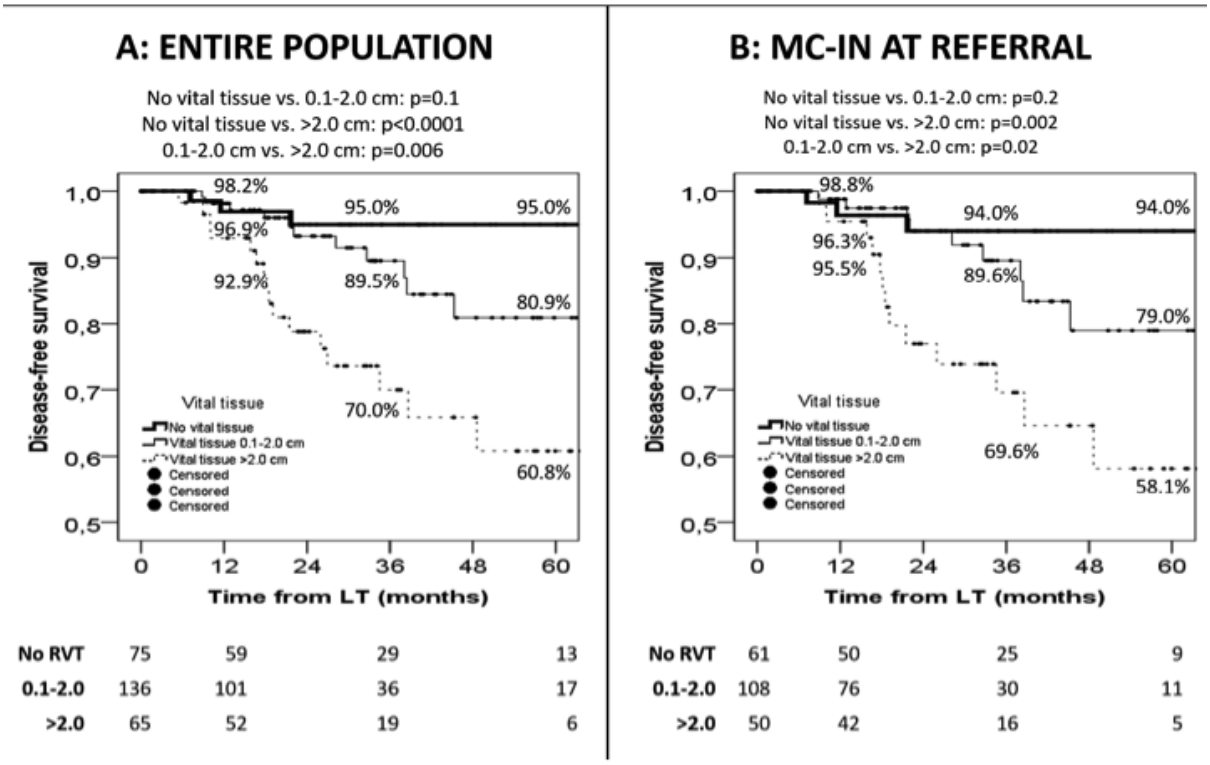

Figure 3