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Intention-to-Treat Analysis of Liver Transplantation in Selected, Aggressively Treated HCC Patients Exceeding the Milan Criteria

U. Cillo[a], A. Vitale[b,*], F. Grigoletto[c], E. Gringeri[a], F. D'Amico[a], M. Valmasoni[a], A. Brolese[a], G. Zanus[a], N. Srsen[a], A. Carraro[a], P. Burra[d], F. Farinati[d], P. Angeli[e] and D. F. D'Amico[a]

[a] *Unità di Chirurgia Epatobiliare e Trapianto Epatico, Dipartimento di Chirurgia Generale e Trapianti d'Organo, Azienda Ospedaliera di Padova*
[b] *Unità di Chirurgia Oncologica, Istituto Oncologico Veneto, IOV-IRCCS, Padova*
[c] *Unità di Biostatistica ed Epidemiologia, Dipartimento di Medicina Ambientale e Sanità Pubblica, Università di Padova*
[d] *Divisione di Gastroenterologia, Dipartimento di Scienze Chirurgiche e Gastroenterologiche, Università di Padova*
[e] *Clinica Medica V, Dipartimento di Medicina Clinica e Sperimentale, Università di Padova*
* *Corresponding author: Alessandro Vitale, alessandro.vitale@unipd.it*

This prospective study analyzed the dropout probability and intention-to-treat survival rates of patients with hepatocellular carcinoma (HCC) selected and treated according to our policy before liver transplantation (LT), with particular attention to those exceeding the Milan criteria. Exclusion criteria for LT were macroscopic vascular invasion, metastases, and poorly differentiated disease at percutaneous biopsy. A specific multi-modal adjuvant algorithm was used to treat HCC before LT. A total of 100 HCC patients were listed for LT: 40 exceeded the Milan criteria in terms of nodule size and number (MILAN OUT) either at listing or in list, while 60 patients continued to meet the criteria (MILAN IN). The Milan criteria did not prove to be a significant predictor of dropout probability or survival rates using Cox's analysis. Cumulative dropout probability at 6 and 12 months was 0% and 4% for MILAN OUT, and 6% and 11% for MILAN IN. The intention-to-treat survival rates at 1 and 3 years were 95% and 85% in MILAN OUT, and 84% and 69% in MILAN IN. None of the 68 transplanted patients had recurrent HCC after a median 16-month follow-up (0–69 months). In conclusion, LT may be effective for selected, aggressively-treated HCC patients exceeding the Milan criteria.

Key words: Aggressive therapy, hepatocellular carcinoma, liver transplantation, tumor biology

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Introduction

Liver transplantation (LT) is an appealing treatment for hepatocellular carcinoma (HCC) because it achieves the widest possible resection margins for the cancer, removing the remaining liver tissue, which risks developing *de novo* tumors and restoring liver function (1).

Over the last decade, most studies (2–9) have focused mainly on the crucial importance of the tumor's characteristics in determining the efficacy of LT for HCC patients. The most important predictors of HCC recurrence after LT were differentiation (grade), vascular invasion (macroscopic and microscopic), tumor size and number of nodules.

In recent years, however, other variables have reportedly been able to strongly influence the outcome of LT for HCC. Longer times on the waiting list due to the shortage of organs was seen as the most important prognostic factor when LT for HCC was considered on an intention-to-treat basis (10). Tumor progression before LT, in fact, can mean that HCC patients are removed from the waiting list because they exceed the listing criteria (11–13). Locoregional therapies, e.g. transarterial chemoembolization (TACE), percutaneous ablation procedures (PAP) and liver resection (LR), may be used to control HCC progression until a donor liver becomes available (14–16), so therapy before LT was seen as another crucial variable in influencing the outcome of LT for HCC (17). Neoadjuvant therapy may reduce the risk of dropout (18) before LT, but it may also downstage the tumor and change its oncological potential before or while patients are on the waiting list (19).

Selecting HCC patients for LT is consequently a dynamic process depending on the interaction between tumor biology, time to LT and bridging therapy strategies. It begins when patients are listed and continues as long as they remain on the waiting list because tumor growth may mean they exceed the established LT criteria at some point (and have to drop out).

The United Network for Organ Sharing (UNOS) and the majority of transplant units worldwide currently use the Milan criteria (single nodule <5 cm, 2–3 nodules <3 cm) for listing and delisting HCC patients (20). The intrinsic nature of the Milan criteria carries a significant risk of

exclusion, however, both before and while on the waiting list, particularly for patients with borderline size and/or number of nodules (12,13). Since exclusion, in most cases, inexorably means progression to death, the survival figures for LT for HCC are unsatisfactory when an intention-to-treat analysis is applied (10,11).

On the other hand, several reports in the literature (5–9) show that a significant proportion of patients excluded by such a policy may be cured by LT. As in the University of California San Francisco (UCSF) experience (21), a controlled expansion of the selection criteria could mean lower dropout rates without significantly increasing the risk of post-LT recurrence (12).

As previously published (22), our policy is based on the exclusion of tumors with aggressive features (poorly-differentiated, vascular invasion, extrahepatic spread), while size and number of nodules are not considered as absolute selection criteria. Moreover, all listed patients are treated with an aggressive multimodal adjuvant protocol to contain tumor progression prior to LT (23–26).

Such a policy inevitably leads us to select a significant number of patients for LT who exceed the Milan criteria both at the time of listing and while on the waiting list.

In this context, we started a prospective observational study in January 2000 to assess the dropout probability and the intention-to-treat survival rates of HCC patients exceeding the Milan criteria who were selected for LT according to our policy.

Patients and Methods

Patient population and listing criteria

We prospectively evaluated all consecutive patients referred to our institution and listed for LT between January 2000 and January 2006 either with a known diagnosis of HCC on cirrhosis at the time of listing or with a diagnosis of HCC established after listing for LT for liver cirrhosis.

Whenever feasible, we performed ultrasound-guided percutaneous biopsies for histological confirmation of the diagnosis of HCC and to determine its degree of differentiation according to the Edmonson-Steiner criteria (27). As previously reported (22,23), we adopted a restrictive LT listing policy including only selected tumors without aggressive features such as extrahepatic spread, macroscopic vascular invasion, or poor differentiation at pre-LT biopsy.

Over 6 years, 158 HCC patients with no general contraindications to transplant (i.e. age > 65 years, severe extrahepatic disease or recent malignancies) were evaluated; 32 of them (20%) underwent radical resection or ablation, while 26 (16%) were excluded because they had aggressive tumor features (macroscopic vascular invasion, metastases or poorly differentiated tumor). Thus, a total of 100 patients with cirrhosis and HCC (64%) were enrolled and prospectively followed up; 74 had a known HCC before listing, whereas in 26 the histological diagnosis of HCC was obtained after listing for LT for end-stage liver disease. In 10 patients (10%), all within Milan criteria, it was not possible to perform the biopsy due to technical problems; the diagnosis in these patients was based on two imaging techniques showing the typical features of HCC, as suggested by the AASLD guidelines (20).

HCC treatment strategy while awaiting LT

All patients received aggressive adjuvant therapy according to a well-defined treatment protocol (Figure 1) (22–26), on the basis of which the rate of anticancer therapeutic aggressiveness was determined mainly by liver function parameters such as Child Pugh class, the presence of clinically relevant hypertension (gastroesophageal varices, splenomegaly with a platelet count of less than 100000/ml, ascites) and, in the last 3 years, the MELD score, whereas nodule size and number were not absolute criteria for assigning adjuvant strategy at our Institution. Whenever possible, patients with a well preserved liver function had laparotomic or laparoscopic treatment using multiple procedures (resection and/or ablation with radiofrequency and/or high alcohol volumes). The laparotomic/laparoscopic approach was only used in selected patients with a moderately deteriorated liver function, while PAP and TACE in association was the main strategy. Percutaneous ethanol injection (PEI) and radiofrequency ablation (RF) were used mainly for nodules smaller than 5 cm without specific contraindications (ascites, critical location). Both ethanol injection and RF were also used for larger nodules, using high volumes of alcohol and/or multiple sessions, and/or association with Pringle maneuver (stop flow technique). PEI was preferred to RF for nodules close to relevant vascular or biliary structures, or in patients with a severe coagulopathy (PT <50 %, platelet count <30 000). TACE was used alone or in association with ablation therapies in the event of multinodular tumors without severe cirrhosis (Child C) or vascular anomalies.

Based on the tumor's imaging features and AFP levels, response to adjuvant therapy was classified as complete, partial, stable or progressive disease. Patients with a complete response were followed up closely, those with a partial/stable response underwent another cycle of treatment, and those with progression underwent full tumor re-staging and repeat biopsy of the largest lesion.

Dropout and allocation criteria

Macroscopic vascular invasion, extrahepatic spread and poor differentiation at percutaneous biopsy were considered absolute contraindications to LT (dropout criteria).

Biopsy of the largest nodule was repeated after 1 year on the waiting list or whenever tumor progression was apparent at treatment-specific imaging follow-up. In the present cohort, a patient's exclusion from the waiting list was confirmed on histological grounds wherever possible, or on 2 consistent sequential imaging studies.

According to Italian policy, each organ is assigned to a given liver transplant unit on the basis of geographical criteria, and each Liver Unit selects a suitable recipient from its own waiting list. Only patients listed for emergency re-LT or those with a pre-operative diagnosis of acute liver failure take national priority as status 1 patients.

At our Liver Unit, priority depends on the following criteria: (1) ABO and body size matching; (2) clinical characteristics of recipients classified according to the severity of their cirrhosis (Child Pugh and MELD score) and the presence of HCC; (3) donor characteristics. Among HCC patients of the same blood group, the main criterion concerns response to adjuvant therapy (complete, partial, stable or progressive disease) and priority is given to patients with a progressive disease irrespective of nodule size and number, unless complete re-staging reveals exclusion criteria.

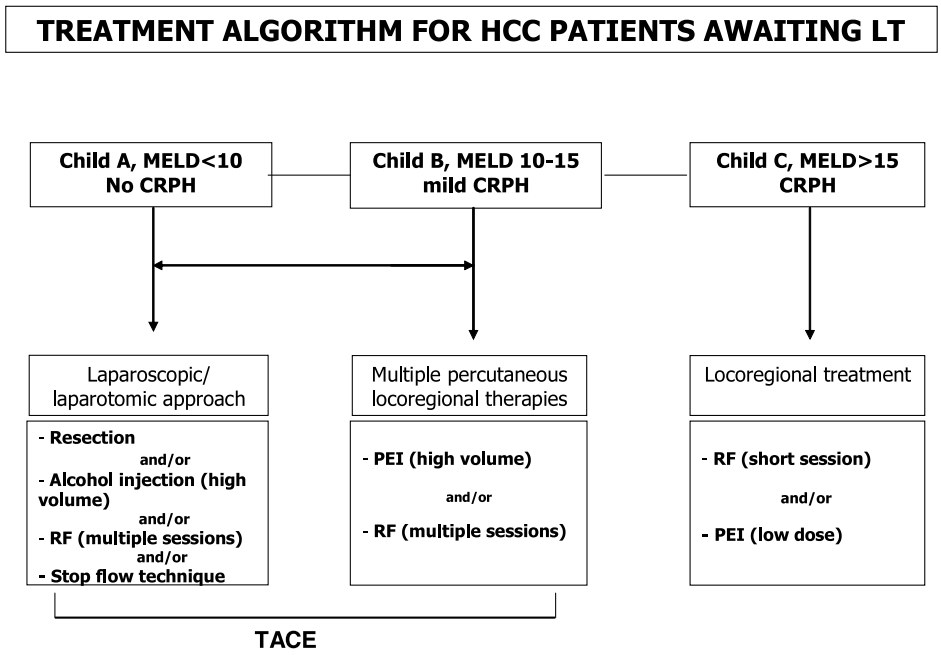

Figure 1: Treatment schedule for HCC patients listed for LT at Padua University.

HCC staging and follow-up before LT and study groups

All patients were initially staged by abdominal ultrasonography (US), hepatic Lipiodol-arteriography or angio-MRI, total body computed tomography (CT) and total-body bone scintigraphy. The whole staging procedure was repeated if patients remained on the transplant waiting list for more than 6 months or when there was evidence of tumor progression after adjuvant therapy. Minimum follow-up consisted in a clinical visit every 3 months with blood chemistry, AFP and abdominal US. Moreover, a specific follow-up was adopted after each treatment cycle including blood chemistry, AFP and CT abdomen or MRI 1 month after the last therapeutic procedure.

For the purposes of this prospective study, patients who were diagnosed as already exceeding the Milan criteria in terms of nodule size or number, either at the time of listing or while on the waiting list, were recorded as MILAN OUT in a prospective database.

On the other hand, patients who continued to meet the Milan criteria in terms of nodule size and number while on the waiting list were classified as MILAN IN. The 10 patients with no biopsy were all in the MILAN IN group.

Post-LT treatment and follow-up

No patients had chemotherapy after LT for HCC. Posttransplant immunosuppressive therapy consisted of cyclosporine or tacrolimus in association with steroids, which were tapered off within 3 months of transplantation. Follow-up at 1, 3 and 6 months after transplantation, and every 6 months thereafter, always included liver US and AFP assay. Total-body CT was performed a year after transplantation or whenever tumor recurrence was suspected.

Statistical analysis

The patients' baseline characteristics are expressed as mean ± standard deviation (SD) and median with inter-quartile range for continuous data, and as frequency for categorical data. The relationship between the binary variable expressing whether or not the patient met the Milan criteria and the other variables was analyzed by logistic regression, the chi-squared or Fisher's exact tests, as appropriate.

Length of follow-up and survival are expressed as median (range). Dropout was defined as removal from the waiting list due to exclusion or death before LT. Probability curves for LT, dropout, and survival were calculated according to the Kaplan-Meier method. In the analysis of LT probability, LT was considered as an event, whereas patient dropout from the waiting list was considered as a censor point. In the analysis of dropout probability, patient dropout was considered as an event, whereas LT was considered as a censor point. In the intention-to-treat analysis, survival was calculated from the day of listing or of the diagnosis of HCC after listing until death or latest follow-up (which continued after dropout or LT, up until latest follow-up or death). Follow-up data were collected up until March 31, 2006, when our initial data analysis was performed.

The probability of LT, the dropout probability and the intention-to-treat survival rates at various time points were expressed together with their 95% confidence intervals (95% CI).

Cox's univariate proportional hazards models were used to identify potential predictors of LT, dropout, and survival probabilities. A Cox's multivariate model was then created, including only variables with $p < 0.1$ at univariate analysis, in order to find any independent parameters.

To further verify the appropriateness of our strategy, we retrospectively stratified MILAN OUT patients according to UCSF criteria. Patients meeting UCSF criteria (21) had the following tumor features: 1 nodule < 6.5 cm, < 3 nodules < 4.5 cm, total diameter < 8 cm.

Table 1: Baseline patient and tumor characteristics

Variable	MILAN OUT (n = 40)	MILAN IN (n = 60)	All patients (n = 100)
Age (years)	55.1 ± 6.4	56.2 ± 5.7	55.8 ± 6.0
	56.7 (8.2)	56.7 (8.3)	56.7 (8.5)
Sex (female)[1]	3 (8%)	18 (30%)	21 (21%)
Etiology			
Hepatitis C	25 (63%)	41 (68%)	66 (66%)
Hepatitis B	8 (20%)	10 (17%)	18 (18%)
Alcohol	7 (17%)	8 (13%)	15 (15%)
Other	–	1 (2%)	1 (1%)
Blood groups B-AB	8 (20%)	9 (15%)	17 (17%)
Portal hypertension	20 (50%)	33 (55%)	53 (53%)
Child pugh classes			
A	5 (13%)	11 (18%)	16 (16%)
B	26 (65%)	40 (67%)	66 (66%)
C	9 (22%)	9 (15%)	18 (18%)
Meld score	13.9 ± 4.3	12.0 ± 3.8	12.7 ± 4.1
	14.0 (4.5)	12.0 (4.5)	13.0 (5.0)
Biochemistry			
AST (U/L)[1]	96.0 ± 63.6	72.5 ± 44.3	81.4 ± 53.3
	73.0 (97.0)	62.5 (43.5)	67.0 (55.0)
ALT (U/L)	86.2 ± 59.3	71.1 ± 46.6	76.7 ± 51.9
	68.0 (80.5)	60.0 (58.0)	64.0 (64.8)
Bilirubin (mg/dL)[1]	2.4 ± 1.6	1.6 ± 1.0	1.9 ± 1.3
	1.6 (2.9)	1.3 (1.3)	1.5 (1.5)
INR	1.4 ± 0.2	1.3 ± 0.1	1.3 ± 0.2
	1.3 (0.3)	0.9 (0.3)	1.3 (0.3)
Creatinine (mg/dL)	0.9 ± 0.2	0.9 ± 0.3	0.9 ± 0.2
	0.9 (0.2)	0.9 (0.3)	0.9 (0.3)
AFP levels (ng/mL)[1]	97.0 ± 143.3	14.8 ± 20.0	54.0 ± 107.1
	33.0 (111.0)	9.0 (10.9)	15.0 (37.8)
Number of tumor nodules[1]	3.0 ± 1.2	1.4 ± 0.7	1.8 ± 1.0
	2.0 (3.0)	1.0 (1.0)	1.0 (1.0)
Size of largest[1] nodule (cm)[1]	4.0 ± 1.6	2.4 ± 0.9	2.9 ± 1.3
	4.0 (2.0)	2.0 (1.0)	3.0 (1.0)

AST = aspartate aminotransferase; ALT = alanine aminotransferase; AFP = alpha-fetoprotein.
[1]$p < 0.05$ in the correlation between study groups.

For continuous variables, the cut-off was identified using the ROC curve method. Statistical significance was set at $p < 0.05$. The calculations were done with the JMP package (1989–2003 SAS Institute Inc.).

Results

Patient characteristics

The baseline and tumor characteristics of patients meeting or exceeding the Milan criteria are illustrated separately in Table 1. There were 40 MILAN OUT and 60 MILAN IN patients.

There was a significantly larger proportion of females in the MILAN IN group. Blood AST, total bilirubin and alpha-fetoprotein (AFP) levels were significantly higher in MILAN OUT patients.

Treatment of HCC while awaiting LT

HCC treatments while on the waiting list for LT are shown in Table 2: 83 patients received at least one treatment; 17 received no therapy because HCC had only just been diagnosed (3 cases), or because they died before any treatment could be administered (3 cases), or due to severe hepatic impairment contraindicating any attempt at therapy (4 cases), or due to the short interval between the diagnosis of HCC and LT (7 cases).

Table 2: Therapies before LT

Variable	MILAN OUT (n = 40)	MILAN IN (n = 60)	All patients (n = 100)
No. of patients treated[1]	39 (98%)	44 (73%)	83
Overall no. of therapies	135	96	231
No. of therapies for each patient treated[1]	3.3 ± 2.6	1.6 ± 1.6	2.8 ± 2.1
	2.0 (4.0)	1.0 (3.0)	2.0 (2.0)
Treatment strategy			
Single therapy			
TACE	13	14	27
PEI	2	14	16
RF	2	3	5
Multi-modal therapy[1]			
PEI + RF	4	1	5
TACE + PEI/RF	12	10	22
PEI/RF + resection	2	2	4
PEI/RF + TACE + resection	4	–	4

TACE = transarterial chemoembolization; PEI = percutaneous ethanol injection; RF = radiofrequency.
[1]$p < 0.05$ in the correlation between study groups.

Fifty patients had been given HCC therapy already before listing. Overall, we administered 231 treatments with a mean number of 2.8 ± 2.1 per patient. In 35 treated patients (42%), more than one type of therapy was used in a sort of multimodal aggressive approach: RF + PEI (5 cases), RF and/or PEI + TACE (22 cases), RF and/or PEI + resection (4 cases), RF and/or PEI + TACE + resection (4 cases). The remaining 48 patients received only one type of treatment, i.e. 27 had TACE, 16 had PEI and 5 had RF.

The MILAN OUT group included a significantly larger percentage of treated patients and number of procedures per patient than the MILAN IN group (Table 2). Twenty-two MILAN OUT patients (55%) received multi-modal treatment as opposed to 13 MILAN IN patients (22%); this difference was also statistically significant.

Probability of LT

***Overall analysis*:** During the study period, 68 of the 100 patients underwent LT, 12 were removed from the waiting list, and 20 were still awaiting LT. The cumulative probabilities of LT at 6, 12 and 24 months were 29% (95% CI: 19, 39), 50% (95% CI: 40, 60) and 76% (95% CI: 66, 86), respectively (Figure 2A). The median time on the waiting list for the whole cohort of HCC patients was 11.8 months (range 0.2–59.5).

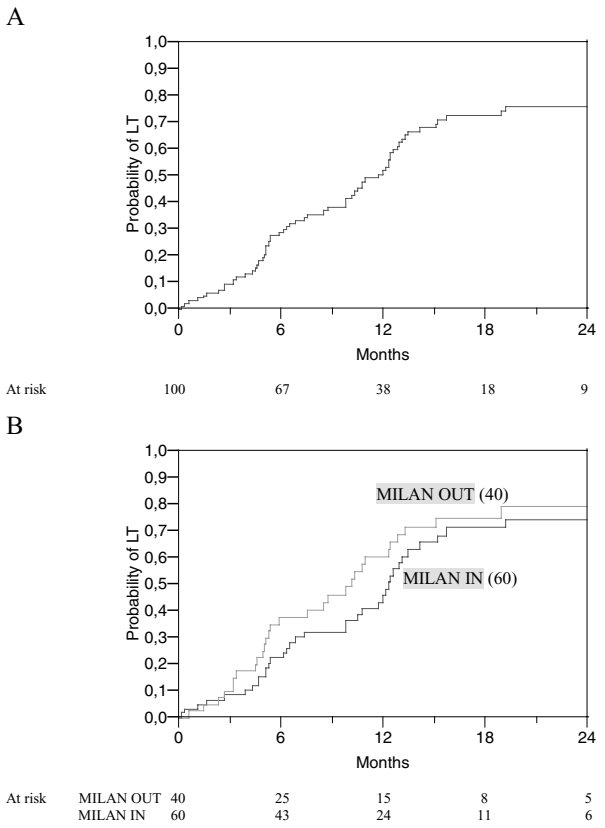

Figure 2: Kaplan-Meier probability of LT in the whole cohort of patients (A) and in the MILAN OUT and MILAN IN groups of patients (B).

***Predictors of LT probability*:** Cox's multi-variate analysis selected only B-AB blood groups and portal hypertension as independent predictors of LT probability (Table 3).

***Analysis based on Milan criteria*:** A larger proportion of MILAN OUT patients ($p = 0.07$) had LT (78%) than MILAN IN patients (62%). Table 3 shows that tumor characteristics did not affect the probability of LT in this series; this was confirmed by the Kaplan-Meier curves (Figure 2B). The probabilities of LT at 6, 12 and 24 months were 38% (95% CI: 22, 54), 60% (95% CI: 44, 76) and 79% (95% CI: 65, 93), with a median 10.2 months to LT (range, 0.7–34.7) for MILAN OUT patients, and 22% (95% CI: 11, 33), 43% (95% CI: 29, 57) and 74% (95% CI: 58, 90) with a median 12.4 months to LT (range, 0.2–59.5) for MILAN IN patients.

Removal from the waiting list

***Overall analysis*:** Dropout probabilities at 6, 12 and 24 months were 4% (95% CI: 0, 8), 8% (95% CI: 2, 14) and 33% (95% CI: 13, 53) (Figure 3A). Among the 12 dropouts, 2 died on the waiting list (1 of gastrointestinal bleeding, 1 of suicide), 6 were excluded due to neoplastic portal thrombosis confirmed by percutaneous biopsy, 2 were excluded due to poor differentiation at rebiopsy, 1 was ruled out due to peritoneal HCC metastases found after laparotomy for LT, and 1 due to histologically confirmed bone metastases. Eight of the 10 dropouts due to tumor progression died within 2 months of their removal from the list, 1 died after 2 years, 1 was still alive 6 months after being excluded. All 9 deaths were related to tumor growth. Three dropouts occurred within 6 months, 3 between 6 and 12 months, 2 between 12 and 18 months, 3 between 18 and 24 months, and 1 after more than 24 months on the waiting list for LT.

Table 3: Predictors of the probability of LT

Variables	Univariate $LR\chi^2$ (p)	Multivariate $LR\chi^2$ (p)
Female sex	1.3 (0.2520)	–
Age > 55 years	0.4 (0.4955)	–
Blood groups B-AB	15.9 (0.0001)	18.7 (0.0000)
HCV	3.3 (0.0683)	1.44 (0.2300)
AST > 68 U/L	3.2 (0.0719)	8.1 (0.0045)
ALT > 65 U/L	0.9 (0.3405)	–
Creatinine > 1 mg/dL	3.3 (0.0695)	2.4 (0.1164)
Bilirubin > 2mg/dL	1.7 (0.1921)	–
Child C	2.0 (0.1564)	–
Portal hypertension	5.0 (0.0249)	9.7 (0.0018)
MELD > 14	1.3 (0.2307)	–
AFP > 20 ng/mL	0.7 (0.3870)	–
Nodule size > 3cm	1.2 (0.2742)	–
Multinodular	0.1 (0.6954)	–
MILAN IN	0.9 (0.3263)	–
Pre-LT therapy	1.0 (0.3167)	–
>3 pre-LT procedures	3.0 (0.0829)	–

For continuous variables, the cut-off was selected using the ROC curves method. The multi-variate model only includes variables with $p < 0.1$ at univariate analysis.
$LR\chi^2$ = likelihood ratio chi-squared; AST = aspartate aminotransferase; ALT = alanine aminotransferase; AFP = alpha-fetoprotein.

***Dropout probability predictors*:** Cox's model found no significant dropout predictors among the variables analyzed in Table 3.

***Analysis according to Milan criteria*:** Dropouts were evenly distributed, 5 in the MILAN OUT group (12%) and 7 in the MILAN IN group (12%). The cumulative dropout probabilities at 6, 12 and 24 months (Figure 3B) were 0% (95% CI: 0), 4% (95% CI: 0, 12) and 42% (95% CI: 8, 76) for MILAN OUT patients, and 6% (95% CI: 0, 12), 11% (95% CI: 1, 21) and 25% (95% CI: 5, 45) for MILAN IN patients ($p > 0.05$).

Survival according to intention-to-treat analysis

***Overall analysis*:** Median follow-up was 21.4 months (range 3–75 months). Kaplan-Meier 1, 3 and 5-year survival rates according to intention-to-treat analysis were 88% (95% CI: 82, 94), 76% (95% CI: 66, 86) and 73% (95% CI: 61, 85), respectively (Figure 4A). Twenty-one patients died (21%), 11 before and 10 after LT (Table 4). Among pre-LT deaths, 9 were related to HCC progression, 1 to

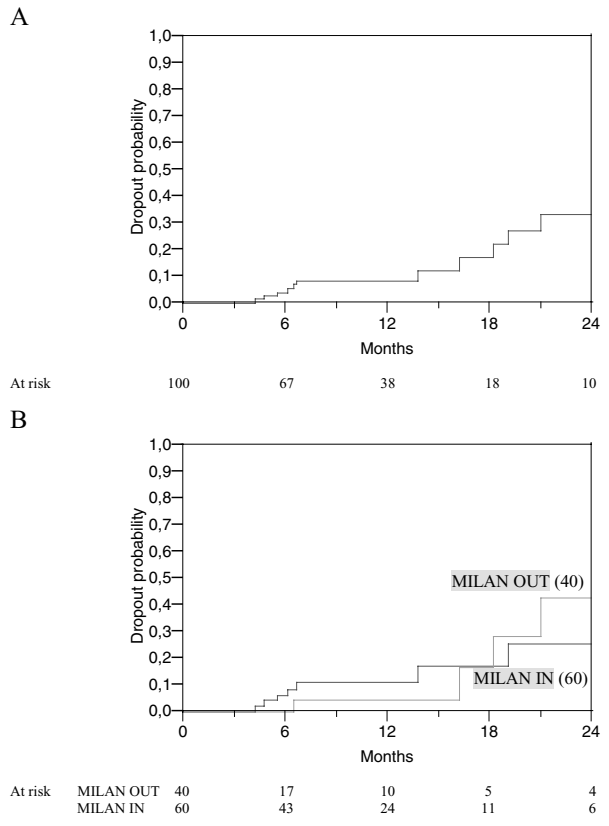

Figure 3: Kaplan-Meier dropout probability in the whole cohort of patients (A) and in the MILAN OUT and MILAN IN groups of patients (B).

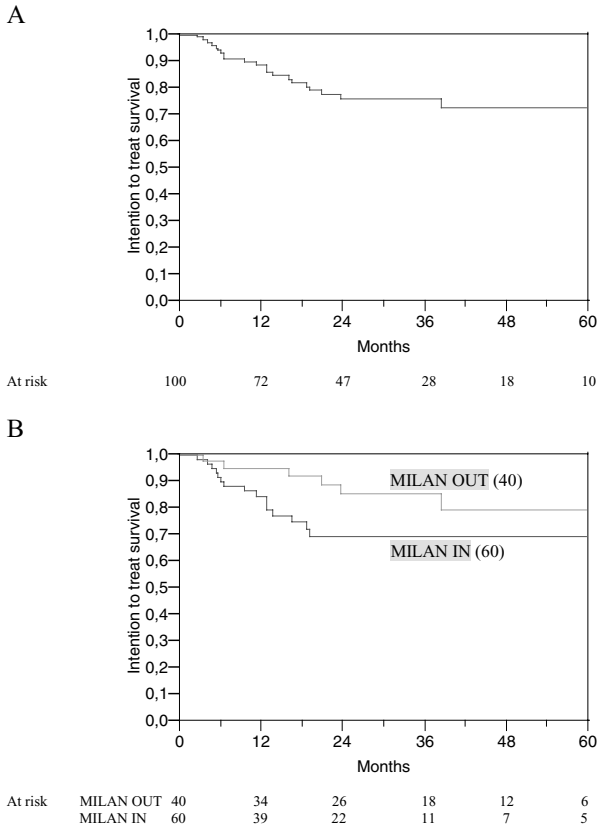

Figure 4: Kaplan-Meier intention-to-treat survival rates in the whole cohort of patients (A) and in the MILAN OUT and MILAN IN groups of patients (B).

gastrointestinal bleeding and 1 to suicide. Among post-LT deaths, 9 occurred within 3 months of LT and were due to primary graft dysfunction (2 cases), sepsis (4 cases), intraoperative bleeding (1 case), and acute cerebral or cardiac events (2 cases). One patient died a year after LT of recurrent HCV.

***Predictors of survival*:** Cox's model found no significant survival predictors among the variables analyzed in Table 3.

***Analysis according to Milan criteria*:** Survival probabilities at 1, 3 and 5 years according to intention-to-treat analysis (Figure 4B) were 95% (95% CI: 87, 100), 85% (95% CI: 73, 97), and 79% (95% CI: 63, 95) for the MILAN OUT group and 84% (95% CI: 74, 94), 69% (95% CI: 55, 83), and 69% (95% CI: 55, 83) for the MILAN IN group ($p > 0.05$). Among the specific causes of death (Table 4), there were more post-LT deaths in the MILAN IN group, though the difference was not statistically significant.

HCC characteristics at the time of LT and post-LT outcome

At histological assessment, MILAN OUT patients confirmed a significantly higher proportion of larger, multinodular tumors exceeding the Milan criteria than MILAN IN patients (Table 5). The prevalence of microscopic vascular invasion was 22% overall, and was similar in the 2 groups. Poorly-differentiated tumor was found in only 8 cases (12%). The proportion of poorly differentiated tumors in MILAN OUT patients (16%) did not significantly differ with respect to that in the MILAN IN group (8%).

Median post-LT survival for the 68 transplanted patients was 16 months (range 0–69). Post-LT 1-, 2- and 3-year survival rates were 86% (95% CI: 78, 94), 84% (95% CI: 74, 94) and 84% (95% CI: 74, 94), respectively (Figure 5A). There was a trend ($p = 0.07$) toward higher survival rates for MILAN OUT patients than for MILAN IN patients (Figure 5B). None of the patients developed recurrent HCC after LT.

Analysis of MILAN OUT patients according to UCSF criteria

We retrospectively analyzed our prospective database to see when MILAN OUT patients also exceeded UCSF criteria. We identified 24 patients (60%) who exceeded UCSF criteria (UCSF OUT) and 16 (40%) who did not (UCSF IN).

Table 4: Causes of death

Overall LT process	MILAN OUT (n = 40)	MILAN IN (n = 60)	All patients (n = 100)
Before LT	4 (10%)	7 (12%)	11 (11%)
Tumor progression	4	5	9
GI bleeding	–	1	1
Other	–	1	1
After LT	2 (5%)	8 (13%)	9
Intra-operative	–	1	1
Sepsis	–	4	4
Primary graft dysfunction	1	1	2
Cerebral/cardiac event	1	1	2
HCV recurrence	–	1	1
Overall mortality	6 (15%)	15 (25%)	21 (21%)

Table 5: Explant histology for patients who underwent LT

Variable	MILAN OUT (n = 31)	MILAN IN (n = 37)	All patients (n = 68)
Complete necrosis	1 (3%)	5 (14%)	6 (9%)
Beyond milan histological criteria[1]	21 (68%)	7 (19%)	28 (41%)
Mean number of nodules ± SD[1]	4.0 ± 3.2 3.0 (3.7)	2.0 ± 1.6 1.0 (1.8)	2.9 ± 2.7 2.0 (3.3)
Mean size of largest nodule ± SD (cm)[1]	3.5 ± 2.3 3.0 (1.9)	2.6 ± 1.1 2.5 (1.6)	3.0 ± 1.8 2.6 (1.5)
Mean sum of diameters ± SD (cm)[1]	6.6 ± 4.0 5.7 (7.3)	3.6 ± 1.8 3.9 (2.9)	5.0 ± 3.3 4.4 (3.7)
Vascular invasion			
Microscopic	8 (26%)	7 (19%)	15 (22%)
Macroscopic	1 (3%)	1 (3%)	2 (3%)
Tumor grade[1]			
Well differentiated	4 (13%)	15 (41%)	19 (28%)
Moderately differentiated	21 (68%)	14 (38%)	35 (51%)
Poorly differentiated	5 (16%)	3 (8%)	8 (12%)

[1]$p < 0.05$ in the correlation between study groups.

The main clinical characteristics of the 40 MILAN OUT patients for the purposes of the UCSF criteria are compared in Table 6. The only significant differences lay in the number of nodules and the total tumor diameter, which were both higher in the UCSF OUT group. Among the 24 UCSF OUT patients, 18 (75%) underwent LT, 3 (12%) dropped out due to tumor progression, and 3 (13%) are still waiting for LT. Among the 16 UCSF IN patients, 13 (81%) underwent LT, 2 (12%) dropped out, and 1 (7%) is still on the waiting list for LT. The median time on the waiting list was 10.4 months (range 1.5–34.7) for UCSF OUT, and 9.9 months (range 0.7–25.0) for UCSF IN patients. The Kaplan-Meier probability of LT, dropout probability and intention-to-treat survival rates for these 2 groups are given in Figure 6. In particular, the survival rates at 1, 3 and 5 years by intention-to-treat analysis (Figure 6C) were 96% (95% CI: 88, 100), 85% (95% CI: 69, 100) and 76% (95% CI: 54, 98) in the UCSF OUT group and 94% (95% CI: 82, 100), 85% (95% CI: 65, 100) and 85% (95% CI: 65, 100) in UCSF IN group. In the light of these results, meeting the UCSF criteria does not seem to be a significant variable in relation to the probability of LT, dropout probability or intention to-treat survival rates.

Discussion

In this study, we prospectively observed the outcome of HCC patients selected and treated before LT according to our policy. The study pays particular attention to those exceeding the Milan criteria because such patients are normally not considered for LT at the majority of centers (20). This is consequently not a randomized controlled trial comparing 2 treatment groups, it is an observational study designed to ascertain the outcome of our strategy. We chose to report our results separately for patients exceeding or meeting the Milan criteria merely for descriptive purposes. In fact, our aim was to demonstrate that applying our selection and treatment protocol enables patients failing to meet the MILAN criteria to be transplanted successfully without any negative fallout on MILAN IN patients.

Since the prognosis for HCC patients enrolled for LT depends not only on the criteria adopted for listing and delisting, but also on adjuvant treatment strategies and time on the waiting list (1,20), we discussed these 3 issues separately.

The selection criteria issue

The risk of a tumor-related death before and after LT is higher in unselected patients with large and multi-nodular tumors (28), but a careful extension of the inclusion and dropout criteria has been shown to determine similar, or even fewer dropouts from the waiting list than the Milan criteria without increasing the probability of post-LT recurrence (12,21,29).

As previously reported (22,23), of the HCC patients referred to our Institution for transplantation, only those with aggressive tumor features (poor differentiation at percutaneous biopsy, macroscopic vascular invasion or extrahepatic spread) are denied the chance of a transplant, irrespective of nodule size and number. This particular listing/delisting policy enabled us to include a sizable group of patients exceeding the Milan criteria in the present analysis.

In several reports in which nodule size and number were used as dropout criteria, most patients were still alive after their removal from the waiting list as at the latest follow-up (10–13), whereas in our study the majority of patients delisted due to tumor progression died within 2 months of their exclusion (Table 4). This suggests that our policy carries a low risk of rejecting patients with a good prognosis and a potential for curative LT, whereas a dropout policy based on macro-morphological parameters alone seems likely to rule out patients whose HCC has a more heterogeneous biological behavior.

On the other hand, keeping MILAN OUT patients on the waiting list did not increase the tumor progression rate prior to LT. The overall probability of patients dropping out was less than 10% at 1 year (Figure 3A), considerably lower

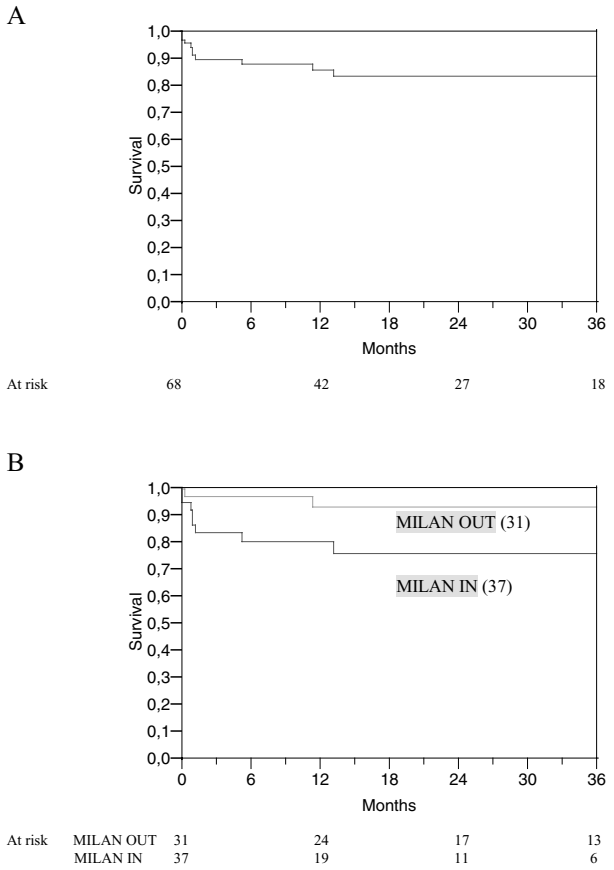

Figure 5: Kaplan-Meier post-LT survival rates in the whole cohort of transplanted patients (A) and in the MILAN OUT and MILAN IN groups of patients (B).

than the 20–50% reported by centers using nodule size and number as dropout criteria (10–13,20). Our policy is therefore associated with a low dropout probability, but it carries the intrinsic risk of arriving at LT with more advanced tumors, with a potentially greater chance of post-LT recurrence and death. No post-LT recurrences have been detected so far, however, and—after a median follow-up of 21.4 months (range 3–75)—the 5-year intention-to-treat survival of the study group was 73% (95% CI: 61, 85), better than the one described in other recent series (10–13,15). In particular, our MILAN OUT patients had a very low post-LT mortality (Table 4), and their intention-to-treat survival did not differ significantly from the situation in MILAN IN patients (Figure 4B).

Our strategy was further tested by stratifying MILAN OUT patients according to the UCSF criteria (Table 6), which revealed a poor capacity for discrimination in terms of dropout and intention-to-treat survival probabilities (Figure 6).

It has to be said, however, that the relatively short post-LT median follow-up carries the risk of underestimating tumor recurrence in our study. Since the majority of aggressive HCC recurrences occur in the first 2 years (30), the total absence of such events in the present study nonetheless points to a low risk of post-LT tumor recurrence for patients exceeding the Milan criteria and listed according to our policy.

Table 6: Main patient and tumor characteristics of patients in the MILAN OUT group stratified according to UCSF criteria

Variable	UCSF OUT (n = 24)	UCSF IN (n = 16)
Mean age ± SD (years)	55.1 ± 6.4	56.2 ± 5.7
	54.6 (10.6)	58.2 (5.5)
Sex (female)[1]	1 (4%)	2 (12%)
HCV etiology	13 (54%)	12 (75%)
Blood groups B-AB	5 (21%)	3 (19%)
Portal hypertension	11 (46%)	9 (56%)
Child pugh class C	7 (29%)	2 (12%)
MELD score	13.8 ± 5.3	14.0 ± 2.0
	13.0 (6.5)	14.0 (3.0)
AFP levels (ng/mL)	123.7 ± 156.8	72.7 ± 132.6
	51.5 (175.3)	23.8 (39.0)
Mean number of tumor nodules ± SD[1]	3.9 ± 0.9	2.7 ± 1.1
	4.0 (1.7)	3.0 (1.8)
Mean size of largest nodule ± SD (cm)	4.1 ± 1.5	4.2 ± 1.4
	4.0 (2.8)	4.0 (1.6)
Total tumor diameter ± SD (cm)[1]	9.3 ± 1.3	6.9 ± 0.6
	9.0 (2.0)	7.0 (0.5)

UCSF OUT = patients exceeding UCSF criteria; UCSF IN = patients meeting UCSF criteria; HCV = hepatitis C virus; AFP = alpha-fetoprotein; SD = standard deviation.
[1]$p < 0.05$ in the correlation between study groups.

The histological analysis of the tumors in patients who underwent LT (Table 5) further supports our previous considerations. Although MILAN OUT patients included a significantly higher proportion of tumors exceeding the Milan criteria, at histological assessment the group actually had a similar proportion of tumors with microscopic vascular invasion and poorly differentiated type. It is important to emphasize, moreover, that the proportion of such aggressive features in both groups was far lower than was recently reported by Pawlik et al. in patients with both small and large tumors (31). This suggests that, even allowing for the risk of false negatives, pre-LT biopsy in association with an aggressive treatment schedule, may reduce the number of poorly-differentiated tumors undergoing LT. Since tumor grade and vascular invasion have proved the most important predictors of post-LT recurrence (5), the low proportion of such aggressive features at the time of LT is, in itself, an important result of any selection and treatment strategy. Moreover, as previously shown (22), such a strategy has probably indirectly set a limit more for the size rather than for the number of nodules and this could explain the characteristics of our population that in many cases only slightly overcomes the UCSF criteria (Tables 5 and 6). Notably, no cases of tumor seeding were observed after percutaneous biopsy, nor of any other major complications.

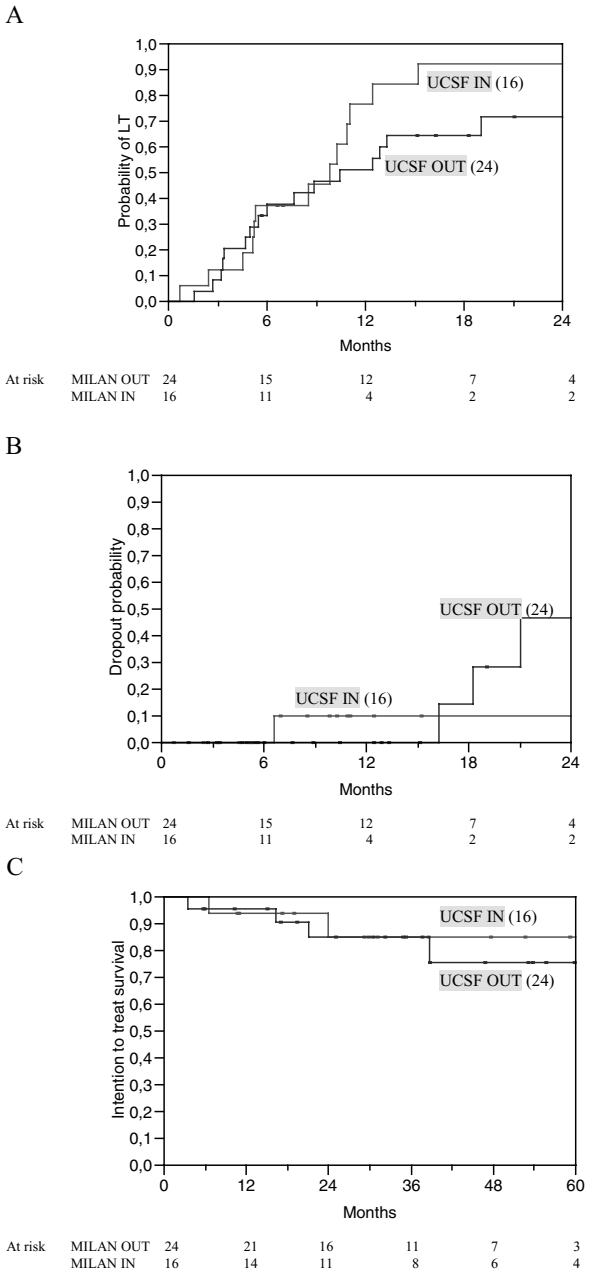

Figure 6: Kaplan-Meier probability of LT (A), dropout probability (B), and intention-to-treat survival rates (C) in the UCSF OUT and UCSF IN groups. UCSF OUT: patients exceeding UCSF criteria: UCSF IN: patients meeting UCSF criteria.

The treatment strategy issue

A second constitutive feature of this prospective study was the adoption of a pre-determined treatment schedule (Figure 1) applied to all patients to aggressively control tumor growth before LT. Several studies in recent years (14–18) have shown the utility of locoregional therapies in containing tumor progression before LT, and the recent guidelines of the American Association for the Study of the Liver (20) have recommended the use of such therapies if the expected waiting time is longer than 6 months. The role of locoregional therapies has been largely evaluated only in HCC patients meeting the Milan criteria (14–19), whereas the present study reports clinical data on such treatments in patients exceeding the criteria too. The overall number of procedures per patient, their complexity and multimodality were significantly higher in MILAN OUT group (Table 2). Since response to adjuvant therapy was the main criterion for deciding on any further treatment, irrespective of nodule size and number, such an asymmetrical distribution in the 2 groups was due mainly to a predictably higher rate of good responses in the MILAN IN than in the MILAN OUT group. Given the priority for LT assigned in our policy for patients a progressive disease, this fact probably also explains the trend toward shorter waiting times for the MILAN OUT than for MILAN IN group in our study. All these considerations might be indicative of a policy that is excessively unbalanced in favor of MILAN OUT patients, but the low probability of dropouts in the MILAN IN group, with better survival figures than those reported in other recent publications (11–13,15–17) demonstrated that this subgroup of patients was not damaged by our policy. Despite our aggressive pre-LT treatment strategy, complete necrosis was found in only a minority of our patients, with a similar distribution in the 2 groups (Table 5). We did see extensive tumor necrosis in most patients, however, which reduced the overall neoplastic burden. It is impossible to say whether this tumor reduction might have been responsible for the small proportion of aggressive features (e.g. vascular invasion and poorly differentiated type) that we encountered. The absence of a control group given no such treatment prevents us from drawing any definitive conclusions on the prognostic effect of pre-LT adjuvant therapy, though it was probably extremely important in achieving our results. As recently suggested by Yao et al. (17), in fact, such a positive effect is probably greater for patients exceeding than for those meeting the Milan criteria.

The time before LT issue

It has been amply demonstrated (10–13) that a long waiting time increases the risk of tumor growth before LT, but the time to LT may paradoxically become an indirect selection tool, since an adequate follow-up period and response to adjuvant therapy are often fundamental to the identification of tumors with a worse biology and higher risk of post-LT recurrence (20). Our relatively long median waiting time for HCC patients (Table 3, Figure 2) probably had a positive synergic interaction with our treatment algorithm (Figure 1) and inclusion-dropout policy in selecting less aggressive HCC cases for LT. In this line, some criticism with regard to the extremely short waiting times reported for HCC patients in the UNOS–MELD area emerged in recent studies (20,32,33).

In conclusion, we prospectively showed that, in patients failing to meet the Milan criteria, excluding only tumors

found poorly differentiated at percutaneous biopsy and cases of macroscopic vascular invasion or extrahepatic spread, and adopting an aggressive multimodal adjuvant protocol was associated with a low dropout probability before LT and excellent intention-to-treat survival figures, comparable with those obtained for patients meeting said selection criteria. In terms of effective tumor bulk, such a policy apparently seems to represent only a gentle tilt beyond UCSF criteria, but this result is obtained completely readdressing the critical issue of patient selection for LT from tumor size and number to tumor grade and response to therapy used as biological selection criteria.

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