Pre-transplant prediction of microvascular invasion of hepatocellular carcinoma

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Abstract

Introduction: Microvascular invasion (MVI) has been demonstrated to be a strong predictor of tumor recurrence and poor survival after liver transplantation (LT) and liver resection for HCC.[1,2] As MVI cannot be determined preoperatively, it is, therefore, of great importance to try to identify predictors of MVI prior to LT.

Methods: A retrospective analysis of preoperative and pathological data of 79 consecutive patients who underwent living donor liver transplantation (LDLT) between 2002 and 2009 for HCC was conducted. MVI was defined as pathological evidence of microscsopic involvement of the vessels (portal vein or hepatic vein) within the peritumoral liver tissue. The chi-square test and Student t test were used for univariate analysis. Overall survival and disease-free survival rates were analyzed using Kaplan-Meier estimates.

Results: Patients were divided into two groups. Group I had no MVI and included 55 (70.6%) patients and Group II had MVI and included 24 (30.4%) patients. Recurrence in group II (MVI group) was significantly higher than in group I (25% Vs 4%, P = 0.008). Among the preoperative factors, the tumors beyond Milan criteria, number, size and tumor grade were significant predictors of MVI. MVI was 6.7% in well differentiated HCC in comparison to 46.8% in moderately and poorly differentiated HCC, respectively (P=0.002) and was 26% versus 83.3% when tumor number was less than 3 and more than 3, respectively (P=0.009). MVI was 25.6% in tumors less than 5 cm and 71.4% in tumors more than 5 cm in size (P=0.02). HCC within Milan criteria had statistically significant lower incidence of MVI than those beyond Milan criteria (P=0.004).

Conclusion: Microvascular invasion associated with higher HCC recurrence rate. Tumor grade, number and size were useful in predicting MVI before LT for HCC. LT for patients within Milan criteria is associated with lower incidence of pathologically evident MVI.

Introduction:

Liver transplantation (LTX) is an established therapeutic option in patients with hepatocellular carcinoma (HCC) and especially in patients with cirrhosis.[3] Multiple studies on liver transplantation (LT) for hepatocellular carcinoma (HCC) have reported various prognostic determinants of "high-risk pathology" for tumor recurrence and decreased patient survival.[4,5] Tumor size, tumor number, lobar distribution, vascular invasion, tumor differentiation, and alpha-fetoprotein (AFP) levels are among the most frequently encountered ones.[6] Micro vascular tumor embolism is an independent predictor of HCC recurrence after liver transplantation. Although LT is a safe and effective treatment for HCC within the Milan criteria, the presence of microvascular tumor embolism at pathologic examination can predict its recurrence.[7] The

microscopic vascular invasion detected histologically has the similar prognostic significance to that of the macroscopic vascular invasion detected by gross examination.[8] Micro vascular invasion is a good predictive parameter, but it is impossible to detect preoperatively.[9] The objective of this study is to detect possible predictors of micro vascular invasion in patients undergoing LDLT for HCC.

Patients and methods:

Patient population:

From January 2002 through December 2009, 79 consecutive patients underwent living donor liver transplantation (LDLT) for HCC at three centers of liver transplantation with the same surgical teams. They include 73 male and 6 female with a median age of 51 years (range from 37 to 64). Tumors within and beyond Milan criteria were included. Tumors beyond Milan criteria and not responding to down-staging (either with radiological evidence or persistent elevated AFP) were excluded.

Preoperative evaluation:

All tumors were assessed with triphasic computed tomography (triphasic CT) and CT portography to confirm diagnosis of HCC and to know tumor number, size (largest and total tumor burden), site, satellite, diffuse or localized type and macro-vascular invasion. Extra-hepatic assessment included routine bone scan and chest CT. AFP was done routine with other tumor marker esp. CEA and CA19,9. Routine assessment of liver (synthetic function and portal hypertension) and other co-morbidity was done. Child and MELD scoring was calculated for all patients.

Down-staging of all tumors beyond Milan criteria was done routinely with either radiofrequency ablation (RFA), chemo-embolization (TACE) or both. Reassessment with triphasic CT and AFP was done after 1 and 3 months to assess response. Tumor progression after down-staging (either radiological or persistent elevated AFP) was considered a contraindication for transplantation. Tumors within Milan criteria were treated with local ablative therapy as a bridge if transplantation was expected to be delayed **Figure(1)**.

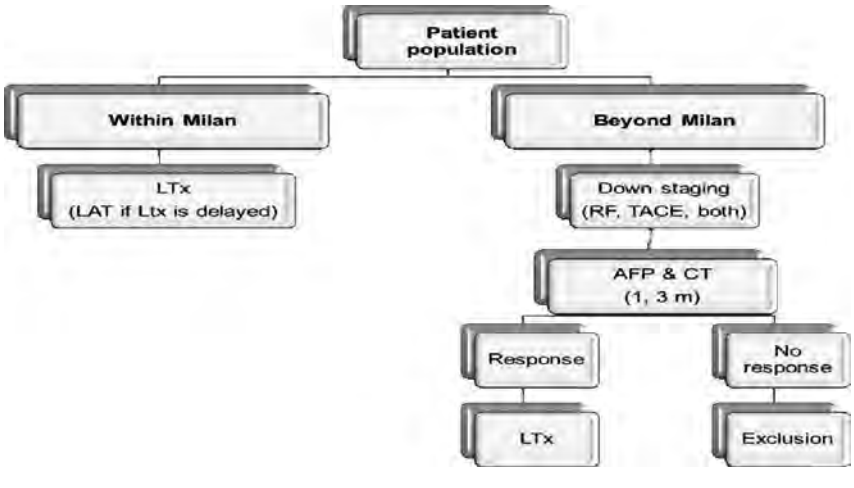

Figure (1): Protocol of management of HCC before LDLT. LTx: liver transplantation, LAT: Local Ablative Therapy, RF: Radiofrequency, TACE: Trans Arterial Chemo-Embolization, AFP: Alpha FetoProtein, CT: Computed Tomography.

Operation:

Recipient operation started with exploration of the whole abdomen, cytology from ascetic fluid and complete hilar lymph node dissection for intra-operative pathological assessment to exclude extra-heptaic spread. Two patients had hilar lymph node positive for malignancy and transplantation was aborted.

All patients had a Right Liver Graft (RLG) with a GRWR between 0.8 and 1.2 with no special precaution regarding type and number of vascular and biliary anastmoses.

Pathological assessment:

All explants were examined histo-pathologically to confirm diagnosis of HCC, accurate assessment of number, site, size

(largest and overall), satellite, grade, macroscopic or microscopic vascular invasion. Microscopic vascular invasion was defined as pathological evidence of microscopic involvement of the vessels (portal vein or hepatic vein) within the peritumoral liver tissue.

Predictors of micro-vascular invasion:

Patients were divided into two groups according to the presence or absence of micro-vascular invasion histo-pathologically in the explant. Group I had no MVI and Group II had MVI. Both groups were compared regarding pre-operative data including age, sex, etiology of cirrhosis, Child score, MELD score, tumor number, size (largest and overall), within or beyond Milan criteria, AFP and grade of the tumor detected pathologically.

Statistical analysis:

We compared the demographic, clinical, and tumor characteristics of the 2 groups. The collected data are shown as mean values and standard deviation. The chi-square test and Student t test were used for univariate analysis of categorical and normally distributed continuous variables respectively. $P<0.05$ was considered significant. Diagnostic accuracy of predictive risk factors was evaluated using receiver operating characteristic (ROC) analysis. Overall survival and disease-free survival rates were analyzed using Kaplan-Meier estimates with comparisons performed using the log-rank test.

Results:

This study includes 79 patients, 73 male (92.4%) and 6 female (7.6%). Patients were divided into two groups. Group I had no MVI and included 55 (70.6%) patients and Group II had MVI and included 24 (30.4%) patients. HCV was the most common cause of cirrhosis in both groups (95.8% vs 94.5%). Patients in group I were Child A in 8 patients (14.5%), Child B in 15 patients (27.3%) and Child C in 32 patients (58.2%), while in group II two patients (8.3%) were Child A, 10 patients (41.7%) were Child B and 12 patients (50%) were Child C. MELD score was nearly similar for both groups **Table(1)**.

Table (1): Demographic and clinical data of patient population.

	No MVI (55pts)	MVI (24pts)
Age (Median ± SD)	50 ± 5.5	52 ± 6
Sex (M/F)	(51/4)	(22/2)
Etiology of cirrhosis		
HCV	52(94.5%)	23 (95.8%)
HBV	2 (3.6%)	1 (4.2%)
HCV+HBV	1 (1.8%)	0
CHILD		
A	8 (14.5%)	2 (8.3%)
B	15 (27.3%)	10 (41.7%)
C	32 (58.2%)	12 (50%)
MELD (Median ± SD)	14 ± 7	14 ± 4

SD: Standard of deviation, M: Male, F: Female, HCV: Hepatitis C Virus, HBV: Hepatitis B Virus.

Microscopic vascular invasion and recurrence

The follow-up time ranged from 1 to 104 months. Sixteen patients died in the course of follow-up. The overall mortality was 20.3%. Recurrences occurred in 8 patients (10.13%) at a mean time of 10 months (range, 6 to 15 months).

Microvascular invasion was detected histo-pathologically in 24 patients (30.4%). Recurrence of HCC post LT occurred in 2 patients (4%) in the group of no MVI and in

6 patients (25%) in the group of MVI and (P=0.008) **Figure(2)**, however, there was no statistical difference in overall survival between both groups. There was a difference (with a trend to be significant) between recurrence free survival between both groups **Figures(3,4)**.

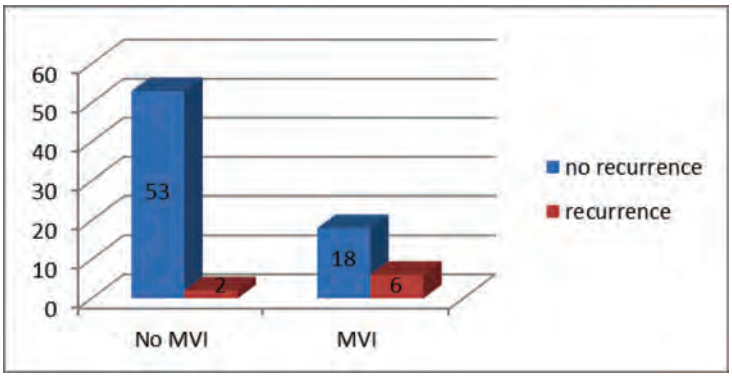

Figure (2): Increased incidence of HCC recurrence with MVI.

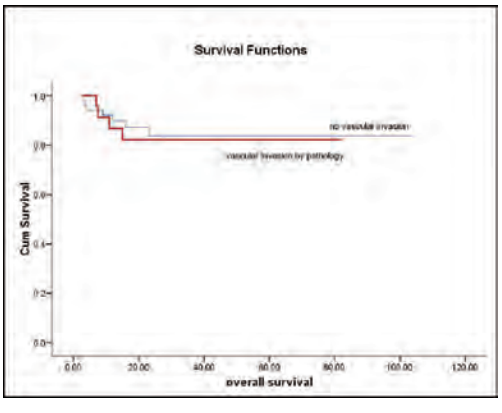

Figure (3): Comparison of overall survival between group I (no MVI) and group II (with MVI).

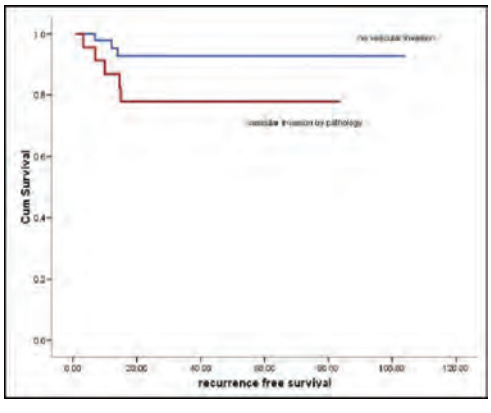

Figure (4): Recurrence free survival between both groups showing trends to be significant difference but still yet not significant.

Predictors of Microscopic vascular invasion Table(2)

Univariate analysis revealed that tumour number, size, grade and extension beyond Milan criteria were significantly associated with microscopic vascular invasion **Table(2)**. Other variables, including sex, age, preoperative hepatic function including Child-Pugh classification and model of end-stage liver disease (MELD) score and AFP, did not significantly affect incidence of MVI.

Tumor number

Tumor number was significantly associated with higher incidence of MVI. Statistical analysis showing a tumor number of three was considered as a cutoff value. Microvascular invasion occurred in 5 out of 6 patients (83.3%) with tumor number more than 3, while it occurred in 19 out of 73 patients (26%) when tumor number was less than 3 (P= 0.009).

Tumor size

Overall tumor size was insignificantly related to microscopic vascular invasion (MVI). Forty six patients had single tumor. In comparison between the incidence of MVI in group of single tumor, tumor size more than 5 cm was significantly associated with MVI (5 out of 7 patients - 71.4%) than when tumor size was less than 5 cm (10 out of 39 patients - 25.6%) (P= 0.02).

Tumor grade

Eleven patients had a well ablated tumor, so grade of these tumors could not be assessed. Micro vascular invasion occurred in 1 of 15 (6.7%) of well differentiated tumors and in 22 of 47 (46.8%) patients with moderately or poorly differentiated tumors (P=0.002).

Milan criteria

Fifty five patients were transplanted for HCC within Milan criteria and 24 patients had tumors beyond Milan criteria. The incidence of MVI in tumors within Milan was much lower than in tumors beyond Milan (20% Vs 54%, P= 0.004).

Table (2): Univariate analysis of clinico-pathological factors in the 2 groups.

	Group 1 (no MVI)	Group 2 (MVI)	P value
Age (Median ± SD)	52 ± 6	50 ± 5.5	NS
Sex			
M	22	51	0.595
F	2	4	NS
Etiology of cirrhosis			
HCV	23(95.8%)	52 (94.5%)	
HBV	1 (4.2%)	2 (3.6%)	0.798
HCV+HBV	0	1 (1.8%)	NS
CHILD			
A	2 (8.3%)	8 (14.5%)	
B	10(41.7)	15 (27.3%)	0.474
C	12 (50%)	32 (58.2%)	NS
MELD (Median ± SD)	14 ± 4	14 ± 7	NS
Tumor data			
Number			
<3	54	19	
>3	1	5	0.009*
Largest tumor size			
<5cm	29	10	0.02*
>5cm	2	5	
Grade			
Well differentiated.	14	1	
Moderate +Poorly differentiated.	25	22	0.002*
Milan criteria			
Within	44	11	
Beyond	11	13	0.004*
Pretransplantation treatment			
No	25	12	
Yes			0.756
RFA	19	6	
TACE	7	4	
RFA + TACE	3	1	
Missed	1	1	

**: Statistically significant.*

Discussion:

Orthotopic liver transplantation (OLT) is the preferred treatment for selected patients with hepatocellular carcinoma (HCC) and end-stage liver disease. While transplantation offers the theoretic advantage of complete tumor excision with removal of the diseased liver, recurrence of HCC following OLT is the rate-limiting factor for long-term survival. Several studies have chronicled the actual incidence of recurrent HCC after transplantation **Table(3)**. Mechanisms of cancer recurrence include the presence of microscopic extrahepatic foci at the time of transplantation. Thus, HCC may resurface in the form of metastatic foci in distant organs, such as the lungs, brain, bone, and in the transplanted allograft.[10] In this study 79 patients were transplanted for HCC within and beyond Milan criteria (with good response to downstaging). Recurrences occurred in 8 patients (10.13%) at a mean time of 10 months (range, 6 to 15 months).

Several clinical variables have been identified that independently influence tumor recurrence and patient survival. Early observations by Iwatsuki and colleagues, identified lymph node metastasis and vascular invasion of the tumor as significant negative predictors. Subsequent experience has confirmed that both microvascular and macrovascular invasion portend a worse outcome and correlate with an increased incidence of post-OLT tumor recurrence.[10,11]

Table (3): HCC recurrence following orthotopic liver transplantation.[10]

Source	No. of Patients	HCC Recurrence %	Study Description/ Outcome
Marsh et al[12]	178	40	Artificial neural network model to predict the likelihood of HCC recurrence following OLT
Roayaie et al[13]	311	18.3	>1 y to recurrence, without bone metastasis, surgical treatment of metastasis associated with longer survival
Leung et al[14]	144	15.3	AFP of ≤10 ng/mL and pathologic UCSF criteria are predictors of recurrence free survival
Yoo et al[15]	985	7.6	5-y survival for patients with HCC was 42.3% and 71.7% in patients without HCC (from UNOS data set)

Microvascular invasion (MVI) has recently been demonstrated to be a very strong predictor of tumor recurrence and poor survival after liver transplantation (LT) and liver resection for HCC.[1,2] While major vascular invasion can be identified preoperatively in the majority of cases, microscopic vascular invasion is impossible to rule out before transplantation.[16]

This study confirmed the relation between recurrence and microvascular invasion. Recurrence of HCC post LT occurred in 2 out of 55 patients (4%) in group of no MVI and in 6 out of 24 patients (25%) in group of MVI (P = 0.008). There was a difference (with a trend to be significant) between recurrence free survival between both groups.

Poorly differentiated HCC have a malignant potential, such as high-frequent micro-vascular invasion even if the tumor size is equal or smaller than 3 cm in diameter.[17] A recent report by Esnaola indicated a strong association between poor HCC differentiation grade and

presence of micro-vascular invasion.[18] Moderately and poorly differentiated tumor in this study was associated with increasing incidence of MVI in comparison to well differentiated tumors (46.8% Vs 6.7%, P= 0.002), so liver biopsy at the time of ablation in high risk patients for recurrence may be beneficial and need to be studied.

Tumor size and the number of HCC nodules have been shown to influence patient survival and recurrence.[19,20] A previous multivariate analysis from the registry has shown that only tumor size and tumor grade are independent predictors of outcome.[21,22] Postoperative recurrence-free survival was significantly diminished by tumor differentiation grade, size greater than 5 cm, or macro- and microvascular invasion.[23,24] In spite of that a lot of works discuss the relation between size and number of HCC with the incidence of the recurrence; very few data analyze the relation between them and microvascular invasion.

In this study, the relation between incidences of microvascular invasion, tumor number and size was studied. Eighty three percent of patients with tumor number more than 3 had microvascular invasion, in comparison to twenty six percent of patients with tumor number less than 3 (P= 0.009). Largest tumor size also showed a significant difference in the incidence of microvascular invasion. Microvascular invasion occurred in seventy one percent of patients with largest tumor size more than 5 cm, in comparison to twenty five percent of patients with largest tumor size less than 5 cm (P= 0.02).

Milan criteria were accepted by the United Network of Organ Sharing to guide patient selection for HCC. Significantly increased 5-year survivals of 85% have been achieved in Child class B and C as the best situation for treatment of small HCC in cirrhotic patients. In other studies, scientists had shown that the Mazzaferro criteria may be too restrictive; some transplant teams have proposed other staging systems with expanded criteria.[25]

In this study Milan criteria still had a lower incidence of MVI. Microvascular invasion occurred in 20% of tumors within Milan criteria and in 54% of tumors beyond Milan criteria (P= 0.004). We believe that Milan criteria are the most safe criteria but do not meet the increasing burden of increasing incidence of HCC all over the world.

Conclusion:

Tumor grade, number and size are useful in predicting the presence or absence of micro vascular invasion before liver transplantation for HCC. Liver transplantation for patients within Milan criteria markedly decrease incidence of pathologically evident MVI with expected lower incidence of recurrence. Liver biopsy at the time of ablation in high risk patients for recurrence may be beneficial and need to be studied.

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