Semmelweis University

Doctoral School of Pathological Sciences

LIVER TRANSPLANTATION FOR HEPATOCELLULAR CARCINOMA IN CIRRHOSIS- EXPANDING THE RECIPIENT POOL

PhD Thesis

Prof. Dr. Georgios C. Sotiropoulos, MD

Supervisor:	Dr. László Kóbori, MD, PhD
Reviewers:	Dr. Szücs Ákos, MD, PhD
	Dr. Hitre Erika, MD, PhD
Final exam Committee:	Prof. Dr. György Reusz, Dsc, MD, PhD
	Dr. Katalin Monostory, PhD
	Dr. Péter Sótonyi, MD, PhD

Budapest

2011

TABLE OF CONTENTS

	Abbreviations ……..……....	5
1.	Introduction ……..……..……...	7
1.1	Incidence of hepatocellular carcinoma …….	7
1.2	Surveillance ……..	7
1.3	Diagnosis of hepatocellular carcinoma ……	8
1.4	Treatment plan ……...	9
1.5	Hepatic resection ……..…….	9
1.6	Liver transplantation …….……	11
1.7	Organ shortage and increasing transplant demand ……...……	13
2.	Research objectives ……...	16
3.	Material and methods ……..……..	18
	Evaluation and follow-up protocol for patients with HCC in cirrhosis ….	18
3.1	Bridging treatments ……..	19
3.1.1	LT without bridging treatments - Monotherapies as treatment for HCC in cirrhosis ……..……	19
3.1.2	LT after bridging treatment ……..	20
3.1.2.a	Tumor down-staging/down-sizing with TACE ……	20
3.1.2.b	LT after complete tumor necrosis following bridging treatment ……	21
3.1.2.c	AFP decrease to undetectable or very low (<-30ng/mL) levels …….	21
3.2	Ethical issues …….	22
3.2.1	LT for alcoholic liver disease and HCC – place for adult LDLT? …….	22
3.3	Pre-transplant radiological assessment ……..	23
3.3.1	Radiological versus pathological staging for HCC in cirrhosis …….	23
3.3.2	Incidentally found HCC …….	24
3.4	Extended listing criteria …….	26
3.4.1	LT for patients beyond Milan/ within UCSF criteria …….	26
3.4.2	LT for HCC and portal vein thrombosis ……..	26
3.4.3	Pulmonary modules at risk in patients undergoing LT for HCC ……	28

3.5	Expanding the donor pool …….	29
3.5.1	Extended criteria donor for LT for HCC – rescue organ offers ……	29
3.5.2	Split LT for HCC …..……	30
3.5.3	LDLT for HCC ……..	31
3.5.4	Institutional results …….	32
4	Results ……..……..…..……	34
4.1	Bridging treatments ……...	34
4.1.1	LT without bridging treatments - Monotherapies as treatment for HCC in cirrhosis ……..……	34
4.1.2	LT after bridging treatment ……..……..	40
4.1.2.a	Tumor down-staging/down-sizing with TACE …….	40
4.1.2.b	LT after complete tumor necrosis following bridging treatment ……..	42
3.1.2.c	AFP decrease to undetectable or very low (<-30ng/mL) levels ……	44
4.2	Ethical issues …….	51
4.2.1	LT for alcoholic liver disease and HCC – place for adult LDLT? …….	51
4.3	Pre-transplant radiological assessment ……..	55
4.3.1	Radiological versus pathological staging for HCC in cirrhosis ……	55
4.3.2	Incidentally found HCC …….	59
4.4	Extended listing criteria …….	62
4.4.1	LT for patients beyond Milan/ within UCSF criteria …….	62
4.4.2	LT for HCC and portal vein thrombosis ……	63
4.4.3	Pulmonary modules at risk in patients undergoing LT for HCC ……..	66
4.5	Expanding the donor pool ……..	70
4.5.1	Extended criteria donor for LT for HCC – rescue organ offers ……	70
4.5.2	Split LT for HCC ……...	72
4.5.3	LDLT for HCC ……..	74
4.5.4	Institutional results ……	79
5	Discussion …….	89
5.1	Study results ……..	89
5.2	Meta analyses ……	97

5.2.1 Impact of hilar lymph nodes metastases on tumor recurrence and survival in patients with HCC undergoing LT …… 97
5.2.2 Tumor recurrence after LT for HCC based on 1,198 cases …… 102
5.2.3 LT for HCC: Metaanalysis of prognostic factors ……. 112

6 Conclusion ……..……..…. 119

7 Summary ……...…….. 122

8 Összefoglalás …….…… 123

9 Publications ……..……..……..…… 124

10 Acknowledgements …….…..…. 127

11 References …….…..……... 128

ABBREVIATIONS

AFP	Alpha fetoprotein
AHPBA	American Hepato-Pancreato-Biliary Assosiation
AJCC	American Joint Commission on Cancer
ALD	Alcoholic liver disease
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMI	Body Mass Index
CI	Confidence interval
CLIP	Cancer of the Liver Italian Program Investigators
CIT	cold ischemia time
CT	computed-tomography scans
CTM	cirrhosis related thrombotic material
CTP	Child-Turcotte-Pugh score
DDLT	deceased donor liver transplantation
EASL	European Association for the Study of the Liver
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
ICU	intensive care unit
iHCC	incidentally found HCC
INR	international normality ratio for prothrombin time
IPF	initial poor graft function
LDLT	live donor liver transplantation
LN	lymph nodes
LT	liver transplantation
MELD	Model for End stage Liver Disease
MIC	Milan criteria
MRI	Magnetic resonance imaging
NS	not significant
NTM	no thrombotic material
OR	Odds Ratio

pkHCC	previously known HCC
PET	Positron Emission Tomography scanning
PNF	primary graft nonfunction
PVT	portal vein thrombosis
PVTT	portal vein tumor thrombi
RFA	radiofrequency ablation
SLT	Deceased donor split liver transplantation
TACE	transarterial chemoembolization
TNM	tumor-node-metastasis classification
TPM	Truncated product method
TTM	tumor related thrombotic material
UCSF	University of California San Francisco
UICC	Union International Contre le Cancer

1. Introduction

1.1 Incidence of hepatocellular carcinoma

Hepatocellular carcinoma (HCC) ranks as the fifth most common cancer in the world with an increasing incidence, and represents the third most common cause of death from cancer worldwide (1-2). HCC has a dismal prognosis because the majority of cases are diagnosed at advanced tumor stage where the median survival totals only 6 to 9 months. The areas of highest incidence are Asia and sub-Saharan Africa, however, the incidence of HCC has rapidly increased also in the USA and Europe over the last 20 years (3). Chronic infection with hepatitis B or C viruses (HBV and HCV) dramatically increases the risk of HCC and is the main etiology (85%) of HCC worldwide. Chronic HBV infection is the predominant cause of HCC in China and sub-Saharan Africa, whereas chronic HCV liver disease is the main reason in Europe, Japan, Egypt and the USA. The incidence of HCC is expected to increase significantly in the next decade since there are about 300 million individuals infected with HBV and around 170 million people infected with HCV worldwide. Alcohol abuse in the setting of chronic viral hepatitis substantially increases the risk of HCC compared with viral infection alone. Consumption of aflatoxin-contaminated foods is an additional risk factor for HCC development, especially in sub-Saharan Africa, Southeast Asia and China, that can act synergistically with chronic viral hepatitis. In Europe and USA, obesity and type II diabetes, often associated with chronic nonalcoholic fatty liver disease, are emerging independent risk factors for the development of HCC (3). Most HCC patients have underlying liver cirrhosis, which *per se* represents a risk factor for cancer development independently from underlying chronic liver disease. Although the risk factors for HCC are well characterized, the molecular and cellular mechanisms that are responsible for malignant transformation of hepatocytes differ and are poorly understood for these various etiologies (3).

1.2 Surveillance

Patients at high risk for HCC should be considered for, and offered to be entered into surveillance programmes (4). These include all cirrhotic HBV carriers; non-cirrhotic patients with high HBV DNA concentration; patients with HCV-related or alcocholic cirrhosis, as well as patients with several rare disorders. Surveillance should

be performed using ultrasonography at 6- to 12-month intervals, associated or not with a-fetoprotein (AFP) determination, in order to detect early HCC amenable to surgical treatment with curative intent [II, B]. Despite correct surveillance, there are, however, still no data confirming that these advantages in detection of earlier lesions produce an improvement in long-term survival, and cirrhotic patients Child–Pugh B and C may have rather limited options for curative treatment.

1.3 Diagnosis

According to the European Association for the Study of the Liver (EASL) (5) and the American Association for the Study of Liver Diseases (AASLD) (6) guidelines diagnosis of HCC can be made if the following criteria are fulfilled: 1) liver biopsy-proven or 2) AFP>400ng/ml and hypervascular liver lesion detectable in one imaging technique (magnetic resonance imaging (MRI), spiral computed tomography (CT), angiography) or 3) hypervascular liver lesion detectable in 2 different imaging techniques. Based on the unique dynamic radiological behavior (contrast up-take in the arterial phase and rapid wash out in the venous/late phase) one imaging technique in nodules >2cm, and coincidental findings by two imaging techniques in nodules between 1 and 2cm are considered diagnostic for HCC in cirrhotic liver, thus making a liver biopsy dispensable (7). Surveillance of high-risk individuals such as patients with chronic HBV infection as well as all patients with liver cirrhosis should comprise abdominal ultrasound at 6-12 month intervals (6). The clinical value of tumor markers has been disappointing so far. For instance, although established as a serum marker for tumor surveillance AFP has a diagnostic value below that of imaging (8). Therefore, other biomarkers such as *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3), des-gamma-carboxy prothrombin (DCP), glypican-3 (GPC3), chromogranin-A(CgA), transforming growth factor beta 1 (TGF-β1), alpha-l-fucosidase (AFU) and Golgi protein 73 (GP73) have been developed to facilitate an early diagnosis and consequently improve the prognosis of HCC patients. However, the question of which molecular marker(s) will prove to be the most useful for selecting treatment for individual patients with HCC remains yet unanswered (9). Proteomic research has raised hope, but valid tumor markers have still not reached a common standard of clinical practice (10). Subtle genomic characterization may allow a molecular diagnosis of HCC, but the proposed

gene-set needs proper validation (11). This emphasizes the pivotal need for a better understanding of the molecular pathomechanisms underlying HCC development (12).

1.4 Treatment plan

The treatment plan in patients with HCC is based on the presence or absence of liver cirrhosis, tumor extent, tumor growth pattern, hepatic functional reserve and patient's performance status. The most commonly used therapeutic scheme has been proposed by the Barcelona Liver Cancer Clinic (BCLC) and has been adopted by EASL/AASLD guidelines (13). The widely applicable treatment possibilities include surgical (resection, transplantation), loco-regional (radiofrequency ablation, RFA; transarterial chemoembolization, TACE; selective internal radiotherapy, SIRT) and medical (chemotherapy) modalities or conservative management of symptoms. However, only hepatic resection (HR) and liver transplantation (LT) can meet the conditions of potentially curative treatment. There is evidence that RFA may be also potentially curative in patients with HCC at very early stage 0 (14-15). Given that more than 80% of patients with HCC have underlying cirrhosis, and of these patients only 10%-15% have potentially resectable tumors, the role of HR in the treatment of HCC is limited mainly to patients without underlying liver cirrhosis. Patients with liver fibrosis or with Child-Turcotte-Pugh (CTP) class A cirrhosis are candidates for partial HR only if they are in a good general condition and minor liver resection has to be undertaken. CTP class B cirrhotic patients are the most prevalent candidates for a multimodal treatment, including a combination of local and systematic therapies. In the era of the model of end stage liver disease (MELD)-score orientated allocation policy in the USA and most European countries, patients with CTP class C cirrhosis have good chances to receive LT if their tumor stage meets current listing criteria.

1.5 Hepatic resection

The value of HR in the treatment of HCC is usually focused on operative risk and curability. In the non-cirrhotic/non-fibrotic liver, HCC typically occurs in predominantly female patients without chronic hepatitis B or C infection and without any other apparent chronic inflammation of the liver. In such cases the diagnosis is usually made at advanced stage, when patients have already developed symptomatic

large tumors. HR is considered the treatment of choice in these instances, whenever technically feasible. Historical data from the 80ies showed 5-year survival rate after partial HR of 45% compared to 12% for patients being transplanted (16). The differences in outcome after partial HR and LT for HCC in the early era of LT may be well explained, since at the time many patients with HCC were transplanted for unresectable tumors. Besides, immunosuppressive therapy needed after LT may facilitate outgrowth of micrometastases as a result of a reduced anti-cancer immune surveillance. Several investigators have tried to identify variables that are associated with poor outcome after HR for HCC in the non cirrhotic liver; these variables include the presence of vascular invasion, multiple tumors, absence of tumor capsule, lymph node involvement and perioperative administration of blood transfusion (17-20). Reported 5-year survival rates vary between 30% and 68%, with a 5-year tumor free survival between 28% and 58%. However, tumor size *per se* does not consistently have a negative impact on outcome (20). In our institutional series of 83 resections for HCC in non-cirrhotic liver, vascular invasion, R1 resection (microscopic evidence of tumor in the resection margin), UICC tumor stage and tumor grading were prognostic factors for tumor recurrence (21). Eighteen patients had positive hepatitis serology. Mean tumor size was 9.0 cm, with solitary tumors evident in 66% of the instances. R0 resections (resections with tumor-free margins) were achieved in most operations (76%). Vascular invasion was present in 54% of the cases, with a proportional of 12% macrovascular invasion. After a median follow-up of 25 months, tumor recurred in 40 out of 63 (63%) patients after R0 resection in our series. Five-year overall survival was 30%. The achievement of a R0 HR (resection with tumor-free boundaries) was a prerequisite for optimal long-term outcome (21).

However, HR for HCC in the non-cirrhotic/non-fibrotic liver is not a common scenario, since HCC develops prevalently in a diseased liver in most of cases. Hence, the resectability rate remains very low, even in regard to the cases presented and evaluated in surgical departments. In a retrospective evaluation of 333 consecutive cases presented for surgery in our department, the estimated resectability rate was 35% (22) a systematic review of the literature showed an overall resectability rate of 30% in surgical departments worldwide, with 1808 resections reported in 6108 cases (22). Resectability rates were significantly higher in Japanese and Eastern series when

compared to American and Western studies, respectively ($p<0.001$). Indeed, HR for HCC in liver cirrhosis in the western world is either very infrequent, or is characterized by inferior results. A recent study showed 5-year overall survival rates after HR for HCC in the non cirrhotic versus cirrhotic liver of 50% and 17%, respectively ($p=0.032$) (23), the corresponding 5-year recurrence-free survival rates were 20% and 4%, respectively ($p=0.016$). In contrast, some Asian groups report acceptable 5-year survival rates between 44%-50% after liver resection for HCC in the cirrhotic liver, comparable to those of non-cirrhotic patients (24). Considering the evolution of the local ablative therapies and the modern interdisciplinary combination of them, HR for HCC in liver cirrhosis remains a matter of very careful evaluation in the western world (25).

1.6 Liver transplantation

LT appears ideally suited for HCC, as it involves complete oncological resection and correction of the underlying liver dysfunction. However, the results of early studies of LT for HCC were disappointing. More than 60% of patients developed tumor recurrence within the first two transplant years (26) and reported 1-year and 5-year survival rates were 42-71% and 20-45%, respectively (27). This was the consequence of the fact, that in the early era of LT, many patients with HCC were transplanted for unresectable tumors. At that time, extension of tumor to adjacent organs and macrovascular or lymph node involvement were not yet recognized as risk factors of very poor outcome after LT. In 1993, Bismuth et al. (28) retrospectively compared the results of resection and transplantation, and reported that patients with small tumors (no larger than 3cm, no more than two nodules, and no portal vein invasion) had better survival with transplantation than with resection. In 1996, on the basis of a retrospective review of 48 patients who underwent transplantation, Mazzaferro et al. (29) reported that in patients with a single tumor no larger than 5cm or with up to three tumors, none larger than 3cm, in the absence of vascular invasion, transplantation resulted in a 4-year overall survival rate of 75% and a 4-year recurrence-free survival rate of 83%. The survival rate was almost comparable to those of patients transplanted for non malignant indications (30-31). These proposed criteria made the break-through in the history of LT for HCC in cirrhosis. Most transplant centers and the United Network for Organ

Sharing (UNOS) have since adopted these criteria, which are now commonly referred to as the "Milan criteria."

Currently, there are 1-year survival rates up to 80%, 5-year survival rates up to 70%, and recurrence rates of 10-15% in patients fulfilling the Milan criteria (32). Thus, nowadays LT has been recognized as the most effective means of treating HCC patients at early stage, although the reduced anti-cancer immune surveillance due to inevitable medical immunosuppression may hamper a favourable outcome. Under MELD allocation, patients must meet the Milan criteria to qualify for exceptional HCC waiting list consideration. MELD was developed in 2002 by the Organ Procurement and Transplantation Network, along with the United Network of Organ Sharing, to prioritize patients on the waiting list. The new system leaves no room for subjective criteria favoritism, as it is based on a mathematical equation. In the Eurotransplant countries, Child Pugh Turcotte score was replaced by the MELD score in December 2006.

Patients may be registered at a MELD score equivalent to a 15% probability of pre-transplant death within 3 months. Patients will receive additional MELD points equivalent to a 10% increase in pre-transplant mortality to be assigned every 3 months until these patients receive a transplant or become unsuitable for LT due to progression of their HCC. The listing center must enter an updated MELD score exception application in order to receive additional MELD points. Pre-listing the patient should undergo a thorough assessment to rule out extrahepatic spread and/or vascular invasion. Evaluation of the potential transplant candidate is a complex and time consuming process that requires a multidisciplinary approach. This process must identify extrahepatic diseases that may exclude the patient from transplantation or require treatment before surgical intervention. The assessment should include CT scan or MRI of the abdomen and chest and a bone scan. Trimonthly routine follow-up examinations (MRI or CT scan) of wait-listed HCC patients for early detection of disease progression are recommended. Accurate discrimination of HCC patients with good and poor prognosis by appropriate criteria (genomic or molecular strategies) is highly warranted but still in the exploratory phases (33). In patients with alcohol-related liver disease and HCC, a multidisciplinary approach and thorough work-up of both alcoholic and oncologic problem is mandatory.

1.7 Organ shortage and increasing transplant demand for HCC patients

The increasing incidence of hepatitis C related cirrhosis in the Western world during the past 2 decades led to a corresponding increase of the related HCC-new cases. The potential cohort of new transplant candidates with HCC has increased rapidly. Additionally, due to improvements in the medical/conservative therapy of patients with advanced liver cirrhosis on the one hand, and the expanding application of a MELD-based organ allocation policy ("the sickest first") in more transplant registries on the other, the "gap" between new registrations in the waiting list and transplanted patients shows a continuous rise worldwide. In Germany, within a 10-year period of time, new registrations in the liver transplant waiting list increased annually from about 1000 cases in the year 2000 to more than 1800 cases in the year 2009. Simultaneously, the number of liver transplanted patients increased from about 800 cases in the year 2000 to only 1200 cases in the year 2009. This resulted to a consecutive increase of the "gap" between new registrations and transplanted patients from 200 cases to 600 cases annually within these 10 years (34). The resulting rising scarcity of organs led to efforts to "expand" the donor pool worldwide, in order to offer LT to more cirrhotic patients.

Concerning the HCC patients, these efforts were shared to 3 directions: 1) taking advantage of the technical innovations in liver surgery and liver transplantation, with the use of partial liver grafts, 2) taking advantage of the innovations in perfusion solutions, allocation rules, intraoperative management and intensive care unit treatment, with the use of expanded criteria donor grafts, and 3) expanding the well-established Milan criteria to less conservative ones.

The application of deceased donor split LT, aimed at adult patients with HCC combined with paediatric recipients, was an alternative option which has failed to fulfil its promise due to both logistical problems that permitted this procedure to be performed only in major transplant centers with active paediatric transplant programs, and because of complications associated with this technique (35). This alternative could not receive *per se* wide application. Live donor liver transplantation (LDLT) is an attractive alternative for the expansion of the organ pool for adult patients with HCC and end-stage liver disease (36), and is in part associated with an effort to expand the

Milan criteria (37). In these instances the strong will to donate among relatives plays a leading role in decision-making (38). However, the indications for transplantation of HCC patients in the era of LDLT are still being debated. While some centers proposed expansion of the current listing criteria (39-40), other centers remained conservative (41), emphasizing donor risks as well as the potential to return to the suboptimal transplant results of the 1980s. Besides, the whole LDLT setting presupposes an experienced high volume transplant center and the above reported limitations regarding donor safety and long-term oncological results do not allow the wide application of this technique in the western world. Acceptance of extended criteria donor grafts for stable patients with HCC became another potential solution. Its application, however, seems possible mostly in large volume transplant centers and is limited by local allocation rules.

The expansion of the tumor-specific criteria for LT beyond Milan is discussed controversially worldwide (40-41). In both Western and Eastern transplantation centers, some expanded criteria based on tumor morphology have been proposed, because the Milan criteria may be too restrictive. However, expansion of the criteria naturally carries a risk of increased posttransplant recurrence. Therefore, expanded criteria can be justified only if the criteria show acceptably low recurrence rates. Some of the proposed staging systems and extended criteria, are listed below: 1) Okuda staging system, based on tumor size (<50% or >50% of liver), presence of ascites, albumin level (cut-off 3g/dl), and bilirubin level (cut-off 3mg/dl) (42), 2) Cancer of the Liver Italian Program (CLIP) scoring, based on CTP score, tumor morphology (unilodar and extension <50% of liver, multinodular and extension <50% of liver, massive or extension ≥50% of liver), alpha fetoprotein level (cut-off 400ng/ml), presence of macrovasular invasion (43), 3) the Barcelona clinic liver cancer (BCLC) staging classification, based on tumor stage, Okuda stage and liver function status (44), 4) University of California San Francisco (UCSF) criteria, a solitary tumor ≤6.5cm in size or 3 or fewer nodules with the largest lesion ≤4.5cm in size and a total tumor diameter ≤8cm (45), 5) the Tokyo criteria, tumor number ≤5 and all tumors ≤5cm in size (46), 6) the Kyushu criteria, all tumors <5cm in size or protein induced by vitamin K absence or antagonist-II (PIVKA-II) level <300mAU/ml (47), 7) the Kyoto criteria, a tumor number ≤10, all tumors ≤5cm in size, and PIVKA-II level ≤400mAU/ml (48), and 8) the Up to 7 criteria, sum of the

size of the largest tumor in centimetres and the number of tumors ≤7 in the absence of microvascular invasion (49). Expansion of criteria in the LDLT setting is even more challenging due to the donor risk and the risk of selection of tumors with unfavorable biology following the concept of fast-tracking (50).

The existence of several scoring systems in this era of LT shows on the one hand the widely conviction of the transplant community that the well-established Milan criteria are too restrictive, bereaving from many HCC patients the LT opportunity; on the other hand, this situation reflects some limitations of the existing pre-transplant radiological evaluation (51). Multiple reports in the radiology literature address nodule detection in cirrhotic livers by means of CT, MRI, or ultrasonography. Many of them conclude that contrast-enhanced MRI is the most sensitive technique for detecting liver nodules (52-53). Krinsky et al. (54) showed that MRI depicted only 39 of 118 HCC in cirrhosis, for an overall sensitivity of 33%. Detection of small tumors was inadequate, with only 11 (52%) of 21 lesions between 1 and 2 cm and 3 (4%) of 72 lesions <1cm correctly classified. Lopez-Hanninen et al. (55), evaluating the sensitivity of CT, reported a sensitivity of 20% for small tumors between 1 and 2 cm. Similar findings were reported by Bhartia et al. (56), who concluded that the identification of tumors <1cm is still limited. The presence of microvascular invasion and, in some cases, macrovascular invasion of segmental branches can usually be determined by pathologic inspection of the explanted liver (57). This, together with the inaccurate tumor detection, leads to upgrading of the tumor stage or the classification according to the different sorts of criteria in the posttransplant period, in comparison to the assumed stages by radiological evaluation. More important, however, is the fact that many patients are not given the opportunity to undergo LT on the basis of inaccurate radiological and clinical preoperative staging (58). Besides, between a third and half of patients currently receive pre-transplantation bridging treatments such as TACE and RFA. The resulting necrosis can significantly affect tumor behaviour (59), making the objective assessment of tumour size and microvascular invasion very difficult. Tumor grade usually worsens while patients are waiting for transplantation (60), and AFP concentrations do not always correlate with tumor aggressiveness.

2. Research objectives

The Milan criteria on the one side can guarantee optimal long-term outcomes for patients with HCC in cirrhosis, but on the other side they are very restrictive; since as a large volume hepatobiliary and transplant centre we have evaluated increasing number of HCC patients during the past decade, we have tried to explore the existing possibilities to offer the transplant modality to more HCC patients. For this purpose we have built a corresponding database in December 2000, including retrospectively all HCC patients evaluated for LT since April 1998 in our centre, and prospectively developing and expanding this database from December 2000 up to September 2007. Since LT is the best treatment option for HCC patients in cirrhosis, but it is not possible to be offered to every HCC patient, we have tried to open up the Milan criteria, in order to transplant more HCC candidates and to determine prognostic factors outside the Milan criteria, which can offer an acceptable long-term outcome. In our research objectives following aspects were examined:

Bridging treatments

1. LT without bridging treatment – monotherapies for HCC
2. LT after bridging treatment
    a. Down staging / down sizing with TACE
    b. Complete tumor necrosis
    c. AFP decrease to negative or very-low levels (<30ng/mL)

Ethical issues

LT for alcoholic liver disease and HCC – LDLT?

Pre-translant radiological assessment

1. Accuracy of radiological staging
2. “incidentally” found HCC

Extended listing criteria

1. Patients outside Milan / within UCSF criteria
2. Portal vein thombosis

3. Pulmonary nodules- granulomas?

Expansion of donor pool

1. Extended criteria donors- rescue organ offers
2. Split LT
3. LDLT
4. Institutional results

Meta analyses

1. Hilar lymphadenectomy for hilar lymphadenopathy
2. Prognostic factors for tumor recurrence
3. Prognostic factors for survival

3. Material and methods

Evaluation and follow-up protocol for patients with HCC in cirrhosis

1056 patients with HCC were presented to our outpatient clinic for assessment of surgical resection or LT during the study period. The standard initial evaluation addressed the exclusion of extrahepatic liver disease and operability/operative risk. Criteria for partial hepatectomy included anatomically resectable disease and adequate reserve liver function. In cases of end-stage liver disease, LT was discussed as a possible therapeutic option. Patients with live donors were evaluated on a case-by-case basis according to their age, severity of liver disease, AFP levels, and HCC characteristics at the time of presentation. Milan criteria (29) were not considered an absolute contraindication in cases of LDLT (38). Whereas patients within Milan criteria at first evaluation and no further contraindications were further evaluated to LT without any additional tumor-related treatment, patients outside Milan criteria underwent bridging treatment in most cases, such as TACE or RFA either in our institution, or elsewhere. Portal vein thrombosis, arterio-portal fistula, extrahepatic disease, and decompensated liver cirrhosis were considered contraindications for TACE. The decision to perform RFA or TACE was made on an individual basis according to tumor characteristics, anatomical considerations, CTP Class and the general condition of each patient. Pre-operative tumor evaluation for potential candidates to LT included abdominal ultrasonography, thoraco-abdominal CT and/or magnetic resonance imaging, as well as bone scintigraphy. The diagnosis was confirmed in the majority of the patients by percutaneous liver biopsy. Serial levels of AFP were obtained prior to and after LT. The most recent AFP serum levels (normal range: <10ng/mL) prior to LT were considered for data analysis. Perioperative mortality was defined as patient death irrespective of cause within 45 days after LT.

Careful pathologic study (1cm slices) of all livers removed at transplantation was performed by a single pathologist. Pre and postoperative information on number of tumors, lobar distribution, maximal tumor diameter, presence or absence of microvascular and macrovascular invasion, and extrahepatic tumor spread, was computed. Total tumor diameter for patients with multiple tumor nodules was calculated by adding the greatest diameter of each lesion. Tumor classification was made according to the 6th Edition of the TNM System of classification of the UICC

(61). Of special interest were the extent of tumor necrosis and the viability (if any) of the ablated tumors. Nodules with a preoperative diameter less than 2 cm and no confirmatory biopsy that showed complete necrosis after ablation were not considered tumors as stipulated by the Barcelona criteria (5).

Follow-up studies included CT scans of the abdomen and chest, and measurement of AFP levels every 4 months during the first year after transplantation, every 6 months during the second year, and yearly thereafter. No patient received adjuvant chemotherapy after LT in these series.

3.1 Bridging treatments

3.1.1 LT without bridging treatments - Monotherapies as treatment for hepatocellular carcinoma in cirrhosis

Since HCC usually arises in the setting of cirrhosis, several treatment modalities have been developed to better address both pathologies i.e. malignancy and end-stage liver disease. Surgical procedures, ablative treatments, and systematic therapies have been evaluated in both single- and multi center trials. Liver resection, TACE, and LT are the most popular options. RFA represents another widely applied method in this task. Although many studies have compared results with various combinations of them, little information is available on outcomes as monotherapies at a single center.

Analysis of our institutional data was performed, in order to evaluate and compare long term results after liver resection, TACE, and LT when implemented as monotherapies in patients with HCC and cirrhosis (62). A total of 185 consecutive HCC patients with cirrhosis and no prior tumor treatments were included in this study. Patients were evaluated to HR or LT according to our evaluation protocol. TACE was considered for patients who did not qualify for liver resection or LT. Patients with prior tumor specific treatments, bridging treatments prior to LT, and re-admission for HCC recurrence were excluded from the study. Data were divided into 3 groups, according to the therapy received (group 1: liver resection; group 2: TACE; group 3: LT) and evaluated for 1) demographic and preoperative characteristics, 2) operative and intervention related details, 3) morbidity and mortality, 4) pathologic findings including number of tumors, tumor size, tumor stage and grading, 5) outcome as determined by

tumor recurrence and survival, and 6) analysis of prognostic factors for overall and disease-free survival.

Statistical methods used included nonparametric Fisher's exact test (one-tail) for categorical variables, Mann-Whitney U test for quantitative variables, and Power analysis (one and two-sided) for sample size calculations. Life tables were constructed with the Kaplan-Meier product limit method. Gehan's Wilcoxon test was employed to compare survival. Univariate and multivariable Cox proportional hazard regression analyses were used for predictors of morbidity and mortality. Significance was assigned at 0.05. Statistica release 7 (Statsoft) was used for statistical analysis.

3.1.2 LT after bridging treatment

3.1.2.a Tumor down-staging/down-sizing with TACE

In this study we evaluated the efficacy of TACE in patients with HCC who underwent LT by correlating the pathological findings in the explanted livers with the clinical classification of the tumors prior to TACE (63).

The data of 22 patients with liver cirrhosis undergoing TACE for HCC prior to LT according to our institutional TACE protocoll were reviewed. Twenty-one patients underwent one to three sessions of TACE prior to LT. Eleven patients underwent DDLT. LDLT was performed in 10 patients. Mean hospital stay associated with TACE was 3 days (range 2-5 days). Patients evaluated for LT who underwent TACE but died while waiting, were excluded from this study because no autopsy was performed.

Most patients (16/21) had virus-associated liver cirrhosis as the underlying disease (8 cases with hepatitis B and 8 with hepatitis C). Eight patients had solitary nodules with a median diameter of 4 cm (range 1.5-7 cm), 9 patients had 2-3 tumors with a median total diameter of 5.9 cm (range 3-9 cm) and 5 patients had a multifocal tumors prior to TACE. Six patients were within the Milan criteria before TACE was performed. Median waiting time to LT was 52.5 days (range 0-158 days). Two patients in whom LDLT was carried out after TACE had initially been on the waiting list for deceased donor LT for 111 and 273 days, respectively.

Tumor up-staging in pathology was documented as tumor-progression, whereas "steady disease" was considered when the same tumor stage was described both in

radiology and pathology. Tumor regression was characterized by down-staging or by the absence of vital tumor by pathological exam.

3.1.2.b LT after complete tumor necrosis following bridging treatment

Analysis of our institutional data was performed, in order to evaluate the course of the disease in transplanted patients with HCC and cirrhosis, in whom pathologic examination showed complete tumor necrosis after performance of TACE or RFA during the waiting time to LT (64).

Data corresponding to 10 patients with liver cirrhosis who underwent bridging treatments for HCC prior to LT and showed complete tumor necrosis in the explanted liver were reviewed. Bridging treatments included computed-tomography guided RFA and segmental or bilobar TACE. No patient received adjuvant chemotherapy after LT in these series.

3.1.2.c AFP decrease to undetectable or very low (<-30ng/mL) levels

Many investigators from different study groups considered the predictive value of various tumor characteristics in an effort to further understand the natural history of HCCs and to "expand" the listing criteria. AFP serial levels were evaluated both independently and as part of multivariate systems together with other parameters such as tumor size, tumor number, lobar distribution, tumor differentiation and vascular invasion (43). Various "cut-off values", such as 10ng/mL, 20ng/mL, 30ng/mL, 100ng/mL, 200ng/mL, 400ng/mL and 1000ng/mL, were proposed as predictors of both patient survival and tumor recurrence. Although the prognostic value of AFP has already been recognized, little information is available about the specific outcome of HCC patients with undetectable (<10ng/mL) or very low (11-30ng/mL) AFP levels who undergo LT.

The purpose of this study was to evaluate the outcome after LT of HCC patients with undetectable (<10 ng/mL) and/or very low (11-30 ng/mL) AFP values, both in our series and in the literature (65). Fifty one transplanted patients with histologically-proven HCC and low serial pre-transplant AFP levels (<30ng/mL), were included in this study. Patients with HCC were evaluated to LT and followed-up post-LT according to our protocol. Analysis of tumor characteristics was made according to pathological

findings. Perioperative mortality was considered in every survival and recurrence-rate analysis reported in this study in order to present "real data" without "optimizations," and in an effort to provide an "intent-to-treat" perspective for this analysis.

Search strategy and selection criteria

On June 2006, PubMed and Medline were accessed and searched to estimate the outcome after LT for HCC. Special emphasis was placed on patients having undetectable (<10ng/mL) or very low (11-30ng/mL) AFP levels. "Hepatocellular carcinoma", "liver transplantation", "tumor recurrence" and "alpha fetoprotein" were entered as keywords both independently and in multiple combinations. Only English language papers published between 1985 and 2006 were considered. In instances of multiple studies from a single institution, the most recent or more informative publication was chosen.

Statistical analysis

Mean values with standard deviation and median values with ranges were used for numerical data. The significance of differences was accessed by chi-square, *t*-test, and analysis of variance. Survival curves were estimated by the Kaplan-Meier method and compared with log-rank test. Differences of $p<0.05$ were considered to be statistically significant. Statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC) and StatXact (Cytel Software Corp., Cambridge, MA).

3.2 Ethical issues

3.2.1 LT for alcoholic liver disease and HCC – place for adult LDLT?

Alcoholic liver disease (ALD) is one of the most common causes of cirrhosis and constitutes one of the major indications for LT in Europe and North America. Five-year survival rates are approximately 70% (66). The reported survival rates after LT for ALD are similar to that in other forms of chronic liver disease and actually better than the corresponding survival rates after LT for viral hepatitis (66). Based on our latest experience in evaluating cirrhotic patients with HCC in ALD for LDLT, relevant ethical issues based in the evaluation of the donor, a family member of the recipient in most of

cases, led us to review both our center experience and the existing literature about LT for HCC in alcoholic liver cirrhosis and in the absence of viral hepatitis (67).

Patients with ALD and HCC who received live and DDLT were included in our study. Recipients with viral hepatitic infections were excluded. All transplant candidates were evaluated by Psychosomatic Medicine and Psychotherapy as part of our routine protocol for psychosocial and compliance clearance requirements. Patients were required a six month history of abstinence prior to listing with "Eurotransplant" (Austria, Belgium, Germany, Luxembourg, the Netherlands, and Slovenia). When such documentation was not attainable, participation in therapy groups was recommended prior to reconsideration. In cases of non-compliance during the waiting time period, patients were classified as "non transplantable" until re-evaluation and clearance by Psychosomatic Medicine and Psychotherapy was obtained. No abstinence contract was required prior to transplantation.

Patients with HCC were evaluated to LT and followed-up post-LT according to our protocol. Alcohol levels were only obtained in cases of suspected relapse. No patient was lost to follow-up.

Search strategy and selection criteria

A computer search of the Medline database for the years 1985 to 2005 was carried out using the MeSH headings: "Hepatocellular carcinoma," "liver transplantation," "alcoholic liver disease," and "alcoholic cirrhosis". The combined set was limited to English-language publications on human subjects. All titles and abstracts were scanned, and appropriate citations reviewed. Consultation with a content expert and a manual search of the bibliographies of relevant papers was also carried out to identify trials for possible inclusion. In instances of multiple studies from a single institution, the most recent or more informative publication was chosen.

3.3 Pre-transplant radiological assessment

3.3.1 Radiological versus pathological staging for HCC in cirrhosis

In spite of advances in imaging technology, detection of small HCCs, especially in end-stage cirrhotic livers, remains problematic. Prominent discrepancies between clinical and pathological staging continue to be observed. In this study, we evaluated the

accuracy of radiological imaging techniques by comparing pre-and post-operative findings in patients who underwent LT at our centre with diagnosis of HCC (68). Thirty five of them underwent LDLT, 32 orthotopic full-size DDLT and the remaining three deceased donor split LT (SLT). Sixty six of the seventy patients had a diagnosis of cirrhosis. The remaining 4 had recurrent HCC after having undergone hepatic resection in the absence of cirrhosis.

Patients with HCC were evaluated to LT and followed-up post-LT according to our protocol. MRI was additionally performed in 30 patients. These studies were interpreted independently by two different radiologists. Clinical classification was based on the morphological description of the tumor according to imaging studies. Thirty four patients underwent tumor specific bridging treatment for HCC (21 TACE, 13 RFA) prior to transplantation. Patients with live donors usually underwent LDLT 4 weeks after evaluation. The gap period between the last pre-operative imaging study and the transplant procedure was 7 days in those who received live donor livers, and a mean of 68 days (range 2-179 days) in those with deceased donor livers.

Statistical analysis was carried out using the chi-square test with Yates' correction for two-way contingency tables. Fisher's exact test was used when expected frequencies were low. Normality was checked by Kolmogorov-Smirnov test. $P<0.05$ was regarded as significant.

3.3.2 Incidentally found HCC

Despite improvements in imaging techniques, the diagnosis of small HCC in cirrhosis during the pre-transplant evaluation remains difficult, resulting in a considerable rate of undetected HCC in liver explants. Unsuspected HCC found at the time of histological examination of liver explants of patients transplanted for benign diseases is defined as "incidental HCC" (69). While there is no current worldwide consensus as to the "optimal" listing criteria for patients with known HCC and cirrhosis, some have suggested that undetected "incidentally found" HCC is associated with a better prognosis (70), similar to that of patients transplanted for non-malignant diseases.

The purpose of this study was to describe our experience with LT and incidental HCC (iHCC) and to present the findings of a literature search about LT and iHCC (71). Five patietns in our transplant series, transplanted with no preoperative diagnosis of

malignancy, had HCC detected "incidentally" in the liver explants (group 1). All patients had a diagnosis of liver cirrhosis and were evaluated for liver transplantation according to our standard evaluation protocol. Since tumor markers were obtained only once during the evaluation process in cases where no tumor was suspected, serial values were not available for those patients with incidental HCC. By suspicion of HCC during the waiting time to LT, patients were further evaluated with thoracic CT, bone scintigraphy and re-evaluation of AFP-levels. Patients with a suspicion of HCC during the waiting time to LT were not included in this group.

An additional 31 patients with a preoperative diagnosis of HCC received deceased-donor organs during the study period (group 2). In this patients' group AFP was estimated every 3 months during the waiting time to LT. Abdominal CT was performed at the time of evaluation to LT, whereas abdominal ultrasonography was routinely repeated at least every 3 months. If there was a suspicion for further tumor growth during the waiting time, abdominal and thorax computed tomography, as well as bone scintigraphy were performed. Ten patients underwent TACE as bridging treatment prior to LT, whereas 10 additional patients underwent RFA. According to the radiological imaging, all patients were meeting the Milan criteria at the time of listing and during the waiting time to LT.

All patients received a deceased donor full-size LT. The outcome of the patients with incidental tumors (group 1) was compared to that of the 31 patients with clinically detectable HCC prior to LT (group 2).

Search strategy and selection criteria

On March 2005, PubMed and Medline were accessed and searched to estimate the incidence and the outcome of iHCC in liver explants after LT. "Hepatocellular carcinoma", "liver transplantation", "incidentally found" and "liver explants" were entered as keywords both independently and in multiple combinations. Only English language papers published between 1985 and 2005 were considered. In instances of multiple studies from a single institution, the most recent or more informative publication was chosen.

Statistical Analysis

Survival was calculated using the Kaplan-Meier method, with statistical significance determined by the log rank test based on the exact permutation distribution. The Cochran-Mantel-Haenszel-Test was used to compare the two groups based on the data found in the literature. For the combination of p-values the truncated product method (72) was used with tau=0.05. Thus, studies that report a non significant result without giving the actual p-value could be included in the meta-analysis. *P* values ≤0.05 were considered significant. The software SAS (SAS Institutute Inc., Cary, NC, USA) and StatXact (Cytel, Cambridge, ass., USA) were used to perform the statistical analysis.

3.4 Extended listing criteria

3.4.1 LT for patients beyond Milan/ within UCSF criteria

The Milan criteria have been expanded in some major LT centers, especially when performing LDLT for HCC, and the outcomes have remained somewhat comparable, especially regarding patient survival (39-41, 45). Although some studies have addressed comparison of survivals according to the Milan and the UCSF criteria (45, 73), only limited information is available about the real benefit for patients when expanding the listing criteria from the Milan to the UCSF guidelines.

The purpose of this study was to retrospectively evaluate the results after LT for HCC patients beyond the Milan but within the UCSF criteria based on pathological findings (74). Data corresponding to patients transplanted for HCC were reviewed. Patients with HCC were evaluated to LT and followed-up post-LT according to our protocol. Of special interest was the classification of the HCC according to both the Milan criteria and the UCSF criteria.

3.4.2 LT for HCC and portal vein thrombosis

Various determinants of “high risk pathology” for tumor recurrence and decreased patient survival after LT for HCC such as tumor size, tumor number, lobar distribution, vascular invasion, tumor differentiation and AFP levels, have already been identified. However, there is still little information on the outcome of transplant

recipients with HCC and portal vein thrombosis (PVT). Although tumoral invasion of the portal vein constitutes an "absolute" contraindication to LT even at transplant centers with expanded guidelines for living donor LT (39-40), the etiology of portal vein thrombosis is not always evident. Portal vein thrombus may develop secondary to HCC "compression" of the portal vein, as a result of direct tumor (macrovascular) invasion, or due to stagnation associated with the underlying cirrhosis. Furthermore, sometimes PVT in the presence of HCC is "artificial", i.e. an apparent vascular complication following ablative bridging treatments prior to LT (75). Since a thrombophilic genotype is reported to be present in almost 70% of patients with PVT in the absence of hepatocellular carcinoma (76), the distinction between benign and malignant portal vein thrombi is essential when deciding whether to proceed with transplantation.

The purpose of this study was to further evaluate the outcome of recipients with portal vein thrombosis in deceased and live donor liver transplantation (77).

Selection criteria, evaluation and follow-up

Prospectively collected data on HCC patients transplanted during the period 04/98-09/05 in our center were analyzed, with special interest in the documentation of PVT. Only HCC patients with partial PVT in the explant were included in this study.

Patients with HCC were evaluated to LT and followed-up post-LT according to our protocol. Both DDLT and LDLT recipients were considered. Evidence of tumor related PVT in the pre-transplant radiological imaging was considered as a contraindication to LT. In cases of doubtful findings regarding portal vein invasion, the decision whether or not to proceed with LT was made on an individual basis according to various tumor characteristics (AFP, tumor grading, tumor size, lobar distribution, tumor number). Although the Milan criteria and, more recently, the UCSF criteria are generally used as listing criteria for deceased donor LT in our center, some cases with debatable portal vein invasion could be transplanted either with living donor grafts, or with grafts which were repeatedly refused from other centers as "marginal" (40, 78). Data collection was completed by September 30, 2006. Minimum follow-up was either 1 year or until death. No patient was lost to follow-up.

Study Design

Data corresponding to transplanted HCC patients with PVT documented by pathological exam were reviewed by two experienced pathologists. Special consideration was given to the presence of thrombotic material within the portal vein, as well as to the benign or malignant nature of the thrombus. An experienced radiologist re-studied the most recent pre-transplant radiological imaging studies (CT and/or MRI) of all cases where PVT was identified in the explant liver.

3.4.3 Pulmonary modules at risk in patients undergoing LT for HCC

The recent availability of sophisticated radiological imaging during the pre-transplant evaluation has led to a new clinical problem in the treatment of HCC patients: the diagnosis of small pulmonary nodules. In fact, the problem is more global, since the detection of nodules as small as 1-2mm in diameter has become routine in the late 1990s. However, the majority of small nodules are benign, of which 80% are granulomas or intrapulmonary lymph nodes (79). The doubling time of most malignant solid nodules is between 30 and 400 days. Nodules displaying more rapid or slower doubling times are typically benign in origin. Radiological stability, either on chest radiography or CT, over a period greater than 2 years implies a doubling time of at least 730 days, which is generally considered to be a reliable indicator of a benign lesion (79). Although there are already some guidelines for the management of small indeterminate pulmonary nodules, according to their prior probability of malignancy (80), in patients with a suspected or known cancer the nodule could be secondary to a pulmonary metastasis and must therefore be managed according to a protocol adapted to the clinical situation. In such a situation, repeated surveillance CT examinations may be indicated to study the growth of the nodule (80).

We analyzed data collected prospectively on patients transplanted at our Center with a diagnosis of HCC between April 2001 and July 2005. We specifically addressed pulmonary nodules detected by pretransplant imaging studies and categorized as granulomas. The term ‘granuloma’ was used to avoid the terms mass or lesion, which is automatically associated with malignancy. Granulomas are benign lesions caused by infection or inflammation and can be seen as postinflammatory residua. In general, the

criteria for benign lesions were: diffuse dense calcification, diagnostic criteria of hamartoma (round shape, smooth, regular contours, containing fat density, ±popcorn calcification) or benign-type calcification (i.e. central, target, laminated, concentric). All nodules included in this study showed either calcification, smooth contours or connection to vessels and size smaller than 10 mm (81). Positron Emission Tomography (PET) scanning was not applied in these series, as all the patients showed multiple, smaller than 10-mm pulmonary nodules. Both DDLT and LDLT recipients were considered. Data collection was completed by July 31, 2007. Minimum follow-up was either 2 years or until death.

3.5 Expanding the donor pool

3.5.1 Extended criteria donor for LT for HCC – rescue organ offers

We herein describe our experience with seven "livers that nobody wanted" within Eurotransplant, which had been official rejected on 34 different occasions by other transplant centers (81). These livers were implanted onto patients in good clinical condition with HCC and cirrhosis, who probably would not have survived long enough to receive another allograft offer, or who would have otherwise been dropped-out during the expected waiting time of 6-8 months because of tumor progression.

Seven patients, with a diagnosis of HCC and cirrhosis, received allografts that had been initially offered to and rejected by at least 3 transplant centers each in accordance to the allocation policies of "Eurotransplant". These grafts were classified as "rescue offers" (78). Data were obtained from both the "Eurotransplant" database and patient records. Weight of the donor, BMI, age, gender, macrovesicular steatosis, ICU stay of donor, prolonged hypotensive episodes, cold ischemia time, peak serum sodium, sepsis, viral infections, alcoholism, bilirubin levels, ALT levels, AST levels, abnormal liver vascular anatomy, vascular injuries during procurement, and evidence of extrahepatic neoplasia were reviewed. During the time period of the study, frozen sections were not routinely performed at the time of procurement. The assessment of donor livers by the donor surgeon was based on visual inspection, palpation, and appearance after flushing with preservation solution. Graft quality was then characterized as good, acceptable or poor.

The clinical status of the recipients at the time of LT was scored according to the standard grading used by Eurotransplant (based also on waiting time and Child-Turcotte-Pugh scoring): 1) T1: fulminant hepatic failure/acute graft failure, 2) T2: chronic liver disease with acute deterioration (corresponding to the former UNOS status 2A), 3) T3: chronic liver disease with complications (corresponding to the former UNOS status 2B), 4) T4: chronic liver disease without complications (corresponding to the former UNOS status 3) and 5) T5: temporarily not transplantable. Age, gender, waiting time to LT, etiology of liver failure, bridging treatment prior to LT, levels of bilirubin, albumin, creatinine, CTP score, and MELD score at the time of LT were also reviewed. TNM classification and Milan criteria were based on the pathologic findings of the explanted liver.

Postoperatively, liver function tests were measured on arrival at the ICU and subsequently every 3 hours during the ICU stay. Prostaglandin E-1 was not routinely used to optimize graft function. After discharge from the ICU, liver function tests were measured daily. AST or ALT levels >1500U/mL on two consecutive measurements within the first 72 hours after LT were considered to indicate IPF (9). PNF was defined as poor function of the allograft culminating in either death of the recipient or retransplantation. Vascular and biliary complications leading to re-operation, renal insufficiency requiring dialysis, severe infections leading to sepsis, cardiac complications and acute rejection were considered. ICU stay, total hospital stay, tumor recurrence, chronic rejection, and survival were also reviewed.

All the patients were followed pre- and postoperatively at our outpatient liver transplant clinic. Because of the small number of patients included in this study no statistical analysis was performed.

3.5.2 Split LT for HCC

The ever-increasing disparity between demand for transplants and supply of organs from deceased donors has meant that many HCC patients who might benefit from transplantation either die before an organ becomes available or must be removed from the waiting list as the result of tumor progression. Alternative options aimed at reducing this shortfall include expanded donors/expanded grafts for HCC, domino

transplantation, live donor liver transplantation and deceased donor split-liver transplantation (SLT). We herein describe our experience with deceased donor SLT for HCC (83).

The data of 6 patients with HCC in liver cirrhosis undergoing SLT were reviewed. Patients with HCC were evaluated to LT and followed-up post-LT according to our protocol. Our concurrent paediatric liver transplant program constituted the leading reason for us to perform SLT. The presumed higher rate of complications associated with adult SLT prompted us to consider also adult tumor patients in stable condition as recipients of the right allograft remnant whenever possible, according to the allocation rules of "Eurotransplant." Such allocation allowed for a reduced waiting time to LT, and a potential decrease in the drop-out rate from the waiting list. In situ splitting of the deceased donor liver was performed whenever the organ was procured by our team. Otherwise, the accepted liver was divided ex situ. Vascular and biliary complications leading to re-operation, renal insufficiency requiring dialysis, severe infections leading to sepsis, cardiac complications, acute rejection, ICU stay, total hospital stay, tumor recurrence, and survival were reviewed.

3.5.3 LDLT for HCC

Being part of a transplant center with an initially "liberal" policy concerning LDLT for HCC, and after critically evaluating our preliminary observations (40), we present in this study our current results. Based on these findings, we also propose prognostic parameters for a better selection of living donor liver transplant candidates with HCC (84).

Data from 57 patients with histologically-proven HCC were reviewed from our prospectively collected database. Patients with HCC were evaluated to LT and followed-up post-LT according to our protocol. The median "gap-period" between the last computed tomography scan and LDLT was 0.7 months (range 0-1.2 months).

Analyses were performed in two arms, including and excluding perioperative mortality, in order to provide an "intent-to-treat" perspective as well as to identify risk factors for perioperative mortality.

Statistical analysis

Mean values with standard deviation, and median values with ranges, were used for numerical data. The significance of differences was accessed by chi-square, *t*-test, and analysis of variance. Survival curves were estimated by the Kaplan-Meier method and compared with Gehan's Wilcoxon test. Univariate and multivariable Cox proportional hazard regression was used in order to estimate the prognostic value of the individual parameters. Categorical data were represented by corresponding dummy variables. Continuous variables were transformed into categorical variables. Differences of $p<0.05$ were considered to be statistically significant. We used the structural equation modelling and bootstrap method (85) for internal validation of the score system. Bootstrap validation is a method of random re-sampling from a given set of samples to simulate the effect of drawing samples from the same population. A re-sampled data set of the same size as the original (training) data set was obtained by random sampling with replacement- in other words, each sample can be drawn more than once or not at all. Differences in three and five year survival rates were calculated between each pair of contiguous stages (for example, between scores 2 and >2) using Gehan's Wilcoxon test. Statistical analyses were performed using Statistica (Version 7.0 Statsoft).

3.5.4 Institutional results

We report herein our experience with 100 consecutive LT for HCC performed at our Department, during an eight-year period (86). Transplanted patients with complete necrosis of the HCC after bridging treatment and no preoperative diagnostic biopsies (n=2), children (n=2), and patients with a post transplant diagnosis of cholangiocellular carcinoma (n=2) were excluded.

Patients with HCC were evaluated to LT and followed-up post-LT according to our protocol. Magnetic resonance imaging of the abdomen was performed in 34 patients and total body positron emission tomography in 15 patients to further outline unclear findings and improve staging. HCC invasion into the portal vein was classified as Vp0 (none), Vp1 (microscopic), Vp2 (into the segmental branch), and Vp3 (into the lobar vein or the main trunk). Perioperative mortality was considered in an effort to provide an “intent-to-treat” perspective to this analysis. Recurrence rate was defined as

percentage of HCC recurrences between all HCC transplanted patients during the follow-up period.

4. Results

4.1 Bridging treatments

4.1.1 LT without bridging treatments - Monotherapies as treatment for hepatocellular carcinoma in cirrhosis

Demographic and preoperative characteristics

There were 185 patients, of which 149 were males and 36 females (ratio 4.14:1). Median age was 62.4 years (range 17.7–80.5). The aetiology of cirrhosis was HCV in 36% of cases, HBV in 17%, alcohol abuse in 21%, cryptogenic in 12%, steatohepatitis in 3%, HCV and alcohol abuse in 2%, HBV and HCV in 2%, and other causes or combinations in 7%. Median MELD score was 9 (range 2–42). Sixty two patients met the Milan criteria. The remaining 123 were beyond these criteria. Median AFP level was 19ng/ml (range 1–64200ng/ml).

Operative and intervention related details

Sixty one (n=61) patients underwent liver resection, 64 TACE, and 60 LT. Among the resection group (n=61), 20 underwent simple segmentectomies, 14 bisegmentectomies, 8 right hemihepatectomies, 5 left lateral sectionectomies, 5 left hemihepatectomies, 4 atypical resections, 3 trisegmentectomies, one right hemihepatectomy plus segment II excision, and one right hemihepatectomy plus caudate lobectomy. Among the TACE group (n=64), 10 patients underwent one session, 15 patients 2 sessions, 5 patients 3 sessions, 8 patients 4 sessions, and 24 patients 5-14 sessions. Among the LT group (n=60), 30 patients underwent living donor LT, 27 deceased donor LT, and 3 split graft LT. Median ICU and hospital stays for the resection, TACE and LT groups were 1 and 16 days, 0 and 2 days and 6 and 29 days, respectively.

Morbidity and Mortality

Morbidity rates for the resection, TACE, and LT groups were 38%, 11% and 38% respectively. Resection group complications included biliary leaks (n=2), abdominal abscesses (n=2), evisceration (n=1), pulmonary failure (n=2), multiorgan failure (n=5), liver insufficiency (n=9), massive ascites (n=1) and peritonitis (n=1). TACE complications included encephalopathy (n=1), emesis (n=1), fever (n=1), super-

infection of the ablated liver requiring CT guided drainage (n=3), and extensive liver necrosis (n=1). LT complications included encephalopathy (n=1), bleeding/hematoma (n=5), hepatic artery thrombosis (n=4), bile leaks (n=2), pulmonary embolism (n=1), pancreatitis (n=1), renal insufficiency (n=1), poor primary function/small-for-size syndrome (n=2), pneumonia (n=2), central pontine myelinolysis (n=2), and abscess (n=1).

Predictors of complications were defined by general linear models analyses of covariance. Age, MELD score, AFP levels, and tumor diameter constituted continuous variables. Extent of surgery in the resection group, number of sessions in the TACE group, deceased versus living donors in the transplantation group, UICC stage, tumor multifocality, and meeting/exceeding the Milan criteria constituted categorical variables. None of the above variables reached statistical significance as an independent predictor of complications by multivariate analysis (Sigma-restricted parameterization). The corresponding model (Test of SS Whole Model vs. SS Residual) was not able to predict complications in any of the three groups (resection group; p=0.109, TACE group; p=0.231, LT group; p=0.740).

Thirty-day mortality for the resection, TACE and LT groups was 23%, 0% and 8% respectively. Fourteen resection group patients died within 30 postoperative days: 12 as a result of multiorgan failure**,** one due aspiration pneumonia, and one of a massive pulmonary embolism. There were 5 30-days deaths in the LT group: 2 patients died secondary to multiorgan failure, and one each as a result of massive pulmonary embolism, methicillin resistant Staphylococcus aureus pneumonia and right ventricular failure. No early deaths were observed in the TACE group. One-tailed Fisher exact p test showed a significant difference in 30-day mortality among the resection and LT groups (p=0.048).

Pathology

Median tumor diameter was 4cm in the resection group (range 1–19cm), 6cm (range 3–14cm) in the TACE group, and 4cm (range 1-16cm) in the LT group. HCCs were solitary in 101 (55%) patients (50 in group 1, 22 in group 2 and 29 in group 3). Multifocal tumors were encountered in 66% (n=42) of TACE cases, 52% (n=31) of LT instances, and 18% (n=11) of resection patients. UICC stages I, II and >II were

encountered in 66 (group 1=31, group 2=14, group 3=21), 50 (group 1=12, group 2=16, group 3=22) and 69 patients (group 1=18, group 2=34, group 3=17), respectively. Microscopic vascular invasion was detected in 17 patients from group 1, and 12 patients from group 3. Evaluation of tumor grading by pathology revealed moderately differentiated HCCs in about half of the cases in the resection and LT group (36/61 and 35/60, respectively). Tumor characteristics are depicted on Table 1.

Comparisons were made among the three groups by Kruskal-Wallis ANOVA and Median test for the following parameters: Gender (p=0.0366; all comparisons significant), Age (p=0.0001; resection vs TACE=NS), MELD score (p=0.0001; resection vs TACE=NS), AFP (p=0.6769; all comparisons NS), Aetiology of cirrhosis [p=0.0024; significant only TACE (HBV/HCV=10/19) vs LT (HBV/HCV=17/30)], Milan criteria (p=0.988; significant only TACE vs resection and TACE vs LT), Complications (p=0.989; significant only TACE vs resection and TACE vs LT), ICU Stay p=0.0001 (all comparisons significant) and Hospital stay p=0.0001 (all comparisons significant).

		Resection Group n=61	**TACE* group n=64**	**Transplantation Group n=60**
Tumor Grade	*well* *moderate* *poor*	12 36 13		15 35 10
Multiple tumors	*Yes/No*	11/50	42/22	31/29
Maximum tumor diameter (cm)	*Median* *Range*	4 (1-19)	6 (3-14)	4 (1-16)
UICC stage	*I* *II* *IIIA* *IIIB* *IIIC* *IV*	31 12 12 0 5 1	14 16 18 3 12 1	21 22 15 2 0 0
Milan Criteria	*Meeting* *Exceeding*	26 35	10 54	26 34
Lymphatic invasion	*Yes/No*	4/57		1/59
Microvascular invasion	*Yes/No*	17/44		12/48
R-Class	*R0/R1/R2*	46/7/8		60/0/0

Table 1: Tumor characteristics; *all data for TACE group are based on clinical diagnostic work up.

Survival and recurrence

Seventy one (n=71) patients are currently alive after a median follow up of 15.3 months (range: 0.2–144). Twenty one (n=21) resection patients are alive, 14 without recurrence, in the resection group. Thirteen (n=13) patients are alive, 3 without current evidence of vital tumor, in the TACE group. Thirty seven (n=37) recipients are alive, 35 without recurrence, in the LT group. Forty of 185 total patients (22%) were followed for more than 3 years. Five-year overall and recurrence-free survival rates for the entire series (n=185 patients) were 29% and 32%, respectively (Figure 1).

Median survival after resection, TACE and LT was 11 months (range: 0.2-67.4), 14 months (range: 1.2-143.9), and 23 months (range: 0.3-105) respectively. One-, 3-, and 5-year cumulative survival rates after resection, TACE and LT were 52%,43%,23%; 60%,13%,10%; and 78%,69%,59%, respectively (Table 2). LT recipients had significantly increased survival (Figure 2) (p=0.001). Survival differences among resection and TACE (p=0.754) achieved statistical significance (p=0.006) only when 30-day mortality was excluded.

Median disease-free survival for the resection and LT groups was 15.5 months (range: 0.2-67.4) and 29 months (range: 0.3-105) respectively. TACE patients were considered to have remnant tumor since no pathological evidence of clear margins or complete tumor necrosis could be produced. One-, 3-, and 5-year cumulative disease free survival among resection and LT patients was 73%;50%;15% and 90%;84%;77% , respectively (Table 2). There was a significant difference (Figure 3) favouring the LT group (p=0.002).

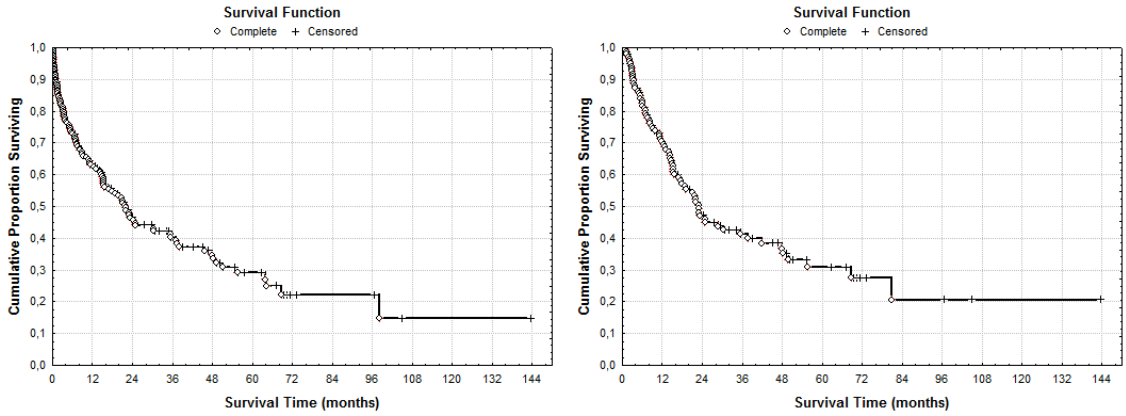
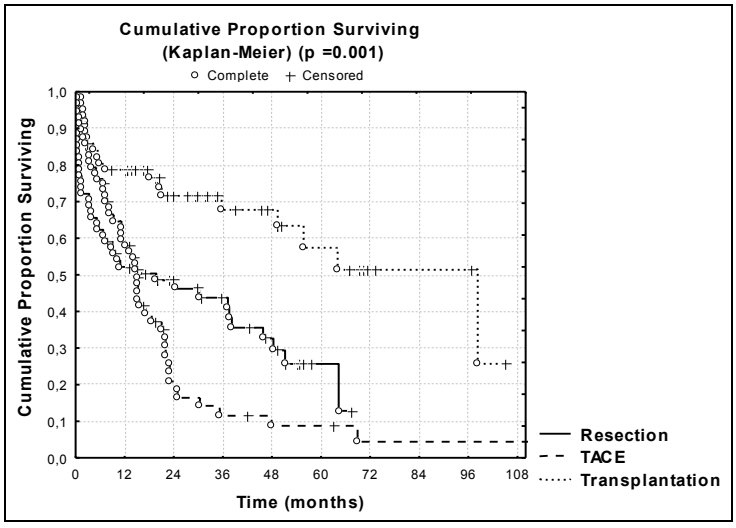


Fig. 1: Overall (left) and recurrence-free (right) survival for the entire series (n=185).

	Cumulative proportion surviving (%)		
Year	***Resection group***	***TACE group***	***LT group***
1	51.66	59.67	78.37
2	47.83	24.24	71.84
3	43.17	13.46	68.72
4	32.03	10.47	68.72
5	22.87	10.47	58.54
	Cumulative proportion disease free surviving (%)		
Year	***Resection group***	***TACE group***	***LT group***
1	72.97	-	89.79
2	55.80	-	84.18
3	49.60	-	84.18
4	40.33	-	84.18
5	14.66	-	77.44

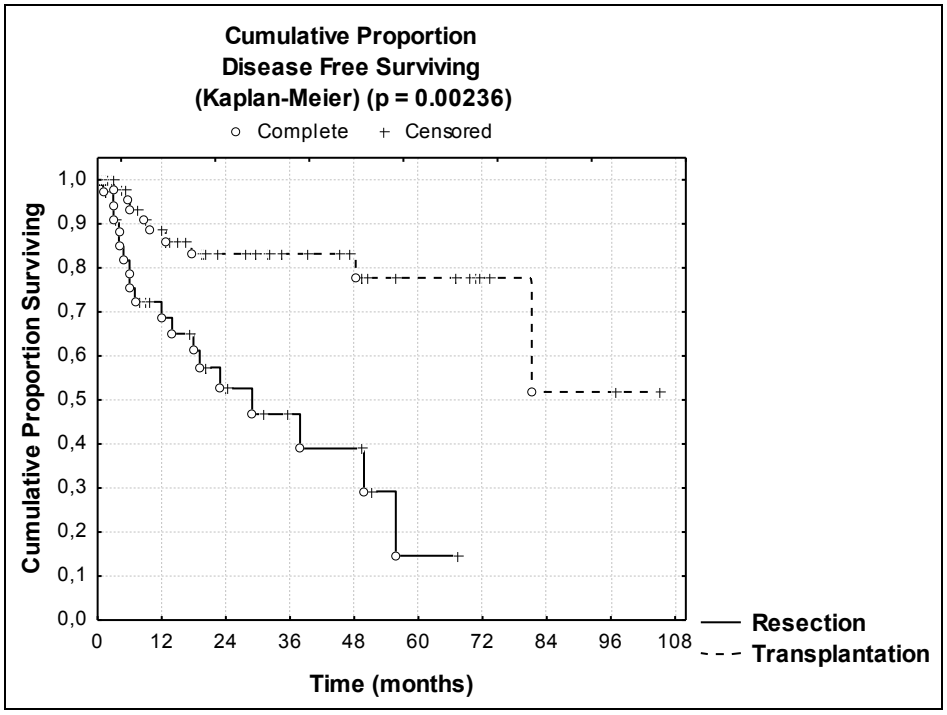
Table 2: Yearly cumulative proportion surviving for patients of the three groups.



Patients at Risk

Months after treatment	0	12	24	36	48	60	72	84
LT	60	44	28	19	16	11	5	4
Resection	61	31	23	17	11	3	-	-
TACE	64	38	11	7	5	4	3	2

Fig. 2: Cumulative Proportion Surviving (Kaplan-Meier curves) by groups.



Patients at Risk

Months after treatment	0	12	24	36	48	60	72	84
LT	60	37	25	18	15	9	4	2
Resection	61	19	11	6	5	1	-	-

Fig. 3: Cumulative Proportion of disease Free Surviving (Kaplan-Meier curves) for the resection and transplantation groups.

Analysis of prognostic factors for overall and disease-free survival

We performed a univariate analysis for each group, taking into account age, gender, MELD score, AFP, cause of cirrhosis, tumor grade, lymphatic invasion, microvascular invasion, resection margin status, UICC stage, tumor multifocality, maximum tumor diameter, meeting or exceeding the Milan criteria, and postoperative complications. Resection margins status (p=0.024), tumor multifocality (p=0.012) and complications (p=0.007) were significant predictors of survival in the resection group. MELD score (p=0.0006), maximum tumor diameter (p=0.05) and Milan criteria (p=0.02) reached statistical significance for TACE. AFP level (p=0.04), tumor grade (p=0.007), lymphatic invasion (p=0.05), tumor multifocality (p=0.05), maximum tumor diameter (p=0.023), Milan criteria (p=0.048) and complications (p=0.05) gained significance in cases of LT.

A further multivariable analysis followed considering only predictors that reached significance in univariate analysis. The only independent predictor of survival

in the resection group was the presence of complications (p=0.004), MELD score (p=0.0003) and maximum tumor diameter (p=0.05) in the TACE group, and tumor grade (p=0.01) and complications (p=0.004) the LT group.

In order to document a difference in 5 year survival rate between resection group (23%), TACE group (10%) and LT group (59%) we applied a two-sided survival log-rank test under Power analysis with a type I error (Alpha) of 0.05 and a power of 0.95. Forty one patients per treatment arm would be sufficient to detect a difference in survival rates between the three groups. This prerequisite is fully covered by our data.

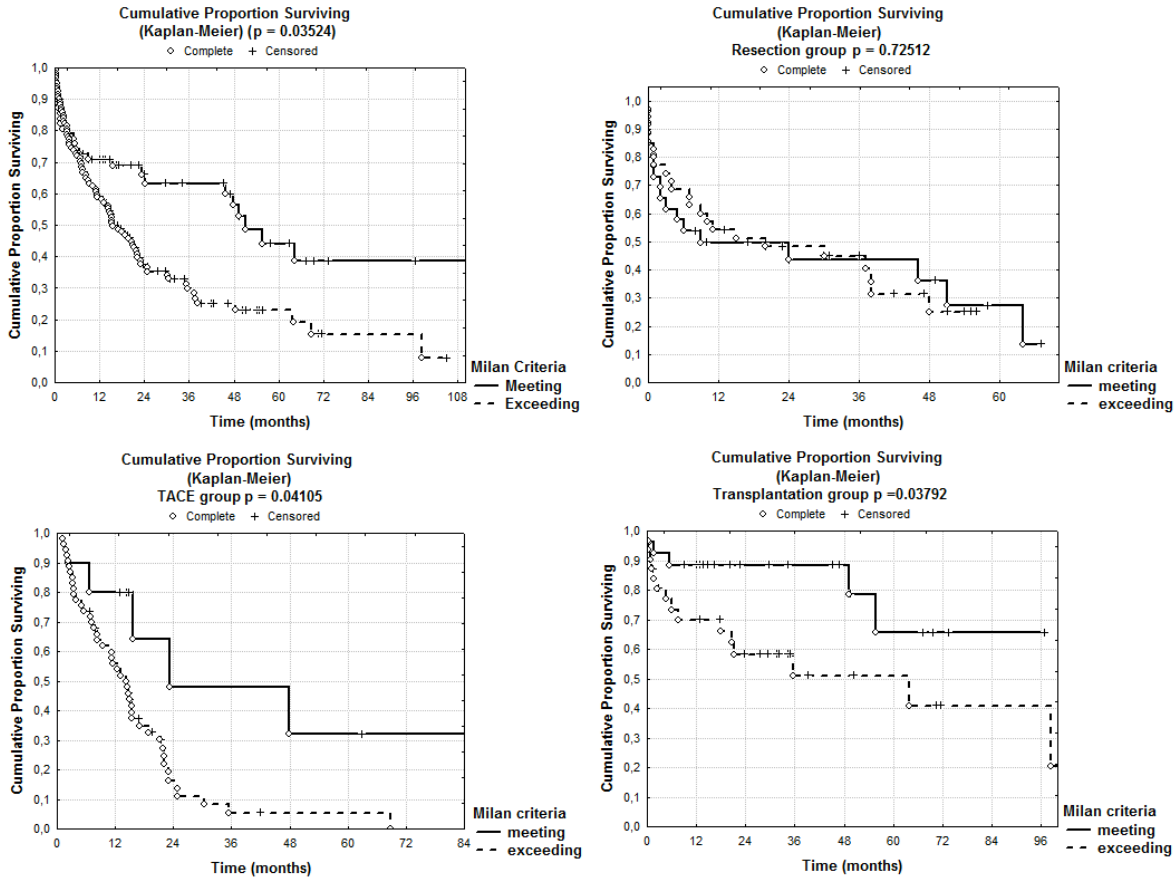


Fig. 4: Cumulative Proportion of disease Free Surviving (Kaplan-Meier curves) according to the Milan criteria for the entire series (a), resection group (b), TACE group (c) and LT group (d).

4.1.2 LT after bridging treatment

4.1.2.a Tumor down-staging/down-sizing with TACE

There was no treatment-related morbidity in these patients' group. Since there were neither lymph node metastases in the hepatic hilum nor intraabdominal tumor

spread at the time of transplantation in any of the patients tumor classification according to the UICC-classification refers to the T-category of the TNM-classification only.

Table 3 illustrates the changes encountered at the time of pathologic examination in reference to the clinical staging. Tumor regression was observed in over half of the patients (11 patients, 52.4%). Tumor regression included under staging of 3 cases from T2 to ypT1, of 2 cases from T3 to ypT2, of 1 case from T3 to ypT1 and the 5 cases (23.8%) with no evidence of vital tumor at all. In the last 5 cases, where no vital tumor was found in pathology, initial staging before the performing of TACE was T1 and T2 in three and two cases, respectively. Three patients (14.3%) exhibited tumor progression including up staging from T1 to ypT2 in one case and from T2 to ypT3 and to ypT4 in the two other cases. In 7 of the patients (33.4%) undergoing TACE there was no change in TNM staging ("steady disease"). Table 4 illustrates the changes in the course of the disease after treatment with TACE prior to LT.

		Pathological classification				
		TACE n=21				
Clinical classification		ypT1	ypT2	ypT3	ypT4	No vital tumor
	T1	2	1	-	-	3
	T2	3	1	1	1	2
	T3	1	2	4	-	-
	T4	-	-	-	-	-

Table 3: Changes in the TNM classification of tumors after treatment with TACE (6thEdition of TNM classification).

From the five patients having a multifocal tumor prior to TACE, only one patient experienced a tumor down-staging (from T2 to ypT1); in 2 patients "steady disease" was found in pathology (T2 to ypT2, T3 to ypT3), whereas in the last 2 patients tumor progression was observed (T2 to ypT3, T2 to ypT4).

The pathological analysis of the explanted livers showed tumor lesions "meeting" the Milan criteria in 11 patients (prior to TACE only 6 patients were within the Milan criteria); however, 10 patients were still "exceeding" these criteria (in comparison to 15 patients prior to TACE).

Course of the disease as proved in pathology		TACE n=21
Tumor progression	Yes	3
	No	18
Tumor regression	Yes	11
	No	10
Steady disease	Yes	7
	No	14
Vital tumor	Yes	16
	No	5

Table 4: Changes in tumor behaviour as a result of treatment with TACE prior to LT.

4.1.2.b LT after complete tumor necrosis following bridging treatment

Pathological exam showed complete tumor necrosis in all instances. Because of the extension of the necrotic areas, no estimation of grading was possible. There were 8 men and 2 women (Table 5). RFA was performed with percutaneous expandable electrode needles under computed tomographic guidance in 4 patients. Five of the 6 patients undergoing transarterial chemoembolization underwent one session, while the remaining patient received a series of 4 sessions prior to LT. Five patients underwent deceased donor full-size orthotopic LT. One received a deceased donor split allograft. LDLT was performed in 4 patients. AFP levels were elevated in all patients at the time of the initial evaluation, with a median value of 76 U/ml prior to bridging treatment (range 58-305 U/ml, normal value <10 U/ml). Median hospital stay associated with the performance of RFA or TACE was 3 days (range 2-5 days).

The etiology of cirrhosis was alcohol in four cases, hepatitis B in two instances, cryptogenic in two other cases, combined hepatitis B and C in one instance, and primary sclerosing cholangitis in the remaining patient. Six patients had solitary nodules with a median diameter of 3.5cm (range 2.5-4.2cm), as determined by radiological evaluation prior to bridging treatments. Four of them underwent RFA, whereas segmental tumor chemoembolization was performed in 2 patients. All these patients fulfilled the Milan criteria prior to the beginning of the treatment. The remaining 4 patients had 2 tumors each with a median total diameter of 4.4cm (range 4.2-6.0cm) prior to TACE and

underwent bilobar chemoembolization with the intent to downstage the HCC. Radiological evaluation after performance of bilobar TACE showed tumor necrosis. LDLT followed in these 4 patients. Median waiting time to LT was 53 days (range 0-859 days). Performance of bridging treatments occurred in a median of 25 days after listing for deceased donor LT (range 2-54 days).

All patients experienced uneventful postoperative courses. No re-transplantation was required. The patient who received the split-liver underwent a second-look laparotomy followed by necrosectomy of the marginal zone of segment IV on postoperative day 13. No bile leakage was detected. Median ICU and hospital lengths of stay were 3 and 28 days, respectively. All patients are alive from 5 to 64 months after LT. Chronic rejection was not observed in any case. No recurrence has been observed so far after a median follow-up of 19 months.

Patient	Age	sex	BT	AFP	Tumor	WT	LT	Follow-up
1	61	m	TACE	175 U/ml	2 lesions 6.0cm*	0 days	LDLT§	5 months
2	31	m	TACE	305 U/ml	2 lesions 4.2cm*	1 days	LDLT§	64 months
3	59	m	TACE	162 U/ml	2 lesions 4.4cm*	0 days	LDLT§	51 months
4	59	m	RFA	75 U/ml	solitary 3.5cm	415 days	DDLT	45 months
5	61	w	RFA	67 U/ml	solitary 4.2cm	40 days	LDLT§	4 months
6	59	w	RFA	103 U/ml	solitary 2.6cm	859 days	DDLT	24 months
7	36	m	TACE	64 U/ml	solitary 3.9cm	64 days	DDLT	30 months
8	68	m	TACE	77 U/ml	solitary 4.1cm	68 days	DDLT	13 months
9	47	m	RFA	58 U/ml	solitary 3.8cm	343 days	DDLT	6 months
10	49	m	TACE	62 U/ml	2 lesions 4.7cm*	42 days	SLT	5 months

Table 5: Patient characteristics. WT: Waiting time; DDLT: Deceased donor full-size liver transplant; SLT: Deceased donor split liver transplant. *:total tumor diameter; §: patients undergoing LDLT were listed in the deceased donor waiting list of "Eurotransplant" after the completion of the evaluation work-up and the performance of bridging treatments.

Similar to the few reports about the efficacy of bridging treatments as documented in pathology, there are only sporadic studies referring to the post-transplant course of HCC patients who had received bridging treatments prior to transplantation. In a literature review (87-93), only 4 recurrences were found out of a total of 192 HCC patients treated with TACE alone or in combination with RFA or percutaneous ethanol injection (PEI) prior to transplantation (Table 6). The median post-transplant follow up

ranged from 15 to 40 months. Unfortunately, there was great disparity in the extent of tumor necrosis documented by pathologic exam. Venook et al. reported a median post-transplant recurrence free survival of 25 months in 4 patients with 100% tumor necrosis after TACE. Graziadei et al. reported complete tumor necrosis in 29% of the patients treated (14/41 patients). Yakamodo et al., on the other hand, reported 100% complete tumor necrosis in their series, regardless of tumor size or morphology, after performing TACE in combination with adjuvant RFA prior to LT. There were no reported local recurrences in nodular lesions during a mean follow-up of 12.5 months. To the best of our knowledge, at the time of publication, this has been the only current study addressing outcome after complete tumor necrosis.

Author	Year	BT	N	CTN	Recurrences	Post-LT follow-up
Venook	1995	TACE	10	40%	0/10	40 m (median)
Harnois	1999	TACE	24	n.i.	0/24	29 m (mean)
Yamakado	2002	TACE+RFA	64	100%	2/64	12.5 m (mean)
Graziadei	2003	TACE	41	29%	1/41	n.i.
Fisher	2004	TACE/RFA/PEI	28	n.i.	1/28	n.i.
Hajashi	2004	TACE	12	n.i.	0/12	35 m (mean)
Moreno	2005	TACE/RFA/PEI	13	n.i.	0/13	15 m (median)
Actual series	2005	TACE/RFA	10	100%	0/10	19 m (median)

Table 6: Review of the literature regarding post-transplant follow-up of HCC patients who underwent bridging treatments prior to LT. BT: Bridging treatment; N: number of patients; CTN: Complete tumor necrosis; PEI: Percutaneous ethanol injection; n.i.: no corresponding information.

3.1.2.c AFP decrease to undetectable or very low (<-30ng/mL) levels

There were 40 men and 11 women with a mean age of 53±8.08 years. Cirrhosis was due to viral infection in 30 patients (HCV, n=17; HBV, n=13) and to nonviral pathologies in the remaining 21. Fifteen patients had preserved liver function (Child-Turcotte-Pugh Class A), twenty were Class B, and sixteen Class C. The majority of patients (n=32) had a MELD score between 10 and 20 prior to LT. Mean MELD score was 14±6.50. Almost half the patients (n=24) received no anticancer therapy before transplantation. In 27 instances tumor specific treatments were attempted, including

TACE (n=13), RFA (n=11), PEI (n=1), combined TACE and RFA (n=1), and hepatic resection with RFA (n=1). Twenty six patients underwent deceased donor full-size LT, 3 received a deceased donor split LT, and the remaining 22 patients live donor LT (right lobe in all instances). HCCs were incidentally found in 4 cases. Twenty nine patients were within the Milan criteria, while 22 patients exceeded them. According to the 6th Edition of the UICC, 13 patients were stage I, 16 patients stage II, 11 patients stage IIIA, and 2 patients stage IIIB. In 9 instances no tumor staging was possible because of extended areas of tumor necrosis after performance of bridging treatments. Vascular invasion was present in 3 patients. In two instances the tumor had locally infiltrated the visceral peritoneum (Stage IIIB). With the exception of the 9 instances with extensive tumor necrosis, where no tumor grading was possible, all but one case showed well (n=21) or moderate (n=20) tumor differentiation. Two patients died during the first 45 days because of pancreatitis and septic complications leading to multi-organ-failure, respectively. Patients were followed up to November 30, 2006, providing a minimum follow-up period of at least 11 months for each patient. Median and mean follow-up periods were 35 months (range, 11-95 months) and 37±24.80 months respectively. Only two patients (4%) developed tumor recurrence. The recurrences occurred in the transplant liver/abdominal lymph nodes and in bone at 20 and 12 months post LT, respectively. The first patient underwent a surgical resection and is currently alive, 48 months post transplant. The second recipient ultimately died 17 months post LT. Both these patients had undergone bridging treatments, i.e. TACE and RFA, respectively. No tumor recurrence has been observed in the group of patients with undetectable/very low AFP values who did not undergo bridging treatments.

AFP values: undetectable (<10ng/mL) versus very low (11-30ng/mL)

Comparison of HCC characteristics in patients with undetectable versus very low AFP values showed significant differences concerning tumor size (p=0.025), tumor number (p=0.030), lobar distribution (p=0.043), and fulfilment of the Milan (p=0.028) or UCSF criteria (p=0.016). Patients with undetectable AFP values had more favourable tumor characteristics (Table 7).

	Undetectable AFP (<10ng/mL)	very low AFP (11-30ng/mL)	p-value
No. of patients	35	16	
Age (mean±SD, years)	51.6±9.01	50.8±5.76	ns
Gender			ns
Male	28	12	
Female	7	4	
Child-Turcotte-Pugh classification			ns
A	8	7	
B	14	6	
C	13	3	
Serology			ns
Negative	14	3	
HBV	9	4	
HCV	12	9	
MELD Score			ns
≤20	32	15	
>20	3	1	
Bridging Treatment			
Yes	19	8	ns
No	16	8	
Tumor size			**0.025**
≤2 cm	10	0	
>2, ≤5 cm	11	4	
>5 cm	14	12	
UICC system			
I	11	2	ns
II	10	6	
IIIA	5	6	
IIIB	1	1	
Not possible*	8	1	
No tumors			**0.030**
1	21	4	
2	7	3	
3	1	0	
≥4	6	9	
Lobar distribution			**0.043**
Unilobar	27	7	
Bilobar	8	9	
Differentiation			
Well	10	11	ns
Moderate	16	4	
Poor	1	0	
Not possible*	8	1	
Milan criteria			**0.028**
Within	24	5	
Beyond	11	11	
UCSF criteria			**0.016**
Within	25	5	
Beyond	10	11	

Table 7: Demographic data of transplanted patients with HCC according to AFP values (undetectable versus low). *In 9 instances no differentiation/UICC was possible because of extensive areas of tumor necrosis after tumor specific bridging treatments.

Bridging treatments

Performance of bridging treatments pre-LT (Table 8) was associated with differences in age of recipients (p=0.011), tumor differentiation (p=0.01) and UICC

staging (p<0.001). This was probably due in part to the 9 instances with tumor necrosis after performance of bridging treatments.

	Bridging treatment		
	Yes	**No**	**p-value**
No. of patients	27	24	
Age (mean±SD, years)	54.1±8.82	48.4±6.03	**0.011**
Gender			ns
Male	21	19	
Female	6	5	
Child-Turcotte-Pugh classification			ns
A	9	6	
B	13	7	
C	5	11	
Serology			ns
Negative	11	6	
HBV	5	8	
HCV	11	10	
MELD Score			ns
≤20	25	22	
>20	2	2	
AFP			
<10	19	16	ns
11-30	8	8	
Tumor size			ns
≤2 cm	2	8	
>2, ≤5 cm	10	5	
>5 cm	15	11	
UICC system			
I	1	12	**<0.001**
II	11	5	
IIIA	6	5	
IIIB	0	2	
Not possible*	9	0	
No tumors			ns
1	13	12	
2	6	4	
3	0	1	
≥4	8	7	
Lobar distribution			ns
Unilobar	20	14	
Bilobar	7	10	
Differentiation			
Well	8	13	**0.010**
Moderate	9	11	
Poor	1	0	
Not possible*	9	0	
Milan criteria			ns
Within	15	14	
Beyond	12	10	
UCSF criteria			ns
Within	15	15	
Beyond	12	9	

Table 8: Demographic data of transplanted patients with HCC according to performance of bridging treatment or not. *In 9 instances no differentiation/UICC was possible because of extensive areas of tumor necrosis after tumor specific bridging treatments.

Survival and Recurrence

Survival analysis (Table 9) showed significant differences according to whether or not bridging treatments were performed. Specifically, the 4-year survival of patients with very low and undetectable AFP serial values who did not undergo bridging treatments was 96% and 100%, compared to 67% and 61%, respectively for those who underwent bridging treatments (p=0.0209 and p=0.0372, respectively, Table 9). Overall patient survival was 88% and 81% at one and 4 years, respectively (Fig. 5a). Corresponding overall one and 4 year recurrence-free survival was 88% and 79%, respectively (Fig. 5b). Both patients who developed tumor recurrence post LT had undergone bridging treatments. Post transplant overall and recurrence-free survival for HCC patients with AFP values<30ng/mL according to the performance of bridging treatments and to the Milan criteria is demonstrated in Figure 6. Statistical differences were shown in both analyses concerning bridging treatments (p=0.0209 and p=0.0111, respectively). Four-year overall survival was 96% and 67% for recipients who had not and had undergone bridging treatments, respectively. The corresponding overall survival rates for patients within or beyond the Milan criteria were 90% and 68%. Four-year recurrence-free survival was 95% and 63% for patients with and without bridging treatments, and 90% and 62% for patients within or beyond the Milan criteria, respectively. Given the few instances of recurrent tumors (n=2) no further specific recurrence analysis was performed for the purposes of this study.

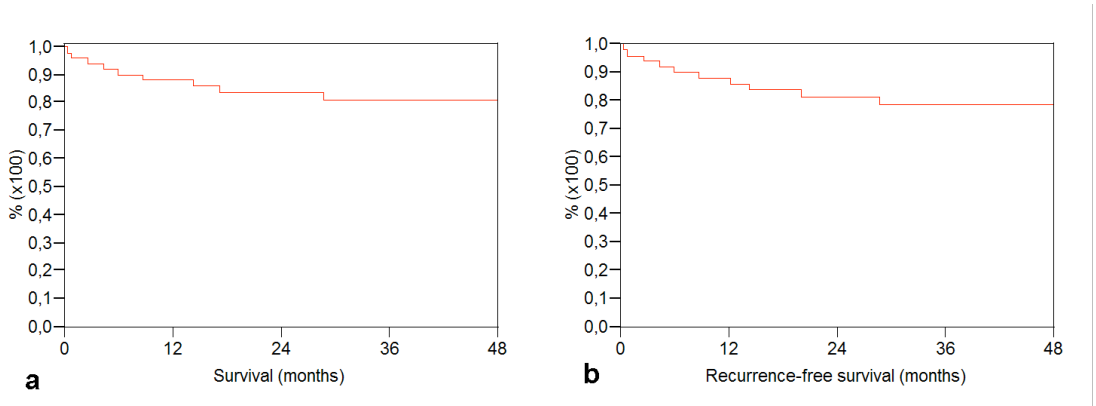


Fig. 5: Post transplant overall and recurrence free survival rates in HCC patients with AFP values<30ng/mL.

		Survival		
	No	**1 Year**	**4 Years**	**p-value**
Gender				
Male	40 (28)	90% (86%)	81% (80%)	ns
Female	11 (7)	82% (72%)	82% (72%)	(0.6493)
Virus				
Negative	17 (14)	82%(78%)	73% (65%)	ns
HBV	13 (9)	100% (100%	92% (100%)	(0.1514)
HCV	21 (12)	86% (74%)	80% (74%)	
AFP				
<10	35	83%	79%	ns
11-30	16	100%	75%	
MELD Score				ns
≤20	47 (32)	89% (84%)	81% (79%)	(0.8585)
>20	4 (3)	75% (67%)	75% (67%)	
Child stage				
A	15 (8)	87% (75%)	87% (75%)	ns
B	20 (14)	89% (84%)	78% (84%)	(0.8641)
C	16 (13)	87% (84%)	79% (75%)	
Bridging Treatment				
Yes	27 (19)	78% (68%)	67% (61%)	**0.0209**
No	24 (16)	100% (100%)	96% (100%)	(**0.0372**)
Graft type				
DDLT	29 (19)	89% (84%)	80% (77%)	ns
LDLT	22 (16)	86% (81%)	81% (81%)	(0.8643)
Lobar distribution				
Unilobar	34 (27)	91% (89%)	87% (84%)	ns
Bilobar	17 (8)	82% (63%)	69% (63%)	(0.1879)
No. tumors				
1	25 (21)	92% (90%)	88% (84%)	ns
2	10 (7)	90% (86%)	90% (86%)	(0.3147)
3	1 (1)	100% (100%)	n.e. (n.e.)	
≥4	15 (6)	80% (50%)	66% (50%)	
Tumor size				
≤2 cm	10 (10)	90% (90%)	90% (90%)	ns
>2, ≤5 cm	15 (11)	100% (100%)	100% (100%)	(**0.0413**)
>5 cm	26 (14)	81% (64%)	67% (54%)	
Vascular invasion				
Yes	3 (2)	100% (100%)	50% (0%)	ns
No	48 (33)	87% (82%)	83% (82%)	(0.3593)
Differentiation				
Well	21 (10)	91% (80%)	85% (80%)	ns
Moderate	20 (16)	90% (87%)	78% (79%)	(0.9650)
Poor	1 (1)	100% (100%)	n.e. (n.e.)	
Not possible*	9 (8)	78% (74%)	78% (74%)	
UICC				
I	13 (11)	92% (91%)	92% (91%)	ns
II	16 (10)	94% (90%)	87% (90%)	(0.1437)
IIIA	11 (5)	82% (60%)	68% (0%)	
IIIB	2 (1)	100% (100%)	n.e.% (n.e.)	
Not possible*	9 (8)	78% (75%)	78% (75%)	
Milan criteria				
Yes	29 (24)	90% (88%)	90% (88%)	ns
No	22 (11)	86% (73%)	68% (54%)	(0.2041)
UCSF criteria				
Yes	30 (25)	90% (88%)	90% (88%)	ns
No	21 (10)	86% (70%)	67% (47%)	(0.1029)

Table 9: Univariate analysis of patient survival. *In 9 instances no differentiation/UICC was possible because of extensive areas of tumor necrosis after tumor specific bridging treatments. In parenthesis, the corresponding results of patients with undetectable AFP (<10ng/mL, n=35) are addressed.

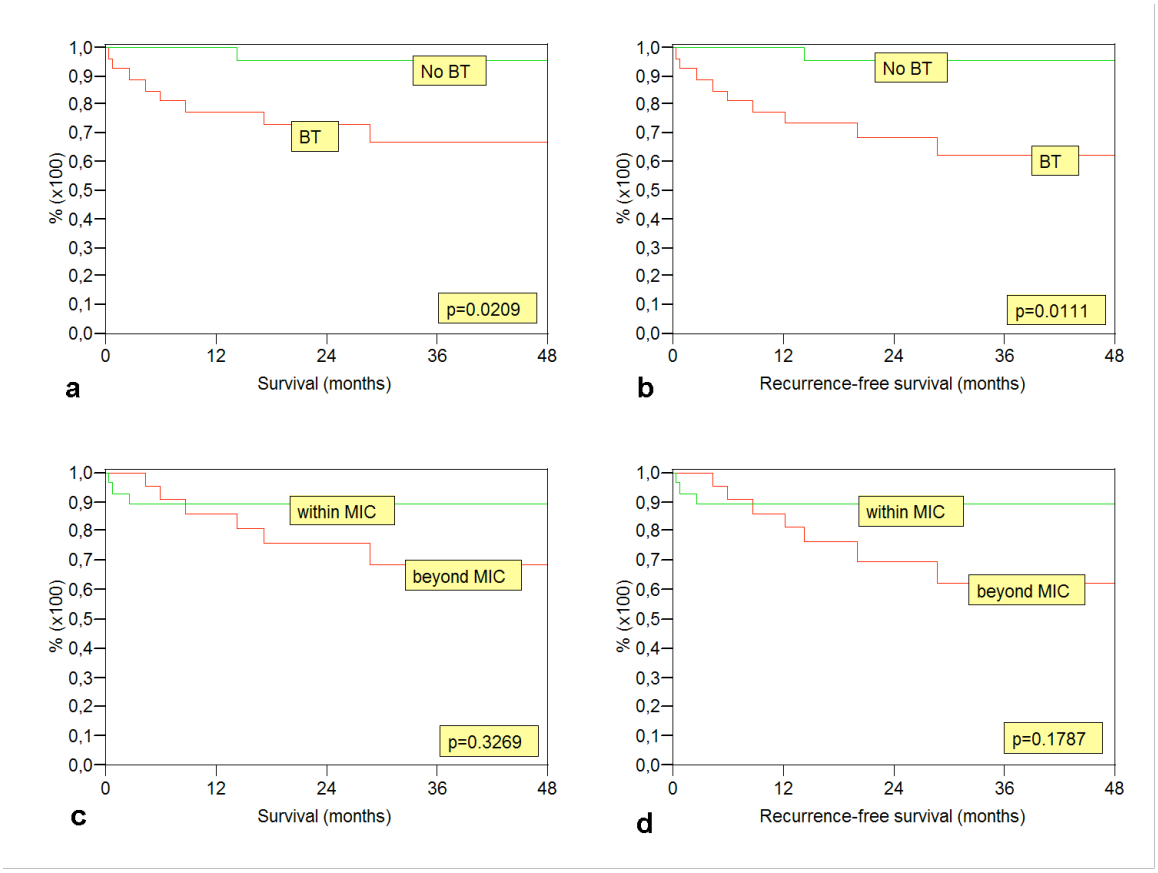


Fig. 6: Post transplant overall and recurrence free survival rates in HCC patients with AFP values<30ng/mL according to the performance of bridging treatments (a and b) or to Milan criteria (c and d). Statistical differences were demonstrated in both analyses concerning bridging treatments (p=0.0209 and p=0.0111, respectively). 4-year survival regarding whether bridging treatments were performed or not was 96% and 67%, respectively. The corresponding survival rates for patients within or beyond the Milan criteria were 90% and 68%. 4-year recurrence-free survival was 95% and 63% with and without bridging treatments, and 90% and 62% for patients within or beyond the Milan criteria, respectively.

Literature Review

Out of the multiple reports in the literature on outcomes after LT for HCC, we could only find 9 series with data on patients with undetectable or very low AFP values prior to transplantation (50, 94-101). Five of them (94, 98-101) were from the study group in Milan, Italy. Overall survival rates for HCC patients with very low serial AFP levels (<30ng/mL) are shown in Table 10. The corresponding survival rates according to the Milan criteria were also entered in the table. According to these data, post LT survival of HCC patients with very low AFP serial values was comparable to the

survival of patients fulfilling the Milan criteria (3-year survival of 73% and 78%, respectively in the study of Cillo et al. 75% and 70%, respectively in the study of Todo et al.). 5-year survival was reported to be as high as 64% and 85% in the studies of Leung et al. and Ravaioli et al., respectively, when the analysis was performed according to AFP values, and 51% and 78%, respectively, when it was performed according to the Milan criteria. We did not encounter any specific information on the outcome of patients with undetectable or very low AFP values and bridging treatments.

Author	PubYear	lowAFP N	lowAFP 3-YS (5-YS)	MIC N	MIC 3-YS (5-YS)
De Carlis	2003	61*	n.i. (77.5%)		
Cillo	2004	15†	73% (73%)	15	78% (72%)
Leung**	2004	-†	88% (64%)	74	64% (51%)
Ravaioli	2004	44‡	n.i. (85%)	55	n.i. (78%)
Todo**	2004	125*	75% (n.i.)	137	79% (n.i.)
Hwang**	2005	121*			91%
Actual series	2006	51‡	96%	29	90%

Table 10: Review of the published literature addressing the post LT outcome of patients with HCC having pre-operative very low serial values of AFP. In the same studies, corresponding results according to patients meeting the Milan criteria (MIC) are reported. YS: year-survival; *values ≤20ng/mL; † values ≤10ng/mL; ‡ values ≤30ng/mL; **: multicenter study; n.i.: no information available.

4.2 Ethical issues

4.2.1 LT for alcoholic liver disease and HCC – place for adult LDLT?

Ten (n=10) men and 2 women with a median age of 59 years (range 50-68 years) underwent LT for HCC in the setting of alcoholic liver cirrhosis. Median waiting time to LT was 54 days (range 1-859 days). Two patients underwent RFA as tumor specific bridging treatment while in the waiting list. TACE was performed in 4 other patients and RFA in combination with surgery in one patient. The remaining 5 patients received no tumor specific bridging treatments. Three patients were Child-Pugh-Turcotte class A, 7 class B, and the remaining 2 class C prior to LT. Between the 3 patients with CTP class A, indication for LT was either central located HCC, requiring major liver resection with high risk for postoperative liver insufficiency (n=2) or recurrent HCC after liver resection (n=1), where anatomical considerations made a

second liver resection not technically feasible. Median MELD score at the time of LT was 10 (range 5-21). Median AFP level prior to LT was 9 U/ml (range 1.8-213).

Patient	Age (years)	sex	BT	WT (days)	MELD score	AFP U/ml	LT	UICC Stage	Milan criteria	Follow-up (months)	Rec.
1	52	w	No	45	8	82.9	DDLT	I	meeting	84	No
2	61	m	RFA	372	17	12.4	DDLT	II	meeting	51†	No
3	59	m	TACE	1	5	9.2	LDLT	NT‡	meeting	63	No
4	59	w	RFA	859	7	7.5	DDLT	NT‡	meeting	36	No
5	50	m	No	316	17	3.4	DDLT	I	meeting	35	No
6	59	m	TACE	37	5	67.4	SLT	IIIA	exceeding*	29	Yes
7	60	m	LR+RFA	99	10	1.8	DDLT	II	exceeding*	28	No
8	68	m	TACE	47	17	5.4	DDLT	NT‡	meeting	25	No
9	60	m	TACE	39	8	8.7	LDLT	II	exceeding*	9†	No
10	52	m	No	32	12	213	DDLT	IIIA	exceeding§	21†	Yes
11	63	m	No	62	9	56.8	DDLT	I	meeting	23	No
12	56	m	No	210	15	6.3	LDLT	I	meeting	12	No

Table 11: Patient characteristics. BT: Tumor specific bridging treatment; WT: Waiting time; MELD-Score: Model for end-stage liver disease; LT: LT procedure; UICC: Union International Contre le Cancer Stage, 6th Edition; ‡: total tumor necrosis after performance of bridging treatment; *:multifocal tumor on pathologic exam; §: multifocal tumor with vascular invasion on pathologic exam; † patient died; Rec.: Tumor recurrence.

Three (n=3) recipients underwent LDLT, one (n=1) deceased donor SLT, and eight (n=8) DDLT. Pathologic evaluation of the explanted livers showed tumors within the Milan criteria in 8 cases. In the remaining 4 cases, the HCCs exceeded the Milan criteria because of multifocality alone (n=3) or in combination with vascular invasion (n=1). Median hospital stay after LT was 23 days. Nine of the ten recipients had uneventful postoperative courses. One patient experienced renal failure and methicillin resistant Staphylococcus aureus sepsis that ultimately led to multi-organ-failure and death 9 months after LDLT. Two additional patients died at 21 and 51 months after DDLT. One died as a consequence of HCC recurrence in the liver allograft and abdominal lymph nodes (patient exceeded the Milan criteria due to multifocality and vascular invasion). The other developed metastatic cancer of the tongue. The remaining 9 patients are alive after a median follow up of 29 months (range 12-84 months). Only one of them had been diagnosed with recurrent HCC, both in the transplant liver and in the lungs, 14 months post LT. This recipient, currently 29 months post transplant, was

beyond the Milan criteria due to tumor multifocality at the time of the surgery, is at the present time. Three year overall and recurrence free survival rates are 82% and 73%, respectively (Fig. 7ab). Overall patient survival rates were 100% at both 1 and 2 years among those who fulfilled the Milan criteria, versus 75% and 50% at 1 and 2 years respectively among these who did not (p=0.0443, Fig. 8a). Recurrence-free survival also showed significant differences (p=0.0070), with 1 and 2 year rates of 100% for patients within the Milan criteria versus 75% and 25% at 1 and 2 years respectively for patients beyond them (Fig. 8b).

All patients described their quality of life as good in the post transplant period. There were no episodes of chronic rejection, ischemic bile duct injuries, or re-listing for LT.

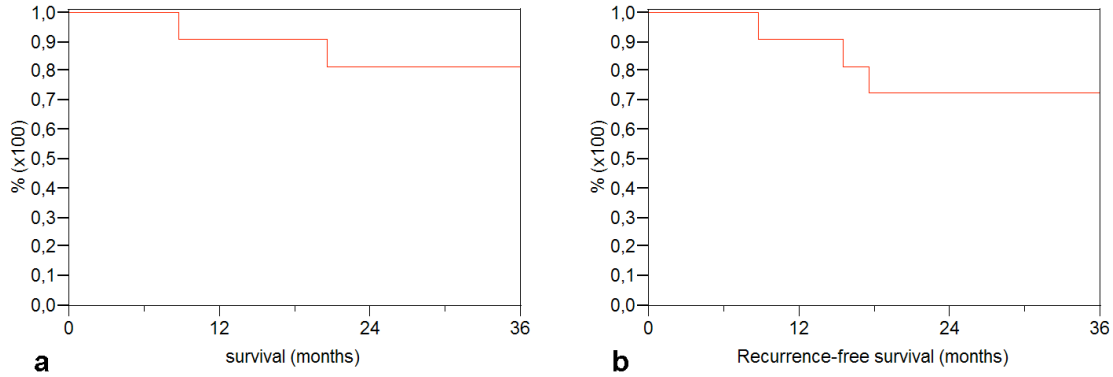


Fig. 7: Overall (a) and recurrence-free-survival (b) rates in recipients of LT for HCC in ALD.

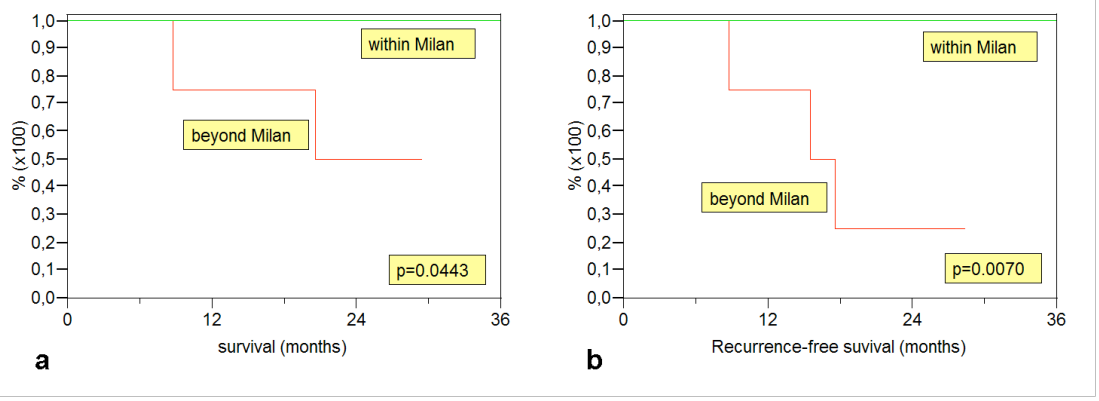


Fig. 8: Overall (a) and recurrence-free survival (b) rates in recipients within and beyond the Milan criteria.

Literature Review

Although there are numerous reports in the world literature addressing outcomes after liver transplantation for HCC, few provide information on HCC and ALD in the absence of viral hepatitis. We identified only 17 studies fulfilling the criteria described in "Patients and Methods" (28, 31, 39, 45, 95, 98, 102-112) (Table 12).

Author	Year of publication	HCC patients (n*)	ALD+HCC (n†)	ALD+HCC (%)
Yokoyama	1990	71	2	3
Bismuth	1993	60	6	10
Ojogho	1996	26	2	8
Michel	1997	113	25	27
Llovet	1998	58	6	10
Yamamoto	1999	270	46	17
Hemming	2001	112	15	13
Herrero	2001	47	12	26
Yao	2001	70	6	9
Margarit	2002	103	14	13
Bigourdan	2003	17	3	18
Kaihara	2003	56	2	4
Cillo	2004	48	2	4
Roayaie	2004	311	26	8
Shetty	2004	109	9	8
Zavaglia	2005	155	13	8
Loehe	2005	93	34	37

Table 12: Review of the published literature addressing the incidence of patients with HCC in ALD undergoing LT. N*: total number of HCC patients undergoing LT; n†: number of HCC patients with ALD; %: Percentage refers to all HCC patients transplanted.

HCC in ALD was encountered in 3%-37% of cases. In the two reports from Eastern countries, the incidence was 4% and 3% respectively (39, 98). In Germany, the reported incidence in the study of Löhe et al. was as high as 37% (112). Unfortunately, no specific information regarding survival, tumor recurrence, or relapse of alcoholism was available. These findings seem to suggest that HCC in ALD is rare in Eastern countries, and that very limited attention has been directed to this group of patients, frequently included under the denomination "others". Zavaglia et al. observed no survival difference when considering underlying liver disease (virus related versus alcohol related versus other causes) by univariate Cox analysis (p-value= 0.69, Hazard ratio 0.93 with 95% confidence interval from 0.64 to 1.34) (98). Two further studies

identified tumor recurrence rates of 14% and 15%, respectively in this group of recipients (104, 110). In the multicenter study of Roayaie et al. (110), tumor recurrence occurred later in patients with ALD than in those with an underlying diagnosis of viral hepatitis (mean time to recurrence in months 33.3±18.3 for ALD, 17.1±14.2 for hepatitis C, 13.5±19.7 for hepatitis B, 12.5±8.7 for cryptogenic cirrhosis and 13.3±8.6 for other reasons, p-value=0.043).

4.3 Pre-transplant radiological assessment

4.3.1 Radiological versus pathological staging for HCC in cirrhosis

Table 13 shows differences observed in tumor characteristics when pathological and imaging findings were compared. A diagnosis of HCC was suspected or confirmed preoperatively in 55 of the 70 patients. In 15 cirrhotics, HCCs were first identified at the time of examination of the explanted specimen. Radiological underestimation of tumor diameter by more than 1cm was observed in 36 (51.4%) cases. Overestimation of 1cm or more was encountered in 14 (20%) patients. Identical tumor size between imaging studies and pathological exam was found in only 10 (14.3%) of the 70 specimens. Tumor number was adequately determined radiologically in 24 (34.2%) patients. Overestimation by 1 or 2 tumors was seen in 8 patients (11.4%), while 2 patients (3%) had 3 or more tumors than predicted. On the other hand, underestimation by 1 to 2 tumors by imaging evaluation was encountered in 14 (20%) cases, while underestimation by 3 or more tumors was detected in 12 (17.1%) instances. Ten of the patients (14.3%) who underwent tumor specific bridging treatment prior to LT could not have adequate tumor evaluation because of extensive necrosis.

Six patients with radiological diagnosis of macrovascular invasion were shown not to have it at the time of pathological examination of the explanted organ. Additionally, pathology revealed microvascular invasion in eight other patients.

Tumor characteristics according to pathological examination	Patients' groups	
	DDLT n=35 (%)	LDLT n=35 (%)
Same tumor diameter than predicted by imaging studies	4 (5.7%)	6 (8.6%)
Tumor diameter smaller than predicted by imaging studies (Deviation >1cm)	6 (8.6%)	8 (11.4%)
Tumor diameter greater than predicted by imaging studies (Deviation >1cm)	21 (30%)	15 (21.4%)
	n=31	n=29
Same tumor number as predicted by imaging studies	12 (17.1%)	12 (17.1%)
1-2 more tumors than predicted by imaging studies	7 (10%)	7 (10%)
1-2 less tumors than predicted by imaging studies	3 (4.3%)	5 (7.1%)
≥3 more tumors than predicted by imaging studies	8 (11.4%)	4 (5.7%)
≥3 less tumors than predicted by imaging studies	1 (1.5%)	1 (1.5%)
	n=31	n=29
Tumor in area of necrosis, no specific estimation of tumor characteristics possible	4 (5.7%)	6 (8.6%)
	Total n=35	Total n=35

Table 13: Comparison of tumor characteristics according to pathological and radiological findings.

Table 14 shows the correlation between pathological and radiological staging according to the Milan criteria. Table 15 shows a similar comparison, but also includes the TNM classification of the UICC, and the UCSF classification. The Milan and UCSF criteria had at best an accuracy of 60%. In the case of the traditional clinical TNM classification of the UICC, the observed accuracy was 30% and 27.1% for the 5th and 6th edition respectively. There was no significant difference in accuracy between the live donor and deceased donor recipient groups. When all transplant recipients were considered, pathological up-staging was observe in 20%-40% of cases, while down-staging was seen in 18.6%-34.3% of patients.

Classification according to Milan criteria		Pathology	
	n=70	"meeting"	"exceeding"
Radiology	"meeting"	20	15
	"exceeding"	14	21

Table 14: Classification according to the Milan criteria.

			Systems/Criteria of classification			
Results	LDLT-Group, n=35		Milan	UCSF	TNM/UICC (5th ed.)	TNM/UICC (6th ed.)
		Pre-/Post- transplant accuracy	20 (57.1%)	22 (62.8%)	9 (25.7%)	7 (20%)
		Pathological Up-staging	5 (14.3%)	5 (14.3%)	11 (31.4%)	15 (42.9%)
		Pathological Down-staging	10 (28.6%)	8 (22.9%)	15 (42.9%)	13 (37.1%)
Results	CLT-Group, n=35	Pre-/Post- transplant accuracy	22 (62.8%)	20 (57.1%)	12 (34.3%)	12 (34.3%)
		Pathological Up-staging	9 (25.7%)	10 (28.6%)	14 (40%)	13 (37.1%)
		Pathological Down-staging	4 (11.5%)	5 (14.3%)	9 (25.7%)	10 (28.6%)
Results	Oerall LT, n=70	Pre-/Post- transplant accuracy	42 (60%)	42 (60%)	21 (30%)	19 (27.1%)
		Pathological Up-staging	14 (20%)	15 (21.4%)	25 (35.7%)	28 (40%)
		Pathological Down-staging	14 (20%)	13 (18.6%)	24 (34.3%)	23 (32.9%)
P-value in Chi-square test: accuracy v/s no accuracy between LDLT-CLT groups			0.807	0.807	0.602	0.282

Table 15: Accuracy of the clinical classification based on radiological findings in relation to the pathological analysis of the explanted liver.

Table 16 depicts an evaluation of the recipients of live donor grafts according to the CLIP scoring system. This system takes into account the Child-Pugh stage, tumor morphology, tumor extension, levels of serum alpha-fetoprotein, and presence of portal vein thrombosis. In our series, the CLIP accuracy was only 54.3%, with an incidence of pathological down-staging of 31.4%.

	CLIP-Score in the LDLT group	n
LDLT-Group, n=35	Pre-/Post- transplant accuracy	19 (54.3%)
	Pathological Up-staging	5 (14.3%)
	Pathological Down-staging	11 (31.4%)

Table 16: Accuracy of the CLIP Score, based on radiological findings, in relation to the pathological analysis of the explanted liver in the LDLT Group.

Table 17 addresses the sensitivity of imaging studies in our series. Radiology failed to detect all tumors under 1cm and the majority (79%) of those between 1-2cm. The corresponding sensitivies were 0% and 21%. In the case of tumors greater than 5cm, the sensitivity was 100% (all tumors were detected). Overall, imaging studies provided us with a sensitivity of 55.5%.

		Sensitivity of radiological imaging tumor lesions n (%)
Tumor size in pathology	<1cm	0/13 (0%)
	1-2cm	8/38 (21%)
	2-5cm	62/80 (77.5%)
	>5cm	6/6(100%)
	Total n (%)	76/137 (55.5%)

Table 17: Tumor-size-analysis in a tumor-to tumor base of 137 tumors found in pathology in correlation to the radiological imaging. Radiology failed to detect all tumors under 1cm and the majority (79%) of tumors between 1-2cm, whereas all tumors >5cm were correctly estimated, as well as the majority (77.5%) of tumors between 2-5cm.

4.3.2 Incidentally found HCC

HCC was incidentally found in 4 men and 1 woman in the group 1. Three patients had been transplanted for cirrhosis associated with viral hepatitis (2 for hepatitis C and one for hepatitis B), and 2 for alcoholic cirrhosis. Median age was 52 years (range 43-61 years) and median waiting time to LT was 316 days (range 45-358 days). AFP values prior to LT were normal in all cases. The last computed tomography study was performed a median of 90 days prior to LT. HCC was not suspected at any time during the evaluation process. All patients were CTP class B at the time of transplantation. Histology showed solitary HCC in 3 cases (tumor diameters of 1.4cm, 1.8cm and 2.5cm respectively) and multifocal bilobar tumors <1cm in diameter in the remaining 2 cases. None had vascular invasion detected. HCC was moderately differentiated in 4 cases and well differentiated in one case. Three cases were stage I and two stage II according to the classification criteria of the UICC. Based on the pathological findings, the three solitary tumors met the Milan criteria whereas the two multifocal ones exceeded them. All five patients experienced uneventful post-transplant courses and were discharged a median of 21 days after transplantation (range 18-25 days). All patients are alive 83, 73, 43, 23, and 23 months after LT, with a median follow-up of 43 months. No patient developed tumor recurrence.

In the second group of the 31 patients with a preoperative diagnosis of HCC mean waiting time to LT was 154±185 days. There were 22 men and 9 women with a mean age of 54.5±11.69 years. Cirrhosis was documented in all these patients, and was attributable to HCV infection, HBV infection, combination of HBV/HCV and alcohol abuse in 42% (13/31 patients), 32% (10/31 patients), 6% (2/31) and 10% (3/31 patients) of such cases, respectively. The remaining 10% had cirrhosis of unknown etiology. Pathological evaluation of the explanted livers showed tumors meeting the Milan criteria in only 39% (12/31) of the instances. In 19 patients pathology revealed tumors exceeding the Milan criteria, showing a corresponding underestimation of these tumors in the radiological evaluation. Twenty two patients are at the moment alive. The overall survival rates at 1 and 3 years were 84% and 68%, respectively (Figure 9) with a median follow up of 28 months (range 13-77 months).

The overall survival of patients with iHCC showed no statistically significant

difference when compared to that of patients with known HCC (pkHCC) prior to transplantation (p=0.1674 in log-rank test, Figure 9).

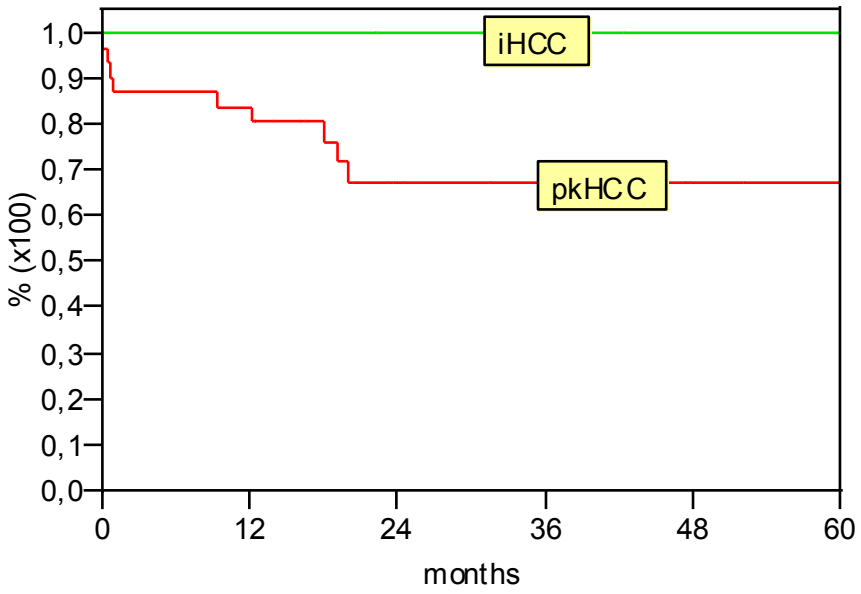


Fig. 9: Comparison of outcomes between patients with iHCC and patients with previously known HCC (pkHCC) ; p=0.1674 in log-rank test.

Review of the literature with reference to incidentally found HCC in liver explants

Although there are many reports in the literature on outcome after liver transplantation for HCC, there is limited information on iHCC. Thirty one studies (ours included) fulfilling the criteria described in Patients and Methods were identified (28, 45, 69-70, 94-95, 102-104, 113-133) (Table 12). The incidence of such tumors among transplanted patients with no known HCC ranged from 1% - 17.5% in liver explants. Incidental HCC 3-5cm in mean diameter was observed in a total of 25 patients by Yao et al. and Leung et al. (45, 129). Tumor recurrence was observed in 146 patients out of 382 patients with iHCC with adequate date (Table 18). Survival of patients with iHCC was documented to be significantly better than that of patients with pkHCC in only 24 (3%) of the 705 patients with iHCC reviewed in the present study (121, 124). On the other hand, the outcome of patients with iHCC was not found to be significantly better

in 343 (49%) patients. In the remaining 338 (48%) patients of Table 1 no comparison of outcome was reported.

The incidence of recurrences was higher in the group of pkHCCs and this difference was significant in Cochran-Mantel-Haenszel-Test ($p<0.0001$ between 14 studies with adequate information). Statistical analysis showed as well a significant better survival in the group of iHCCs ($p<0.05$ using the truncated product method between the 12 studies with corresponding data in the present literature review).

Author	Year	N	%	Survival			Vascular invasion	Recurrence	Statistical survival comparison iHCC vs pkHCC
				1-year	3-years	5-years			
Iwatsuki	1985	13	2.8	-	-	-	-	0/13	-
Penn	1991	31	7.2	74%	57%	57%	-	4/31	
Bismuth	1993	6	-	-	-	-	-	-	-
Chung	1994	6	-	-	-	-	-	-	-
Mion	1996	14	17.5	-	-	-	-	0/14	-
Ojogho	1996	12	2.5	-	83%	-	-	0/12	0.42
Achkar	1998	39	-	-	-	-	31%	1/39	-
Bechstein	1998	15	2.4	93%	-	60%	-	-	-
Klintmalm	1998	169	-	70%	50%	45%	16%	127/169	0.3107
Liovet	1998	7	1.8	-	-	-	-	1/7	n.s.
Otto	1998	6	-	-	-	-	-	-	-
Chui	1999	6	1.5	-	-	-	-	-	-
Yao	2000	23	-	-	-	-	30%	3/23	n.s.
Figueras	2001	81	-	-	-	-	-	-	-
Tamura	2001	13	-	100%	100%	-		0/13	P<0.001
Margarit	2002	15	4.0	-	-	-	13%	5/15	-
Moya	2002	13	2.5	-	-	-	-	3/13	-
Bassanello	2003	15	4.0	-	-	-	-	-	-
De Carlis	2003	28	-	-	-	72%	-	-	0.3
Fernández	2003	11	-	82%	-	82%	-	-	P<0.05
González-Uriarte	2003	13	-	92%	77%	75%	0%	1/13	n.s.
Khakhar	2003	33	-	79%	63%	59%	30%	-	0.65
Nart	2003	11	-	-	-	-	-	-	-
Pérez Saborido	2003	25	-	-	-	-	-	-	-
Cillo	2004	15	4.6	100%	92%	92%	0%	1/15	n.s.
Leung	2004	38	6.7	-	-	-	-	-	n.s.
Ravaioli	2004	36	-	-	-	-	-	-	-
Stadlbauer	2004	2	-	-	-	-	-	-	-
Wong	2004	2	-	-	-	-	-	-	-
Yedibela	2004	2	1.0	-	-	-	-	-	n.s.
Actual series	2005	5	1.2	100%	100%	100%	0%	0/5	n.s.
Overall		705	2.65* (1-17.5)	92%*	80%*	72%*	15%*	146/382	

Table 18: Review of the published literature addressing incidentally found HCC in liver explants after liver transplantation. The percentage is referring to all patients transplanted for benign disease; iHCC: incidentally found HCC; pkHCC: previously known HCC. *P* values ≤0.05 were considered significant in all studies mentioned. *median values; n.s.: not significant.

4.4 Extended listing criteria

4.4.1 LT for patients beyond Milan/ within UCSF criteria

Four 4 patients exceeding the Milan but meeting the UCSF criteria were identified. All four recipients underwent first-time LT, receiving a graft either from a deceased (n=2) or a live donor (n=2). Recipient characteristics were age of 59 years, waiting time to LT of 45 days, MELD score of 8, and AFP serum levels of 144 ng/ml (median values, Table 1). Two patients were CTP class A, one class B, and one class C. Two patients had a diagnosis of cirrhosis secondary to hepatitis B infection, one secondary to hepatitis C infection, and one due to autoimmune hepatitis. One patient underwent transarterial chemoembolization as tumor bridging treatment prior to LT. Three patients had no tumor specific treatment prior to LT.

Pathological evaluation of the explanted liver showed multifocal tumors without vascular invasion in all patients. Hepatocellular carcinomas were moderately differentiated in 3 patients and poorly differentiated in one patient. In all cases, HCC characteristics were beyond the Milan criteria, classified as 2-3 tumor lesions >3cm, but within the UCSF criteria, classified as 2-3 tumor lesions with a total diameter ≤8cm (Table 19). In all instances HCCs were UICC stage II.

All patients experienced uneventfully postoperative courses. Three of them are alive after a median follow up of 57 months. One patient died 20 months post-transplant due to liver failure from hepatitis B re-infection. One patient was re-evaluated for LT 4.8 years post transplant because of recurrent hepatitis C cirrhosis. Two years after the new listing and 6.7 years post transplant, this patient developed HCC recurrence in the mediastinal lymph nodes. He was treated surgically and removed from the "Eurotransplant" waiting list. Currently, he remains in stable clinical condition, 5 months postoperatively and 86 months post transplant. The remaining 2 patients enjoy an excellent quality of life without evidence of HCC recurrence, 56 and 57 months post transplant, respectively.

Patients	Age/MELD	Diagnosis	AFP (ng/ml)	BT	Tumor characteristics	TTD	Rec.	Follow-up (months)
1.	63 / 5	HBV	3000	TACE	2 les., 3.5+2.5cm	6cm	No	20†
2.	64 / 8	HCV	174	No	3 les., 4.5+1+1.5cm	7cm	Yes§	86
3.*	55 / 8	HBV	114	No	2 les., 4+2.5cm	6.5cm	No	57
4.*	44 / 24	AUCI	87	No	2 les., 4+1.5cm	5.5cm	No	56

Table 19: Patients' characteristics. All patients were in UICC stage II, having HCCs without vascular invasion. *: LDLT; MELD: Model for End Stage Liver Disease; HBV: Hepatitis B viral cirrhosis; HCV: Hepatitis C viral cirrhosis; AUCI: cirrhosis due to autoimmune hepatitis; BT: Bridging treatment; TACE: transarterial chemoembolization; les.: tumor lesions; Rec.: Tumor recurrence; §: Recurrence to mediastinal lymph nodes, 81 months post-LT.

4.4.2 LT for HCC and portal vein thrombosis

Patients and outcome

There were 9 men and 3 women with a mean age of 59 years (range 36-68 years). Cirrhosis was due to viral infection in 8 patients (HCV, n=5; HBV, n=3) and nonviral causes in 4. Five patients (n=5) had preserved liver function (CTP Class A), while four (n=4) were CTP Class C. Median value for MELD score was 14 (range 5-24). The majority of patients (7/12, 58%) received bridging treatment prior to LT, such as TACE (n=4), RFA (n=1), percutaneous ethanol injection (n=1) or combinations of TACE with RFA (n=1). Six patients underwent deceased donor full-size LT, 1 deceased donor split LT, and the remaining 5 patients live donor LT (right lobe in all instances). Only 2 patients were within the Milan criteria according to pathological findings, whereas 10 patients were exceeding them. According to the 6th Edition of the UICC, 1 patient was stage I, 1 patient stage II, 8 patients stage IIIA, and 1 patient stage IIIB. In one instance no tumor staging was possible because of extended areas of tumor necrosis associated with bridging treatments. Vascular invasion was present in 7 patients. In one case the tumor had locally infiltrated the visceral peritoneum (Stage IIIB). Except for the case with extensive necrosis, where no grading was possible, all but one of the tumors showed moderate (n=8) or poor (n=2) differentiation. There was no hospital mortality. One year survival was 92%. Six patients (50%) developed tumor recurrence at 5, 8, 9, 10, 14 and 20 months post LT in the transplant liver/abdominal lymph nodes and lungs. One patient underwent partial liver resection 20 months posttransplant. Three patients received palliative chemotherapy. One patient ultimately died 27 months post LT due to disseminated tumor recurrence. Two further patients died as a result of sepsis

and metastatic prostate carcinoma 4.5 and 47 months post LT, respectively. Nine patients (n=9) are currently alive after a median follow-up period of 25 months (range, 13-61 months).

Retrospective comparison of pathological and radiological findings

In eight cases, the portal vein thrombotic material was free of malignancy (Figure 10). In two further cases, although the thrombus was free of malignancy, there was tumoral macrovascular invasion of the portal vein (Figure 11). Tumor thrombi were evident in two patients (Figure 12).

Retrospective radiological re-evaluation, with knowledge of the pathological findings, attributed the PVT to cirrhosis in 7 cases and to malignancy in 3 cases. In the remaining 2 instances, no signs of PVT were evident in the most recent pre-transplant imaging studies. The median "gap-period" between the last CT/MRI scan and LT was 1.1 month for all patients evaluated (range 0-2.3 months).

According to the imaging re-evaluation, the etiology of PVT could be correctly determined by radiological studies in 7/12 patients (58%, Table 20). The corresponding sensitivity and specificity were 50% (1/2 cases) and 80% (10/12 instances) respectively.

Patient	Age (y)	sex	BT	AFP U/ml	LT	UICC Stage	PV inv	Milan criteria	Radiology	Pathology	F-U (m)	Rec.
1	44	w	No	6.5	LDLT	II	No	exceeding*	CTM	CTM	61	No
2	66	m	TACE	36	DDLT	IIIA	Yes	exceeding	TTM	CTM	38	Yes
3	66	m	RFA	9.2	DDLT	IIIA	Yes	exceeding	CTM	CTM	29†	No
4	36	m	TACE	5.7	DDLT	NT‡	No	meeting‡	CTM	CTM	34	No
5	59	m	TACE	67	SLT	IIIA	No	exceeding*	CTM	CTM	31	Yes
6	62	m	No	77	LDLT	IIIA	Yes	exceeding§	CTM	CTM	17†	Yes
7	49	m	No	10	LDLT	I	No	meeting	NTM	CTM	29	No
8	69	w	PEI	187	DDLT	IIIA	No	exceeding*	CTM	CTM	5†	Yes
9	59	w	No	319	LDLT	IIIA	Yes	exceeding	TTM	CTM	14†	Yes
10	59	m	TACE/RFA	79	DDLT	IIIA	Yes	exceeding	TTM	TTM	14	No
11	55	m	No	2.1	LDLT	IIIA	Yes	exceeding*	CTM	TTM	13	Yes
12	46	m	TACE	361	DDLT	IIIB	Yes	exceeding*	NTM	CTM	13	No

Table 20: Patient characteristics. BT: Tumor specific bridging treatment; LT: LT procedure; UICC: Union International Contre le Cancer Stage, 6th Edition; PV inv: Portal vein invasion; F-U: Follow-up; y: years; m: months; CTM: cirrhosis related thrombotic material; TTM: tumor related thrombotic material; NTM: no thrombotic material; ‡: total tumor necrosis after performance of bridging treatment; *:multifocal tumor in pathology; §: multifocal tumor with vascular invasion in pathology; † patient died; Rec.: Tumor recurrence.

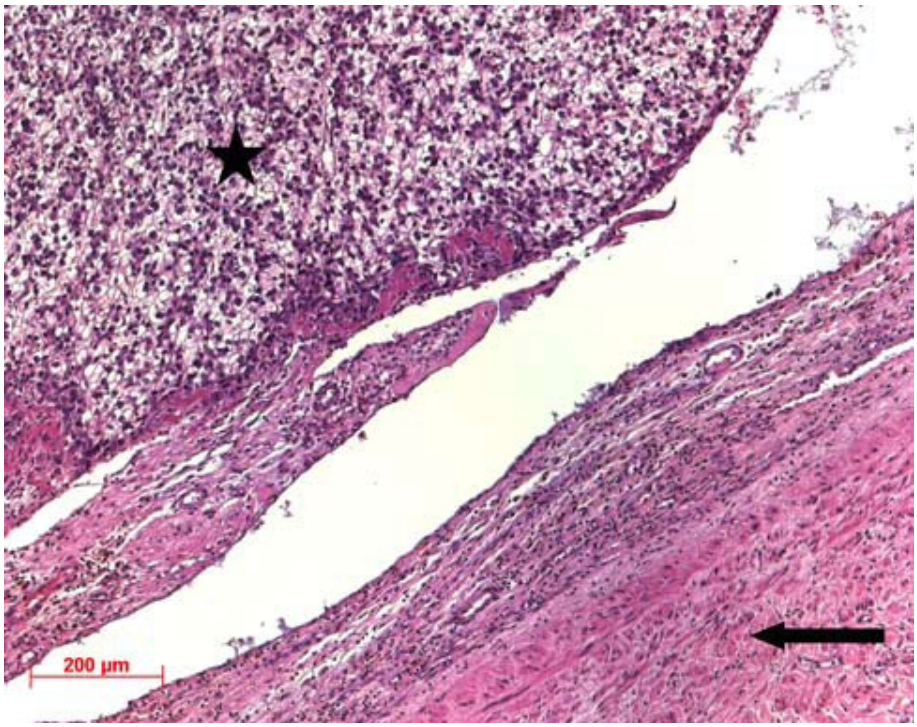


Fig. 10: Light micrograph displaying cirrhosis related thrombotic material in the portal vein. The thrombotic material consists of fibrin, erythrocytes, platelets and degenerating leukocytes (asterisk). Notice the smooth muscle cells of the portal vein (arrow). Hematoxylin-Eosin , Original magnification, x 100.

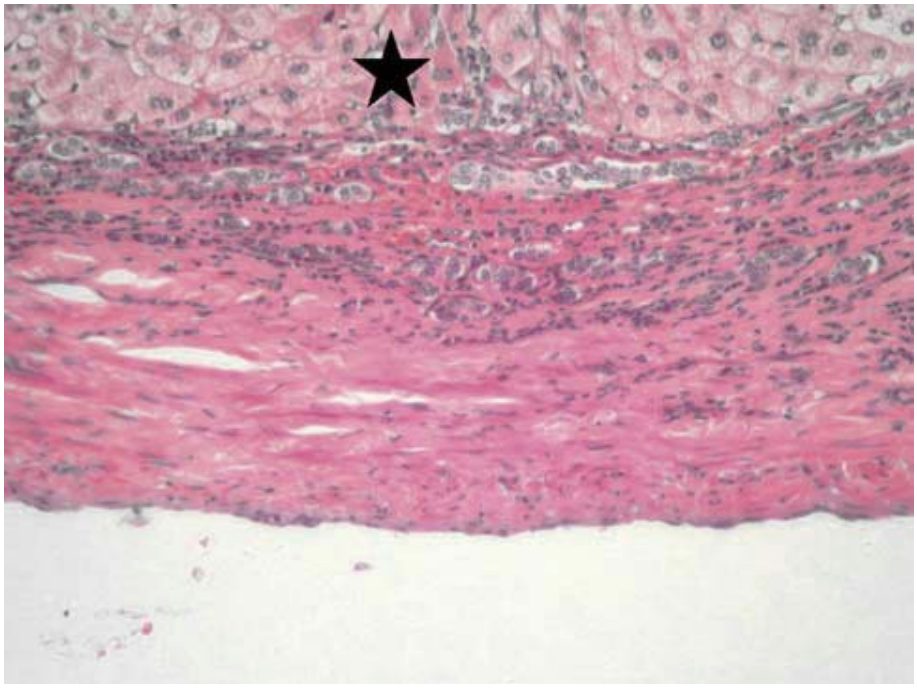


Fig. 11: Image showing portal vein infiltration by an hepatocellular carcinoma. Tumor cells (asterisk) invade the portal vein accompanied by lymphocytic infiltrate. Hematoxylin-Eosin, original magnification, x200.

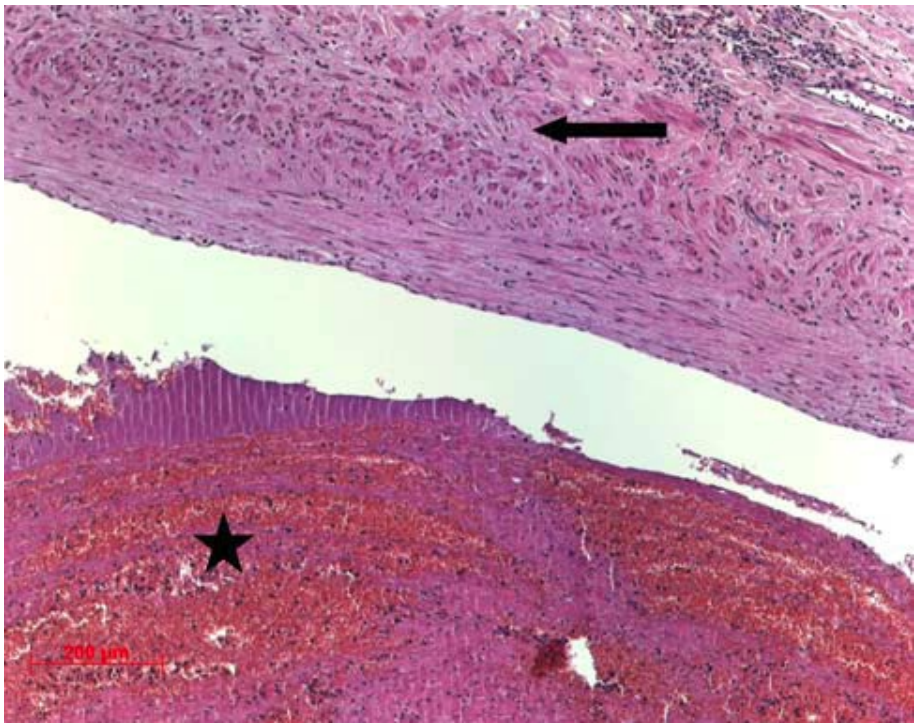


Fig. 12: Light micrograph showing tumor related thrombotic material. The tumor thrombus consists of hepatocellular carcinoma cells exhibiting a clear cytoplasm (asterisk). The portal vein is localized on the right side (arrow). Hematoxylin-Eosin, Original magnification, x 100.

4.4.3 Pulmonary modules at risk in patients undergoing LT for HCC

Data corresponding to 10 transplant patients with HCC and a diagnosis of small pulmonary nodules (all <10mm) by pretransplant CT imaging studies were reviewed. Four additional patients without evidence of small pulmonary nodules in the pretransplant CT evaluation developed pulmonary metastases within the first two post-LT years. One of them, experiencing a prolonged and complicated course after LDLT, developed early pulmonary metastasis, within the first three post-LT months.

There were five men and five women with a median age of 57 years (range, 29–66 years). The etiology of cirrhosis was alcohol in two cases, hepatitis B in two instances, hepatitis C in three instances, and cryptogenic in the remaining two cases. One patient underwent ‘salvage’ living donor LT for HCC recurrence in a noncirrhotic liver after a right trisectionectomy. Median MELD score was 10 (range, 7–42). Four patients had solitary liver nodules with a median diameter of 3 cm (range 1.8–11 cm). Multifocal tumors with a median total diameter of 5.2 cm were detected in six patients. Seven out of the 10 patients fulfilled the Milan criteria prior to LT by imaging studies. TACE (n=1) and radiofrequency ablation (n=2) have been performed in three patients

as ‘bridging treatments’ prior to LT. Both LDLT (n=6) and DDLT (n=4) were included in our series. Median AFP value was 33 U/ml (range, 4–1196U/ml), with half of the patients having levels within normal laboratory range (<10U/ml).

All the patients had uneventful postoperative courses. Two patients were UICC stage I, four patients stage II, and two patients stage IIIA. In two instances, no tumor staging was possible because of extended areas of tumor necrosis after bridging treatments. Vascular invasion was present in two patients. With the exclusion of the two cases with extensive tumor necrosis where no tumor grading was possible, HCCs showed well (n=2), moderate (n=4), and poor (n=2) differentiation. Median follow-up period was 38 months (range 17–76 months). Two patients developed bifocal lung metastases 10 and 6 months post-transplant, respectively. They died from multifocal tumor recurrence 17 and 19 months postoperatively, i.e. 12 and 13 months after the diagnosis of the tumor recurrence in the lungs, respectively. One additional patient died 29 months post-transplantation on account of diffuse metastatic prostate carcinoma. The remaining seven patients are alive with no evidence of tumor after a median follow-up period of 48 months post-transplant (range, 24–75). Patient characteristics are outlined in Table 21.

Patient	**Age (years)**	**Gender**	**AFP U/ml**	**LT**	**UICC Stage**	**Grade**	**Milan criteria***	**IS**	**Follow-up (months)**	**Met.**
1	59	m	9 (wnlr)	LDLT	NT‡	0	exceeding	PT	76	No
2	55	f	114	LDLT	II	2	meeting	PT	72	No
3	43	m	1196	DDLT	II	3	exceeding	PC	19†	Yes
4	66	m	9 (wnlr)	DDLT	IIIA	2	meeting	PC	29†	No
5	46	f	33	LDLT	II	1	meeting	PT	51	No
6	53	f	6 (wnlr)	LDLT	II	1	meeting	PT	48	No
7§	62	m	77	LDLT	IIIA	3	exceeding	PT	17†	Yes
8	63	m	57	DDLT	I	2	meeting	PC	35	No
9	63	f	8 (wnlr)	DDLT	I	2	meeting	PC	41	No
10	29	f	4 (wnlr)	LDLT	NT‡	0	meeting	PT	24	No
p	0.54	0.73	0.19		0.88	**0.0101**	**0.0157**	0.78		

Table 21: Patient characteristics. LDLT: live donor liver transplant; DDLT: deceased donor liver transplant; UICC: Union International Contre le Cancer Stage, 6th Edition; IS: Immunosuppression; ‡: total tumor necrosis after performance of bridging treatment; *: radiological Milan criteria; §:patient with lung metastasis incorrectly interpreted as granuloma; † patient died; wnlr: within normal laboratory range; Met.: Metastases; p: p-value according to the multivariate discriminant regression analysis. Statistical significant values are presented in bold.

All pre- and post-transplant CT imaging studies in patients with identified small pulmonary nodules prior to LT were independently evaluated by an experienced radiologist. Median periods between the first diagnosis of pulmonary granulomas and LT, and the last CT scan and LT, were 62 days (range 18–334 days) and 35 days (range 0–67 days), respectively. A pulmonary metastasis, misdiagnosed as a small fibroma (opaque lesion with 3-mm diameter) prior to LT, was detected in one case (Fig. 13). This patient had bifocal pulmonary metastases diagnosed 10 months post-LT and died in the 17th posttransplant month.

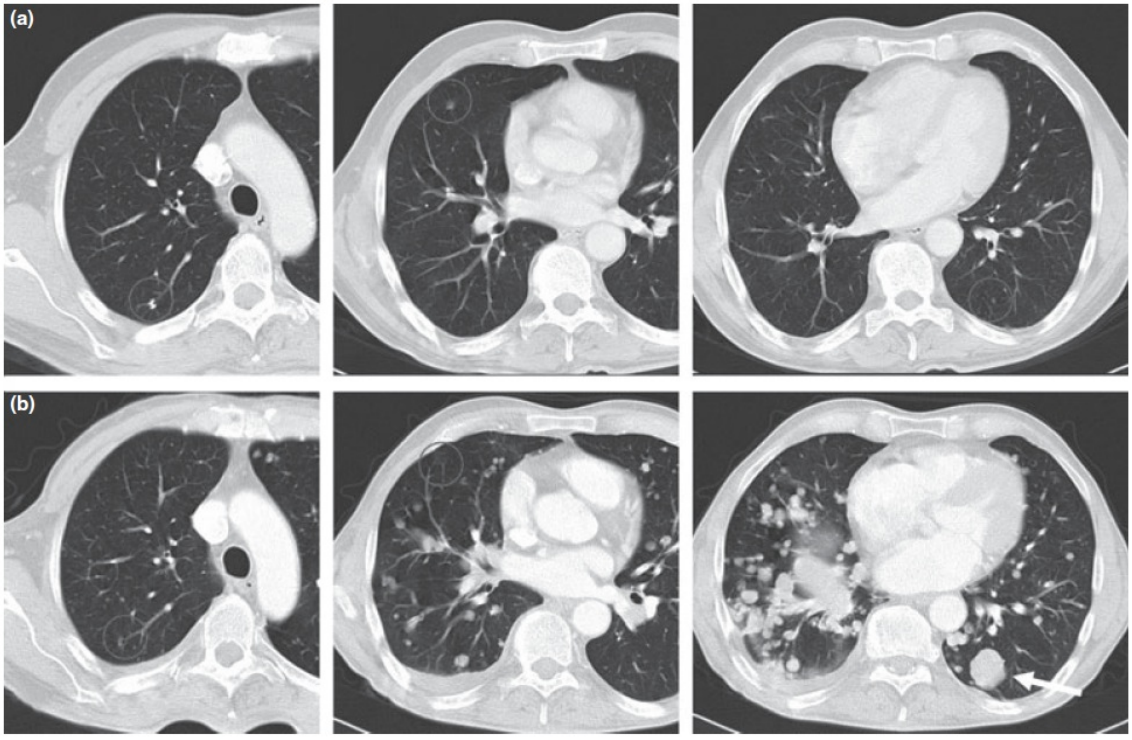


Fig. 13: Small granuloma left and right (rings) 17 days prior to LDLT (a); small granuloma right idem (rings), multiple new intrapulmonary metastases, nodule left is metastasis (arrow), 5 months post LT (Patient Nr 7).

Re-study of the pre- and post-LT images corresponding to the other patient who developed lung metastases 6 months post-LT showed that the existing nodule remained stable and showed no association with the metastases (Fig. 14). The remaining eight patients were found to have stable findings (Fig. 15). The negative predictive value, i.e. the proportion of patients with negative test results who were correctly diagnosed, was 90%. Discriminant function analysis between seven variables (age, gender, AFP, UICC

stage, grade, immuno-suppression and Milan criteria) was performed, giving a predictive value in the parameters tumor grade and Milan criteria (Table 21).

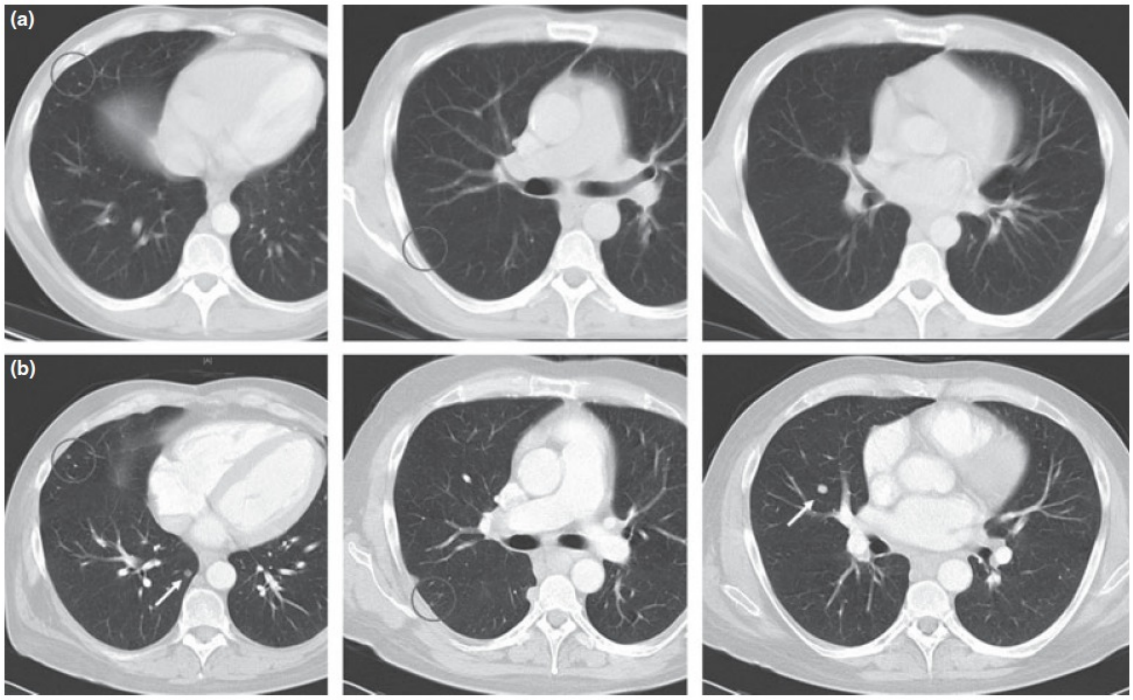


Fig. 14: Small granuloma left and right, 42 days prior to DDLT (a); both granuloma idem (ring), new intrapulmonary metastases (arrows), 6 months post LT (Patient Nr 3).

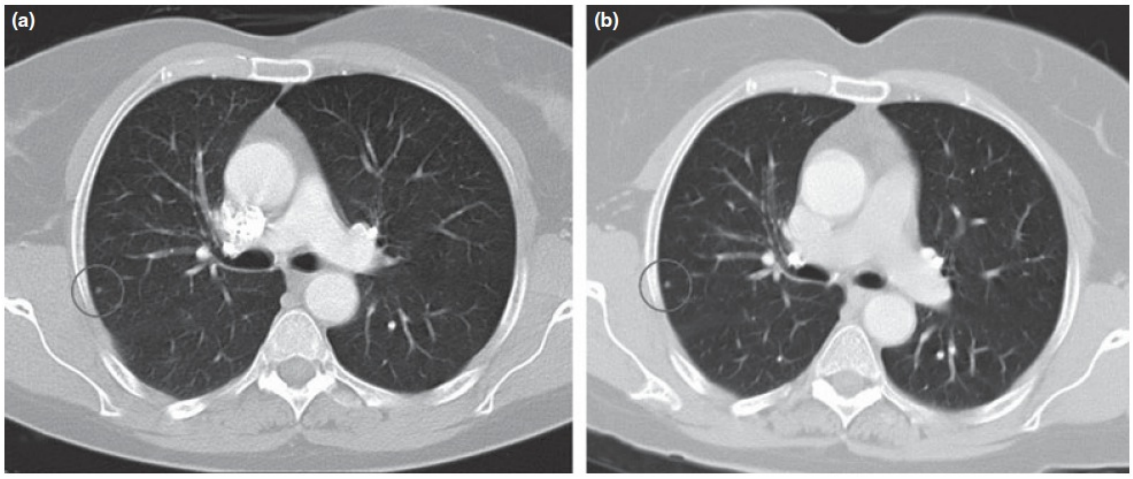


Fig. 15: Small granuloma right upper lobe (ring), 24 days prior to LDLT (a); small granuloma right upper lobe (ring) unchanged, 4 years post LT (Patient Nr 6).

4.5 Expanding the donor pool

4.5.1 Extended criteria donor for LT for HCC – rescue organ offers

A total of 34 official refusals from other transplant centers for higher ranked recipients were registered in "Eurotransplant" for these 7 grafts. Nine refusals were documented as "poor organ quality", 2 as "poor donor quality", 7 as "incompatible age/ size match", 9 as "recipient reasons: non-immunological" and 7 as "organizational reasons: inside the transplant center".

Donor characteristics were age of 45 years, a BMI of 22.5, peak serum sodium of 151 mEq/L and an ICU stay of 8 days (median values, Table 22). There were 2 female and 5 male donors. According to macroscopic observational criteria, the donor surgeon reported good graft quality in 3 cases and acceptable graft quality in the remaining 4 cases. All donors experienced prolonged hypotensive episodes requiring support with high doses of vasopressors. Cardiopulmonary reanimation was documented in one of them. The liver anatomy was referred to as normal in 3 donors. Vascular variations in the liver hilum were present in 3 donors. One donor had sustained an injury of the arteria hepatica at the time of procurement. Multiple liver cysts were described in one of the donors. Biopsies and frozen section examinations were performed in 4 of the 7 allografts. Biopsy results showed 10% macrovesicular steatosis, 40% macrovesicular steatosis, siderosis, and no steatosis. Median cold ischemia time was 480 minutes.

Donor	Sex	Age	Cause of death	ICU-stay (days)	BMI	History of hepatitis	Hypotension*	CPR	Peak Na	AST/ALT	Macroscopy	Biopsy	CIT (min)	Documentation of official refusal Eurotransplant
Nr 1	M	35	ICB	16	22.9	No	Yes	No	151	24/16	Acceptable	No steatosis	612	3x "poor organ quality" 1x "incompatible age/size match"
Nr 2	F	43	SAB, ICB	6	20.8	No	Yes	No	165	11/19	Good	Siderosis	360	2x "poor donor quality" 1x "recipient reasons:non-immunological"
Nr 3	M	23	ICB	9	21.9	No	Yes	Yes	157	33/53	Acceptable	-	752	4x "poor organ quality" 3x "recipient reasons:non-immunological"
Nr 4	M	52	ICB	8	29.4	No	Yes	No	148	34/70	Acceptable	40% steatosis	360	2x "poor organ quality"
Nr 5	M	72	ICB	2	22.5	No	Yes	No	139	99/31	Acceptable	10% steatosis	419	4x "incompatible age/size match"
Nr 6	M	49	SAB	15	26.1	No	Yes	No	151	69/48	Good	-	480	4x "recipient reasons:non-immunological"
Nr 7	F	45	SAB	2	22.5	no	Yes	No	143	12/16	Good	-	810	7x "organizational reasons:inside Txp center" 2x "incompatible age/size match" 1x "recipient reasons:non-immunological"

Table 22: Donors' characteristics. ICB: intra-cranial bleeding, SAB: subarachnoidal bleeding. CPR: cardiopulmonary resuscitation

All recipients underwent first-time, full-size LT. Recipient characteristics were

age of 57 years, waiting time to LT of 64 days, MELD score of 10, international normality ratio (INR) for prothrombin time of 1.1, bilirubin of 1 mg/dl and creatinine of 0.9 mg/dl (median values, Table 23). All the patients were CTP class B. Four patients were T3-Status. The remaining three were T4-Status prior to LT. Two patients had a diagnosis of cirrhosis secondary to hepatitis B infection, 2 secondary to hepatitis C infection, 2 secondary to a combination of hepatitis B and C, and one due to alcohol abuse. Three patients underwent transarterial chemoembolization as tumor specific bridging treatment prior to LT. One patient underwent partial hepatectomy and radiofrequency ablation while waiting, and another one received chemotherapy. Two patients had no tumor specific treatment prior to LT.

Recipient	Sex	Age	Waiting time (days)	T-status	MELD-score	Diagnosis	Bridging treatment	CTP	INR	Bilirubin	Creatinine	Initial liver function	TNM Milan (pathology)	ICU-stay (days)	Hospital-stay (days)	Follow-up (months)
1	M	57	238	T3	14	HBV HCV	-	B	1.1	5.6	0.95	Normal	pT2N0M0 within	14	28	35
2	M	67	62	T4	7	HBV HCV	TACE	B	1.1	1	0.69	Normal	pT3N0M0 beyond	3	21	27
3	F	39	40	T4	6	HBV	TACE	B	0.95	1	0.67	PNF	pT1N0M0 within	3	3	0.1†
4	M	47	152	T3	10	HCV	-	B	1.4	1	0.68	Normal	pT1N0M0 within	8	45	16
5	M	37	64	T3	24	HBV	TACE	B	1.83	15.9	0.9	Normal	pT1N0M0 within	1	22	11
6	M	61	99	T4	10	ALCI	Partial hepatectomy RFA	B	1.02	0.7	1.45	IPF	pT2N0M0 beyond	3	21	7
7	F	64	3	T4	8	HCV	CTx	B	0.9	0.4	1.19	Normal	pT1N0M0 within	10	40	7

Table 23: Recipients' characteristics. Tumor recurrence in the transplant liver was observed in the second case, 23 months after LT; an atypical resection followed. HBV: hepatitis B viral infection, HCV: hepatitis C viral infection, ALCI: alcoholic cirrhosis, TACE: transarterial chemoembolization, RFA: radiofrequency ablation, CTx: chemotherapy; †patient death

Table 24 demonstrates the peak levels of ALT, AST and bilirubin during the first 5 post-transplant days. One patient had biochemical evidence of IPF in the first 3 post transplant days, but recovered with supportive treatment. The patient who received the allograft from the donor with cardiopulmonary resuscitation developed PNF as a result of outflow complications because of big-for-size syndrome; although a high-urgency liver request was asked in Eurotransplant, no suitable organ was found and the patient died in the third post-transplant day**.** One patient developed hepatic artery thrombosis that was treated successfully with thrombectomy and reconstruction of the

arterial anastomosis. Another patient was re-operated in order to evacuate a sub-hepatic hematoma. No re-transplantations were required. Median ICU and hospital lengths of stay were 3 (range 1-14) and 22 days (range 3-45) respectively. One patient developed renal insufficiency requiring hemodialysis during the first three post-operative months. Among other predisposing factors, this patient had received the liver with 40% steatosis. Median follow-up is 15.3 months. Six patients are alive from 7 to 35 months after LT. Chronic rejection was not observed in any of the cases. One patient, whose initial histology showed HCC with vascular invasion, developed recurrent tumor in the allograft 23 months after LT. He underwent an atypical liver resection and was discharged without further incidents.

Patient	ALT Day 1	ALT Day 2	ALT Day 3	ALT Day 4	ALT Day 5	AST Day 1	AST Day 2	AST Day 3	AST Day 4	AST Day 5	Bili Day 1	Bili Day 2	Bili Day 3	Bili Day 4	Bili Day 5
1	1425	1526	840	599	349	982	869	236	64	32	12.9	8.6	9	7.3	8.4
2	289	505	1047	2086	1167	390	489	981	1885	183	1.6	2.7	4.7	8.1	9.1
3	883	3528	3270	-	-	1249	6573	3972	-	-	2	5	5.5	-	-
4	442	517	433	328	241	879	887	430	183	77	3.8	6.0	6.9	7.1	7.8
5	305	305	296	234	143	623	902	541	242	77	9.7	8.6	7.5	8.1	7.2
6	2498	3454	2480	1939	1346	4059	4983	1478	319	132	1.8	2.3	2.9	2.4	2.6
7	525	549	490	352	407	719	961	518	251	294	0.7	2.6	2.0	1.8	2.8

Table 24: Peak levels of alanine aminotranferase, aspartate aminotranferase and bilirubin during the first 5 post-transplant days

4.5.2 Split LT for HCC

SLT was performed in 5 men and one woman with a median age of 60 years (range 49-64 years). Most patients (5/6) had virus-associated liver cirrhosis as the underlying disease (4 cases with hepatitis C and one with hepatitis B). The remaining patient had alcohol induced liver cirrhosis. Median waiting time to LT was 82 days (range 6-245 days). Three patients underwent RFA as tumor specific bridging treatment while in the waiting list. TACE was performed in 2 other patients. One patient received no tumor specific bridging treatment. All recipients were Child-Pugh-Turcotte class A at the time of listing as well as prior to LT. MELD score at the time of LT was 9 in

median value (range 8-15). Three patients were T3-Status (corresponding to the former UNOS status 2B) and 3 were T4-Status (corresponding to the former UNOS status 3).

An extended right split graft (liver segments I, IV-VIII) was transplanted in 5 patients. In 3 cases in situ harvesting was performed. Ex situ harvesting occurred in 2 instances. One patient received a right split graft (liver segments V-VIII) after in situ harvesting. Median cold ischemia time was 10 hours (range 7-13 hours). All patients experienced good primary function of the split liver graft. Median peak bilirubin, aspartate aminotransferase and international normality ratio (INR) values were 3.3 mg/dl (range 2.8-6.2 mg/dl), 986 U/l (range 855-1347), and 1.59 (range 1.5-2.11), respectively. No re-transplantations were required. Median intensive care unit stay was 4 days (range 2-10 days). There were no vascular or biliary complications in this group of patients. Pathologic evaluation of the explanted liver showed tumors meeting the Milan criteria in 4 cases. In the remaining 2 cases HCC exceeded the Milan criteria because of multifocality and microvascular invasion in one case each. Median hospital stay was 32 days. Patients' characteristics are demonstrated in Table 25.

Patient	Age (years)	sex	BT	WT (days)	MELD score	SLT	ICU (days)	HS (days)	Milan criteria	Follow-up (months)	Rec.
1	50	m	RFA	127	9	i.s.	2	21	meeting	66	No
2	64	m	No	7	11	i.s..	10	28	exceeding*	42	No
3	59	m	TACE	37	8	e.s.	2	28	exceeding§	20	Yes
4	64	w	RFA	148	8	e.s.	6†	82	meeting	82 days‡	-
5	61	m	RFA	245	15	i.s.	2	37	meeting	16	No
6	49	m	TACE	6	8	i.s.	7	54	meeting	12	No

Table 25: Patient characteristics. BT: Tumor specific bridging treatment; WT: Waiting time; MELD-Score: Model for end-stage liver disease; SLT: Deceased donor split liver transplant; ICU: Intensive care treatment; HS: Hospital stay; Rec.: Tumor recurrence; i.s.: in situ; e.s.: ex situ: *:multifocal tumor in pathology; §: vascular invasion in pathology.†initial ICU-stay, the patient was re-submitted to ICU and remained there till her death ‡patient died.

The postoperative course was uneventful in half of the patients (3/6 patients), who were discharged after a median stay of 27 days. Two patients developed an abscess caused by necrosis of the marginal zone of segment IV and were treated surgically. They were discharged without any additional problems. The postoperative course was prolonged and complicated in one patient, although the liver function was satisfactory.

This patient experienced a type II heparin induced thrombocytopenia leading to diffuse intraabdominal bleeding, renal insufficiency requiring continuous veno-venous haemodialysis and sepsis due to methicillin resistant Staphylococcus aureus leading to multi-organ-failure. This patient ultimately died 82 days after SLT. The remaining 5 patients are alive, one with recurrence, after a median follow up of 20 months (range 12-66 months). Tumor recurrence in the lungs was observed in one patient 4 months after SLT. This was the only patient with vascular invasion of the tumor detected at the time of pathologic evaluation of the explanted liver. He is currently receiving systemic chemotherapy. None of the patients experienced chronic rejection or ischemic bile duct disease. No patient had to be re-listed for LT.

4.5.3 LDLT for HCC

There were 42 men and 15 women with a median age of 55 years (range 18-67 years). Cirrhosis was due to viral infection in 36 patients (HCV, n=23; HBV, n=13) and to non-viral pathologies in the remaining 21 patients. MELD score was 10 (range 6-34). Tumor specific treatments were undertaken in 22 instances, including TACE (n=13), RFA (n=3), and hepatic resection (n=6). Twenty four patients were within the Milan criteria, while 33 patients exceeded them. Correspondingly, 27 patients were meeting the UCSF criteria, while 30 patients were exceeding them. Vascular invasion was present in 12 patients. Patients' characteristics are demonstrated in Table 26. Seven patients died during the first 45 days as a result of heart failure (n=1), pancreatitis (n=1), pulmonary embolism (n=1), small-for-size syndrome (n=2), and septic complications leading to multi-organ-failure (n=2).

Patients were followed up to July 31, 2007, providing a minimum follow-up period of at least 6 months for each patient. Nine patients developed tumor recurrence after a median of 6 months (range 3-35 months). Six of them died after a median of 13 months post transplant, while the remaining 3 are alive at 8, 19 and 24 months post LDLT. Currently, 33 patients are alive, after a median follow-up period of 30 months (range, 6-91 months). One-, 3- and 5-year overall survival rates are 68%, 62% and 58%, respectively (Figure 16a). The corresponding survival rates after exclusion of the 7 patients who died within 45 days are 76%, 71% and 67%, respectively (Figure 16b).

No. of patients	57
Age (median, years)	55 (range 18-67)
Gender	
Male	42
Female	15
HCV infection	
Yes	23
No	34
Bridging treatment	
Yes	22
No	35
MELD score	
<10	24
≥10, <20	24
≥20	9
Tumor size	
≤8 cm	29
>8 cm	28
Number of tumors	
solitary	33
multifocal	24
Vascular invasion	
V0	45
V1	12
Differentiation	
Well	14
Moderate	36
Poor	7
UICC Stage	
I	23
II	18
>II	16
Milan criteria	
Meeting	24
Exceeding	33
UCSF criteria	
Meeting	27
Exceeding	30

Table 26: Demographic and perioperative characteristics of HCC patients undergoing LDLT

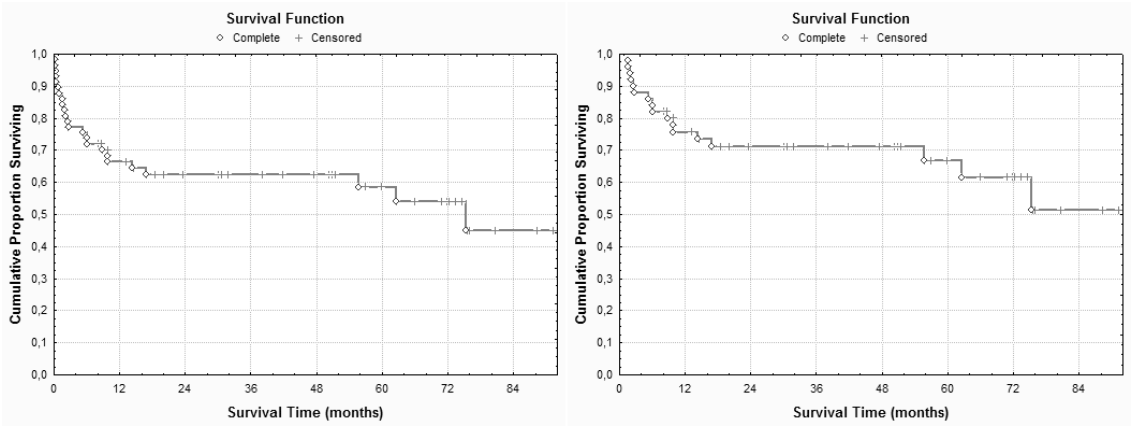


Fig. 16: Cumulative survival inclusive (left) and exclusive (right) of 45 day mortality.

Statistical Analyses

We used survival time as the only end point. Fifteen variables were analysed by univariate analysis: gender, performance of bridging treatments, HCV infection, UICC stage, tumor multifocality, the presence of more than 3 tumors, tumor diameter, tumor diameter> 8cm, tumor grade, vascular invasion, Milan criteria, UCSF criteria, AFP level, age, and MELD score.

When we considered all patients included those who died within 45 days postoperatively (7 out of 57), only age (p=0.049), AFP (p=0.033) and MELD score (p=0.031) were the independent variables that influenced the hazard rate in univariate analysis (Table 27).

Variable	Inclusive of 45 day mortality	Exclusive of 45 day mortality
Age	p=0.049303	p=0.003247
MELD score	p=0.031987	p=0.27
AFP level	p=0.033440	p=0.001623
Gender	p=0.11	p=0.21
Bridging treatment	p=0.29	p=0.036277
HCV infection	p=0.34	p=0.006999
UICC Stage	p=0.92	p=0.033554
Tumor multifocality	p=0.09	p=0.76
>3 tumors	p=0.62	p=0.11
Tumor diameter	p=0.81	p=0.61
Tumor diameter >8cm	p=0.35	p=0.97
Grade	p=0.33	p=0.91
Vascular invasion	p=0.65	p=0.62
Milan criteria	p=0.74	p=0.74
UCSF criteria	p= 0.41	p=0.42

Table 27: Univariate Cox regression analysis.

All three factors showing statistical significance as a predictor were further analysed using a multivariable Cox proportional hazard regression. Age (p=0.005) and MELD score (p=0.016) were included finally in the model as predictors of survival (Table 28).

patients (n=57)	Beta	Standard	t-value	exponent	Wald	p
Age	0,139518	0,049867	2,797796	1,149719	7,827663	**0,005148**
AFP level	0,000127	0,000133	0,957698	1,000127	0,917185	0,338222
MELD score	0,080713	0,033742	2,392096	1,084060	5,722121	**0,016758**

Table 28: Multivariable Cox regression analysis inclusive of 45 day mortality

A new database was created thereafter with exclusion of patients who died within 45 days postoperatively (n=7) and univariate and multivariable analysis were also performed in the same way referred above (Table 2). Age (p=0.003), performance of bridging treatments (p=0.036), diagnosis of HCV (p=0.006), UICC stage (p=0.033) and AFP (p=0.001) were predictors of survival in the former and age (p=0.012) and AFP (p=0.033) in the later (Table 29).

patients (n=50)	Beta	Standard	t-value	exponent	Wald	p
Age	0.129947	0.052203	2.48926	1.138769	6.196427	**0.012806**
Bridging treatment	-0.779194	0.542941	-1.43514	0.458776	2.059611	0.151258
HCV infection	-0.012417	0.540940	-0.02295	0.987660	0.000527	0.981687
UICC Stage	-0.024140	0.208866	-0.11558	0.976149	0.013358	0.907988
AFP level	0.000289	0.000136	2.12064	1.000289	4.497092	**0.033960**

Table 29: Multivariable Cox regression analysis exclusive of 45 day mortality

What is concluded from the above two analyses is the fact that age is a constant predictor of survival when considering 45 days mortality or not. On the contrary MELD score is a predictor of survival in the first case and AFP in the later.

A further analysis was performed. We divided each of these continuous variables (age, AFP and MELD score) into two levelled categorical data by setting one break point. P-values were calculated for each set of break point respectively with univariate and multivariable Cox proportional hazard regression, and the set of break points showing the lowest p-value was retained if the value reached significance. The optimum decision cut-off value for patient age was 60 years (60≤ or >60), for AFP was 400 ng/mL (400≤ or >400), and for MELD score was 22 (≤22 or >22). Cut-off values were identical with or without 45 days mortality inclusion.

The respective patient populations recognized after the establishment of the cut-off values had statistical significant different cumulative proportion surviving (Figure 17a-d): age 60≤ vs >60 p =0.01295, MELD score ≤22 vs >22 p=0.02054 when including 45 days mortality and age 60≤ vs >60 p =0.00772, AFP≤400 vs >400 p=0.00475 when excluding 45 days mortality.

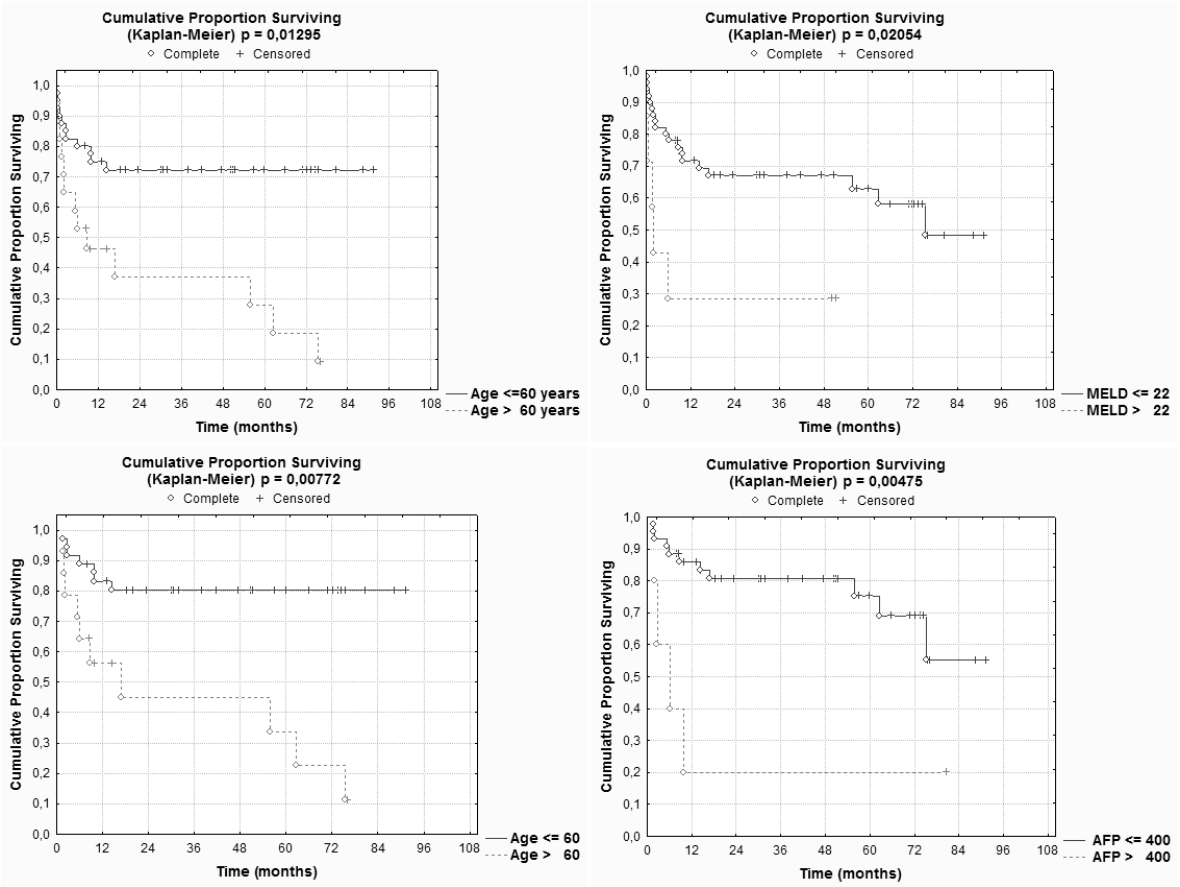


Fig. 17: Patient populations recognized after the establishment of the cut-off values had significantly different cumulative survivals both when 45 day mortality was included (upper set) and excluded (lower set).

In order to validate our data a score (CEB score, dedicated to the leading author of this article) was allocated to each patient as follows: age 60≤ 1 point, >60 2 points; MELD score ≤22 1 point, >22 2 points; and AFP ≤400ng/mL 1 point, >400ng/mL 2 points. When including 45 day mortality, the number of patients having scores =2 and >2 was 37 and 20, respectively. When excluding 45 day mortality, the number of patients having scores =2 and >2 was 31 and 19, respectively. Survival rates when

including 45 day mortality is 72% for both 3 and 5 years for score=2 and 41% and 33% for score>2, respectively (p=0.0146, Figure 18a). When excluding 45 day mortality survival rates at 3 and 5 years were 90% and 84% for score=2, and 32% and 23% for score>2, respectively (p=0.00002, Figure 18b).

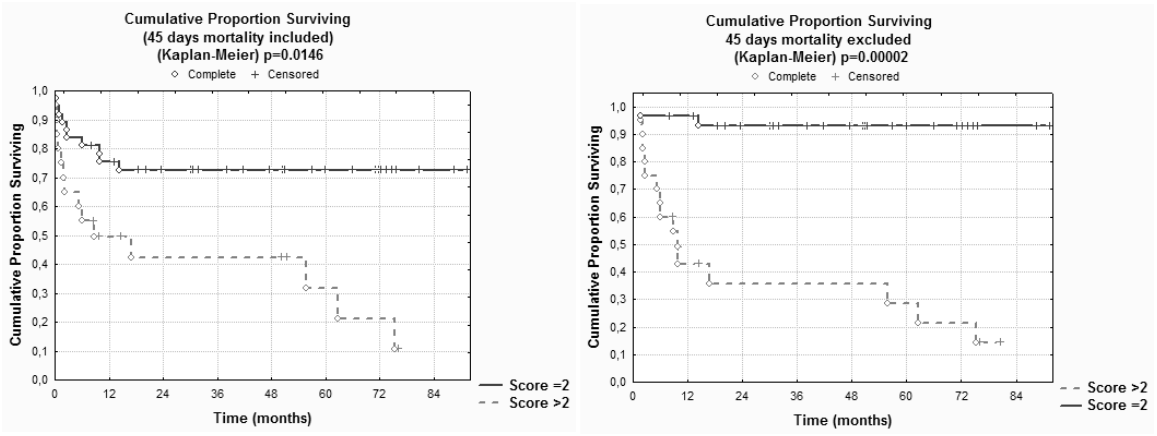


Fig. 18: Cumulative survival according to the CEB score classes when including 45 day mortality (combination of the parameters Age/MELD) (left) and when excluding 45 day mortality (combination of the parameters Age/AFP) (right).

Internal validation

The application of structural equation modelling (two populations in each analysis separated by scoring) depicted the standard error at 0.201 when including 45 day mortality and at 0.210 when excluding 45 day mortality. These results show a population with a multivariable normal distribution. The variables had no kurtosis. Consequently, the estimated standard error obtained under the assumption of multivariable normality is not biased and our model with the stratified predictors fits well to survival.

4.5.4 Institutional results

There were 75 men with a median age of 55 years (range, 31-68 years) and 25 women with a median age of 58 years (range, 18-69 years). Cirrhosis was due to viral infection in 74 patients (HCV, n=45; HBV, n=27; both HCV and HBV n=2) and nonviral causes in 26. Almost half the patients (n=49) had a MELD score between 10 and 20 prior to LT. Median MELD score was 13 (range, 5-42) with a mean value of

13.53±6.58. Just less than half of the patients (n=49) received no anticancer therapy before transplantation. In 51% of cases tumor specific treatments had been attempted, including partial or anatomic resection (n=6), RFA (n=15) or TACE (n=30). Forty nine patients underwent deceased donor full-size LT, 6 deceased donor split LT, and the remaining 45 patients live donor LT (right lobe in all instances). Patients were followed up to June 30, 2006. Median and mean follow-up periods were 31.3 months (range, 6.5-92.3 months) and 38.39±24.34 months respectively.

LT procedure

As shown in Table 30, demographic data among patients undergoing LDLT or DDLT showed no significant differences. MELD score showed a trend (p=0.085) for higher values among patients undergoing LDLT. Tumor characteristics were comparable among both groups except for unilobar tumor distribution (67% in DDLT versus 58% in LDLT) and small tumors≤2cm (20% in DDLT versus 8% in LDLT). Milan criteria according to pathological findings were fulfilled in 45% and 51% of patients undergoing DDLT and LDLT, respectively. Survival and recurrence rate analysis showed no difference (Fig. 19).

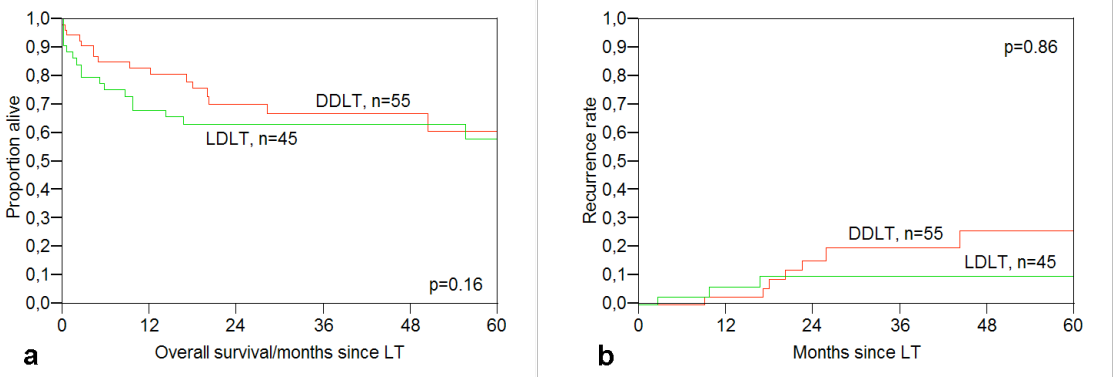


Fig. 19: Patient survival and cumulative recurrence rates according to the LT procedure for n=100 cases; DDLT: deceased donor liver transplantation; LDLT: live donor liver transplantation.

Survival

Of the 100 recipients, 64 are currently alive, with recurrent tumor in 5 cases. One- and 5 year patient survival was 76% and 60%, respectively; 1- and 5-year recurrence-free survival was 75% and 54%, respectively (Fig. 20). Thirty six patients

died, 14 of them within 3 months after LT. Early deaths were attributable to sepsis secondary to medical and surgical complications (n=9), fulminant pulmonary embolism (n=2), pancreatitis (n=2), and intracranial hemorrhage (n=1). Among the 22 deaths that occurred more than 3 months after LT, 6 were caused by tumor recurrence, 8 by sepsis (2 following aspergillus pneumonia, 2 following methicillin-resistant staphylococcus aureous infection, 2 following chronic rejection, one following pneumocystis carinii infection and heart failure after myocardial infarction, respectively), 2 by hepatitis re-infection, and 6 by miscellaneous causes.

	DDLT	**LDLT**	**p-value**
No. of patients	55	45	
Age (mean±SD, years)	53.4±9.1	55.0±10.1	0.24
Gender			0.91
Male	42	33	
Female	13	12	
Child-Turcotte-Pugh classification			0.56
A	19	10	
B	23	24	
C	13	11	
Serology			0.37
Negative	13	13	
HBV	15	12	
HBV/HCV	0	2	
HCV	27	18	
MELD Score			0.09
<10	24	19	
>10, ≤20	29	20	
>20, ≤30	0	5	
>30	2	1	
Tumor size			0.29
≤2 cm	11	4	
>2, ≤5 cm	14	14	
>5 cm	30	27	
No tumors			0.96
1	26	20	
2	9	8	
3	2	1	
≥4	18	16	
Lobar distribution			0.44
Unilobar	37	26	
Bilobar	18	19	
AFP			0.71
≤20	31	22	
>20, ≤200	12	14	
>200, ≤1000	8	5	
>1000	4	4	

	DDLT	LDLT	p-value
Milan criteria			0.72
Within	25	23	
Beyond	30	22	
UCSF criteria			0.66
Within	27	25	
Beyond	28	20	
Perioperative mortality	5	9	0.20

Table 30: Demographic Data of patients with HCC who underwent DDLT or LDLT.

Only tumor differentiation (p=0.0154) was significantly associated with patient survival by univariate analysis (Table 31). It retained its significance also by multivariable analysis (p=0.028). Excluding the HCC-non-related deaths from the analysis, 5-year survival of 87% was achieved for a total of n=73 cases (Fig. 27).

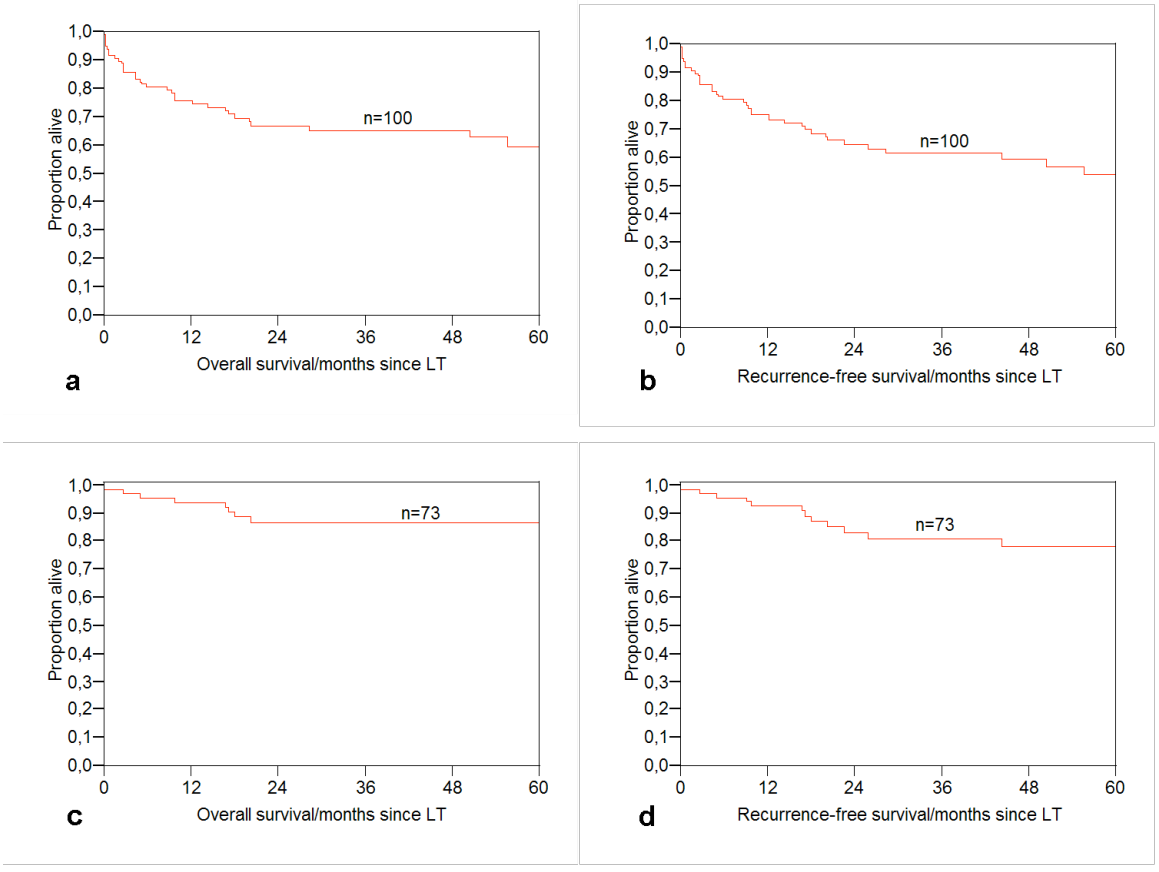


Fig. 20: Overall patient (a) and recurrence-free-survival (b) rates after LT for n=100 cases. Kaplan-Meier curves c and d present corresponding survival rates censored for n=73 cases, excluding the HCC-non-related deaths. In this instance, 5-year overall and recurrence-free survival of 87% and 78%, respectively, was achieved.

		Survival			
	No	**1 Year**	**3 Years**	**5 years**	**p-value**
Gender					
Male	75	75%	62%	55%	0.49
Female	25	76%	76%	76%	
Virus					
Negative	26	69%	69%	53%	0.92
HBV	27	82%	69%	68%	
HBV/HCV	2	100%	100%	100%	
HCV	45	76%	62%	57%	
AFP					
≤20	53	87%	76%	55%	0.14
>20, ≤200	26	76%	72%	72%	
>200, ≤1000	13	84%	65%	65%	
>1000	8	50%	25%	25%	
MELD					
≤10	43	99%	67%	67%	0.41
>10, ≤20	49	76%	65%	52%	
>20, ≤30	5	40%	40%	-	
>30	3	79%	69%	-	
Child-Turcotte-Pugh classification					
A	29	89%	78%	68%	0.16
B	47	79%	64%	52%	
C	24	65%	58%	54%	
Preop treatment					
None	49	87%	77%	70%	0.08
TACE	30	62%	52%	52%	
RFA	15	67%	50%	38%	
Hepatectomy	6	83%	83%	83%	
Graft type					
DDLT	55	83%	67%	61%	0.17
LDLT	45	68%	63%	58%	
Lobar distribution					
Unilobar	63	77%	73%	64%	0.24
Bilobar	37	74%	52%	52%	
No. tumors					
1	46	78%	72%	64%	0.31
2	17	82%	75%	66%	
3	3	100%	100%	100%	
≥4	34	69%	50%	50%	
Tumor size					
≤2 cm	15	86%	86%	43%	0.25
>2, ≤5 cm	28	78%	78%	71%	
>5 cm	57	73%	54%	54%	
Portal invasion					
Vp0	82	78%	69%	62%	0.39
Vp1	7	57%	57%	57%	
Vp2	5	80%	53%	-	
Vp3	6	76%	37%	37%	
Differentiation					
Well	28	73%	69%	62%	**0.0154**
Moderate	47	82%	71%	63%	
Poor	10	58%	0%	0%	
Not possible*	15	73%	73%	73%	
UICC					
I	26	73%	68%	54%	0.47
II	30	83%	67%	59%	
IIIA	23	75%	59%	59%	
IIIB	6	62%	41%	41%	
Not possible*	15	74%	74%	74%	
Milan criteria					
Yes	48	77%	77%	66%	0.22
No	52	76%	55%	54%	
UCSF criteria					
Yes	52	79%	76%	67%	0.10
No	48	73%	52%	52%	

Table 31: Univariate analysis of patient survival using the Kaplan-Meier method.*In 15 instances, also included in the analysis, no differentiation/UICC was possible because of extensive areas of tumor necrosis after tumor specific bridging treatments.

HCC Recurrence

Fourteen patients developed HCC recurrence; 5 are currently alive. The cumulative incidences of tumor recurrence after LT were 1 (8%) by 3 and by 6 months, 3 (23%) by 1 year, 6 (46%) by 18 months, 8 (62%) by 2 years, 9 (69%) by 3 years, 10 (77%) by 5 years and 13 (100%) by 7 years. The initial presentation of the recurrence was confined to the liver in 2 patients (15%), to the lungs in 3 patients (23%), to abdominal and thoracic lymph nodes in one case each (8% and 8%, respectively), and to bones in one patient (8%). Recurrence presented in multiple sites in 5 patients (38%). Pretransplant serum AFP levels, vascular (portal) invasion, tumor differentiation, Milan and UCSF criteria were independent risk factors for recurrence (Table 32). On multivariable analysis, only tumor differentiation (p=0.006) and UCSF criteria (p=0.035) were independent risk factor for recurrence. Poorly differentiated HCCs were associated with higher recurrence rates than well and moderately differentiated ones.

		Recurrence Rate			
		No	**1 Year**	**3 Years**	**5 Years**
	p-value				
Gender					
	Male	75	5%	17%	21%
	0.19				
	Female	25	0%	7%	7%
Virus					
	Negative	26	5%	13%	13%
	0.62				
	HBV	27	9%	14%	14%
	HBV/HCV	2	0%	0%	0%
	HCV	45	0%	19%	26%
AFP					
	≤20	53	0%	3%	3%
	0.0003				
	>20, ≤200	26	3%	13%	22%
	>200, ≤1000	13	0%	13%	13%
	>1000	8	32%	77%	77%
MELD					
	≤10	43	3%	16%	23%
	0.39				
	>10, ≤20	49	3%	10%	10%
	>20, ≤30	5	25%	25%	-
	>30	3	0%	33%	-
Child-Turcotte-Pugh classification					
	A	29	4%	25%	25%
	0.76				
	B	47	4%	8%	15%
	C	24	5%	16%	16%
Preop treatment					
	None	49	0%	13%	13%
	0.46				
	TACE	30	6%	16%	30%
	RFA	15	18%	28%	28%
	Hepatectomy	6	0%	0%	0%

		Recurrence Rate *(continued)*			
		No	**1 Year**	**3 Years**	**5 Years**
	p-value				
Graft type					
	DDLT	55	2%	19%	26%
	0.87				
	LDLT	45	6%	10%	10%
Lobar distribution					
	Unilobar	63	4%	7%	12%
	0.12				
	Bilobar	37	4%	32%	32%
No. tumors					
	1	46	6%	6%	13%
	0.10				
	2	17	0%	0%	0%
	3	3	0%	0%	0%
	≥4	34	4%	40%	40%
Tumor size					
	≤2 cm	15	0%	0%	0%
	0.19				
	>2, ≤5 cm	28	9%	9%	9%
	>5 cm	57	3%	23%	28%
Portal invasion					
	Vp0	82	2%	10%	10%
	0.0001				
	Vp1	7	33%	67%	67%
	Vp2	5	0%	33%	-
	Vp3	6	0%	0%	0%
Differentiation					
	Well	28	5%	5%	5%
	<0.0001				
	Moderate	47	0%	11%	17%
	Poor	10	13%	-	-
	Not possible*	15	9%	9%	9%
UICC					
	I	26	0%	0%	0%
	0.30				
	II	30	0%	20%	20%
	IIIA	23	6%	25%	38%
	IIIB	6	25%	25%	25%
	Not possible*	15	9%	9%	9%
Milan criteria					
	Yes	48	3%	3%	3%
	0.0148				
	No	52	5%	27%	33%
UCSF criteria					
	Yes	52	3%	3%	3%
	0.0092				
	No	48	6%	31%	39%

Table 32: Univariate analysis of tumor recurrence factor using the Kaplan-Meier method.*In 15 instances, also included in the analysis, no differentiation/UICC was possible because of extended areas of tumor necrosis.

Patient selection criteria

Of the 100 recipients, 48 met the Milan criteria and 52 did not. Demographic data and profiles are shown in Table 33. Significant differences among the two groups (within/beyond Milan criteria) were found for lobar distribution of lesions (more cases of bilobar HCC in patients beyond the Milan criteria, $p<0.001$), and for tumor differentiation (more instances of poorly differentiated HCCs in patients beyond the Milan criteria). AFP serial levels were not significant ($p=0.099$). Cirrhosis related parameters were almost identical.

Patient survival among patients within the Milan criteria was 77% and 66% at 1 and 5 years, versus 76% at 1 year and 54% at 5 years among those beyond the criteria (p=0.22, Fig. 3). Among patients who met the Milan criteria, only 2 (4%) had tumor recurrence, while 12 (23%) of those who exceeded the criteria had recurrence. Cumulative HCC recurrence in patients meeting or exceeding the Milan criteria was 3% and 5% at 1 year, 3% and 23% at 2 years, and 3% and 27% at 3 years, respectively (Fig. 28). These differences in cumulative recurrence rates were significantly different (p=0.0148).

	Within Milan/UCSF	**Beyond Milan/UCSF**	**p-value**
No. of patients	48/52	52/48	
Gender			0.48/0.81
Male	38/40	37/35	
Female	10/12	15/13	
Child-Turcotte-Pugh classification			0.77/0.75
A	13/15	16/14	
B	22/23	25/24	
C	13/14	11/20	
Serology			0.38/0.40
Negative	16/17	10/9	
HBV	13/14	14/13	
HBV/HCV	1/1	1/1	
HCV	18/20	27/25	
MELD Score			0.90/0.63
<10	22/25	21/18	
>10, ≤20	23/23	26/26	
>20, ≤30	2/3	3/2	
>30	1/1	2/2	
AFP			0.10/0.24
≤20	31/32	22/21	
>20, ≤200	11/12	15/14	
>200, ≤1000	3/4	10/9	
>1000	3/4	5/4	
Lobar distribution			**<0.001/<0.001**
Unilobar	45/48	18/15	
Bilobar	3/4	34/33	
Differentiation			
No grade possible*	15/15	0/0	**<0.001/<0.001**
Well	14/14	14/14	
Moderate	18/21	29/26	
Poor	1/2	9/8	
Perioperative mortality	9/9	5/5	0.20/0.20

Table 33: Demographic data of patients according to the Milan/UCSF criteria. *In 15 instances no grade/was possible because of extended areas of tumor necrosis after tumor bridging treatments.

Of the 100 recipients in our study, 52 met the UCSF criteria and 48 did not. Patient survival among those who met the criteria was 79% at one and 67% at 5 years, versus 73% at 1 year and 52% at 5 years among these who did not (p=0.1007, Fig. 28). Among those patients who met the UCSF criteria, 3 (6%) had tumor recurrence. Eleven (23%) of those who exceeded the UCSF criteria had recurrence. Cumulative HCC recurrence in patients meeting or exceeding the UCSF criteria was 3% and 6% at 1 year, 3% and 26% at 2 years, and 3% and 32% at 3 years, respectively (Fig. 21). These differences in cumulative recurrence rates were significantly different (p=0.0092). On multivariable analysis, UCSF criteria (p=0.035) were found to be an independent risk factor for recurrence.

There was a 71% concordance among pretransplant imaging studies and pathologic analyses of the explanted livers for the Milan criteria. Imaging studies underestimated 16% of cases and overestimated the remaining 13%. Considering the UCSF criteria, the corresponding concordance among pretransplant imaging studies and pathologic analyses of the explanted livers was 69%. In these instances, imaging studies underestimated 18% of cases and overestimated the remaining 13%.

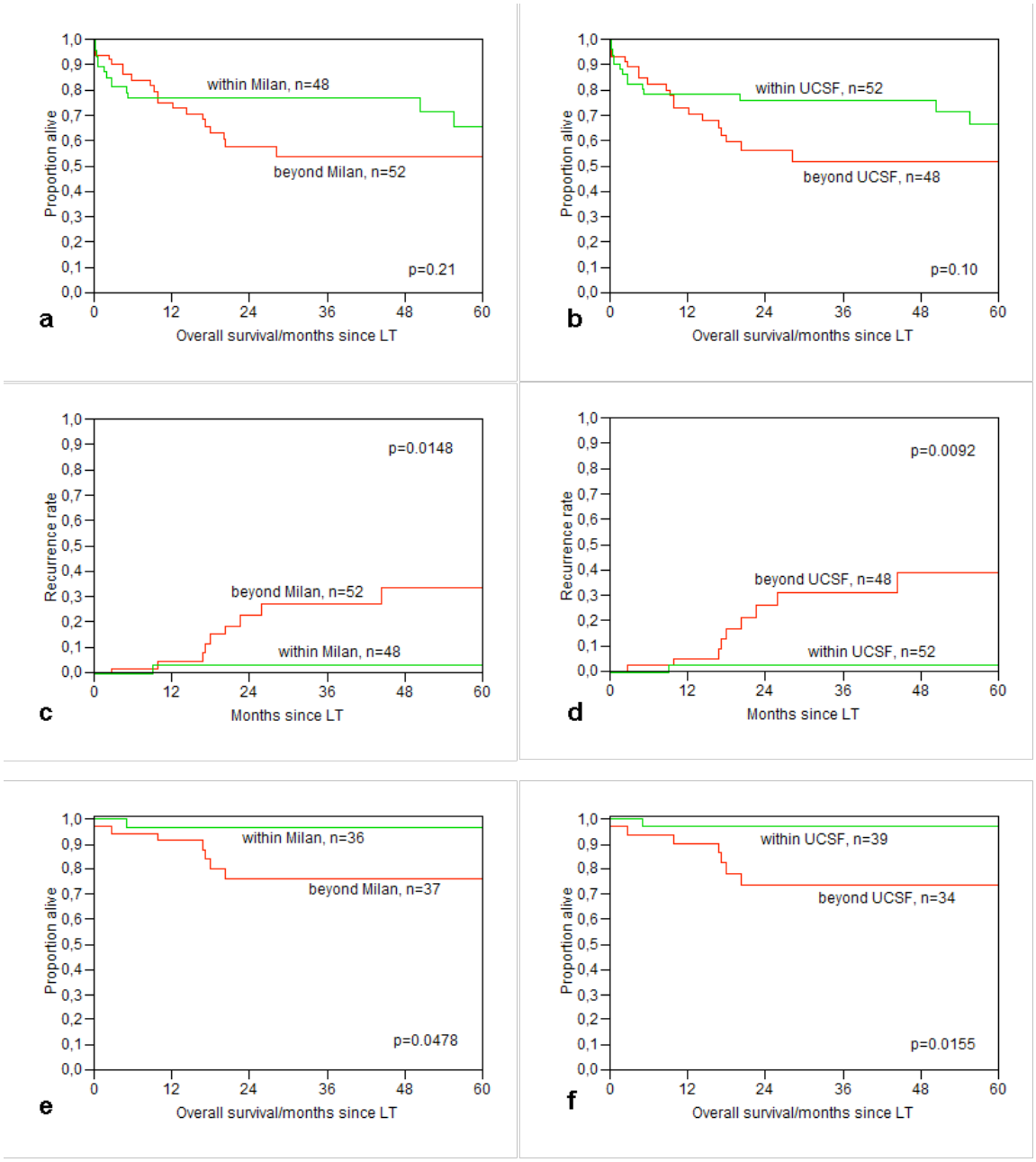


Fig. 21: Patient survival and cumulative recurrence rates according to the Milan (a and c, respectively) and UCSF criteria (b and d, respectively) for n=100 instances. Kaplan-Meier curves e and f present corresponding survival rates censored for n=73 cases, excluding the HCC-non-related deaths. Under this presupposition, 5-year overall survival of 77% and 74%, for patients beyond the Milan or the UCSF criteria, respectively, was achieved.

5. Discussion

5.1 Study results

LT for HCC in cirrhosis is doubtless the best therapy option for patients with HCC meeting the worldwide adapted Milan criteria. However, due to the reasons reported in the introduction of this work, regarding organ scarcity, rising HCC incidence, accuracy of radiological methods, improvements of surgical techniques, peri-operative management and immunosuppression, innovations in allocation rules, and experience gained with outcomes of patients with HCCs exceeding the Milan criteria in pathological evaluation, many transplant centres have tried to explore some possibilities to expand the organ pool for adult patients with HCC. Representing a large volume transplant centre, with longstanding experience in all above mentioned issues, we have focused in a prospective developing database of patients with HCC in cirrhosis in several concepts of potential expansion of the recipient pool, from less conservative to more liberal ones. The results of the above reported studies can be summarized as follows:

LT without bridging treatments - Monotherapies as treatment for hepatocellular carcinoma in cirrhosis

LT represents the best therapy option for patients with HCC in the setting of cirrhosis and in the absence of extrahepatic disease and/or macrovascular invasion, even for patients exceeding the Milan criteria. From the “transplant” point of view, restricted HCC criteria have to be applied for LT, given the scarcity of available organs, in order to provide same long term outcomes, as by patients transplanted for non tumor indications. From the “oncological” point of view, HCC criteria have to be more liberal for LT, since LT itself provides the longest overall and recurrence-free survival to these patients. The best alternative seems to be liver resection, itself hampered by postoperative morbidity and mortality, at least at the western world. TACE, with a low rate of complications, remains an option especially in cases of cirrhotic patients within the Milan criteria. In such cases, it may even excel liver resection.

LT after bridging treatment: Tumor down-staging/down-sizing with TACE

TACE provided acceptable local tumor control as bridging treatment before LT. Although the majority of our patients (15/21, 71.4%) had 2 or more tumor lesions at the beginning of treatment, tumor progression was observed in only a minority (14.3%) of patients. However, multifocal tumors could not be successfully under-staged through this treatment and, furthermore, vital tumor was always observed in pathology; the useless of TACE in multifocal disease has to be re-estimated.

LT after bridging treatment: Complete tumor necrosis

In our series of biopsy-proven HCC patients with complete tumor necrosis after bridging treatments followed by LT, no recurrence was observed after a median follow-up of 19 months. Although our actual series was small in number of patients, the "rarity" of similar reports in the literature coupled with the encouraging results achieved could lead to further discussion and consideration.

LT after bridging treatment: AFP decrease to undetectable or very low (<-30ng/mL) levels

According to the results of this study: 1) patients with a true low AFP (i.e. non-AFP-producing tumors) do better than those patients with elevated AFPs that are reduced via tumor-ablative procedures and 2) this holds true regardless of initial tumor stage. The message is reminiscent of the studies that address "downstaging" of tumors that exceed standard LT criteria. "Big" tumors with low AFP can behave like smaller tumors, and tumors of any size with elevated AFP behave according to the "peak" AFP, even if they are "treated" and "brought down" to low or non-detectable AFP values at the time of transplant. It seems that HCC patients with very low "real" AFP values, independent of UICC staging, represent a subgroup with very favourable tumor biology, so that an extension of the Milan listing criteria in these special cases is justified.

Ethical issues: LT for alcoholic liver disease and HCC – place for adult LDLT?

As this study shows, patients who undergo LT for HCC in the setting of ALD experience a very favourable overall and recurrence-free 3 year survival of 82% and 73%, respectively. Of special interest is the fact that all those patients who fulfilled the Milan criteria are currently alive with no evidence of recurrence after 3 years. Since to

the best of our knowledge this was the first formal report on LT for HCC in ALD (at least at the time of publication), we were able to derive several conclusions from our experience. First of all, there is an excellent outcome for patients meeting the Milan criteria. Second, there seems to be a lower carcinogenic potential and associated longer recurrence-free survival periods when compared to transplant recipients with other causes of liver failure. The third observation is that there is a definite risk of developing a *de novo* carcinoma, potentially associated with prolonged pre-transplant exposure to tobacco and alcohol (one patient died on metastatic tongue carcinoma, 51 months post transplant). The risk for *de novo* oropharyngeal cancer is reported to be 25 times higher in patients with ALD compared with non-ALD and the general population (134). Unfortunately, and although it is being currently addressed, we have no definitive information on the abstinence status of these patients. However, this could be the focus for a prospective study.

Although outcomes of this patients' group were excellent, the evaluation of patients with ALD and HCC constitutes a challenging topic in transplantation surgery, especially when LDLT is considered. Cases must be considered independently, with all parameters evaluated. A multi-disciplinary approach and a structured consideration of both the alcoholic and oncologic problems are mandatory.

Pre-transplant radiological assessment: Radiological versus pathological staging for HCC in cirrhosis

Correlating pathological findings and radiological imaging, we found that only 10 of the 70 patients (14.3%) were correctly evaluated concerning tumor diameter. Only 24 patients (34.2%) were adequately classified with respect to tumor number. Fifty patients (71.4%) showed a discrepancy of more than 1cm in tumor diameter, and 36 patients (51.5%) had discrepancies in the number of tumors. In our specific series the best accuracy of clinical and imaging classification was for both the Milan and UCSF criteria, with an overall percentage of only 60%. Forty percent of patients were incorrectly classified, and 14 of them (20%) could have lost the possibility to undergo LT because of "exceeding" the recommended parameters. The CLIP scoring system showed a marginal accuracy (54.3%) in the LDLT group. No statistical significance was found between the two patient groups (DDLT and LDLT) concerning

clinical/pathological staging and the accuracy of the various systems/criteria of classification. These results don't seem to point toward any difference associated with the timing difference from last imaging to transplantation seen among live and deceased donor groups.

Radiological and clinical evaluations remain the most widely available means to evaluate patients with HCC. However, accuracy of results still remains suboptimal. Given the wide acceptance of transplantation for the treatment of small HCC in cirrhosis, and the lack of accurate information provided by imaging studies, we believe that patients with HCC in the setting of cirrhosis and without extrahepatic disease should be candidates to liver transplantation wherever possible. LDLT can be an additional rescue therapy for many of these patients.

Pre-transplant radiological assessment: Incidentally found HCC

HCCs will continue to be found incidentally after LT for "benign disease." This trend will persist as long as imaging techniques are not able to detect small tumors in cirrhotic livers, and as long as patients with cirrhosis and no known malignancy are not evaluated and re-staged as intensively as tumor patients. Expansion of the tumor staging for all patients with cirrhosis waiting for LT seems relatively impracticable. Given the above mentioned "problem of definition", many of the reported in the literature "incidental" HCCs are rather "undetected" HCCs because of the long waiting time and the absence of intensive tumor staging. As a consequence of this situation, the self-evident "better survival" of patients with iHCC is not unequivocally documented in the literature.

According to the results of our review, the widely accepted definition of iHCC as "unsuspected HCC found at the time of histological examination of liver explants of patients transplanted for benign diseases" seems insufficient to distinguish between iHCC and undetected HCC. This definition could result to misinterpretation that patients with iHCC and pkHCC share same outcomes, situation which is not in accordance with the clinical experience. A consensus determining a concrete art of objective radiological evaluation (CT or MRI) and an acceptable intervening period of time between last radiological evaluation and LT could guide to a better separation between iHCC and undetected HCC and a better assessment of the patients' survival.

Extended listing criteria: LT for patients beyond Milan/ within UCSF criteria

Our results, in accordance to the results of Leung et al. (96), showed that only a small percentage of HCC patients undergoing LT have tumor findings "beyond Milan but within UCSF criteria" (4% and 7%, respectively). Furthermore, these patients have an acceptable long survival (median of 57 months in our series, 32 months in the report of Leung et al.), and probably not necessarily a higher recurrence rate (only one patient in our series, 81 months post transplant, with no corresponding information available from Leung et al.). These findings could add some encouraging information in the discussion about the possible expansion of the Milan to the UCSF criteria. However, large-volume patients' series, intention- to-treat analysis based on the radiological findings and multi-center prospective studies are required, in order to further explore the outcome of patients "beyond Milan but within UCSF" and in order to better define the risk/benefit ratio of a potential expansion of the current listing criteria.

Extended listing criteria: LT for HCC and portal vein thrombosis

To the best of our knowledge, the current series was at the time of publication the first one in the era of transplant surgery to evaluate and correlate radiological imaging results, pathological findings, and overall and recurrence-free survival in this group of patients. Our observations suggested that:

1. The origin of PVT in HCC patients cannot be accurately determined by currently available pre-transplant imaging studies (accuracy of 58% with corresponding sensitivity and specificity of 50% and 80%, respectively).
2. Thrombotic tumor material is found in only a minority (17%) of patients.
3. A respectable proportion of patients with HCC and PVT (42% in our series) do not show tumoral invasion of the portal vein on pathologic exam of the liver explant.
4. Proven portal vein invasion was evident in the lobar vein or main trunk (33%) and in the segmental branches (25%).
5. Only a minority of patients (17%) meet the Milan criteria.

6. One third of the patients experience rapid tumor recurrence within the first posttransplant year (median recurrence-free survival 12 months, range 5-61 months).
7. One third of the patients become long term-survivors, without evidence of tumor recurrence (median survival of 36 months in our series).

PVT in the setting of HCC is characterized by poor patient outcome. However, a respectable number of instances are not accurately evaluated preoperatively, making the decision to exclude these patients from LT sometimes a challenging dilemma.

Extended listing criteria: Pulmonary modules at risk in patients undergoing LT for HCC

Since images frequently cannot clearly differentiate between benign lesions and very small HCC metastasis, follow-up studies are usually recommended in the clinical praxis, but are not helping in making a decision "to transplant or not to transplant". Besides, short waiting periods for patients with HCC and cirrhosis (135) render such "wait and see" policies impractical. However, since there are no corresponding data on this special topic, we have adopted a "waiting" policy of at least 3 months, before proceeding with a LT.

To the best of our knowledge, our study was the first to address this clinical problem (at least at the time of publication), describing the accuracy of the radiological diagnosis and providing information on the long-term outcome of HCC patients transplanted with such pulmonary nodules. We have included only patients with a minimum post transplant follow-up of 2 years, which is considered to be a reliable indicator of a benign lesion. Although stable imaging results were detected in 9 out of 10 patients in a median follow-up period of 38 months, we believe that small pulmonary lesions presumed to be granulomas should be characterized as "*nodules at risk*", given that no guidelines about the surveillance of these nodules exist yet. The importance of the 2 predictors, which have been detected in the discriminant function analysis (tumor grade and Milan criteria) should be validated from other study groups. Detection and close observation of these nodules during the pre and post transplant period is mandatory. Further reports from other centers may lead to the development of a corresponding transplant policy.

Expanding the donor pool: Extended criteria donor for LT for HCC – rescue organ offers

After 34 official refusals, the livers in our study qualified as "livers that nobody wanted". Eurotransplant policy permitted us to select as recipients for these allografts whichever patients we thought would benefit the most among those in our transplant waiting list. We opted to select patients in stable condition but with unfavourable prognosis because of tumor characteristics. Initial poor graft function was observed in one of the 7 patients, but recovered spontaneously. PNF due to big-for-size syndrome led to the death of a patient. There was one arterial complication requiring reoperation. ICU stay and hospital stay were similar to those of patients transplanted with standard donors. One patient developed transient renal dysfunction immediately after the LT and recovered 3 months later. One patient underwent an atypical liver resection because of recurrent tumor in the transplant liver 23 months after the LT. Six of the seven patients (86%) are alive from 7 to 35 months after the LT. All patients are followed at our outpatient liver transplant clinic.

LT with such "livers that nobody wants" constitutes an additional option for patients with HCC and cirrhosis. The potential risks of using "marginal livers" as well as the potential benefits of transplanting patients unlikely to survive the waiting list period, must be evaluated on an individual basis.

Expanding the donor pool: Split LT for HCC

One of the main limitations of our series was the small size of the study group. However, the ongoing international discussion about treatment options for HCC, and the "originality" of this study being the first to address SLT in HCC patients prompted us to evaluate this treatment possibility for HCC patients in stable condition but with unfavourable prognoses because of tumor characteristics. The benefit/risk ratio was for the time being positive in our series, considering the short waiting time to LT (82 days in median), the 0% rate of primary non function or vascular/biliary complications, and the 83% patient survival after a median follow up of 20 months.

Deceased donor SLT constitutes an additional option for patients with HCC and cirrhosis. The potential risks of using "split livers" as well as the potential benefits of

transplanting patients unlikely to survive the waiting list period must be evaluated on an individual basis. A concurrent paediatric liver transplant program is of vital importance when considering this option.

Expanding the donor pool: LDLT for HCC

Our study provides us with several learning points. The first one was a message of caution when transplanting patients over sixty years of age. The second one is the association of MELD scores greater than 22 with early post-transplant mortality. Three and 5 year survival rates for patients with MELD scores ≤22 were 67% and 63%, respectively, whereas the corresponding rates for patients with MELD scores>22 were 28% in both instances. The survival rate at 3 years when including 45 day mortality (i.e. age, MELD) was 72% for score=2 and 41% for score>2, respectively.

When 45-day mortality was excluded from the analysis, AFP was found to be of predictive value, with a cut-off point ≤400ng/mL. Three and 5 year survival rates for patients with AFP levels≤400ng/mL were 80% and 75%, respectively, whereas the corresponding rates for patients with AFP levels>400ng/mL were 20% in both instances, respectively. When excluding 45 day mortality survival at 3 years was 90% for score=2 and 32% for score>2, respectively (p=0.00002). At a time when the role of AFP as a part of scoring systems is being re-considered, our results provide further evidence on its behalf.

As a final remark, we would like to mention that patients within the widely accepted listing criteria have the best post-transplant life expectancy, and that there would be no discussion at all if it wasn't for the current organ scarcity and HCC epidemic. Although our overall long terms results are hampered by our liberal tumor criteria, we believe that our results could further improve current guidelines on age, MELD score, and AFP levels when selecting patients with HCC for LT.

Expanding the donor pool: Instiutional results

Our study provided almost equivalent data for both listing criteria (48 cases within the Milan criteria versus 52 beyond, 52 within the UCSF criteria versus 48 beyond, respectively) and for LT procedures (55 DDLT versus 45 LDLT). Overall 5 year patient and recurrence-free survival rates were 60% and 54%, respectively. When

excluding the HCC-non-related deaths, corresponding 5-year overall and recurrence-free survival rates of 87% and 78% were achieved, respectively. Survival analysis demonstrated no significant differences between the indexed parameters except for tumor differentiation. Although the 54% 5-year survival for patients exceeding the Milan criteria was lower than the 66% 5-year survival for patients meeting the Milan criteria, this difference did not reach statistical significance (p=0.21). Similar results were obtained when the UCSF criteria were considered (5-year survival rates of 52% and 67% for patients exceeding and meeting these criteria, respectively, p=0.10). The recurrence rate however, was significantly higher for patients exceeding the Milan and the UCSF criteria. AFP levels, tumor differentiation, and portal vein invasion were also found to be associated with HCC recurrence. In the multivariable Cox regression analysis, tumor differentiation retained its statistical significance both for survival (p=0.028) and tumor recurrence (p=0.006), while UCSF criteria were associated with tumor recurrence (p=0.035).

Although many studies report on various determinants of "high risk pathology" for tumor recurrence and decreased patient survival, no systematic analysis of the literature was available until now. In the present study a meta-analysis was performed to better define "high risk" characteristics and to "compose" a compilation of the past 20 year's experience of study groups on LT for HCC.

5.2 Meta analyses

5.2.1 Impact of hilar lymph nodes metastases on tumor recurrence and survival in patients with HCC undergoing LT

Hilar lymph node (LN) involvement in cases of HCC constitutes a contraindication for LT. As such, patients suspected of having LN metastases during the pre-transplant radiological evaluation are being removed from waiting lists. However, this situation is complicated by the frequent presence of LN enlargement due to chronic inflammation in cases of hepatitis-C induced cirrhosis (136-137). The differential diagnosis of LN swelling in patients with HCC arising in hepatitis-C cirrhosis remains difficult (136-137), and the decision of whether to proceed with a LT has been considered only in transplant centers performing live donor LT for extended tumor

indications (36). In such cases, the option of an elective surgery with evaluation of LN by frozen sections of resected LN facilitates the decision making.

The goal of our study was to determine the prognostic significance of hilar LN metastases on patient survival and tumor recurrence, and to evaluate the significance of hilar LN sampling at the time of LT (138).

Literature Search

A computer search of the Medline database for the years 1985 to 2005 was carried out using the MeSH headings: "hepatocellular carcinoma", "liver transplantation", "tumor recurrence", "lymph nodes", and "tumor staging". The combined set was limited to English-language publications on human subjects. All titles and abstracts were scanned, and appropriate citations reviewed. Consultation with a content expert and a manual search of the bibliographies of relevant papers was also carried out to identify trials for possible inclusion.

Inclusion Criteria

Inclusion criteria for this analysis were clinical studies of any size on LT for HCC. Special emphasis was placed on the effect of LN metastasis on tumor recurrence and patient survival. Outcome of patients with positive hilar LN (study group) was compared to that of patients with no LN metastases (reference group).

Data collection

Critical appraisal and data extraction were conducted independently by the authors, and discrepancies resolved by consensus. In instances of multiple studies from a single institution, the most recent or more informative publication was chosen.

Analyses

Comparisons of results across studies were pooled for tumor recurrence and mortality. All analyses were conducted on a personal computer using Review Manager 3.0 (The Cochrane Collaboration, Software Update, Oxford). A fixed effects model was applied. Statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC)

and StatXact (Cytel Software Corp., Cambridge, MA). The summary statistic used was the odds ratio, which represents the odds of an event (tumor recurrence, mortality) occurring in the group of patients with positive hilar LN divided by odds of the control group. Odds ratios >1 display the higher risk in the tumor positive LN group, and the point estimate of the odds ratio is considered statistically significant at the alpha=0.05 level only if the 95% confidence interval (95% CI) does not include the vertical bar at 1. Any value lying within the 95% CI is considered to be consistent with the data, in the sense that it cannot be rejected at the 0.05 level. Because the sample size was relatively small in some studies exact statistical methods were applied (139). The exact confidence interval for the odds ratio of a single study was computed. Homogeneity of odds ratios across different studies was tested using the exact homogeneity test. If this test was not significant, no evidence for heterogeneity was considered, i.e. for systematic differences between the studies. In that case a confidence interval for the common odds ratio was calculated.

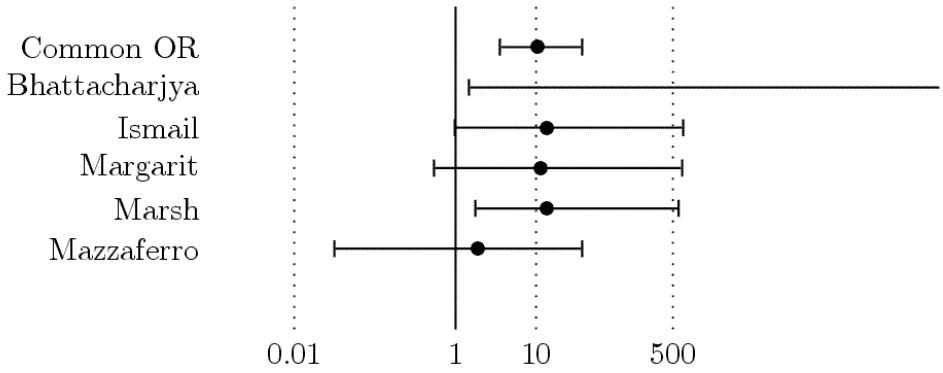
Results

Among 34 retrospective clinical studies screened (138), 5 fulfilled the criteria described in Patients and Methods (104, 140-143). The studies dated from 1990 to 2004 and contained from 20 to 178 patients, yielding a total of 397 patients for this analysis. Ismail et al. reported 10 recurrences after LT for HCC, 6 of them in the presence of tumor positive LN. Since the Milan criteria for liver transplantation for HCC had not been yet established, Ismail's study encompassed patients with more advanced tumor stages. Mazzaferro et al. reported a total of 17 post-transplant tumor recurrences in their series of 80 patients, one of them in the study group. Seventy one further cases of tumor recurrence were referred by Marsh et al., the majority of them (n=63) in patients without LN tumor metastases. A total of 19 cases of post-transplant tumor recurrence were reported in the series of Margarit et al. and of Bhattacharjya et al., with 2 cases of recurrences in the study group for each of the studies. Characteristics of the studies are summarized in Table 34.

	Ismail	Mazzaferro	Marsh	Margarit	Bhattacharjya	Total
Year	1990	1994	1997	2002	2004	
Total number of subjects	20	80	178	89	30	397
Study group (positive LN)	7	3	9	3	2	24
Reference group (negative LN)	13	77	169	86	28	373
Recurrences in study group	6	1	8	2	2	19
Recurrences in reference group	4	16	63	13	2	98

Table 34: Characteristics of clinical studies evaluating tumor recurrence after LT for HCC according to LN status in the liver hilum.

Evaluation of the data extraction showed 100% agreement among the reviewers. Study-specific and common odds ratios for the outcomes are displayed in Figure 22. The estimates of effect size (odds ratio of LN infiltration vs. the control group) on recurrence was 10.44 (exact estimation of common odds ratio, 95% CI 3.431 to 38.59), showing a significant correlation between LN infiltration during LT and recurrence of HCC. The width of the horizontal bars reflects the 95% CI expressed on a logarithmic scale. The test of heterogeneity for each comparison revealed no significant differences between the studies (exact p=0.4638), permitting pooling of the data using a fixed effects model. The point estimates of odds ratio for recurrence ranged from 1.906 to infinity, suggesting that LN infiltration is associated with an increased recurrence rate.



Study	OR	lower bound of 95% CI	upper bound of 95% CI
Common OR	10.44	3.431	38.59
Bhattacharjya	infinity	1.422	infinity
Ismail	13.5	0.9488	687.9
Margarit	11.23	0.525	671.2
Marsh	13.46	1.715	602.7
Mazzaferro	1.906	0.0304	38.46

Fig. 22: Odds ratios and confidence intervals (CI) of clinical studies evaluating tumor recurrence after LT for HCC according to hilar LN status. Meta-analysis resulted to a common odds ratio of 10.44.

Between the 34 studies screened (138), 5 retrospective clinical studies that evaluated patient survival after liver transplantation for HCC according to the hilar LN status were identified (16, 117, 133, 140, 144). The studies dated from 1990 to 2004 and contained from 20 to 387 patients, yielding a total of 606 patients for this analysis (Table 35). Selby et al. showed a statistically worse patient survival (p=0.0054) in the presence of LN metastases (mean survival of 10.5 months) when compared to a mean survival of 49.7 months of the reference group (negative LN). Ringe et al. demonstrated also a worse patient survival (p=0.0004) in the presence of LN metastasis. In their series, the median survival of 1.5 months and the 5-year survival of 0% were statistically inferior to the corresponding median survival of 10.8 months and 5-year survival of 19% in cases of tumor negative LN. Klintmalm also showed a significantly better patient survival (p=0.0014) in the absence of LN metastases (5-years survival of about 45%) when compared to tumor positive LN (5-year survival of about 25%). In the series of Ismail et al. median patient survival was also better in the reference group (13 months for tumor negative and 8 months for tumor positive LN, respectively). Note that in the series of Yedibela et al., the 5-year survival was 100% for patients with positive LN (n=2) and 77% in the group of 31 patients with no LN metastases. However, sample sizes were small and the difference is far from being significant (p=0.6012).

	Ismail	Ringe	Selby	Klintmalm	Yedibela	Total
Year	1990	1991	1995	1998	2004	
Total number of subjects	20	61	105	387	33	606
Study group (positive LN)	7	12	9	25	2	55
Reference group (negative LN)	13	49	96	362	31	551
p-value	0.5627	0.0004	0.0054	0.0014	0.6012	<0.001

Table 35: Characteristics of clinical studies evaluating patient outcome after LT for HCC according to LN status in the liver hilum.

Data analyses using the Fisher-combination test (55-56) yield $\chi^2 = 41.40$ (df = 10), which corresponds to a p-value <0.0001. That corresponds to a significant correlation between LN infiltration during LT for HCC and decreased survival.

Comments

According to the results of this meta-analysis, systematic hilar lymphadenectomy during LT for HCC should routinely be undertaken, especially in the

context of coexisting hepatitis C or secondary biliary cirrhosis. In cases where frozen sections results are available at the time of transplantation, the decision to proceed with total hepatectomy and LT should be based on the presence of tumor involvement. In institutions with the capacity to perform frozen sections at all times, a laparoscopic hilar lymphadenectomy could be initially performed, with subsequent conversion to an open surgery, if the sampled LN contain no tumor. A back-up recipient, preferably with no evident tumor in order to minimize preservation time, would be transplanted in those cases where the primary recipient has positive LN. In Institutions where a pathologist is not continuously on duty, the removed LN could serve to better define patients at risk for early recurrence, and correspondingly inform, follow-up and eventually provide oncologic therapy. The question, which remains open is what to do, if enlarged LN are present in the context of coexisting hepatitis C or secondary biliary cirrhosis and in the case that no pathologist is available. In this task, the experience of the transplant surgeon is mandatory for the decision-making, although exclusion of the HCC patient from the LT without pathological documentation is challenging.

5.2.2 Tumor recurrence after LT for HCC based on 1,198 cases

Recurrence of HCC after liver transplantation for HCC in the setting of liver cirrhosis is a frequent occurrence. Although some series report on prognostic factors resulting in higher rates of tumor recurrence, no systematic analysis exists until now in the published literature. The goal of our study was to systematically review tumor characteristics leading to HCC recurrence after LT (145).

Literature Search

A computer search of the Medline database for the years 1985 to 2005 was carried out using the MeSH headings: “hepatocellular carcinoma”, “liver transplantation”, “tumor recurrence”, “tumor staging”. The combined set was limited to English-language publications on human subjects. All titles and abstracts were scanned, and appropriate citations reviewed. Consultation with a content expert and a manual search of the bibliographies of relevant papers was also carried out to identify trials for possible inclusion.

Inclusion Criteria

Inclusion criteria for this analysis consisted of clinical studies of any size reporting on tumor recurrence after liver transplantation for HCC, under the prerequisite that adequate data about recurrence rate according to the examined tumor parameters listed bellow were available.

Data collection

Critical appraisal and data extraction were conducted independently by the authors, and discrepancies resolved by consensus. In instances of multiple studies from a single institution, the most recent or more informative publication was chosen.

Analyses

Common analyses - if feasible - of results across studies were carried out for the presence of tumor recurrence. The following tumor characteristics were examined, according to pathological findings in the explanted liver: 1) no vascular versus vascular invasion, 2) solitary versus multifocal tumors, 3) well differentiated versus not well differentiated tumors, 4) tumors meeting the Milan criteria (single tumor ≤5cm, or 2-3 tumors none of them >3cm, no vascular invasion) versus tumors exceeding them, 5) tumors ≤5cm versus tumors>5cm. Although the parameters of vascular invasion and tumor >5cm are somewhat included in the Milan criteria, we examined them independently since there were analyses in the literature that considered them as such prior to 1996, the year in which the Milan criteria were first proposed. Data analyses were conducted using Review Manager 3.0 (The Cochrane Collaboration, Software Update, Oxford). A fixed effects model was applied. Statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC) and StatXact (Cytel Software Corp., Cambridge, MA). The summary statistic used was the odds ratio, which represents the odds of an event (tumor recurrence) occurring in the group of patients with “unfavourable pathology” divided by odds of the control group. Odds ratios >1 display the higher risk in the group with “unfavourable pathology” (i.e. vascular invasion, multifocal HCC, not well differentiated HCC, beyond the Milan criteria, HCC>5cm, respectively), and the point estimate of the odds ratio is considered statistically significant at the alpha=0.05 level only if the 95% confidence interval (95%

CI) does not include 1. Any value lying within the 95% CI is considered to be consistent with the data, in the sense that it cannot be rejected at the 0.05 level. Because the sample size was relatively small in some studies, exact statistical methods were applied (139). The exact confidence interval for the odds ratio of a single study was computed. Homogeneity of the odds ratios across the different studies was tested using the exact homogeneity test. If this test was not significant, no evidence for heterogeneity was considered, i.e. for systematic differences between the studies. In that case a confidence interval for the common odds ratio was calculated.

Results

Of 45 retrospective clinical studies screened (145), 9 providing special information about tumor recurrence post LT according to the examined tumor parameters described in Patients and Methods were identified (29, 97, 102, 106, 108, 110, 146-148). The studies dated from 1989 to 2004 and ranged from 21 to 316 patients, yielding a total of 1198 patients. Information on HCC recurrence after LT was available for 1065 recipients. On review of the data extraction, there was 100% agreement among the authors who performed the literature review. Of the two studies from the Mount Sinai Medical Center in New York (110, 146), information on vascular invasion and tumor grading was obtained from the larger/most recent series (Roayaie et al, 2004), while data for recurrences according to number of tumor lesions, tumor size, and Milan criteria, was procured from the smaller/initial one (Gondolesi et al, 2002) since such information was not available in the former series. Although hospital mortality was for the most part not considered in the recurrence analysis (97, 102, 106, 147-148), in some instances it was not considered at all (108, 110), or was included in the recurrence analysis (29, 146).

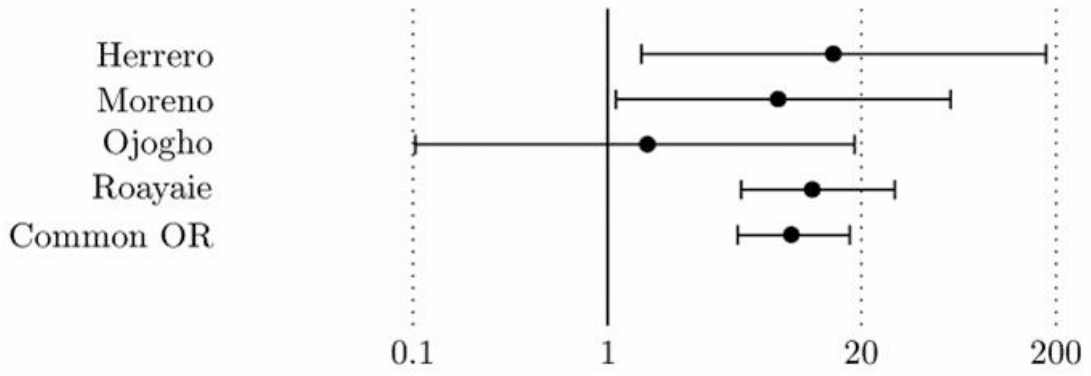
1) HCC-Recurrence according to the presence of vascular invasion in the explanted liver (no vascular versus vascular invasion)

Six studies were identified, resulting in a total of 483 patients (Table 36). There were 19 recurrences among 290 patients with no vascular invasion (6.5%). On the other hand, 65 recurrences were identified among 193 patients presenting vascular invasion in the liver explants (33.7%). The study of Mazzaferro et al., having no control group, was

not included in the meta-analysis. The test of heterogeneity revealed no significant differences between the studies (exact p=0.2937), permitting for a common analysis of the data using a fixed effects model. The study-specific as well as the common odds ratios for the outcomes are displayed in Figure 23. The width of the horizontal bars reflects the 95% CI expressed on a logarithmic scale. The estimate of size effect (odds ratio of vascular infiltration vs. the control group) on recurrence was 8.727 (exact estimation of common odds ratio, 95% CI 4.557 to 17.72), showing a significant correlation between vascular invasion and recurrence of HCC.

	Gondolesi	Herrero	Mazzaferro	Moreno	Roayaie	Ojogho	Total
Year	2002	2001	1996	1995	2004	1996	
Total number of subjects	27	47	48	31	311	22	483
Study group (no vascular invasion)	11	38	48	19	162	15	290
Reference group (vascular invasion)	16	9	0	12	149	7	193
Recurrences in study group	0	2	4	3	7	3	19
Recurrences in reference group	2	4	0	7	50	2	65
p-value in chi-square test	0.512	0.027	1.000	0.144	<0.001	1.000	<0.001

Table 36: Characteristics of clinical studies evaluating tumor recurrence after LT for HCC according to vascular invasion.



Study	OR	lower bound of 95% CI	upper bound of 95% CI
Common OR	8.727	4.557	17.72
Herrero	14.4	1.468	180.8
Moreno	7.467	1.085	58.47
Ojogho	1.6	0.1012	18.77
Roayaie	11.18	4.758	30.2

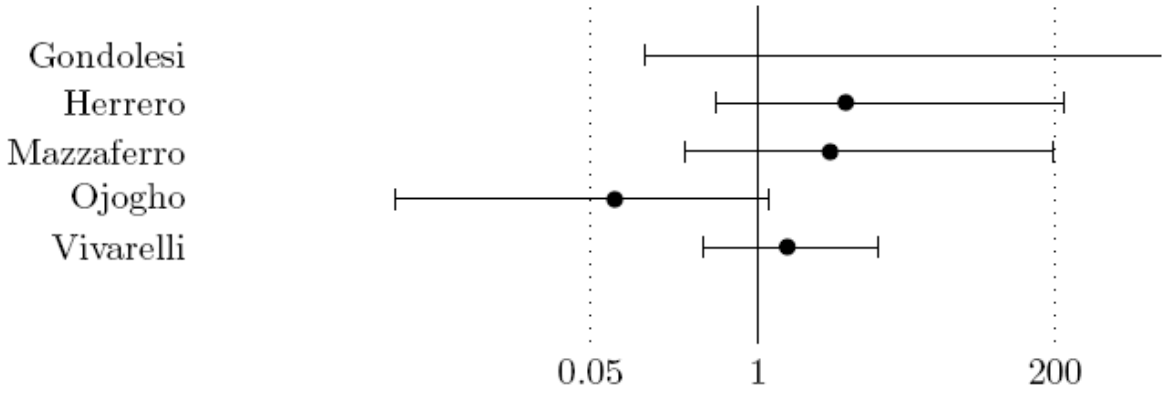
Fig. 23: Odds ratios and confidence interval (CI) of clinical studies evaluating tumor recurrence after LT for HCC according to vascular invasion. Meta-analysis resulted in a common odds ratio of 8.727.

2) HCC-Recurrence according to number of HCC lesions in the explanted liver (solitary versus multifocal tumors)

Five studies were identified, resulting in a total of 226 patients (Table 37). There were 10 recurrences among 107 patients with solitary HCCs (9.3%). On the other hand, 17 recurrences were identified among 119 patients with multifocal HCCs in their liver explants (14.3%). The study-specific odds ratios for the outcomes are displayed in Figure 24. The point estimates of odds ratio for recurrence ranged from 0.0769 to infinity, so that no common trend could be seen. Moreover the test of heterogeneity revealed significant differences among the studies (exact p=0.0428), impeding the conduction of a common analysis of the data by means of a fixed effects model.

	Gondolesi	Herrero	Mazzaferro	Ojogho	Vivarelli	Total
Year	2002	2001	1996	1996	2002	
Total number of subjects	27	47	48	22	82	226
Study group (solitary HCC)	11	21	25	8	42	107
Reference group (multifocal HCC)	16	26	23	14	40	119
Recurrences in study group	0	1	1	4	4	10
Recurrences in reference group	2	5	3	1	6	17
p-value in chi-square test	0.512	0.382	0.610	0.139	0.739	0.417

Table 37: Characteristics of clinical studies evaluating tumor recurrence after LT for HCC according to number of HCC lesions.



Study	OR	lower bound of 95% CI	upper bound of 95% CI
Gondolesi	infinity	0.1288	infinity
Herrero	4.762	0.4589	236.3
Mazzaferro	3.6	0.2586	196.7
Ojogho	0.0769	0.0015	1.216
Vivarelli	1.676	0.3598	8.736

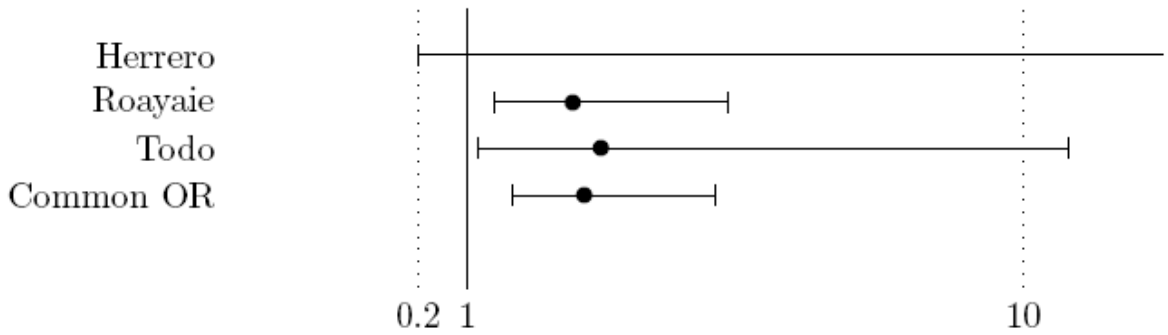
Fig. 24: Odds ratios and confidence interval (CI) of clinical studies evaluating tumor recurrence after LT for HCC according to number of tumors. Due to heterogeneity of the studies, no common odds ratio could be calculated.

3) HCC-Recurrence according to tumor grading in the explanted liver (well differentiated versus not well differentiated tumors)

Three studies were identified, resulting in a total of 630 patients (Table 38). There were overall 24 recurrences among 256 patients with well differentiated HCCs (9.4%). On the other hand, 76 recurrences were identified among 374 patients with not well differentiated HCCs in the liver explants (20.3%). The test of heterogeneity revealed no significant differences among the studies (exact p=1.00), permitting a common analysis of the data using a fixed effects model. The study-specific as well as the common odds ratios for the outcomes are displayed in Figure 25. Meta-analysis resulted in a common odds ratio of 2.89 (exact estimation of common odds ratio, 95% CI 1.708 to 5.036), showing a significant correlation between not well differentiated tumors and recurrence of HCC.

	Herrero	Roayaie	Todo	Total
Year	2001	2004	2004	
Total number of subjects	40	311	279	630
Study group (well differentiated tumor)	6	165	85	256
Reference group (not well differentiated tumor)	34	146	194	374
Recurrences in study group	0	19	5	24
Recurrences in reference group	6	38	32	76
p-value in chi-square test	0.579	0.010	0.051	0.002

Table 38: Characteristics of clinical studies evaluating tumor recurrence after LT for HCC according to tumor grading.



Study	OR	lower bound of 95% CI	upper bound of 95% CI
Common OR	2.89	1.708	5.036
Herrero	infinity	0.1905	infinity
Roayaie	2.704	1.425	5.241
Todo	3.16	1.157	10.75

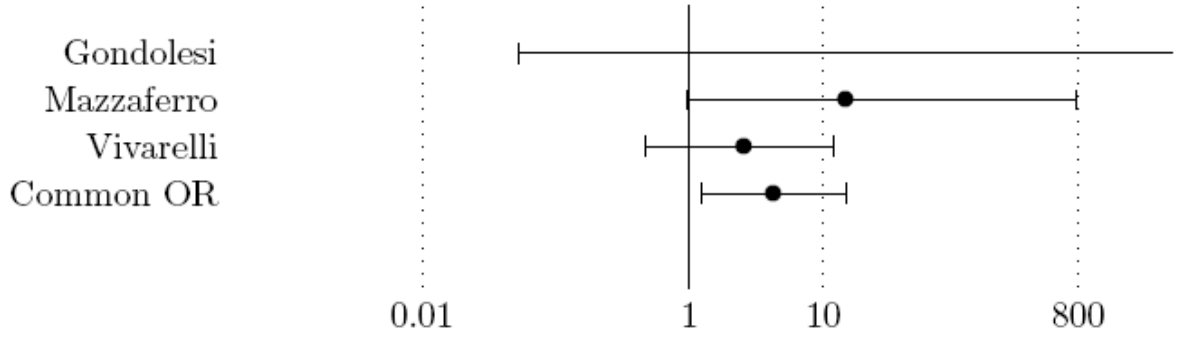
Fig. 25: Odds ratios and confidence interval (CI) of clinical studies evaluating tumor recurrence after LT for HCC according to tumor grading. Meta-analysis resulted in a common odds ratio of 2.89.

4) HCC-Recurrence according to the pathological Milan criteria (tumors meeting versus tumors exceeding the Milan criteria)

Three studies were identified resulting in a total of 154 patients (Table 39). There were overall 7 recurrences among 104 patients meeting the Milan criteria (7.2%). On the other hand, 9 recurrences were identified among 50 patients exceeding these criteria in the liver explants (18%). The test of heterogeneity revealed no significant differences among the studies (exact p=0.5761), permitting a common analysis of the data using a fixed effects model. The study-specific as well as the common odds ratios for the outcomes are displayed in Figure 23. The estimate of size effect (odds ratio for patients beyond the Milan criteria vs. the control group) on recurrence was 4.205 (exact estimation of common odds ratio, 95% CI 1.188 to 15.33), showing a significant correlation between HCC beyond the Milan criteria and tumor recurrence.

	Gondolesi	Mazzaferro	Vivarelli	Total
Year	2002	1996	2002	
Total number of subjects	27	48	82	154
Study group (within Milan criteria)	6	35	63	104
Reference group (exceeding Milan criteria)	21	13	19	50
Recurrences in study group	0	1	6	7
Recurrences in reference group	2	3	4	9
p-value in chi-square test	1.000	0.081	0.261	0.104

Table 39: Characteristics of clinical studies evaluating tumor recurrence after LT for HCC according to the Milan criteria.



Study	OR	lower bound of 95% CI	upper bound of 95% CI
Common OR	4.205	1.188	15.33
Gondolesi	infinity	0.0512	infinity
Mazzaferro	14.57	0.9255	786.3
Vivarelli	2.533	0.4586	12.2

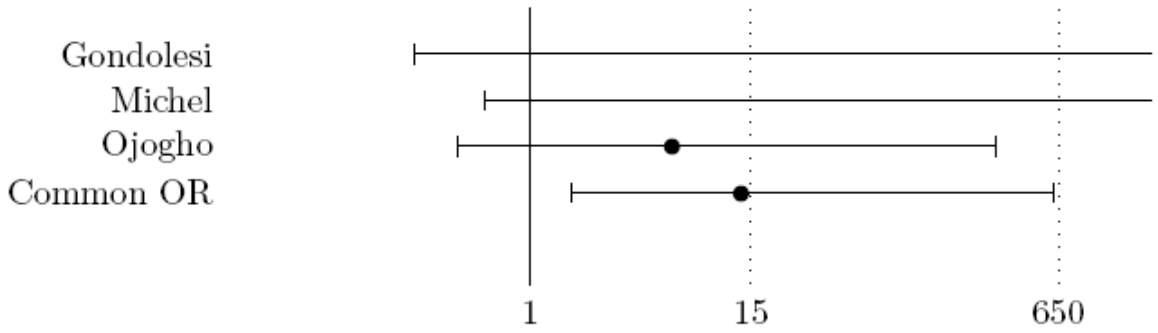
Fig. 26: Odds ratios and confidence interval (CI) of clinical studies evaluating tumor recurrence after LT for HCC according to the Milan criteria. Meta-analysis resulted in a common odds ratio of 4.205.

5) HCC-Recurrence according to tumor size in the explanted liver (tumors ≤5cm versus tumors >5cm in diameter)

Three studies were identified, resulting in a total of 62 patients (Table 40). There was 1 recurrence among 33 patients with HCCs≤5cm (3%). On the other hand, 9 recurrences were identified among 29 patients exceeding these criteria in the liver explants (31%). The test of heterogeneity revealed no significant differences among the studies (exact p=1.00), permitting a common analysis of the data using a fixed effects model. The study-specific as well as the common odds ratios for the outcomes are displayed in Figure 27. Meta analysis resulted in a common odds ratio of 13.32 (exact estimation of common odds ratio, 95% CI 1.634 to 622.1), showing a significant correlation between HCC >5cm and tumor recurrence.

	Gondolesi	Michel	Ojogho	Total
Year	2002	1995	1996	
Total number of subjects	27	13	22	62
Study group (tumor ≤5cm)	15	7	11	33
Reference group (tumor >5cm)	12	6	11	29
Recurrences in study group	0	0	1	1
Recurrences in reference group	2	3	4	9
p-value in chi-square test	0.224	0.212	0.342	0.015

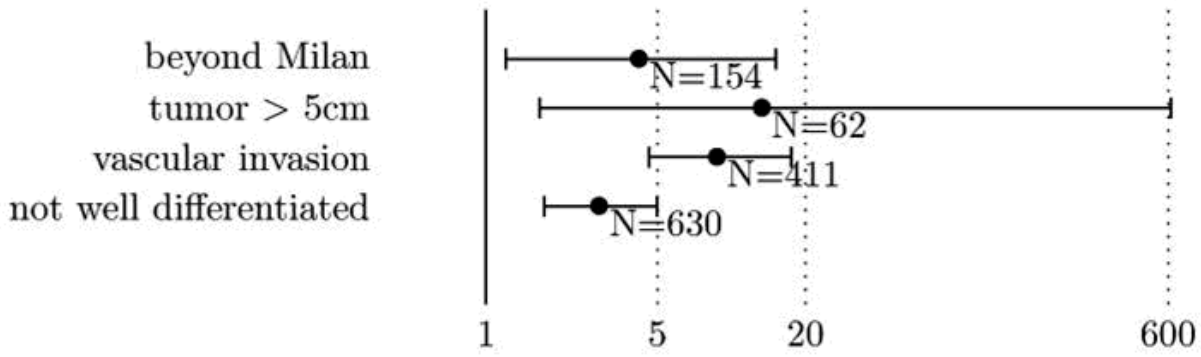
Table 40: Characteristics of clinical studies evaluating tumor recurrence after LT for HCC according to tumor size.



Study	OR	lower bound of 95% CI	upper bound of 95% CI
Common OR	13.32	1.634	622.1
Gondolesi	infinity	0.2399	infinity
Michel	infinity	0.5631	infinity
Ojogho	5.714	0.4057	308.9

Fig. 27: Odds ratios and confidence interval (CI) of clinical studies evaluating tumor recurrence after LT for HCC according to tumor size. Meta-analysis resulted in a common odds ratio of 13.32. Due to the small sample size of the corresponding studies (overall n=62) there is a greater uncertainty of the common OR. Two of the ORs are infinity, resulting in a very high common OR.

Our systematic review of the literature and meta-analysis of 45 clinical studies on HCC recurrence after LT for HCC showed that the parameters vascular invasion, not well differentiated HCC, tumor size >5cm, and HCC exceeding the Milan criteria constitute significant negative prognostic factors for post-transplant recurrences (common odds ratio by 8.727, 2.89, 13.32 and 4.205 for the above mentioned parameters, respectively, Fig. 28). As demonstrated in Figure 28, the effect was statistically more significant for the parameters “vascular invasion” and “not well differentiated HCC”, which represent the higher-volume meta-analyses of the present study (411 and 630 patients reviewed, respectively). For the parameter solitary versus multifocal HCC, no homogeneity of the studies was provided, preventing us from calculating a common odds ratio. Given that the parameters tumor size>5cm and presence of vascular invasion are now nearly automatically considered as “exceeding” the Milan criteria, we could summarize the results of our meta-analysis by reporting that high risk pathology for HCC recurrence is characterized by tumors beyond the Milan criteria and by HCCs not well differentiated. Potential clinical applications of these data could serve to optimize patient selection, including tumor grading in the evaluation/listing criteria, as well as to better survey for early, eventually surgically treatable recurrence.



Parameter	COR	lower bound of 95% CI	upper bound of 95% CI	p-value	N
Beyond Milan	4.205	1.188	15.33	0.024	154
Tumor>5cm	13.32	1.634	622.1	0.0045	62
Vascular invasion	8.727	4.557	17.72	<0.0001	411
Not well differentiated HCC	2.89	1.708	5.036	<0.0001	630

Fig. 28: Common OR and confidence interval (CI) of clinical studies evaluating tumor recurrence after LT for HCC according to the parameters "Milan criteria", "tumor size", "vascular invasion" and "grading". For the parameter "solitary/multifocal lesions" no Common OR was calculated because the corresponding studies lacked homogeneity. Exact p-value was calculated for Common OR=1. In the cases of bigger sample size meta-analysis (for example "vascular invasion" n=411, "tumor differentiation" n=630), the statistical effect was more significant.

Comments

Our systematic review of the literature and meta-analysis of 45 clinical studies on HCC recurrence after LT for HCC showed that the parameters vascular invasion, not well differentiated HCC, tumor size >5cm, and HCC exceeding the Milan criteria constitute significant negative prognostic factors for post-transplant recurrences (common odds ratio by 8.727, 2.89, 13.32 and 4.205 for the above mentioned parameters, respectively). As demonstrated in Figure 30, the effect was statistically more significant for the parameters "vascular invasion" and "not well differentiated HCC", which represent the higher-volume meta-analyses of the present study (411 and 630 patients reviewed, respectively). For the parameter solitary versus multifocal HCC, no homogeneity of the studies was provided, preventing us from calculating a common odds ratio. Given that the parameters tumor size>5cm and presence of vascular invasion are now nearly automatically considered as "exceeding" the Milan criteria, we could summarize the results of our meta-analysis by reporting that high risk pathology for HCC recurrence is characterized by tumors beyond the Milan criteria and by HCCs not

well differentiated. Potential clinical applications of these data could serve to optimize patient selection, including tumor grading in the evaluation/listing criteria, as well as to better survey for early, eventually surgically treatable recurrence.

5.2.3 LT for HCC: Metaanalysis of prognostic factors

We present herein the findings of a literature review, intended to systematically expose and combine the statistical findings of other study groups during the past 2 decades (86).

Search strategy and selection criteria

In June 2006, PubMed and Medline were accessed and searched to estimate the outcome of patients with HCC undergoing LT. “Hepatocellular carcinoma”, “liver transplantation”, “tumor recurrence” and “prognostic factors” were entered as keywords both independently and in multiple combinations. Only English language papers published between 1985 and 2006 were considered. From the clinical studies identified, the following tumor characteristics were examined: 1) Serial levels for AFP, 2) tumor number, 3) tumor size, 4) lobar distribution, 5) vascular invasion, 6) tumor differentiation, 7) Milan criteria and 8) UCSF criteria.

Statistical analysis

Mean values with standard deviation and median values with ranges were used for numerical data. The significance of differences was assessed by chi-square, *t*-test, and analysis of variance. Survival curves were estimated by the Kaplan-Meier method and compared with log-rank test. Multivariable stepwise regression analysis was performed with the Cox proportional hazard model for the variables which were significant in the univariate analysis. Differences of $p<0.05$ were considered to be statistically significant. Statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC) and StatXact (Cytel Software Corp., Cambridge, MA). For the combination of p-values the truncated product method (72) was used with tau=0.05. Thus, studies that report a non significant result without giving the actual p-value could be included in the meta-analysis.

Results

Of the 92 retrospective clinical studies screened (86) p-values of 36 studies (16, 30-31, 37, 39, 45, 73, 95-99, 101-102, 104, 108, 112, 117, 118, 120-122, 130, 141, 147, 149-159) were entered in the corresponding tables. These p-values represented statistical results of data analysis for the 8 categories of tumor characteristics described in "patients and methods" either after performing a univariate analysis for patient survival (Table 41) and tumor recurrence (Table 42) or after performing a multivariable Cox regression analysis for patient survival (Table 43) and tumor recurrence (Table 44). In the case of studies evaluating different values for tumor size, for example 3cm as well as 5cm (96, 118, 120, 149), 2cm as well as 5cm (52), or 5cm as well as 8cm (23), the results for the 5cm value were used for this meta-analysis, since this is currently the value mostly interpreted. Regarding the parameter "vascular invasion", in studies where p-values both for macrovascular as well as for microvascular invasion were independently addressed (73, 98, 120, 149), the values corresponding to the macrovascular invasion were further selected for the meta-analysis, since they were considered as having better predictive value for patient evaluation. We applied this policy for the parameters "tumor size" and "vascular invasion", in order to independently combine the p-values of the 36 studies with the corresponding data in the truncated product method as well as in the Tippet's method.

Author	PubYear	Study-period	N	AFP	Tumor number	Tumor size	Lobar distribution	Vascular invasion	Grading	Milan criteria	UCSF criteria
Iwatsuki	1991	1980-1989	105	-	**0.0143**	**0.0034**	**0.0003**	**0.0002**	-	-	-
Ringe	1991	01.74-12.88	61	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	-	-
Mc Peake	1993	05.68-12.86	87	-	-	**0.0006**	-	-	-	-	-
Ojogho	1996	03.88-12.93	27	-	0.19	0.56	-	0.53	-	-	-
Figueras	1997	01.90-12.95	38	0.10	0.5	**0.0011**	0.62	**0.0029**	-		-
Klintmalm	1998	09.97	422	-	-	**0.0221**	-	**0.0005**	**0.0001**	-	-
Otto	1998	06.87-06.96	50	-	0.25	**0.0128**	-	**0.0004**	-	-	-
Figueras	2001	01.90-12.00	307	**0.003**	0.14	0.4	0.08	**0.0004**	**-**	**-**	-
Jonas	2001	01.89-02.00	120	-	**0.03**	**0.03**	-	**0.02**	**0.01**	-	-
Hemming	2001	1985-2000	112	>0.05	>0.05	>0.05	-	**0.03**	>0.05	-	-
Tamura	2001	06.91-01.99	53	0.72	0.05	**0.02**	**0.02**	**0.04**	**<0.001**	-	-
Yao	2001	02.88-02.00	70	**-**	-	0.27	-	-	-	0.12	**0.0005**
Moya	2002	01.91-01.00	104	**0.05**	-	-	-	-	-	-	-
Vivarelli	2002	04.86-01.01	82	0.68	0.41	-	-	-	-	0.97	-
De Carlis	2003	12.85-12.99	154	**0.002**	0.06	**0.01**	-	**0.002**	**0.002**	-	-
Cillo	2004	07.91-05.02	48	>0.05	>0.05	>0.05	>0.05	-	>0.05	>0.05	-
Leung	2004	09.92-03.03	144	**0.015**	-	>0.05	-	>0.05	>0.05	>0.05	**0.04**
Ravaioli	2004	11.86-08.01	63	**<0.001**	>0.05	>0.05	-	>0.05	>0.05	>0.05	-
Roayaie	2004	09.88-09.02	57	**-**	**0.0273**	**0.0041**	-	**-**	**0.015**	**-**	-
Todo	2004	10.89-12.03	316	**<0.0001**	0.47	**0.0102**	0.09	**0.0173**	**0.0497**	**0.0222**	-
Hwang/LDLT	2005	08.92-12.02	176	**-**	-	-	-	-	**--**	**<0.001**	**<0.001**
Hwang/CDLT	2005	08.92-12.02	61	-	-	-	-	-	-	0.18	0.19
Löhe	2005	06.85-12.03	93	-	-	**<0.01**	**--**	**<0.001**	**<0.05**	**<0.01**	-
Zavaglia	2005	01.89-12.02	155	**0.003**	0.34	0.88	0.37	**0.003**	**0.0004**	0.48	0.27
Takada	2006	02.99-09.04	93	0.23	0.71	**0.0259**	0.70	**0.0026**	**0.0001**	0.65	-
Actual series	2006	04.98-03.06	100	0.13	0.30	0.25	0.24	0.38	**0.0154**	0.21	0.10
TPM p-value			3098	**<0.0001**	**0.0164**	**<0.0001**	**0.0067**	**<0.0001**	**<0.0001**	**0.0023**	**<0.0001**

Table 41: Survival according to univariate analysis. TPM p-value: p-value resulting from the truncated product method analysis (24) of listed p-values.

The results of the present systematic review are demonstrated in tables 41-44. The combination of the p-values using the TPM gave significant p values in all 8 categories of tumor characteristics evaluated by univariate analysis for both patient survival and tumor recurrence (Tables 41 and 42). In contrast, the Tippet's method for the p-values of the multivariable analyses for patient survival showed no statistical

significance for the parameters “AFP”, “tumor number”, “lobar distribution”, “Milan criteria” and “UCSF criteria” (Table 43). However, these parameters gained statistical significance after combination of the p-values of the multivariable analyses for tumor recurrence, with the exception of “Milan criteria” and “UCSF criteria” (Table 44).

Author	PubYear	Study-period	N	AFP	Tumor number	Tumor size	Lobar distribution	Vascular invasion	Differentiation	Milan criteria	UCSF criteria
Otto	1998	06.87-06.96	50	-	>0.05	**0.038**	-	**0.017**	-	-	-
Klintmalm	1998	09.97	152	-	-	>0.05	-	>0.05	**0.0009**	-	-
Figueras	2001	01.90-12.00	307	**0.04**	0.8	0.52	0.65	**0.01**	**-**	-	-
Molmenti	2002		374	-	-	**0.0019**	-	-	**0.0163**	-	-
De Carlis	2003	12.85-12.99	154	**0.02**	>0.05	>0.05	-	>0.05	**0.03**	-	-
Leung	2004	09.92-03.03	144	**0.04**	-	>0.05	-	>0.05	>0.05	>0.05	0.09
Roayaie	2004	09.88-09.02	57	-	>0.05	**0.017**	-	-	**0.012**	-	-
Zavaglia	2005	01.89-12.02	155	>0.05	>0.05	>0.05	>0.05	**0.02**	**0.0009**	>0.05	>0.05
Löhe	2005	06.85-12.03	93	-	-	>0.05	-	**<0.001**	>0.05	>0.05	-
Actual series	2006	04.98-03.06	100	0.13	0.30	0.25	0.24	0.38	**0.0154**	0.21	0.10
Significant according to Tippet				no	no	**yes**	no	**yes**	**yes**	no	no

Table 43: Survival according to multivariable Cox regression analysis. The analysis of the multivariable results was performed according to the Tippet’s Method of listed p-values.

Author	PubYear	Study-period	N	AFP	Tumor number	Tumor size	Lobar distribution	Vascular invasion	Differentiation	Milan criteria	UCSF criteria
Klintmalm	1998	09.97	151	-	-	**0.0133**	-	-	**0.0134**	-	-
Otto	1998	06.87-06.96	46	-	>0.05	**0.023**	-	>0.05	-	-	-
Hemming	2001	1985-2000	112	>0.05	>0.05	>0.05	-	**0.03**	>0.05	-	-
Molmenti	2002		370			**<0.0001**	**0.0002**	**0.0003**	-	-	-
De Carlis	2003	12.85-12.99	154	**0.0001**	>0.05	>0.05	-	**0.0005**	**0.0002**	-	-
Leung	2004	09.92-03.03	144	**0.03**	-	>0.05	-	>0.05	>0.05	>0.05	**0.03**
Hwang	2005	08.92-12.02	274	0.95	>0.05	**<0.001**	0.22	**0.049**	**0.012**	>0.05	-
Takada	2006	02.99-09.04	93	>0.05	**0.002**	>0.05	>0.05	>0.05	**0.003**	>0.05	-
Actual series	2006	04.98-03.06	100	0.09	-	-	-	0.27	**0.006**	0.90	**0.035**
Significant according to Tippet				**yes**	**yes**	**yes**	**yes**	**yes**	**yes**	no	no

Table 44: Recurrence according to multivariable Cox regression analysis. The analysis of the multivariable results was performed according to the Tippet’s Method of listed p-values.

Author	PubYear	Study-period	N	AFP	Tumor number	Tumor size	Lobar distribution	Vascular invasion	Grading	Milan criteria	UCSF criteria
Mazzaferro	1994	04.84-09.93	80	>0.05	>0.05	-	-	**<0.001**	-	-	-
Ojogho	1996	03.88-12.93	27	-	**<0.04**	0.31	-	1	-	-	-
Colella	1997	12.85-06.96	71	**<0.0001**	**0.02**	**0.02**	**0.002**	**0.0001**	-	-	-
Otto	1998	06.87-06.96	46	-	0.07	**0.02**	-	**0.0263**	-	-	-
Regalia	1998	06.87-12.96	21	>0.05	>0.05	**<0.05**	-	>0.05	-	**<0.03**	
Schlitt	1999	1972-1994	69	>0.05	**0.0011**	**0.0015**	-	**0.0001**	>0.05	-	-
Marsh	2000	08.81-08.97	231	-	-	**<0.0001**	**<0.0001**	**<0.0001**	-	-	-
Figueras	2001	01.90-12.00	307	**0.0001**	**0.0001**	**0.0002**	**0.0008**	**0.0001**	-	-	-
Hemming	2001	1985-2000	112	>0.05	>0.05	**0.04**	-	**0.03**	**0.03**	-	-
Herrero	2001	01.91-06.00	47	-	-	-	**0.03**	**0.02**	-	-	-
Tamura	2001	06.91-01.99	53	0.88	0.13	**0.04**	**0.04**	0.05	**<0.001**	-	-
Margarit	2002	10.98-12.00	93	-	0.81	0.5	0.24	**0.003**	**0.031**	-	-
Moya	2002	01.91-01.00	104	**0.01**	-	-	-	**0.00001**	-	-	-
Roayaie	2002	10.91-01.99	43	-	>0.05	**0.024**	>0.05	**0.036**	>0.05	-	-
Vivarelli	2002	04.86-01.01	82	0.32	0.54	-	-	-	-	0.2	-
De Carlis	2003	12.85-12.99	154	**0.002**	0.1	0.1	-	**0.001**	**0.02**	-	-
Kaihara	2003	02.99-03.02	40	-	0.08	0.28	-	**<0.01**	**<0.01**	0.19	-
Ravaioli	2004	11.86-08.01	63	**<0.05**	>0.05	>0.05	-	>0.05	>0.05	>0.05	-
Cillo	2004	07.91-05.02	48	>0.05	>0.05	>0.05	>0.05	-	>0.05	>0.05	-
Gondolesi	2004	08.98-04.02	36	-	**0.01**	>0.05	**0.03**	>0.05	>0.05	-	-
Leung	2004	09.92-03.03	144	**0.004**	-	>0.05	-	>0.05	>0.05	>0.05	**0.008**
Roayaie	2004	09.88-09.02	57	0.93	0.23	**0.008**	-	0.62	**0.018**	-	-
Todo	2004	10.89-12.03	316	**<0.0001**	**0.0008**	**<0.0001**	**0.0006**	**<0.0001**	**<0.0001**	**<0.0001**	-
Hwang/CDLT	2005	08.92-12.02	61	**0.044**	0.40	0.41	0.32	0.65	**0.019**	0.18	-
Hwang/LDLT	2005	08.92-12.02	176	**<0.001**	0.10	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**	-
Sauer	2005	1987-2004	110	-	-	-	-	-	-	**<0.05**	-
Yao	2005	01.88-12.02	168	**0.003**	-	-	-	**0.0003**	**0.006**	-	**<0.0001**
Zavaglia	2005	01.89-12.02	155	-	0.82	0.33	0.34	**0.000007**	**0.0007**	**0.035**	0.06
Takada	2006	02.99-09.04	93	**0.0015**	**0.0004**	**0.0031**	0.54	**0.0026**	**0.0001**	**0.0190**	-
Actual series	2006	04.98-03.06	100	**0.0003**	0.10	0.18	0.11	**0.0001**	**<0.0001**	**0.0148**	**0.0092**
TPM p-value			3107	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Table 42: Recurrence according to univariate analysis. TPM p-value: p-value resulting from the truncated product method analysis of listed p-values.

Comments

According to the statistical results of this systematic literature review, the following conclusions regarding the 8 tumor parameters evaluated can be reached:

1) AFP: Although not all HCCs are AFP positive, and serial values can be "optimised" because of tumor necrosis after ablative bridging treatments, AFP remained significant for patient survival and tumor recurrence in the most analyses performed.

2) Tumor number: Although statistical significance was demonstrated by the TPM by univariate analysis for both patient survival and tumor recurrence, this parameter was rarely examined in the basis of a multivariable analysis. Combination of p-values of the multivariable analyses showed significance only for tumor recurrence. The difficulty to correctly estimate HCC lesions <2cm in end-cirrhotic livers, reduced the predictive value of this parameter, which could explain the infrequent utilisation of this parameter in multivariable analyses.

3) Tumor size: This parameter, representing the size of the biggest tumor, or in more recent studies, the cumulative size of all HCC lesions, retained its predictive value, as it gained statistical significance in all combination tests performed. Besides, there seems to be a frequent association between tumor size and vascular invasion.

4) Lobar distribution: This parameter showed similar results to "tumor number", i.e. statistical significance by univariate analyses but contradictory results by multivariable analysis. Similar limitations associated with radiological evaluation are also applied.

5) Vascular invasion: Statistical significance proved the predictive value of this parameter by all combination tests performed in this review. Possible limitations include the fact that some authors do not specify whether "vascular invasion" corresponded to micro- and/or macroscopic involvement, and that preoperative imaging of microvascular invasion is virtually impossible.

6) Differentiation: This parameter showed significant associations in all combination tests performed. Since many multivariable analyses independently proved its predictive value, this parameter may be combined with the Milan criteria when considering patients for LT.

7) Milan criteria: The predictive value of the Milan criteria was shown by univariate analyses. However, it failed to reach significance by multivariable analysis.

8) UCSF criteria: Similarly to the “Milan criteria”, significant associations were found only by univariate analyses.

According to the results of the present study, tumor differentiation represents a key prognostic factor in the prognosis of patients undergoing LT for HCC. Routine performance of fine needle biopsies during the evaluation for LT, is currently controversially discussed, not only due to the corresponding risk of bleeding and tumor dissemination or even sampling errors, but also because of the possibility that a HCC lesion may exhibit different grade of cell differentiation. In a recent study, Pawlik et al. showed that the diagnostic agreement of preoperative needle core biopsy grading of HCC versus the final surgical pathologic tumor grade was poor (sensitivity=34.6%, $p<0.0001$), concluding that pre-operative tumor grade may be misleading, as it often does not correlate with grade or presence of microscopic vascular invasion on final pathology (60). However, accurate post-transplant tumor grading is important in determining outcome and should be discussed with the patient.

Considering the patient selection criteria, neither the Milan nor the UCSF criteria were associated with survival or tumor recurrence, as it stipulated by the meta-analysis of the available multivariable studies. A novel prognostic score for the better evaluation of transplant candidates with HCC in cirrhosis is needed.

6. Conclusion

The main disadvantage of the existing criteria/scoring systems for the evaluation of patients with HCC in cirrhosis is that they represent retrospective evaluation of institutional/uni-centric data, having a lot of additional biases because of the time-span of the studies, the changes in transplant policy, imaging and specimen interpretation.

For example, the 'up-to seven criteria' (in the absence of microvascular invasion, seven is the result of the sum of size in cm and number of tumours for any given HCC) were proposed recently in an effort to include additional HCC patients as transplant candidates. These criteria were based on a retrospective multicentric analysis, and were published in a high-impact Journal (49). However, we could express several concerns, as described below (51):

-A considerable number of patients (n=266, 17.2%) were transplanted from 1984-1996, prior to the introduction of the Milan criteria, under earlier TNM Classification editions, when different tumour characteristics were being considered, the total number of tumours was not a priority, and imaging was less developed.

-The 22-year span of this multicenter retrospective study carried considerable bias. Imaging and specimen interpretation by innumerable radiologists and pathologists in various countries, at different centers, and at widely diverse time periods added further bias and made the objective evaluation of the data questionable.

-One-third to one-half of patients currently receives pre-transplant bridging treatments such as TACE and RFA. The resulting necrosis can significantly affect tumour behaviour (59, 64), making the "objective" assessment of tumour size and microvascular invasion very difficult. The authors provided no information on such treatments, adding further bias to their study! It is difficult to base a prognostic model on the "preoperative" detection of microvascular invasion when even pathologists cannot clearly determine such finding in most explants with extensive tumour necrosis. Size, number, grade, and AFP levels also have flaws. Tumour grading usually progresses while on the waiting list (60), and AFP levels do not always correlate with tumour aggressiveness.

-An important message from this paper, as stated by its authors, was the limitations of the Milan criteria. Of 1556 candidates classified "outside" the Milan criteria by radiological findings, 444 (28.5%) were found to fall "within" the criteria by

pathology reports. This difficulty evaluating small HCCs (<2cm or <1cm) is well documented (68, 160), and still represents a major hurdle. The preoperative "objective" identification of the total number of tumours in cirrhotic livers is almost impossible, as can be attested by those who confront this problem daily.

-"The primary aim of the "Up-to-7 criteria"-study was to derive a prognostic model based in objective tumour characteristics" such as tumour size, tumour number, and microvascular invasion. Unfortunately, these are pathology findings. None of them represents a preoperative "objective tumour charasteristic." Consequently, the "up-to-seven criterion" is illusive and not applicable in clinical practice.

The up-to-seven criterion is a retrospective analysis of pathology findings of an extremely inhomogenous patient cohort. Its clinical value is even more limited than that of the Milan criteria, since it nurtures the illusive and impractical clinical identification of "microvascular invasion." The tumour characteristics the authors invoked are not objective but rather rest on subjective, and highly discordant, readings of imaging studies (51).

Novel molecular biology techniques, such as genotyping for HCC, may be relevant for determining recurrence-free survival and improving organ allocation in the future (161). However, liberal and conservative HCC transplant policies in the USA and Europe have shown that parameters such as AFP serum levels, MELD score, and the age of the patient represent applicable and objective evaluation tools (84, 162-163). Although LT represents the best treatment option from oncological point of view also for patients exceeding the Milan criteria, offering a 5-year survival of more than 50%, because of organ scarcity the topic is surely still open to further proposals for optimization of patient selection (4). According to the results of this study, apart of the existing tumor number/size criteria, further oncological parameters, such as AFP and tumor grading and cirrhosis related parameters as MELD score and patient age may be important tools of a scoring system in transplant centres with the possibility of performing LT for extended indications for HCC.

Concrete proposals according to the findings of the study

The present study offers a refinement of prognostic factors for performance of LT for extended HCC criteria. According to our findings, patients with HCC in cirrhosis

outside the radiological Milan criteria may be transplanted with acceptable long term results, if the following presuppositions are covered:

1) No extrahepatic tumor
2) No macrovascular invasion
3) Well or moderate tumor differentation (excluding poor tumor grading)
4) AFP levels ≤400ng/mL
5) recipient age ≤60 years
6) recipient labMELD Score ≤22.

The inclusion of these parameters in a scoring system and the internal and external validation in a large volume patient series will be the next step, in order to offer a scoring system based on preoperatively available criteria, which combine tumor and physical/cirrhosis related characteristics. Performance of LT using grafts from extended criteria donors, or partial liver grafts as deceased split liver grafts or living donor liver grafts represent reliable solutions to this direction.

7. Summary

Background: LT is the best treatment option for cirrhotic patients with HCC within the Milan criteria. Aim of this study was to explore the existing possibilities to offer the transplant modality to more HCC patients.

Methods: A corresponding database was built and most data were prospectively collected. Accuracy of the radiological findings, efficacy of bridging treatments, LT using partial and ECD grafts, LT for special indications and LT for extended indications were evaluated. Additionally, systematic reviews and meta-analyses for related issues were contacted.

Results: LT represents the best therapy option for patients with HCC in the absence of extrahepatic disease/macrovascular invasion as monotherapy, even for patients exceeding the Milan criteria. Current imaging techniques have a low accuracy when evaluating HCC in cirrhosis, especially tumors <2cm. TACE provides acceptable local tumor control before LT. Achievement of complete tumor necrosis by means of bridging treatments is characterized by a very low recurrence rate. LT with split, living donor and ECD grafts represent a reliable option to extend the donor pool and to select over the standard tumor criteria; recipient age, MELD score, and AFP levels could further improve current guidelines. LT can be extended for HCC patients "beyond Milan-within UCSF" or with initial undetected/very low AFP value, irrespective of the Milan criteria. Portal vein thrombosis or pulmonary granulomas are not accurately evaluated preoperatively and reserve further consideration. Poor tumor differentiation represents an additional prognostic factor for HCC recurrence. Neither the Milan nor the UCSF criteria were associated with survival or tumor recurrence, as it stipulated by the meta-analysis of the available multivariable studies.

Conclusion: There is still plenty of space to improve current listing criteria and expand the recipient pool of patients with HCC in cirrhosis. A novel prognostic score for the better evaluation of transplant candidates with HCC in cirrhosis is needed.

8. Összefoglalás

Bevezetés: Napjainkban a májátültetés jelenti a Milánói kritériumokon belüli hepatocelluláris karcinóma legjobb kezelési lehetőségét. Jelen dolgozat célja, azon rendelkezésre álló lehetőségek vizsgálata volt, melyekkel több HCC-s beteg részére válik elérhetővé a májtranszplantáció. **Módszer:** Munkánk alapját egy általunk létrehozott adatbázis képezte, melybe prospektív módon gyűjtöttük az adatokat a radiológiai leletek pontosságáról, a "bridging" terápiák effektivitásáról, a split- és marginális donormájjal végzett átültetésekről, illetve a ritka indikációval végzett transzplantációkról. Munkánkat az irodalomban fellelhető meta-analizisekkel, és átfogó munkákkal vetettük össze. **Eredmények:** A májtranszplantáció jelenti a legjobb terápiás lehetőséget azoknak a HCC-vel rendelkező betegeknek, akiknek májon kívüli tumorpropagációja nem ismert, vagy szövettanilag makrovaszkuláris invázió nem mutatható ki. Ez még akkor is igaz, ha a tumor mérete és a gócok száma meghaladja a Milanói kritérium rendszerben foglaltakat. A jelenleg használt képalkotó eljárások érzékenysége májcirrózisban HCC esetén igencsak vitatható különösen 2 cm tumor átmérő alatti tartományban. A transzarteriális kemoembolizáció egy elfogadható eljárás a tumor lokális kezelésében. Ha sikerül komplett tumornekrózist elérni, akkor a kiújulás valószínűsége is csökken, ami "bridging" eljárásról lévén szó jó eredménynek mondható. A szétválasztott májlebenyekkel, az élődonorból átültetésre kerülő májakkal, a kiterjesztett donorkritériumok alapján választott szervekkel történő májtranszplantáció mind a donor-pool növelését segítik. A recipiensek kiválasztása a standard tumor kritériumokon túl, magasabb életkorral, magasabb MELD pontszámmal, vagy jelentősen emelkedett AFP szinttel mind a jelenleg érvényben lévő útmutatások felülvizsgálatát indokolják. A portatrombózis diagnózisa, az alacsonyan differenciált tumor vagy a tüdőben található göbös elváltozások megléte továbbra is körültekintő preoperatív vizsgálatokat indokolnak. Kialakításukkor sem a Milanói sem a kiterjesztett UCSF kritériumok nem vették figyelembe a beteg túlélést és a tumor kiújulást ezen általunk feldolgozott adatok alapján. **Következtetés:** Még mindig számtalan lehetőség kínálkozik a jelenlegi beteg kiválasztási kritériumok bővítésére, a HCC miatt transzplantációra kerülő betegcsoportban. Következtetésképpen elmondhatjuk egy újabb prognosztikai pontrendszerre lenne szükség az effektívebb posztoperatív eredmények elérése miatt.

9. Publications

Publications for the dissertation based on

- **Sotiropoulos GC**, Drühe N, Sgourakis G, Molmenti EP, Beckebaum S, Baba HA, Antoch G, Hilgard P, Radtke A, Saner FH, Nadalin S, Paul A, Malagó M, Broelsch CE, Lang H. Liver transplantation, liver resection, and transarterial chemoembolization for hepatocellular carcinoma in cirrhosis: which is the best oncological approach? Dig Dis Sci 2009;54:2264-2273.
- **Sotiropoulos GC**, Malagó M, Molmenti E, Paul A, Nadalin S, Brokalaki EI, Verhagen R, Dirsch O, Gerken G, Lang H, Broelsch CE. Efficacy of transarterial chemoembolization prior to liver transplantation for hepatocellular carcinoma as found in pathology. Hepatogastroenterology 2005;52:329-332.
- **Sotiropoulos GC**, Malago M, Molmenti EP, Radtke A, Brokalaki EI, Nadalin S, Lang H, Frilling A, Baba HA, Kühl H, Verhagen R, Broelsch CE. Disease course after liver transplantation for hepatocellular carcinoma in patients with complete tumor necrosis in liver explants after performance of bridging treatments. Eur J Med Res 2005;10:539-542.
- **Sotiropoulos GC**, Malagó M, Molmenti E, Paul A, Nadalin S, Brokalaki E, Kühl H, Dirsch O, Lang H, Broelsch CE. Liver transplantation for hepatocellular carcinoma in cirrhosis: is clinical tumor classification before transplantation realistic? Transplantation 2005;79:483-487.
- **Sotiropoulos GC**, Malagó M, Molmenti EP, Nadalin S, Radtke A, Brokalaki EI, Lang H, Frilling A, Baba HA, Neuhäuser M, Broelsch CE. Liver transplantation and incidentally found hepatocellular carcinoma in liver explants: need for a new definition? Transplantation 2006;81:531-535.
- **Sotiropoulos GC**, Molmenti EP, Omar OS, Bockhorn M, Brokalaki EI, Lang H, Frilling A, Broelsch CE, Malagó M. Liver transplantation for hepatocellular carcinoma in patients beyond the Milan but within the UCSF criteria. Eur J Med Res 2006;11:467-470.
- **Sotiropoulos GC**, Radtke A, Schmitz KJ, Molmenti EP, Schroeder T, Saner FH, Baba HA, Fouzas I, Broelsch CE, Malagó M, Lang H. Liver transplantation in

the setting of hepatocellular carcinoma and portal vein thrombosis: a challenging dilemma? Dig Dis Sci 2008;53:1994-1999.

- **Sotiropoulos GC**, Malagó M, Bockhorn M, Schmitz KJ, Radtke A, Molmenti EP, Schaffer R, Beckebaum S, Cicinnati VR, Fouzas I, Broelsch CE, Lang H. Liver transplantation for hepatocellular carcinoma and cirrhosis in candidates with undetectable or very low alpha-fetoprotein levels: is an expansion of the listing criteria justified? Hepatogastroenterology 2008;55:1671-1677.
- **Sotiropoulos GC**, Beckebaum S, Lang H, Frilling A, Molmenti EP, Cicinnati VR, Saner FH, Erim Y, Baba HA, Malagó M, Broelsch CE. Single-center experience on liver transplantation for hepatocellular carcinoma arising in alcoholic cirrhosis: results and ethical issues. Eur Surg Res 2008;40:7-13.
- **Sotiropoulos GC**, Kuehl H, Sgourakis G, Molmenti EP, Beckebaum S, Cicinnati VR, Baba HA, Schmitz KJ, Broelsch CE, Lang H. Pulmonary nodules at risk in patients undergoing liver transplantation for hepatocellular carcinoma. Transpl Int 2008;21:850-856.
- **Sotiropoulos GC**, Paul A, Molmenti E, Lang H, Frilling A, Napieralski BP, Nadalin S, Treckmann J, Brokalaki EI, Gerling T, Broelsch CE, Malagó M. Liver transplantation for hepatocellular carcinoma in cirrhosis within the Eurotransplant area: an additional option with "livers that nobody wants". Transplantation 2005;80:897-902.
- **Sotiropoulos GC**, Lang H, Frühauf NR, Molmenti EP, Omar OS, Radtke A, Paul A, Nadalin S, Frilling A, Broelsch CE, Malagò M. Split liver transplantation for hepatocellular carcinoma. Hepatogastroenterology 2006;53:764-767.
- **Sotiropoulos GC**, Lang H, Sgourakis G, Nadalin S, Molmenti EP, Radtke A, Paul A, Beckebaum S, Saner FH, Baba HA, Gerken G, Malagó M, Broelsch CE. Liberal policy in living donor liver transplantation for hepatocellular carcinoma: lessons learned. Dig Dis Sci 2009;54:377-384.
- **Sotiropoulos GC**, Malagó M, Molmenti EP, Lösch C, Lang H, Frilling A, Broelsch CE, Neuhäuser M. Hilar lymph nodes sampling at the time of liver transplantation for hepatocellular carcinoma: to do or not to do? Meta-analysis to determine the impact of hilar lymph nodes metastases on tumor recurrence

and survival in patients with hepatocellular carcinoma undergoing liver transplantation. Transpl Int 2007;20:141-146.

- **Sotiropoulos GC**, Molmenti EP, Lösch C, Beckebaum S, Broelsch CE, Lang H. Meta-analysis of tumor recurrence after liver transplantation for hepatocellular carcinoma based on 1,198 cases. Eur J Med Res 2007;12:527-534.
- **Sotiropoulos GC**, Lang H, Nadalin S, Neuhäuser M, Molmenti EP, Baba HA, Paul A, Saner FH, Weber F, Hilgard P, Frilling A, Broelsch CE, Malagó M. Liver transplantation for hepatocellular carcinoma: University Hospital Essen experience and metaanalysis of prognostic factors. J Am Coll Surg 2007;205:661-675.
- **Sotiropoulos GC**, Molmenti EP, Lang H. Milan criteria, up-to-seven criteria, and the illusion of a rescue package for patients with liver cancer. Lancet Oncol 2009;10:207-208.

Other publications

- Jelic S, **Sotiropoulos GC**; ESMO Guidelines Working Group. Hepatocellular carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010 May;21 Suppl 5:v59-64.
- Cicinnati VR, **Sotiropoulos GC**, Beckebaum S. Established and emerging therapies for hepatocellular carcinoma. Minerva Med. 2010 Dec;101(6):405-418.

10. Acknowledgements

This project would not have been possible without the support of many people. The author wishes to express his appreciation and gratitude to Prof. Dr. Christoph Erich Broelsch for his support and encouragement during the whole period 2000-2007. He has provided an optimal working enviroment at the Department of General, Visceral and Transplantation Surgery, University Hospital Essen, Germany.

Deepest gratidute is also due to his supervisor, Dr. László Kóbori, who was abundantly helpful and offered invaluable assistance and guidance. His outstanding personality made a strong impact on this work.

The author is particularly grateful to Prof. Ernesto Molmenti for his continuous assistance and essential contribution during the course of the last 8 years.

Special thanks are dedicated to Prof. Evangellos Papaevangellou, authors' main mentor, who has inspired him to become a liver surgeon.

The author wishes to express his love and gratitude to his parents, children, and above all to his wife Eirini, for their support, patience and understanding during this long period.

11. References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
2. Cicinnati VR, Sotiropoulos GC, Beckebaum S. Established and emerging therapies for hepatocellular carcinoma. Minerva Med. 2010 Dec;101(6):405-418.
3. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132:2557-2576.
4. Jelic S, Sotiropoulos GC. Hepatocellular carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21 Suppl 5:v59-v64.
5. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J; EASL Panel of Experts on HCC. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001;35:421-430.
6. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology 2005;42:1208-1236.
7. Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. J Hepatol 2008;48 Suppl 1:S20-S37.
8. Bruix J, Llovet JM. Major achievements in hepatocellular carcinoma. Lancet 2009;373:614-616.
9. Malaguarnera G, Giordano M, Paladina I, Berretta M, Cappellani A, Malaguarnera M. Serum Markers of Hepatocellular Carcinoma. Dig Dis Sci 2010;55:2744-2755.
10. Diao S, Zhang JF, Wang H, He ML, Lin MC, Chen Y, Kung HF. Proteomic identification of microRNA-122a target proteins in hepatocellular carcinoma. Proteomics 2010;10:3723-3731.
11. Llovet JM, Chen Y, Wurmbach E, Roayaie S, Fiel MI, Schwartz M, Thung SN, Khitrov G, Zhang W, Villanueva A, Battiston C, Mazzaferro V, Bruix J, Waxman S, Friedman SL. A molecular signature to discriminate dysplastic

nodules from early hepatocellular carcinoma in HCV cirrhosis. Gastroenterology 2006;131:1758-1767.

12. Pang RW, Poon RT. From molecular biology to targeted therapies for hepatocellular carcinoma: the future is now. Oncology 2007;72 Suppl 1:30-44.
13. Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, Sherman M, Schwartz M, Lotze M, Talwalkar J, Gores GJ; Panel of Experts in HCC-Design Clinical Trials. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008;100:698-711.
14. Chen MS, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ, Lau WY. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. Ann Surg 2006;243:321-328.
15. Cho YK, Kim JK, Kim WT, Chung JW. Hepatic resection versus radiofrequency ablation for very early stage hepatocellular carcinoma: a Markov model analysis. Hepatology 2010;51:1284-1290.
16. Ringe B, Pichlmayr R, Wittekind C, Tusch G. Surgical treatment of hepatocellular carcinoma: experience with liver resection and transplantation in 198 patients. World J Surg 1991;15:270-285.
17. Fong Y, Sun RL, Jarnagin W, Blumgart LH. An analysis of 412 cases of hepatocellular carcinoma at a Western center. Ann Surg 1999;229:790-799.
18. Grazi GL, Cescon M, Ravaioli M, Ercolani G, Gardini A, **Del** Gaudio M, Vetrone G, Cavallari A. Liver resection for hepatocellular carcinoma in cirrhotics and noncirrhotics. Evaluation of clinicopathologic features and comparison of risk factors for long-term survival and tumour recurrence in a single centre. Aliment Pharmacol Ther 2003;17 Suppl 2:119-129.
19. Dupont-Bierre E, Compagnon P, Raoul JL, Fayet G, de Lajarte-Thirouard AS, Boudjema K. Resection of hepatocellular carcinoma in noncirrhotic liver: analysis of risk factors for survival. J Am Coll Surg 2005;201:663-670.
20. Laurent C, Blanc JF, Nobili S, Sa CA, le BB, Bioulac-Sage P, Balabaud C, Capdepont M, Saric J. Prognostic factors and longterm survival after hepatic resection for hepatocellular carcinoma originating from noncirrhotic liver. J Am Coll Surg 2005;201:656-662.

21. Lang H, Sotiropoulos GC, Brokalaki EI, Schmitz KJ, Bertona C, Meyer G, Frilling A, Paul A, Malagó M, Broelsch CE. Survival and recurrence rates after resection for hepatocellular carcinoma in noncirrhotic livers. J Am Coll Surg 2007;205:27-36.
22. Sotiropoulos GC, Lang H, Frilling A, Molmenti EP, Paul A, Nadalin S, Radtke A, Brokalaki EI, Saner F, Hilgard P, Gerken G, Broelsch CE, Malagò M. Resectability of hepatocellular carcinoma: evaluation of 333 consecutive cases at a single hepatobiliary specialty center and systematic review of the literature. Hepatogastroenterology 2006;53:322-329.
23. Gassmann P, Spieker T, Haier J, Schmidt F, Mardin WA, Senninger N. Prognostic Impact of Underlying Liver Fibrosis and Cirrhosis After Curative Resection of Hepatocellular Carcinoma. World J Surg 2010;34:2442-2451.
24. Poon RT, Fan ST, Lo CM, Liu CL, Ng IO, Wong J. Long-term prognosis after resection of hepatocellular carcinoma associated with hepatitis B-related cirrhosis. J Clin Oncol 2000;18:1094-1101.
25. Jarnagin WR. Management of small hepatocellular carcinoma: a review of transplantation, resection, and ablation. Ann Surg Oncol 2010;17:1226-1233.
26. Ringe B, Wittekind C, Bechstein WO, Bunzendahl H, Pichlmayr R. The role of liver transplantation in hepatobiliary malignancy. A retrospective analysis of 95 patients with particular regard to tumor stage and recurrence. Ann Surg 1989;209:88-98.
27. Busuttil RW, Farmer DG. The surgical treatment of primary hepatobiliary malignancy. Liver Transpl Surg 1996;2:114-130.
28. Bismuth H, Chiche L, Adam R, Castaing D, Diamond T, Dennison A. Liver resection versus transplantation for hepatocellular carcinoma in cirrhotic patients. Ann Surg. 1993;218:145-151.
29. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med 1996;334:693-699.

30. Molmenti EP, Klintmalm GB. Liver transplantation in association with hepatocellular carcinoma: an update of the International Tumor Registry. Liver Transpl 2002;8:736-748.
31. Hemming AW, Cattral MS, Reed AI, Van der Werf WJ, Greig PD, Howard RJ. Liver transplantation for hepatocellular carcinoma. Ann Surg 2001;233:652-659.
32. Yoo HY, Patt CH, Geschwind JF, Thuluvath PJ. The outcome of liver transplantation in patients with hepatocellular carcinoma in the United States between 1988 and 2001: 5-year survival has improved significantly with time. J Clin Oncol 2003;21:4329-4335.
33. Roessler S, Budhu A, Wang XW. Future of molecular profiling of human hepatocellular carcinoma. Future Oncol 2007;3:429-439.
34. http://www.dso.de/grafiken/g49.html, assessed Mar 15, 2011
35. Malago M, Hertl M, Testa G, Rogiers X, Broelsch CE. Split-liver transplantation: future use of scarce donor organs. World J Surg 2002;26:275-282.
36. Malago M, Testa G, Frilling A, Nadalin S, Valentin-Gamazo C, Paul A, Lang H, Treichel U, Cicinnati V, Gerken G, Broelsch CE. Right living donor liver transplantation: an option for adult patients: single institution experience with 74 patients. Ann Surg 2003;238:853-862.
37. Gondolesi GE, Roayaie S, Munoz L, Kim-Schluger L, Schiano T, Fishbein TM, Emre S, Miller CM, Schwartz ME. Adult living donor liver transplantation for patients with hepatocellular carcinoma: extending UNOS priority criteria. Ann Surg 2004;239:142-149.
38. Malago M, Testa G, Marcos A, Fung JJ, Siegler M, Cronin DC, Broelsch CE. Ethical considerations and rationale of adult-to-adult living donor liver transplantation. Liver Transpl 2001;7:921-927.
39. Kaihara S, Kiuchi T, Ueda M, Oike F, Fujimoto Y, Ogawa K, Kozaki K, Tanaka K. Living-donor liver transplantation for hepatocellular carcinoma. Transplantation 2003;75:S37-S40.
40. Malago M, Sotiropoulos GC, Nadalin S, Valentin-Gamazo C, Paul A, Lang H, Radtke A, Saner F, Molmenti E, Beckebaum S, Gerken G, Frilling A, Broelsch

CE. Living donor liver transplantation for hepatocellular carcinoma: a single-center preliminary report. Liver Transpl 2006;12:934-940.

41. Lo CM, Fan ST, Liu CL, Chan SC, Wong J. The role and limitation of living donor liver transplantation for hepatocellular carcinoma. Liver Transpl 2004;10:440-447.

42. Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, Nakajima Y, Ohnishi K. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer 1985;56:918-928.

43. The Cancer of the Liver Italian Program (CLIP) Investigators: A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology 1998; 28:751–755.

44. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 1999;19:329-338.

45. Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. Hepatology 2001;33:1394-1403.

46. Sugawara Y, Tamura S, Makuuchi M. Living donor liver transplantation for hepatocellular carcinoma: Tokyo University series. Dig Dis 2007;25:310-312.

47. Soejima Y, Taketomi A, Yoshizumi T, Uchiyama H, Aishima S, Terashi T, Shimada M, Maehara Y. Extended indication for living donor liver transplantation in patients with hepatocellular carcinoma. Transplantation 2007;83:893-899.

48. Takada Y, Ito T, Ueda M, Sakamoto S, Haga H, Maetani Y, Ogawa K, Ogura Y, Oike F, Egawa H, Uemoto S. Living donor liver transplantation for patients with HCC exceeding the Milan criteria: a proposal of expanded criteria. Dig Dis 2007;25:299-302.

49. Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda

GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P; Metroticket Investigator Study Group. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. Lancet Oncol 2009;10:35-43.

50. Hiatt JR, Carmody IC, Busuttil RW. Should we expand the criteria for hepatocellular carcinoma with living-donor liver transplantation?--no, never. J Hepatol 2005;43:573-577.

51. Sotiropoulos GC, Molmenti EP, Lang H. Milan criteria, up-to-seven criteria, and the illusion of a rescue package for patients with liver cancer. Lancet Oncol 2009;10:207-208.

52. Teefey SA, Hildeboldt CC, Dehdashti F, Siegel BA, Peters MG, Heiken JP, Brown JJ, McFarland EG, Middleton WD, Balfe DM, Ritter JH. Detection of primary hepatic malignancy in liver transplant candidates: prospective comparison of CT, MR imaging, US, and PET. Radiology 2003;226:533-542.

53. Rode A, Bancel B, Douek P, Chevallier M, Vilgrain V, Picaud G, Henry L, Berger F, Bizollon T, Gaudin JL, Ducerf C. Small nodule detection in cirrhotic livers: evaluation with US, spiral CT, and MRI and correlation with pathologic examination of explanted liver. J Comput Assist Tomogr 2001;25:327-336.

54. Krinsky GA, Lee VS, Theise ND, Weinreb JC, Morgan GR, Diflo T, John D, Teperman LW, Goldenberg AS. Transplantation for hepatocellular carcinoma and cirrhosis: sensitivity of magnetic resonance imaging. Liver Transpl 2002;8:1156-1164.

55. Lopez HE, Vogl TJ, Bechstein WO, Guckelberger O, Neuhaus P, Lobeck H, Felix R. Biphasic spiral computed tomography for detection of hepatocellular carcinoma before resection or orthotopic liver transplantation. Invest Radiol 1998;33:216-221.

56. Bhartia B, Ward J, Guthrie JA, Robinson PJ. Hepatocellular carcinoma in cirrhotic livers: double-contrast thin-section MR imaging with pathologic correlation of explanted tissue. AJR Am J Roentgenol 2003;180:577-584.

57. Esnaola NF, Lauwers GY, Mirza NQ, Nagorney DM, Doherty D, Ikai I, Yamaoka Y, Regimbeau JM, Belghiti J, Curley SA, Ellis LM, Vauthey JN. Predictors of microvascular invasion in patients with hepatocellular carcinoma

who are candidates for orthotopic liver transplantation. J Gastrointest Surg 2002;6:224-232.

58. Libbrecht L, Bielen D, Verslype C, Vanbeckevoort D, Pirenne J, Nevens F, Desmet V, Roskams T. Focal lesions in cirrhotic explant livers: pathological evaluation and accuracy of pretransplantation imaging examinations. Liver Transpl 2002;8:749-761.

59. Ravaioli M, Grazi GL, Ercolani G, Fiorentino M, Cescon M, Golfieri R, Trevisani F, Grigioni WF, Bolondi L, Pinna AD. Partial necrosis on hepatocellular carcinoma nodules facilitates tumor recurrence after liver transplantation. Transplantation 2004;78:1780-1786.

60. Pawlik TM, Gleisner AL, Anders RA, Assumpcao L, Maley W, Choti MA. Preoperative assessment of hepatocellular carcinoma tumor grade using needle biopsy: implications for transplant eligibility. Ann Surg 2007;245:435-442.

61. Sobin LH, Wittekind CH: TNM Classification of malignant tumours. Sixth edition. West Sussex: John Wiley & Sons, Ltd.; 2002

62. Sotiropoulos GC, Drühe N, Sgourakis G, Molmenti EP, Beckebaum S, Baba HA, Antoch G, Hilgard P, Radtke A, Saner FH, Nadalin S, Paul A, Malagó M, Broelsch CE, Lang H. Liver transplantation, liver resection, and transarterial chemoembolization for hepatocellular carcinoma in cirrhosis: which is the best oncological approach? Dig Dis Sci 2009;54:2264-2273.

63. Sotiropoulos GC, Malagó M, Molmenti E, Paul A, Nadalin S, Brokalaki EI, Verhagen R, Dirsch O, Gerken G, Lang H, Broelsch CE. Efficacy of transarterial chemoembolization prior to liver transplantation for hepatocellular carcinoma as found in pathology. Hepatogastroenterology. 2005;52:329-332.

64. Sotiropoulos GC, Malago M, Molmenti EP, Radtke A, Brokalaki EI, Nadalin S, Lang H, Frilling A, Baba HA, Kühl H, Verhagen R, Broelsch CE. Disease course after liver transplantation for hepatocellular carcinoma in patients with complete tumor necrosis in liver explants after performance of bridging treatments. Eur J Med Res. 2005;10:539-542.

65. Sotiropoulos GC, Malagó M, Bockhorn M, Schmitz KJ, Radtke A, Molmenti EP, Schaffer R, Beckebaum S, Cicinnati VR, Fouzas I, Broelsch CE, Lang H. Liver transplantation for hepatocellular carcinoma and cirrhosis in candidates

with undetectable or very low alpha-fetoprotein levels: is an expansion of the listing criteria justified? Hepatogastroenterology. 2008;55:1671-1677.

66. Sotiropoulos GC, Beckebaum S, Lang H, Frilling A, Molmenti EP, Cicinnati VR, Saner FH, Erim Y, Baba HA, Malagó M, Broelsch CE. Single-center experience on liver transplantation for hepatocellular carcinoma arising in alcoholic cirrhosis: results and ethical issues. Eur Surg Res 2008;40:7-13.
67. Lim JK, Keeffe EB. Liver transplantation for alcoholic liver disease: current concepts and length of sobriety. Liver Transpl 2004;10:S31-38.
68. Sotiropoulos GC, Malagó M, Molmenti E, Paul A, Nadalin S, Brokalaki E, Kühl H, Dirsch O, Lang H, Broelsch CE. Liver transplantation for hepatocellular carcinoma in cirrhosis: is clinical tumor classification before transplantation realistic? Transplantation. 2005;79:483-487.
69. Mion F, Grozel L, Boillot O, Paliard P, Berger F. Adult cirrhotic liver explants: precancerous lesions and undetected small hepatocellular carcinomas. Gastroenterology 1996;111:1587-1592.
70. Iwatsuki S, Gordon RD, Shaw BW Jr, Starzl TE. Role of liver transplantation in cancer therapy. Ann Surg 1985;202:401-407.
71. Sotiropoulos GC, Malagó M, Molmenti EP, Nadalin S, Radtke A, Brokalaki EI, Lang H, Frilling A, Baba HA, Neuhäuser M, Broelsch CE. Liver transplantation and incidentally found hepatocellular carcinoma in liver explants: need for a new definition? Transplantation 2006;81:531-535.
72. Zaykin DV, Zhivotovsky LA, Westfall PH, Weir BS. Truncated product method for combining p-values. Genet Epidemiol 2002;22:170-185.
73. Hwang S, Lee SG, Joh JW, Suh KS, Kim DG. Liver transplantation for adult patients with hepatocellular carcinoma in Korea: comparison between cadaveric donor and living donor liver transplantations. Liver Transpl 2005;11:1265-1272.
74. Sotiropoulos GC, Molmenti EP, Omar OS, Bockhorn M, Brokalaki EI, Lang H, Frilling A, Broelsch CE, Malagó M. Liver transplantation for hepatocellular carcinoma in patients beyond the Milan but within the UCSF criteria. Eur J Med Res. 2006;11:467-470.
75. Akahane M, Koga H, Kato N, Yamada H, Uozumi K, Tateishi R, Teratani T, Shiina S, Ohtomo K. Complications of percutaneous radiofrequency ablation for

hepato-cellular carcinoma: imaging spectrum and management. Radiographics 2005;25:S57-68.

76. Amitrano L, Brancaccio V, Guardascione MA, Margaglione M, Iannaccone L, D'Andrea G, Marmo R, Ames PR, Balzano A. Inherited coagulation disorders in cirrhotic patients with portal vein thrombosis. Hepatology 2000; 31: 345-348.

77. Sotiropoulos GC, Radtke A, Schmitz KJ, Molmenti EP, Schroeder T, Saner FH, Baba HA, Fouzas I, Broelsch CE, Malagó M, Lang H. Liver transplantation in the setting of hepatocellular carcinoma and portal vein thrombosis: a challenging dilemma? Dig Dis Sci 2008;53:1994-1999.

78. Sotiropoulos GC, Paul A, Gerling T, Molmenti EP, Nadalin S, Napieralski BP, Treckmann J, Lang H, Saner F, Frilling A, Broelsch CE, Malagó M. Liver transplantation with "rescue organ offers" within the eurotransplant area: a 2-year report from the University Hospital Essen. Transplantation 2006;82:304-309.

79. Beigelman-Aubry C, Hill C, Grenier PA. Management of an incidentally discovered pulmonary nodule. Eur Radiol 2007;17:449-466.

80. MacMahon H, Austin JH, Gamsu G, Herold CJ, Jett JR, Naidich DP, Fleischner Society Guidelines for management of small pulmonary nodules detected on CT scans: a statement from the Fleischner Society. Radiology 2005;237:395-400.

81. Sotiropoulos GC, Kuehl H, Sgourakis G, Molmenti EP, Beckebaum S, Cicinnati VR, Baba HA, Schmitz KJ, Broelsch CE, Lang H. Pulmonary nodules at risk in patients undergoing liver transplantation for hepatocellular carcinoma. Transpl Int 2008;21:850-856.

82. Sotiropoulos GC, Paul A, Molmenti E, Lang H, Frilling A, Napieralski BP, Nadalin S, Treckmann J, Brokalaki EI, Gerling T, Broelsch CE, Malagó M. Liver transplantation for hepatocellular carcinoma in cirrhosis within the Eurotransplant area: an additional option with "livers that nobody wants". Transplantation. 2005;80:897-902.

83. Sotiropoulos GC, Lang H, Frühauf NR, Molmenti EP, Omar OS, Radtke A, Paul A, Nadalin S, Frilling A, Broelsch CE, Malagò M. Split liver transplantation for hepatocellular carcinoma. Hepatogastroenterology. 2006;53:764-767.

84. Sotiropoulos GC, Lang H, Sgourakis G, Nadalin S, Molmenti EP, Radtke A, Paul A, Beckebaum S, Saner FH, Baba HA, Gerken G, Malagó M, Broelsch CE. Liberal policy in living donor liver transplantation for hepatocellular carcinoma: lessons learned. Dig Dis Sci. 2009;54:377-384.

85. Davison AC, Hinkley DV. Bootstrap methods and their application. Cambridge: Cambridge University Press, 1997.

86. Sotiropoulos GC, Lang H, Nadalin S, Neuhäuser M, Molmenti EP, Baba HA, Paul A, Saner FH, Weber F, Hilgard P, Frilling A, Broelsch CE, Malagó M. Liver transplantation for hepatocellular carcinoma: University Hospital Essen experience and metaanalysis of prognostic factors. J Am Coll Surg. 2007;205:661-675.

87. Fisher RA, Maluf D, Cotterell AH, Stravitz T, Wolfe L, Luketic V, Sterling R, Shiffman M, Posner M. Non-resective ablation therapy for hepatocellular carcinoma: effectiveness measured by intention-to-treat and dropout from liver transplant waiting list. Clin Transplant 2004;18:502-512.

88. Graziadei IW, Sandmueller H, Waldenberger P, Koenigsrainer A, Nachbaur K, Jaschke W, Margreiter R, Vogel W. Chemoembolization followed by liver transplantation for hepatocellular carcinoma impedes tumor progression while on the waiting list and leads to excellent outcome. Liver Transpl 2003;9:557-563.

89. Harnois DM, Steers J, Andrews JC, Rubin JC, Pitot HC, Burgart L, Wiesner RH, Gores GJ. Preoperative hepatic artery chemoembolization followed by orthotopic liver transplantation for hepatocellular carcinoma. Liver Transpl Surg 1999;5:192-199.

90. Hayashi PH, Ludkowski M, Forman LM, Osgood M, Johnson S, Kugelmas M, Trotter JF, Bak T, Wachs M, Kam I, Durham J, Everson GT. Hepatic artery chemoembolization for hepatocellular carcinoma in patients listed for liver transplantation. Am J Transplant 2004;4:782-787.

91. Moreno Planas JM, Lopez Monclus J, Gomez Cruz A, Rubio Gonzalez E, Perez Aranguena R, Boullosa Grana E, Garcia Suárez A, Pérez-Picouto JL, Fernández Ruiz M, Lucena de la Poza JL, Sánchez Turrión V, Cuervas-Mons Martínez V.

Efficacy of Hepatocellular Carcinoma Locoregional Therapies on Patients Waiting for Liver Transplantation. Transplant Proc 2005;37:1484-1485.

92. Venook AP, Ferrell LD, Roberts JP, Emond J, Frye JW, Ring E, Ascher NL, Lake JR. Liver transplantation for hepatocellular carcinoma: results with preoperative chemoembolization. Liver Transpl Surg 1995;1:242-248.

93. Yamakado K, Nakatsuka A, Ohmori S, Shiraki K, Nakano T, Ikoma J, Adachi Y, Takeda K. Radiofrequency ablation combined with chemoembolization in hepatocellular carcinoma: treatment response based on tumor size and morphology. J Vasc Interv Radiol. 2002;13:1225-1232

94. De Carlis L, Giacomoni A, Pirotta V, Lauterio A, Slim AO, Sammartino C, Cardillo M, Forti D. Surgical treatment of hepatocellular cancer in the era of hepatic transplantation. J Am Coll Surg 2003;196:887-897.

95. Cillo U, Vitale A, Bassanello M, Boccagni P, Brolese A, Zanus G, Burra P, Fagiuoli S, Farinati F, Rugge M, D'Amico DF. Liver transplantation for the treatment of moderately or well-differentiated hepatocellular carcinoma. Ann Surg 2004;239:150-159.

96. Leung JY, Zhu AX, Gordon FD, Pratt DS, Mithoefer A, Garrigan K, Terella A, Hertl M, Cosimi AB, Chung RT. Liver transplantation outcomes for early-stage hepatocellular carcinoma: results of a multicenter study. Liver Transpl 2004;10:1343-1354.

97. Todo S, Furukawa H; Japanese Study Group on Organ Transplantation: Living donor liver transplantation for adult patients with hepatocellular carcinoma: experience in Japan. Ann Surg 2004;240:451-459.

98. Zavaglia C, De Carlis L, Alberti AB, Minola E, Belli LS, Slim AO, Airoldi A, Giacomoni A, Rondinara G, Tinelli C, Forti D, Pinzello G. Predictors of long-term survival after liver transplantation for hepatocellular carcinoma. Am J Gastroenterol 2005;100:2708-2716.

99. Colella G, De Carlis L, Rondinara GF, Sansalone CV, Belli LS, Aseni P, Slim AO, Gelosa F, Iamoni GM, Corti A, Mazza E, Arcieri K, Giacomoni A, Minola E, Ideo G, Forti D. Is hepatocellular carcinoma in cirrhosis an actual indication for liver transplantation? Transplant Proc 1997; 29:492-494.

100. Colella G, Rondinara GF, De Carlis L, Sansalone CV, Slim AO, Aseni P, Rossetti O, De Gasperi A, Minola E, Bottelli R, **Belli LS**, Ideo G, Forti D. Liver transplantation for hepatocellular carcinoma: prognostic factors associated long-term survival. Transpl Int 1996;9 Suppl 1:S109-111.

101. De Carlis L, Giacomoni A, Lauterio A, Slim A, Sammartino C, Pirotta V, Colella G, Forti D. Liver transplantation for hepatocellular cancer: should the current indication criteria be changed? Transpl Int 2003;16:115-122.

102. Ojogho ON, So SK, Keeffe EB, Berquist W, Concepcion W, Garcia-Kennedy R, Imperial J, Esquivel CO. Orthotopic liver transplantation for hepatocellular carcinoma: factors affecting long-term patient survival. Arch Surg 1996;131:935-939.

103. Llovet JM, Bruix J, Fuster J, Castells A, Garcia-Valdecasas JC, Grande L, Franca A, Brú C, Navasa M, Ayuso MC, Solé M, Real MI, Vilana R, Rimola A, Visa J, Rodés J. Liver transplantation for small hepatocellular carcinoma: the tumor-node-metastasis classification does not have prognostic power. Hepatology 1998;27:1572-1577.

104. Margarit C, Charco R, Hidalgo E, Allende H, Castells L, Bilbao I. Liver transplantation for malignant diseases: selection and pattern of recurrence. World J Surg 2002;26:257-263.

105. Yokoyama I, Todo S, Iwatsuki S, Starzl TE. Liver transplantation in the treatment of primary liver cancer. Hepatogastroenterology 1990;37:188-193.

106. Michel J, Suc B, Fourtanier G, Durand D, Rumeau JL, Rostaing L, Lloveras JJ. Recurrence of hepatocellular carcinoma in cirrhotic patients after liver resection or transplantation. Transplant Proc 1995;27:1798-1800.

107. Yamamoto J, Iwatsuki S, Kosuge T, Dvorchik I, Shimada K, Marsh JW, Yamasaki S, Starzl TE. Should hepatomas be treated with hepatic resection or transplantation? Cancer 1999;86:1151-1158.

108. Herrero JI, Sangro B, Quiroga J, Pardo F, Herraiz M, Cienfuegos JA, Prieto J. Influence of tumor characteristics on the outcome of liver transplantation among patients with liver cirrhosis and hepatocellular carcinoma. Liver Transpl 2001;7:631-636.

109. Bigourdan JM, Jaeck D, Meyer N, Meyer C, Oussoultzoglou E, Bachellier P, Weber JC, Audet M, Doffoël M, Wolf P. Small hepatocellular carcinoma in Child A cirrhotic patients: Hepatic resection versus transplantation. Liver Transpl 2003;9:513-520.
110. Roayaie S, Schwartz JD, Sung MW, Emre SH, Miller CM, Gondolesi GE, Krieger NR, Schwartz ME. Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. Liver Transpl 2004;10:534-540.
111. Shetty K, Timmins K, Brensinger C, Furth EE, Rattan S, Sun W, Rosen M, Soulen M, Shaked A, Reddy KR, Olthoff KM. Liver transplantation for hepatocellular carcinoma validation of present selection criteria in predicting outcome. Liver Transpl 2004;10:911-918.
112. Löhe F, Angele MK, Gerbes AL, Lohrs U, Jauch KW, Schauer RJ. Tumour size is an important predictor for the outcome after livertransplantation for hepatocellular carcinoma. Eur J Surg Oncol 2005;31:994-999.
113. Penn I. Hepatic transplantation for primary and metastatic cancers of the liver. Surgery 1991;110:726-735.
114. Chung SW, Toth JL, Rezieg M, Cameron R, Taylor BR, Greig PD, Levy GA, Langer B. Liver transplantation for hepatocellular carcinoma. Am J Surg 1994;167:317-321.
115. Achkar JP, Araya V, Baron RL, Marsh JW, Dvorchik I, Rakela J. Undetected hepatocellular carcinoma: clinical features and outcome after liver transplantation. Liver Transpl Surg 1998;4:477-482.
116. Bechstein WO, Guckelberger O, Kling N, Rayes N, Tullius SG, Lobeck H, Vogl T, Jonas S, Neuhaus P. Recurrence-free survival after liver transplantation for small hepatocellular carcinoma. Transpl Int 1998;11:S189-92.
117. Klintmalm GB. Liver transplantation for hepatocellular carcinoma: a registry report of the impact of tumor characteristics on outcome. Ann Surg 1998;228:479-490.
118. Otto G, Heuschen U, Hofmann WJ, Krumm G, Hinz U, Herfarth C. Survival and recurrence after liver transplantation versus liver resection for hepatocellular carcinoma: a retrospective analysis. Ann Surg 1998;227:424-432.

119. Chui AK, Rao AR, McCaughan GW, Waugh R, Verran DJ, Koorey D, Sheil AG. Liver transplantation for hepatocellular carcinoma in cirrhotic patients. Aust N Z J Surg 1999;69:798-801.

120. Figueras J, Ibañez L, Ramos E, Jaurrieta E, Ortiz-de-Urbina J, Pardo F, Mir J, Loinaz C, Herrera L, López-Cillero P, Santoyo J. Selection criteria for liver transplantation in early-stage hepatocellular carcinoma with cirrhosis: results of a multicenter study. Liver Transpl 2001;7:877-883.

121. Tamura S, Kato T, Berho M, Misiakos EP, O'Brien C, Reddy KR, Nery JR, Burke GW, Schiff ER, Miller J, Tzakis AG. Impact of histological grade of hepatocellular carcinoma on the outcome of liver transplantation. Arch Surg 2001;136:25-30.

122. Moya A, Berenguer M, Aguilera V, Juan FS, Nicolás D, Pastor M, López-Andujar R, Rayón M, Orbis F, Mora J, De Juan M, Carrasco D, Vila JJ, Prieto M, Berenguer J, Mir J. Hepatocellular carcinoma: Can it be considered a controversial indication for liver transplantation in centers with high rates of hepatitis C? Liver Transpl 2002;8:1020-1027.

123. Bassanello M, Vitale A, Ciarleglio FA, Brolese A, Zanus G, D'Amico F, arraro A, Cappuzzo G, Bridda A, Senzolo M, Burra P, Pevere S, D'Amico D, Cillo U. Adjuvant chemotherapy for transplanted hepatocellular carcinoma patients: impact on survival or HCV recurrence timing. Transplant Proc 2003;35:2991-2994.

124. Fernández JA, Robles R, Marin C, Sánchez-Bueno F, Ramirez P, Pons JA, Garre MC, Pérez D, Parrilla A, Navalón JC, Parrilla P. Can we expand the indications for liver transplantation among hepatocellular carcinoma patients with increased tumor size? Transplant Proc 2003;35:1818-1820.

125. González-Uriarte J, Valdivieso A, Gastaca M, Errasti G, Campo M, Hernandez MJ, Montejo M, Bustamante J, Suarez MJ, Testillano M, Fernandez JR, Ortiz de Urbina J. Liver transplantation for hepatocellular carcinoma in cirrhotic patients. Transplant Proc 2003;35:1827-1829.

126. Khakhar A, Solano E, Stell D, Bloch M, Dale C, Burns P, Ghent C, Quan D, McAlister V, Marotta P, Wall WJ. Survival after liver transplantation for hepatocellular carcinoma. Transplant Proc 2003;35:2438-2441.

127. Nart D, Arikan C, Akyildiz M, Yuce G, Demirpolat G, Zeytunlu M, Karasu Z, Aydogdu S, Killi R, Yuzer Y, Tokat Y, Kilic M. Hepatocellular carcinoma in liver transplant era: a clinicopathologic analysis. Transplant Proc 2003;35:2986-2990.

128. Pérez Saborido B, Loinaz Segurola C, Gimeno Calvo A, Meneu Díaz JC, Abradelo de Usera M, Calvo Pulido J, Jiménez Romero C, Gómez Sanz R, García García I, Moreno González E. Liver transplantation for hepatocellular carcinoma: our experience from 1986. Transplant Proc 2003;35:1825-1826.

129. Leung JY, Zhu AX, Gordon FD, Pratt DS, Mithoefer A, Garrigan K, Terella A, Hertl M, Cosimi AB, Chung RT. Liver transplantation outcomes for early-stage hepatocellular carcinoma: results of a multicenter study. Liver Transpl 2004;10:1343-1354.

130. Ravaioli M, Ercolani G, Cescon M, Vetrone G, Voci C, Grigioni WF, D'Errico A, Ballardini G, Cavallari A, Grazi GL. Liver transplantation for hepatocellular carcinoma: further considerations on selection criteria. Liver Transpl 2004;10:1195-1202.

131. Stadlbauer V, Schaffellner S, Kniepeiss D, Jakoby E, Stauber R, Iberer F, Tscheliessnigg KH. Experiences in liver transplantation for hepatocellular carcinoma. Transplant Proc 2004;36:195-198.

132. Wong LL, Tsai N, Limm W, Wong L. Liver transplant for hepatocellular cancer: a treatment for the select few. Clin Transplant 2004;18:205-210.

133. Yedibela S, Fortsch T, Hohenberger W, Meyer T. Liver transplantation for hepatocellular carcinoma: a single European centre experience. Langenbecks Arch Surg 2004;389:104-109

134. Jain A, DiMartini A, Kashyap R, Youk A, Rohal S, Fung J. Long-term follow-up after liver transplantation for alcoholic liver disease under tacrolimus. Transplantation 2000;70:1335-1342.

135. Decaens T, Roudot-Thoraval F, Hadni-Bresson S, Meyer C, Gugenheim J, Durand F, Bernard PH, Boillot O, Sulpice L, Calmus Y, Hardwigsen J, Ducerf C, Pageaux GP, Dharancy S, Chazouilleres O, Cherqui D, Duvoux C. Impact of UCSF criteria according to pre- and post-OLT tumor features: analysis of 479

patients listed for HCC with a short waiting time. Liver Transpl 2006;12:1761-1769.

136. Muller P, Renou C, Harafa A, Jouve E, Kaplanski G, Ville E, Bertrand JJ, Masson C, Benderitter T, Halfon P. Lymph node enlargement within the hepatoduodenal ligament in patients with chronic hepatitis C reflects the immunological cellular response of the host. J Hepatol 2003;39:807-813.

137. Dietrich CF, Lee JH, Herrmann G, Teuber G, Roth WK, Caspary WF, Zeuzem S. Enlargement of perihepatic lymph nodes in relation to liver histology and viremia in patients with chronic hepatitis C. Hepatology 1997;26: 467-472.

138. Sotiropoulos GC, Malagó M, Molmenti EP, Lösch C, Lang H, Frilling A, Broelsch CE, Neuhäuser M. Hilar lymph nodes sampling at the time of liver transplantation for hepatocellular carcinoma: to do or not to do? Meta-analysis to determine the impact of hilar lymph nodes metastases on tumor recurrence and survival in patients with hepatocellular carcinoma undergoing liver transplantation. Transpl Int 2007;20:141-146.

139. Weerahandi S. Exact statistical methods for data analysis. Springer, New York, 1995.

140. Ismail T, Angrisani L, Gunson BK, Hübscher SG, Buckels JA, Neuberger JM, Elias E, McMaster P. Primary hepatic malignancy: the role of liver transplantation. Br J Surg 1990;77:983-987.

141. Mazzaferro V, Rondinara GF, Rossi G, Regalia E, De Carlis L, Caccamo L, Doci R, Sansalone CV, Belli LS, Armiraglio E, et al. Milan multicenter experience in liver transplantation for hepatocellular carcinoma. Transplant Proc 1994;26:3557-3560.

142. Marsh JW, Dvorchik I, Subotin M, Balan V, Rakela J, Popechitelev EP, Subbotin V, Casavilla A, Carr BI, Fung JJ, Iwatsuki S. The prediction of risk of recurrence and time to recurrence of hepatocellular carcinoma after orthotopic liver transplantation: a pilot study. Hepatology 1997;26:444-450.

143. Bhattacharjya S, Bhattacharjya T, Quaglia A, Dhillon AP, Burroughs AK, Patch DW, Tibballs JM, Watkinson AF, Rolles K, Davidson BR. Liver transplantation in cirrhotic patients with small hepatocellular carcinoma: an analysis of pre-

operative imaging, explant histology and prognostic histologic indicators. Dig Surg 2004;21:152-159.

144. Selby R, Kadry Z, Carr B, Tzakis A, Madariaga JR, Iwatsuki S. Liver transplantation for hepatocellular carcinoma. World J Surg 1995;19:53-58.

145. Sotiropoulos GC, Molmenti EP, Lösch C, Beckebaum S, Broelsch CE, Lang H. Meta-analysis of tumor recurrence after liver transplantation for hepatocellular carcinoma based on 1,198 cases. Eur J Med Res. 2007;12:527-534.

146. Gondolesi G, Munoz L, Matsumoto C, Fishbein T, Sheiner P, Emre S, Miller C, Schwartz ME. Hepatocellular carcinoma: a prime indication for living donor liver transplantation. J Gastrointest Surg 2002;6:102-107.

147. Vivarelli M, Bellusci R, Cucchetti A, Cavrini G, De Ruvo N, Aden AA, La Barba G, Brillanti S, Cavallari A. Low recurrence rate of hepatocellular carcinoma after liver transplantation: better patient selection or lower immunosuppression? Transplantation 2002;74:1746-1751.

148. Moreno P, Jaurrieta E, Figueras J, Benasco C, Rafecas A, Fabregat J, Torras J, Casanovas T, Casais L. Orthotopic liver transplantation: treatment of choice in cirrhotic patients with hepatocellular carcinoma? Transplant Proc 1995;27:2296-2298.

149. Figueras J, Jaurrieta E, Valls C, Benasco C, Rafecas A, Xiol X, Fabregat J, Casanovas T, Torras J, Baliellas C, Ibañez L, Moreno P, Casais L. Survival after liver transplantation in cirrhotic patients with and without hepatocellular carcinoma: a comparative study. Hepatology 1997;25:1485-1489.

150. Iwatsuki S, Starzl TE, Sheahan DG, Yokoyama I, Demetris AJ, Todo S, Tzakis AG, Van Thiel DH, Carr B, Selby R, Madariaga J. Hepatic resection versus transplantation for hepatocellular carcinoma. Ann Surg 1991; 214: 221-228.

151. Jonas S, Bechstein WO, Steinmüller T, Herrmann M, Radke C, Berg T, Settmacher U, Neuhaus P. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. Hepatology 2001;33:1080-1086.

152. Marsh JW, Dvorchik I, Bonham CA, Iwatsuki S. Is the pathologic TNM staging system for patients with hepatoma predictive of outcome? Cancer 2000;88:538-543.

153. McPeake JR, O'Grady JG, Zaman S, Portmann B, Wight DG, Tan KC, Calne RY, Williams R. Liver transplantation for primary hepatocellular carcinoma: tumor size and number determine outcome. J Hepatol 1993;18:226-234.

154. Regalia E, Fassati LR, Valente U, Pulvirenti A, Damilano I, Dardano G, Montalto F, Coppa J, Mazzaferro V. Pattern and management of recurrent hepatocellular carcinoma after liver transplantation. J Hepatobiliary Pancreat Surg 1998;5:29-34.

155. Roayaie S, Frischer JS, Emre SH, Fishbein TM, Sheiner PA, Sung M, Miller CM, Schwartz ME. Long-term results with multimodal adjuvant therapy and liver transplantation for the treatment of hepatocellular carcinomas larger than 5 centimeters. Ann Surg 2002;235:533-539.

156. Sauer P, Kraus TW, Schemmer P, Mehrabi A, Stremmel W, Buechler MW, Encke J. Liver transplantation for hepatocellular carcinoma: is there evidence for expanding the selection criteria? Transplantation 2005;80:S105-108.

157. Schlitt HJ, Neipp M, Weimann A, Oldhafer KJ, Schmoll E, Boeker K, ashan B, Kubicka S, Maschek H, Tusch G, Raab R, Ringe B, Manns MP, Pichlmayr R. Recurrence patterns of hepatocellular and fibrolamellar carcinoma after liver transplantation. J Clin Oncol 1999;17: 324-331.

158. Takada Y, Ueda M, Ito T, Sakamoto S, Haga H, Maetani Y, Ogawa K, Kasahara M, Oike F, Egawa H, Tanaka K. Living donor liver transplantation as a second-line therapeutic strategy for patients with hepatocellular carcinoma. Liver Transpl 2006; 12: 912-919.

159. Yao FY, Kinkhabwala M, LaBerge JM, Bass NM, Brown R Jr, Kerlan R, Venook A, Ascher NL, Emond JC, Roberts JP. The impact of pre-operative loco-regional therapy on outcome after liver transplantation for hepatocellular carcinoma. Am J Transplant 2005;5:795-804.

160. Willatt JM, Hussain HK, Adusumilli S, Marrero JA. MR Imaging of hepatocellular carcinoma in the cirrhotic liver: challenges and controversies. Radiology. 2008;24:311-330.

161. Hoshida Y, Toffanin S, Lachenmayer A, Villanueva A, Minguez B, Llovet JM. Molecular classification and novel targets in hepatocellular carcinoma: recent advancements. Semin Liver Dis 2010;30:35-51.

162. Ioannou GN, Perkins JD, Carithers RL, Jr. Liver transplantation for hepatocellular carcinoma: impact of the MELD allocation system and predictors of survival. Gastroenterology 2008;134:1342-1351.
163. Sotiropoulos GC, Molmenti EP, Lang H. Liver transplantation for hepatocellular carcinoma in the MELD era: leading roles of MELD score, AFP level, and recipient age as predictors of survival. Dig Dis Sci. 2009;54:917.