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ORIGINAL ARTICLE

Liver transplantation for hepatocellular cancer: UCL experience in 137 adult cirrhotic patients. Alpha-foetoprotein level and locoregional treatment as refined selection criteria

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alpha-foetoprotein, downstaging, hepatocellular cancer, liver transplantation, locoregional treatment.

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Summary

Liver transplantation (LT) is a validated treatment for selected cirrhotics with hepatocellular cancer (HCC). A retrospective single center study including 137 recipients having proven HCC was done to refine inclusion criteria for LT as well as to look at impact of locoregional treatment (LRT) on outcome. At pre-LT imaging, 42 (30.6%) patients were Milan criteria (MC)-OUT; 28 (20.4%) were University of California San Francisco criteria (UCSFC)-OUT. Pre-LT LRT was performed in 109 (79.6%) patients. Multivariate analysis identified four factors to be independently predictive of recurrence: tumour number >3, AFP level ≥400 ng/ml, microvascular invasion and rejection needing anti-lymphocytic antibodies. When considering pre-transplant variables only, AFP level ≥400 ng/ml (HR = 5.13; $P < 0.0001$) was the unique risk factor for recurrence; conversely, application of LRT was protective (HR = 0.42; $P = 0.04$). MC-IN patients having LRT ($n = 79$) had the best 5-year tumour-free survival (TFS) (91.6%). MC-IN patients without LRT ($n = 16$) and MC-OUT patients with LRT ($n = 30$) had similar good TFS (72.7% vs.77.5%); finally MC-OUT patients without LRT ($n = 12$) had the worst results (45.0%; vs. 1st group: $P < 0.0001$). Immediate pre-LT AFP and aggressive pre-transplant LRT strategy, especially in MC-OUT patients, are both important elements to further expand inclusion criteria without compromising long-term results of HCC liver recipients.

Introduction

Liver transplantation (LT) represents a widely accepted therapeutic option for (early) hepatocellular cancer (HCC) in cirrhotic patients. The Milan criteria (MC), this means a single tumour of ≤5 cm in diameter, or 2–3 tumours of ≤3 cm in diameter and absence of macrovascular invasion, moved LT from a rescue towards a real curative treatment of HCC [1,2]. Five-year overall and disease-free survival rates of 83% and 75%, as reported by Mazzaferro, have been confirmed afterwards by several groups [3,4]. Despite the need to rationalize the use of liver grafts in an era of organ shortage, the MC have the major drawback to be too restrictive, limiting thereby the access to LT to many patients. This consideration is especially important in view of the continuously rising incidence of alcoholic-, non-alcoholic fatty- (NASH) and HCV-related liver diseases, all known to be major risk factors for the development of HCC [5]. The fact that long-term survivals were observed in several patients

harbouring, on the hepatectomy specimen, HCC outside the MC, led to a progressive widening of the inclusion criteria for LT. Yao showed in 2001 that similar results can be obtained in patients harbouring a solitary tumour of ≤6.5 cm, or three or fewer tumours, the largest lesion having a diameter of <4.5 cm and the total tumour diameter being ≤8 cm [6]. The validity of the University of California San Francisco criteria (UCSFC) has been also confirmed during the last years by several groups [7–9]. Some Asian centres, mainly the Seoul, Tokyo and Kyoto groups, further expanded the inclusion criteria for LT even up to ten tumours and a maximal tumour diameter of 5 cm [10–13]. Such aggressive approach towards HCC is based on two fundamental conditions: the factor 'time' is eliminated in the context of the living donor LT and morphologic as well as biological tumour behaviour are taken into account when selecting the potential recipients. By doing so, an excellent 75% 5-year disease-free survival has been obtained. It must however be stressed that broadening of the inclusion criteria is not without danger as clearly shown by the ''Metroticket concept'', elaborated from a multicentre, transcontinental survey of outcome of LT for HCC in 'MC out' patients. Unlimited extension of criteria indeed carries the real risk to transform waiting list drop-out rate (due to tumour progression) into a high post-LT recurrence rate [14].

This single centre, retrospective study analyses the impact of the MC and UCSFC on outcome of LT in the treatment of HCC in cirrhotic patients, looking thereby at possible newer markers as well as at the impact of neo-adjuvant loco-regional therapies (LRT) as potential means to cautiously extend the indication for LT in these patients.

Material and methods

Data collection

During the period March 1987–November 2009, 159 patients were transplanted at our institution having HCC. Fourteen patients presenting incidental small HCC discovered only at pathological specimen and eight patients with unclear pre-LT diagnosis on state of the art imaging were excluded from the analysis. One hundred thirty-seven patients with a pre-operatively proven diagnosis of HCC were thus finally enrolled in the present study. Characteristics of the overall cohort regarding recipient, pre-transplant workup and treatment, allograft and tumour are displayed in Table 1.

Table 1. Demographic data of 137 HCC cirrhotic liver recipients.

Variable	Distribution
Age (median; range) (years)	57 (30–71)
Male gender (%)	114 (83.2)
Aetiology: (%)*	
HCV-related cirrhosis	57 (41.6)
HBV-related cirrhosis	32 (23.4)
Alcohol-related cirrhosis	45 (32.8)
Other liver pathologies	15 (10.9)
CTP class C (%)	26 (19.0)
Lab-MELD ≥15 points (%)**	35 (25.5)
AFP level ≥400 ng/ml (%)	15 (10.9)

*In 8 cases HCV and alcohol, in 3 cases HBV and HCV, in 1 case HBV and alcohol cirrhosis.

**Lab-MELD calculation according to laboratory values without taking into account bonus points attributed to HCC.

HCV, Hepatitis C Virus; HBV, Hepatitis B Virus; CTP, Child-Turcotte-Pugh; MELD, Model for End Stage Liver Disease; AFP, Alpha-foetoprotein.

Diagnosis and HCC staging

Diagnosis of HCC was made if typical features of HCC were present on two different imaging modalities (e.g. Doppler-ultrasound, CT-scan and/or MRI) and/or one imaging modality supported by an alpha-foetoprotein (AFP) level ≥400 ng/ml [15].

In 9 (6.6%) cases in which imaging was insufficient, tumour diagnosis was confirmed by imaging guided biopsy.

In 1996, MC were adopted as selection criteria for registration on the waiting list; since 2001 selection criteria were extended to the UCSFC. The latter criteria were considered the acceptable upper limit of tumour progression during the waiting time. All patients exceeding UCSFC during this period were maintained on the list only in case of good response after LRT.

Liver allocation system

According to the Eurotransplant Foundation, liver allocation was done in relation to recipient urgency status and waiting time during the period 1991–2006; from 2007 onwards the allocation was based on lab- and/or exception MELD scores. Patients with UICC tumour stage II [16] and lab-MELD score below 22 got an initial bonus of 22 points; this score was raised every 3 months by three points.

Treatment of HCC on the LT wait list

Locoregional treatment was performed accordingly to the pre-LT management guidelines proposed by the European Association for the Study of the Liver (EASL) [15]. Several sessions of LRT were applied in accordance to the tolerance of this treatment. LRT consisted of partial liver resection, transarterial chemoembolization (TACE),

percutaneous ethanol injection (PEI), and radiofrequency (RF) destruction.

Assessment of pathological response to neo-adjuvant LRT

Patients were followed-up monthly after LTR by computed tomography (CT) or magnetic resonance (MRI) imaging. Pathological response after LRT was graded into three categories according to the documented necrosis rate at definitive pathological examination of the total hepatectomy specimen: complete response (100%), partial response (99–30%) and poor response (<30%).

Tumour downstaging was defined as a decrease in tumour size corresponding to a total or partial response after LRT in the absence of new lesions, when comparing pre-transplant CT or MRI imaging and histological examination of the liver specimen.

Immunosuppression

Induction and maintenance immunosuppression (IS) varied over the time, according to ongoing protocols. Between March 1987 and December 1996, a cyclosporine-A (CyA) based triple drug IS regimen, including steroids and azathioprine, was used. From 1997 onwards, oral tacrolimus (TAC) was used. Steroid discontinuation was obtained in nearly all patients; CyA or TAC monotherapy regimens were achieved following clinical evolution [17–19]. Mofetil mycophenolate (MMF) was introduced in case of renal dysfunction and rapamycin (RAPA) was used in case of HCC recurrence or *de-novo* tumour development and,recently, as first-line IS regimen in 16 (11.7%) patients included in the multicentre SILVER study [20].

Induction therapies with anti-lymphocytic antibodies using polyclonal rabbit anti-lymphocytic antibodies (R-ATG, Fresenius Biotech, Bad Homburg, Germany), anti-CD2 monoclonal antibodies (BTI 322, Biotransplant, Boston, MA, USA) and anti-CD25 monoclonal antibodies (Lo-TACT, Biotransplant) were all used only in the context of prospective clinical studies. Biopsy-proven corticosteroid-sensitive rejection was treated with five boluses of 200-mg methylprednisolone; corticosteroid-resistant rejection was treated with anti-CD3 monoclonal antibodies (OKT3, Cilag, NJ, USA).

Liver transplant procedure

Surgical and medical management have been described previously in detail [21]. From 1987 to 1991, LT included resection of the inferior vena cava (IVC); since 1991, IVC sparing LT was done in almost all recipients, even if the tumour was located in the right posterior or paracaval segments. Piggy-back implantation technique was used from 1987 to 1994; afterwards the graft was implanted using side-to-side cavo-cavostomy. Veno-venous bypass was used until 1991 in 19 (12%) patients only. In 60 (38%) patients intra-operative blood salvage was applied using Cell-saver device (Sorin group, Arvada, CO, USA)

Patient follow-up

All patients were followed-up in our liver transplant outpatient clinic. Screening for tumour recurrence was done by repetitive measurement of AFP levels and by 3-monthly sonography. Routine CT-scan of the abdomen and chest was performed half yearly or yearly depending on the outcome of the histological examination; additional imaging techniques such as scintigraphy and MRI, were used in case of suspected HCC recurrence. No patient received adjuvant chemotherapy.

As of December 31, 2011, the median follow-up for the entire population was 5.0 years (range: 0.2–22.2).

Statistical analysis

Categorical variables were reported as the number of cases and percentages; continuous variables were given as median (range) values. A univariate analysis to identify prognostic risk factors was performed with a Cox regression model; a multivariate analysis was conducted afterwards. The model was build using the variables who presented a *P*-value of <0.20 at univariate analysis. Goodness of fit for the model was tested with the cAIC (corrected Akaike Information Criterion) test. The risk prediction was reported as *P* value, odds ratios (OR), and 95% confidence intervals (95% CI).Overall survival (OS) was defined as the time interval between date of LT and of death (from any cause). Graft survival (GS) was defined as the time interval between date of LT and of graft loss (from any cause). Tumour-free survival (TFS) was defined as the time interval between date of LT and of HCC recurrence. Survival was analyzed using Kaplan–Meier method and compared with the log-rank test. Statistical significance was reached at $P \leq 0.05$. Statistical analyses and plots were performed with SPSS 19.0 (SPSS, Chicago, IL, USA).

Results

Median age of the 137 patients at time of LT was 57 years (range: 30–71); 85 (62%) patients were older than 55 years. The most common underlying diseases were HCV-related (41.6%), alcoholic (32.8%) and HBV-related (23.4%) cirrhosis.

According to the sixth edition of TNM staging system [15], 75 patients (54.7%) were classified in stage I, 55 (40.1%) in stage II, and 7 (5.1%) in stage III.

According to imaging assessment, 42 (30.6%) patients were MC-OUT and 28 (20.4%) patients were UCSFC-OUT. Before 1996, 11 (38%) of 29 patients exceeded UCSF criteria, whilst only 17 (15.7%) of 108 recipients did after 1996.

One hundred-nine (79.6%) patients underwent at least one LRT with the aim to stabilize or downstage (DS) the tumour during the waiting time. LRT consisted of partial liver resection ($n = 7$), TACE ($n = 84$), PEI ($n = 40$), and RF ($n = 10$). The number of LRT varied from one to seven (median: 2). Sixty-seven (48.9%) patients had at least 2 LRT procedures; 35 (25.5%) patients had one LRT only. During TACE, cisplatin (36% of patients), doxorubicin (26.7% of patients), or an emulsion of both (37.3% of patients) were used as chemotherapeutic agents.

One hundred thirty-seven transplants including 15 re-transplantations [of whom six were done early (<3 months) and nine late early (>3 months)] were performed using a whole liver from a post-mortem donor (90.1%); sequential, right split, right living donor grafts were used in four, five and six cases, respectively. In all but one cases of sequential or living donor LT the recipient exceeded UCSFC at initial radiological examination.

Comparison between clinical and pathological tumour staging is displayed in Table 2.

At pathological analysis, 13 (9.5%) patients presented a poorly differentiated HCC. Macro- and micro-vascular invasion was observed in 3 (2.2%) and in 18 (13.1%) patients, respectively. Lymphatic permeation was found twice and tumour capsule rupture four times. In 32 (23.3%) patients, no residual viable tumour was detected: all had received one or more LRT before LT. The necrotic nodules at histology ranged from 9 to 65 mm.

One, 3- and 5-year OS rates were 88.3%, 75.7%, and 68.6%, whilst the corresponding GS rates were 85.4%, 70.9% and 61.8%, respectively. Forty-seven (34.3%) patients died during the follow-up after a median post-LT interval of 25.2 months (range 0–133). The causes of patient and graft losses are listed in Table 3.

Twenty-four (17.5%) patients presented tumour recurrence after a median delay of 15.3 months (range 6–88).

Table 2. Comparison between clinical and pathological AJCC–UICC staging system (6th ed.).

	Radiology staging	Pathology staging
Stage I	75 (54.7%)	50 (36.5%)
Stage II	55 (40.1%)	46 (33.6%)
Stage III	7 (5.1%)	9 (6.6%)
Stage IV	–	–
No residual viable tumour	–	32 (23.3%)

Table 3. Causes of death and retransplantation after LT for HCC.

	Number of patients	Delay after LT (d) (median/ranges)
Cause of death (%)	$n = 47/137$ (34.3)	
Peri-operative death	1 (2.1)	(–)
Delayed graft function	2 (4.3)	19 (16–21)
Post-LT lymphoproliferative disease	2 (4.3)	479 (144–813)
De novo cancer	2 (4.3)	1833 (1386–2279)
Neurologic event	2 (4.3)	2882 (1780–3984)
Bacteria, viral, fungal infection	4 (8.5)	77 (54–138)
Cardiovascular	5 (10.5)	1462 (129–2230)
Recurrent HBV/HCV infection	9 (19.1)	670 (178–2886)
Recurrent hepatocellular cancer	20 (42.6)	769 (226–2593)
Indication for re-transplantation (%)	$n = 15/137$ (10.9)	
7^{th} day syndrome (acute rejection)	1 (6.6)	7 (–)
Biliary complication	3 (20.0)	185 (52–1208)
Chronic rejection	3 (20.0)	1791 (555–4748)
Primary non-function	4 (26.7)	5 (1–7)
Recurrent viral allograft disease	4 (26.7)	1066 (655–1431)

LT, Liver Transplantation; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus.

One-, 3- and 5-year TFS rates of the entire patient cohort were 91.3%, 84.8%, and 82.6%.

The univariate Cox regression model revealed microvascular invasion ($P < 0.0001$), presence of more than 3 nodules ($P = 0.03$), major nodule diameter of over 5 cm ($P = 0.09$), MC-OUT status ($P = 0.005$), downstaging ($P = 0.003$), 100% tumour necrosis ($P = 0.03$), all at definitive pathology of the hepatectomy specimen, LRT ($P = 0.004$), LT performed before 1996 ($P = 0.002$), AFP level ≥400 ng/ml ($P < 0.0001$), IVC replacement ($P = 0.004$), use of bypass ($P = 0.003$), tacrolimus monotherapy ($P = 0.05$) and corticosteroid-resistant rejection ($P = 0.007$), all as factors significantly influencing the risk of recurrence (Table 4).

In the multivariate analysis, number of tumours >3 (HR = 5.87. $P = 0.05$), AFP level ≥400 ng/ml (HR = 4.86; $P = 0.01$) microvascular invasion (HR = 3.58; $P = 0.01$) and steroid-resistant rejection needing treatment using anti-lymphocytic antibodies (HR = 4.56; $P = 0.05$) finally came out as independent risk factors for the development of HCC recurrence after LT.

When considering the pre-transplant available variables only, AFP level ≥400 ng/ml (HR = 5.13; $P < 0.0001$) was a risk factor for HCC recurrence, conversely overall

Table 4. Results of the univariate analysis assessing the impact on disease-free patient survival in 137 cirrhotic patients transplanted during the period March 1987–November 2009.

Variable	OR (95% CI)	*P*
Male gender	0.60 (0.24–1.50)	0.27
Age >55 years	0.77 (0.34–1.72)	0.53
LT before 1996	3.64 (1.63–8.12)	0.002
HBV-related cirrhosis	1.23 (0.51–2.97)	0.65
HCV-related cirrhosis	0.89 (0.39–2.03)	0.78
Waiting list time >6 months	1.13 (0.50–2.55)	0.77
Medical MELD Score ≥15	1.37 (0.58–3.20)	0.47
CTP class C	0.54 (0.16–1.82)	0.32
>3 nodules at pathology	2.73 (1.08–6.90)	0.03
Pathological major nodule diameter >5 cm	2.55 (0.87–7.47)	0.09
Pathological Milan criteria-out	3.13 (1.40–7.00)	0.005
LRT	0.30 (0.13–0.68)	0.004
>2 LRTs	1.03 (0.46–2.29)	0.95
Downstaging	0.20 (0.07–0.58)	0.003
AFP level ≥400 ng/ml	6.43 (2.80–14.76)	<0.0001
IVC replacement	3.91 (1.55–9.87)	0.004
Veno-venous bypass	3.76 (1.55–9.09)	0.003
IOBS use	0.57 (0.21–1.55)	0.27
100% tumor necrosis at pathology	0.11 (0.01–0.84)	0.03
Tumoral capsular effraction	3.23 (0.76–13.80)	0.11
Microvascular invasion	4.66 (2.02–10.72)	<0.0001
Satellite nodules	1.48 (0.44–4.99)	0.52
Cyclosporine monotherapy	0.79 (0.11–5.88)	0.82
Tacrolimus monotherapy	0.41 (0.17–1.00)	0.05
Corticosteroid resistant rejection needing anti-lymphocytic antibodies	3.97 (1.46–10.78)	0.007

LT, Liver Transplantation; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; MELD, Model for End Stage Liver Disease; CTP, Child-Turcotte-Pugh; LRT, Locoregional Treatment; AFP, Alpha-foetoprotein; IVC, inferior vena cava; IOBS, intraoperative blood salvage.

Table 5. Results of the multivariate analysis assessing the impact on disease-free patient survival in 137 cirrhotic patients transplanted during the period March 1987–November 2009.

	OR (95% CI)	*P*
All variables*		
AFP level ≥400 ng/ml	4.86 (1.36–17.34)	0.01
Microvascular invasion	3.58 (1.34–9.54)	0.01
>3 nodules at pathology	5.87 (1.00–35.14)	0.05
Corticosteroid resistant rejection needing anti-lymphocytic antibodies	4.56 (1.00–21.28)	0.05
Tumoral capsule rupture	4.39 (0.77–25.13)	0.10
100% tumor necrosis at pathology	0.13 (0.01–1.47)	0.10
Pathological Milan criteria-out	0.45 (0.15–1.38)	0.16
Pathological major nodule diameter >5 cm	2.36 (0.57–9.76)	0.24
LT before 1996	1.72 (0.38–7.82)	0.48
Veno-venous bypass	0.43 (0.04–4.88)	0.50
LRT	0.70 (0.19–2.61)	0.59
Downstaging	0.75 (0.19–3.04)	0.69
Tacrolimus monotherapy	0.94 (0.27–3.30)	0.92
IVC replacement	1.09 (0.09–12.55)	0.94
Only pre-operatively available variables**		
AFP level ≥400 ng/ml	5.13 (2.80–14.76)	<0.0001
LRT	0.42 (0.18–0.98)	0.04
LT before 1996	5.70 (0.81–40.33)	0.08
Downstaging	0.49 (0.20–1.21)	0.12

*cAIC=182.71.

**cAIC = 96.85.

AFP, Alpha-foetoprotein; LT, Liver Transplantation; LRT, Locoregional Treatment; IVC, Inferior Vena Cava.

application of LRT (HR = 0.42; $P = 0.04$) was a protective factor (Table 5).

Four different groups were identified when stratifying the whole patient cohort according to MC status and preoperative LRT. MC-IN patients having LRT ($n = 79$) had the best 5-year TFS (91.6%). Surprisingly MC-IN patients without LRT ($n = 16$) and MC-OUT patients with pre-LT LRT ($n = 30$) had similar good TFS of 72.7% and 77.5%. MC-OUT patients without LRT ($n = 12$) had the worst results (45%) (Fig. 1).

Each subgroup was compared to the others using log-rank testing. MC-IN patients treated with LRT did significantly better than MC-IN untreated ($P = 0.05$); MC-OUT treated ($P = 0.03$), and MC-OUT untreated patients ($P < 0.0001$).

No significant differences in TFS were observed between MC-OUT treated and MC-IN untreated patients ($P = 0.93$). MC-OUT patients treated with LRT had a significantly better outcome when compared to MC-OUT untreated patients ($P = 0.06$). Interestingly, no survival difference was reported when untreated MC-IN patients were compared to MC-OUT patients ($P = 0.13$).

When stratifying the population according to the number of LRT, untreated patients ($n = 28$) had the worst 5-year TFS (62.8%), patients who had 1 or 2 LRT ($n = 55$) had the best results (91.4%) and patients who received more than 2 LRT ($n = 54$) had intermediate results (84.0%) (Fig. 2). Comparing these groups using the log-rank test, untreated patients did worse than patients having 1 to 2 and more than 2 LRT ($P = 0.003$ and 0.04, respectively). No statistical significance was reported between the patients treated with 1–2 or more LRT ($P = 0.24$).

The lack of further improvement of results in the latter group can be explained by the fact that this subgroup included significantly more MC-OUT patients (40.7% vs. 14.5%; $P = 0.002$), a greater number of patients with the major lesion >5 cm (16.7% vs. 1.8%; $P = 0.007$) and more patients with AFP ≥400 ng/ml (14.8% vs. 1.8%; $P = 0.01$) than the patient group that had 1 or 2 LRT.

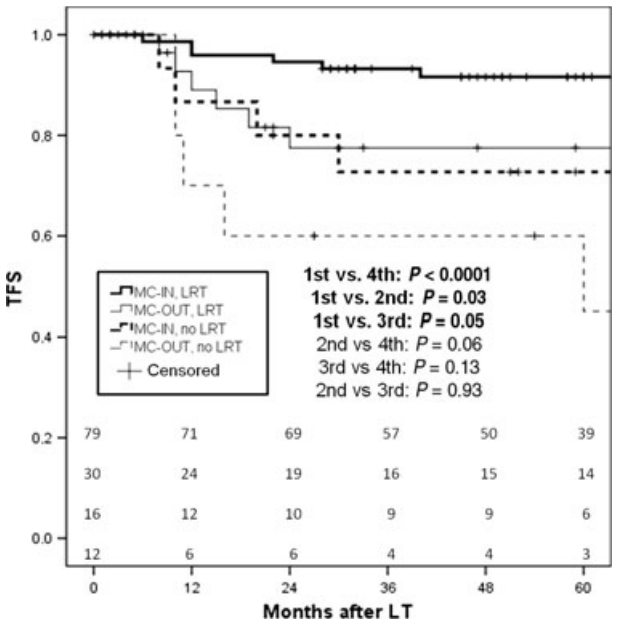

Figure 1 Tumour-free survival according to the MC status and pre-transplant LRT in 137 recipients.

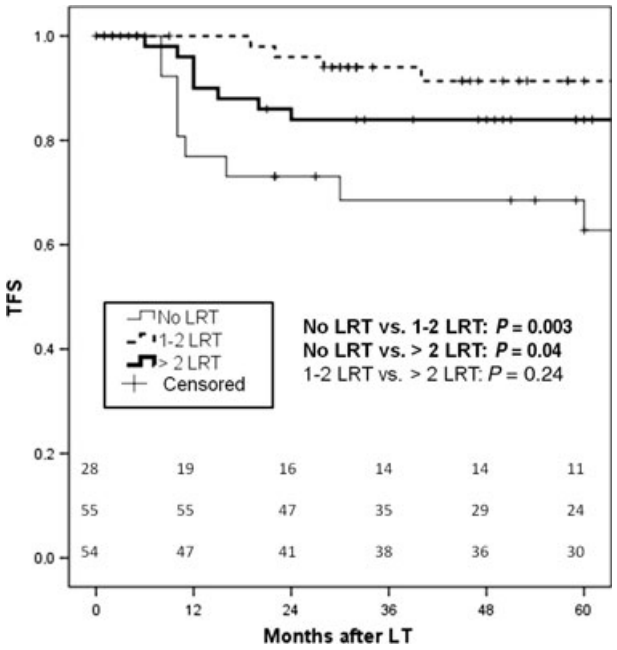

Figure 2 Tumour-free survival according to the pre-transplant LRT number in 137 recipients. The patient group having had more than two LRT had a significantly larger tumour burden.

Discussion

In the 1980s, the outcome after LT for HCC was discouraging due to the fact that the transplant was offered to patients bearing too advanced tumours. Several studies showed that tumour size and number are the two determinant prognostic factors in LT for HCC. Bismuth was the first to demonstrate that size and number of tumours matters; HCC patients with two or fewer lesions, each with a diameter ≤3 cm, had a 3 years tumour-free survival of 83% [22]. Shortly after this observation, Mazzaferro introduced in 1996 the well-known MC in clinical practice [2]. These criteria are nowadays worldwide accepted as the benchmark against which all treatments for HCC must be compared. Recently, numerous proposals have been made in order to expand the MC and thus to broaden the access to LT for more cancer patients. The introduction of the UCSF criteria have indeed shown that the MC were too restrictive, denying unjustifiably the access to LT to an important (around 15–20%) number of patients [23]. Based on the major advantage of the living donor LT, eliminating the tumour time factor, the inclusion criteria for LT have been continuously broadened over the last years in Asia [10–13]. The Tokyo group adheres to the 5 to 5 rule (number of tumours ≤5 and maximum tumour diameter ≤5 cm); the Kyoto group even applies the 10 to 5 rule (number of tumours ≤10, each tumour ≤5 cm) but adding a cut-off level of ≤400 mAu/ml of the biological tumour marker des-carboxyprothrombin (DCP). Finally the Seoul group has adopted an intermediate policy, limiting the number of tumours to six with a maximum tumour diameter of 5 cm. All three Asiatic groups obtained excellent 5-year tumour-free rates of around 80% [11].

Despite these results one should extend the inclusion criteria carefully as such strategy carries the risk to transform the dropout rate on the waiting list into a prohibitive recurrence rate after LT. The debate about the maximum acceptable tumour burden allowing access to LT without compromising the results is still ongoing. The MC remain without any doubt also the gold standard to which every extension of criteria should be weighted off. Inclusion criteria can only be reasonably extended when taking into account better imaging, improved and aggressive bridging and/or downstaging strategies as well as tumour biology, including analysis of molecular tumour markers [24,25]. The value of adjuvant LRT as bridging and/or downstaging strategies is still not yet clearly defined, mainly because of the lack of clear definition of endpoints. Indeed some retrospective studies showed that multimodal adjuvant LRT only confers a moderate survival benefit, but the importance of LRT should be judged in view of the possibility to bring firstly the HCC patients to LT and secondly to judge its impact on long-term survivals after LT [26–29]. It is well known from literature that all loco-regional treatments, including partial hepatectomy, allow to obtain almost similar 3-year survival

rates. What really matters is in fact the long-term TFS rate and without any doubt the combination LRT–LT has the best cards in hand to reach this aim. The value of pre-LT LRT should therefore be analysed in this context.

In the present study, pathological (microvascular invasion, number of nodules) and biological (level of AFP) parameters were significant independent risk factors for post-transplant HCC recurrence at multivariate analysis, whilst LRT and total necrosis following LRT weren't, thereby indicating that, as can be expected, 'tumour-related' features are linked more to risk of recurrence than the 'management-related' ones [30]. However, the analysis of pre-transplant available variables only, sorted LRT out as a protective factor against recurrence. This observation is even of more strength taking into account that about one out of three and one out of five recipients exceeded MC and UCSF criteria respectively at initial imaging; despite this, excellent TFS rates were obtained in both patient groups.

LRT seems to play an important role especially when considering MC-OUT patients for LT. Indeed LRT allowed obtaining a total tumour response in one fourth of treated patients and it also significantly influenced long-term TFS. Patients receiving LRT experienced better outcomes than patients who didn't receive any LRT. In contrast to what could have been expected, a higher number of LRT did not further improve results. It is indeed a basic oncologic principle that more frequent (chemo)therapies allow to interfere with a larger number of cell cycle divisions [31]. The fact that the patient cohort receiving more than two LRT had a larger tumour burden and a higher biological aggressiveness, as documented by higher pre-LT AFP values, possibly explains the findings observed in our patient material.

Nevertheless all these observations, may allow to conclude that LT can be offered safely to extended-criteria patients on the condition that the disease is controlled, this means stabilized or downsized, using an aggressive LRT policy during the waiting period. Data from the Mainz and Innsbruck HCC–LT patient cohorts are in agreement with our findings [26,27]. Further studies need to be done to not only to confirm these results but also to look at the impact of repeated LRT on outcome after LT.

The role of the different types of LRT was not analyzed because of the heterogeneity of the treatment modalities, the small number of patients in each LRT group and of recurrences. Moreover, the present study span of 22 years explains the discrepancy between numbers of PEI ($n = 40$) and of, possibly more effective, RF ($n = 10$). The former method was the preferred one in the 1980 and 1990s, whilst the latter one has been applied more frequently during the last decade. The long time span also implied clear differences in patient selection. More advanced tumours were transplanted before the introduction of MC. Despite the fact that this parameter strongly impacts on risk of recurrence and OS, this patient group remained included in the analysis, being well aware of the introduction of potential statistical biases.

It is very well known that the tumour bulk doesn't always reflect the tumour aggressiveness and behaviour. In our multivariate analysis, AFP level ≥400 ng/ml significantly influenced the risk for HCC recurrence. The use of other criteria to select the candidates for LT, such as AFP dynamics with or without downstaging procedures [25,32], routine use of DCP as a complementary marker for vascular invasion and lower grade differentiation [33], tumour volume and more sophisticated indicators of the tumour proliferative status (molecular biology) [24,31,34,35] will be needed as predictors of outcome after LT.

Adjustment of IS to the oncologic status is another important factor when extending the inclusion criteria for LT [18]. LT is not only a surgical act, but it also implies a life-long medical treatment compromising the immunological status of the patient. Heavy IS contributes to accelerated tumour growth and decreased survival, as showed by several studies [36,37].

Our study pointed out that use of anti-lymphocytic antibodies as treatment for rejection negatively impacts on outcome. The development of targeted HCC therapy will undoubtedly play a pivotal role in the treatment of HCC liver recipients. The results of the 'Silver study' will hopefully confirm the beneficial role of rapamycin demonstrated already previously in non-controlled transplant studies [20]. There is also some evidence that m-Tor inhibitors can be safely associated with sorafenib as adjuvant treatment in recipients at high-risk for recurrence [38].

In conclusion, better knowledge of tumour biology and use of LRT, especially in MC-OUT patients, together with a more precise definition of downstaging following LRT, are all important elements when considering further expansion of the inclusion criteria without compromising the long-term results of LT in HCC patients.

Authorship

OC and JL: study concept and design. OC, PG, FR, CDR, RR and JL: patient care. OC, QL, PF, PG, FR, CDR, CS and JL: acquisition of data. QL, OC, ED and JL: analysis and interpretation of data. OC, QL and JL: drafting of the manuscript. JL: critical revision and supervision.

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