Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Impact of incidentally found hepatocellular carcinoma on the outcome of living donor liver transplantation

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Keywords
hepatocellular carcinoma, incidental tumor, live donor, liver transplantation, microvascular invasion, Milan criteria.

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Received: 6 March 2006
Revision requested: 14 April 2006
Accepted: 18 April 2006

doi:10.1111/j.1432-2277.2006.00338.x

Summary

Hepatocellular carcinoma (HCC) nodules newly found in the explant liver have been observed, but the impact on patient prognosis is not known. Sixty HCC patients who underwent living donor liver transplantation were the subjects of the study. Radiologic findings prior to transplantation and pathologic findings of the explant liver were compared. Histologic characteristics of preoperatively overlooked tumors were examined. The influence of the discrepancy between these findings on tumor recurrence was evaluated. A total of 227 HCC nodules were found in the explant livers. Of these, 91 nodules (40%) were newly found by pathologic examination. They were smaller and more likely to be well differentiated than the others. The number and size of the tumors were underestimated in 50% (30/60) and 32% (19/60), respectively. There was no significant difference in the recurrence-free survival rate between patients who met the Milan criteria both in the pre- and post-transplant evaluation ($n = 29$) and those who met the Milan criteria preoperatively, but exceeded the criteria in the explant ($n = 19$). Nodules newly found in the explant liver had little impact on recurrence-free survival. A decision for liver transplantation according to the Milan criteria based on preoperative evaluation is valuable for securing an excellent outcome.

Introduction

Deceased donor liver transplantation (DDLT) is established as an effective treatment for hepatocellular carcinoma (HCC) [1–3]. Progression of the tumor before transplantation, however, strongly affects outcome. It has become accepted that DDLT should only be performed in selected cases [4,5]. Currently, patient selection according to the Milan criteria [6] is applied world-wide with excellent results [5,7,8].

The existence of incidental HCCs found in the explant liver, but overlooked during the pretransplant evaluation, has been observed and recognized as not adversely affecting the outcome of transplantation [9–13]. Recently, HCC has become a major indication for living donor liver transplantation (LDLT) because the risk of dropout or tumor progression while waiting for an available organ is negligible in LDLT [14–16]. The existence of incidental HCC, however, has also become evident, even in this population which undergoes scheduled transplant surgery after strict preoperative evaluation with a shorter waiting period. The histologic nature of such incidental HCC and its effect on prognosis after LDLT, however, has not yet been described in detail. We retrospectively analyzed the discrepancy between pre- and post-transplantation tumor evaluation in our series and report the results herein.

Patients and methods

Patients

A total of 68 patients underwent LDLT for HCC either as a primary or secondary indication with decompensated liver cirrhosis at our center between June 1998 and

December 2004, including 13 cases that exceeded the Milan criteria. Of these, two patients with more than 10 tumors and six patients with only completely necrotic tumors in the explanted liver were excluded, and the other 60 patients were the subjects of the study. Mean and SD of the follow-up period was 31 ± 20 months.

The etiology of liver cirrhosis was hepatitis C virus-related in 38 (63%), hepatitis B virus-related in 14 (23%), alcoholism in three (5%), primary biliary cirrhosis in two (3%), cryptogenic in two (3%), and hepatitis B and C virus-related in one (2%). The mean and SD of the Child-Pugh score was 9.6 ± 1.9. Twenty-two (38%) and 31 (53%) patients were classified as Child-Pugh B and C, respectively.

Preoperative therapy for HCC included percutaneous ethanol injection therapy or radio-frequency ablation in 29 patients and transarterial chemoembolization in 28, and both in seven patients.

Preoperative evaluation of HCC

The preoperative diagnosis of HCC was based on helical computed tomography (CT) of multi-phase dynamic study with contrast enhancement taken within 1 month before LDLT. Triple-phase contiguous CT scans with 7-mm-thick sections were obtained. First, nonenhanced CT scans were obtained. Early (arterial)-phase CT scans were obtained 30 s after the initiation of the bolus injection of 100 ml of 65% iopamidol; late (portal)-phase scans were obtained 120 s after the initiation of the injection.

The lesions with typical radiologic characteristics or classical HCC, i.e. stained in the arterial phase and detected as low-density lesion in the portal phase, were diagnosed as HCC. The lesions with early staining and not low in the portal phase or low-density lesions in the portal phase without enhancement in the arterial phase were diagnosed as regenerative or dysplastic nodules.

Evaluation of the explant liver

Immediately following explantation of the diseased liver, the location of each tumor identified prior to transplantation was confirmed by *ex situ* hepatic ultrasound (SS-6500; Aloka Co., Tokyo, Japan) at the side table in the operation theater. Tumors newly detected at this stage were recorded and assigned for further study. The liver was then sliced approximately 1-cm thick along the axial plane to check for tumors on the cut surface. Pathologic features, including histologic differentiation, capsular formation, growth pattern (expansive or infiltrative), intrahepatic metastasis, and microvascular invasion were evaluated.

Comparison of pre- and post-transplant staging

Comparison of the number and size of HCC nodules recognized by preoperative radiologic evaluation and pathologic analysis of the explant liver were performed in each case. According to the discrepancy between the pre- and post-transplant findings concerning the number of tumors, the patients were classified into the following three groups: NU, the number of tumors was underestimated; NO, the number was overestimated; NC, the number was correctly diagnosed.

According to the discrepancy in tumor size, the patients were classified into three groups: SU, the size of the tumor was underestimated; SO, the size was overestimated; SC, the size was correctly diagnosed. The over- or underestimation of tumor size was defined as a mismatch of the tumor diameter of 5 mm or more in diameter between the preoperative and postoperative diagnosis.

Follow-up of the patient after transplantation

All patients were followed-up at our department after transplantation according to the following protocol; monthly measurement of alpha-feto protein and protein-induced-by-vitamin-K absence II, abdominal ultrasound performed every 3 months, and dynamic CT every 6 months. Recurrence was defined as the emergence of radiologic findings compatible with classical HCC.

To assess the influence of the discrepancy between pre- and post-transplantation evaluation on recurrence-free survival, patients were stratified into four groups as follows: M, those who satisfied the Milan criteria in both the pre- and post-transplant evaluation; E1, those with extra-Milan HCC in both pre- and postoperative evaluations; E2, those who satisfied the Milan criteria preoperatively, but had HCCs in the explant exceeding the criteria. In addition, patients' preoperative profiles and pathologic tumor characteristics were analyzed to specify the risk factors of tumor recurrence.

Statistics

Each value was expressed as mean and SD. Statistical comparisons were made by Wilcoxon signed rank test for quantitative variables and Pearson's chi-squared test or Fisher's exact test for qualitative variables. Recurrence-free survival was estimated using the Kaplan–Meyer method and compared by log-rank test. Multivariate analysis for the risk factors of tumor recurrence was performed by logistic regression test. A P-value <0.05 was considered to be statistically significant.

Results

Discrepancy between pre- and post-transplantation evaluation

The NC, NO, and NU consisted of 27, 3, and 30 cases, respectively (Table 1). There were no statistical differences in age, sex, or severity of cirrhosis as represented by the Child-Pugh score. When treatment modalities against HCC prior to transplantation were analyzed, however, percutaneous ethanol injection therapy or radio-frequency ablation were performed more frequently in NU patients, although transarterial chemoembolization seemed to have little influence over such discrepancies. In contrast, 26, 15, and 19 patients were classified as group SC, SO, and SU, respectively.

Histologic grade of incidentally found HCCs

A total of 227 HCC nodules were found in the explant livers. Of these, 91 nodules (40%) were newly found by pathologic examination (Table 2). None were poorly differentiated. Among them, 15 were preoperatively detected, but diagnosed as dysplastic nodules.

Table 1. Profiles of the patients stratified by the tumor numbers.

Groups	NC ($n = 27$)	NO ($n = 3$)	NU ($n = 30$)	*P*-value*
Age	54 ± 6.7	57 ± 4.5	55 ± 5.4	0.86
Men/women	20/7	1/2	27/3	0.10
Child-Pugh class (A/B/C)	1/9/17	2/1/0	2/13/15	0.80†
Preoperative treatment				
TACE	8	2	12	0.79
PEIT/RFA	4	1	14	0.03
Both	1	0	6	0.10

TACE, transarterial chemoembolization; PEIT, percutaneous ethanol injection therapy; RFA, radio-frequency ablation; NU, the number of tumors was underestimated; NO, the number was overestimated; NC, the number was correctly diagnosed.
*Comparison between NC + NO and NU.
†Comparison between Child A + B and C.

Table 2. Differentiation of preoperatively detected or undetected tumors.

Preoperative diagnosis	Hepatocellular carcinoma ($n = 136$)	Benign nodule ($n = 15$)	Undetected ($n = 76$)
Well	52	12	43
Moderate	45	3	14
Poor	3	0	0
Combined*	1	0	0
Necrosis	35	0	19

*Hepatocellular carcinoma and cholangiocellular carcinoma.

Table 3. Histological characters of hepatocellular carcinoma nodules.

Preoperative diagnosis	Hepatocellular carcinoma	Benign nodule or undetected	*P*-value
n	101	72	
Size	2.1 ± 1.4	1.1 ± 0.5	<0.0001
Well differentiated	52 (51%)	55 (76%)	0.002
Vascular invasion	15 (15%)	2 (3%)	0.009
Capsular formation	60 (59%)	27 (38%)	0.007
Fc-inf*	34 (34%)	8 (11%)	0.001
Ig†	22 (22%)	15 (21%)	>0.9999
Im‡	7 (7%)	0	0.04

*Tumor infiltration to the capsule.
†Infiltrative growth pattern.
‡Intrahepatic metastasis.

Of the 227 HCC nodules, 54 nodules could not be histologically analyzed because they were essentially necrotic (Table 3). The morphologic characteristics of the other 173 HCCs indicated that the preoperatively undetected HCCs were significantly smaller and more likely to be well differentiated. A total of 47 nodules were 1 cm or less in diameter and 30 (64%) of them were preoperatively undetected.

Analysis of recurrence-free survival

Groups M, E1, and E2 consisted of 29, 12, and 19 patients, respectively. Group E2 included 11 number underestimation, four number underestimation and microvascular invasion, three microvascular invasion (both number and size were correctly estimated), and one underestimation in both number and size. Recurrence occurred in three patients in group E1 at 3, 6, and 14 months after LDLT. In group E2, two patients presented with recurrent HCC at 9 and 16 months after LDLT. No recurrence occurred in group M patients. There was a statistically significant difference between M and E1. There were no significant differences, however, between groups M and E2 (Fig. 1).

An alpha-feto protein level over 300 ng/ml and poorly differentiated HCC negatively affected tumor-free survival rate (Table 4). Multivariate analysis revealed that poorly differentiated HCC was a significant risk factor for recurrence (Table 5).

Discussion

Several articles emphasize the inconsistency of radiologic and pathologic estimation of HCC in cirrhotic liver [8,10,17–19]. These studies revealed that 7–60% of HCC are undetected preoperatively, although most of the reports did not discuss the influence of undetected HCC on exceeding the Milan criteria or on tumor recurrence rate in the context of liver transplantation. Our results indicate

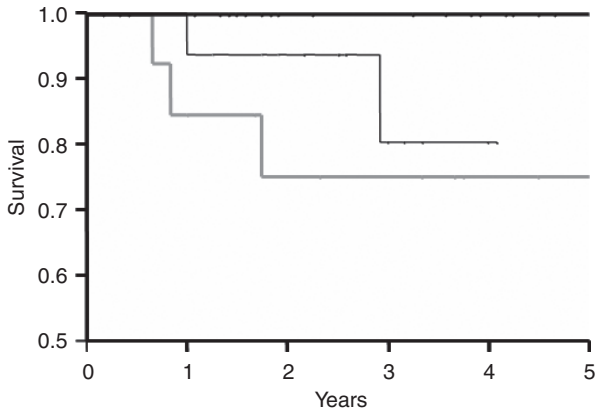

Figure 1 Recurrence-free survival of each group. There was no tumor recurrence during the follow-up period in patients of group M. Significant difference was seen between group M and group E1. Thick line, group M; thin gray line, group E1; thin black line, group E2.

that 40% of HCCs were missed or underestimated as dysplasia or adenomatous hyperplasia. This might be due, at least in part, to preoperative local treatment such as radio-frequency ablation or percutaneous ethanol injection therapy. These results are similar to another report showing the low sensitivity of CT (36%) for detecting residual or recurrence after radio-frequency ablation [20]. Sotiropoulos *et al.* [21] reported that the sensitivity of radiologic detection of HCC of 1–2 cm and <1 cm in diameter was only 21% and 0%, respectively. Zancherl *et al.* [22] described that preoperative CT was inferior in staging HCC in cirrhosis versus intraoperative ultrasonography.

The present analysis indicated that even if patients with extra-Milan criteria were falsely estimated to satisfy Milan criteria preoperatively, their tumor-free survival rate does not significantly change compared with patients who fulfill the criteria. Similar to that were reported by Ravaioli *et al.* [8]. In their analysis of 63 HCC patients who survived more than 1 year after liver transplantation, the rate of exceeding the Milan criteria as assessed by postoperative staging (29%) was significantly higher than that assessed by preoperative staging (13%) because of small nodules undetected preoperatively. Comparison of the recurrence and survival rates between patients meeting and not meeting the Milan criteria, however, revealed a significant difference only in the preoperative staging and not in the postoperative staging. This might be due to the preoperatively undetected nodules being histologically less aggressive. In the present analysis, in 11 of the 19 Group E2 patients, number of the tumors was underestimated. That is, the tumor size underestimation had little influence on the prognosis.

Multivariate analysis revealed that only poorly differentiated HCC was a significant risk factor for tumor recurrence. Unexpectedly, vascular invasion or intrahepatic metastasis, which are recognized as risk factors of tumor recurrence in several previous reports [7,17,23,24], did not significantly affect recurrence rate in our study. This discrepancy might be partly due to the small number of episodes of HCC recurrence. In our five cases of HCC recurrence, there was microvascular invasion in only two.

Table 4. Recurrence-free survival.

Factors	No	Recurrence-free survival (%) 1 year	3 year	*P*-value
Age				
≥60	12	91	91	0.97
<60	48	96	91	
Sex				
Men	48	96	91	>0.9999
Women	12	91	91	
Preoperative RFA/PEIT				
Yes	19	90	84	0.20
No	41	95	95	
Preoperative TACE				
Yes	22	95	90	0.90
No	38	94	91	
AFP*				
≥300 ng/ml	12	83	74	0.02
<300 ng/ml	48	98	95	
PIVKA II†				
≥200 mAu/ml	7	100	80	0.54
<200 mAu/ml	53	94	92	
Maximum tumor size				
≥5 cm	5	80	80	0.26
<5 cm	55	96	92	
Tumor numbers				
≥5	9	89	89	0.78
<5	51	96	91	
Histological differentiation*				
Well/moderate	50	100	95	<0.0001
Poor	3	33	33	
Vascular invasion				
Yes	14	100	85	0.45
No	46	93	93	
Intrahapteic metastasis				
Yes	6	100	100	0.47
No	54	94	90	

AFP, alpha-feto protein; PIVKA II, protein-induced-by-vitamin-K-deficiency II; TACE, transarterial chemoembolization; PEIT, percutaneous ethanol injection therapy; RFA, radio-frequency ablation.

Table 5. Multivariate analysis for the risk factors of tumor recurrence.

Factors	Odds ratio	95% CI	*P*-value
AFP (≥300 ng/ml vs. <300 ng/ml)	5.63	−0.96 to 4.93	0.2
Histological differentiation (poor versus well/moderate)	56.94	1.20–7.78	0.0097

AFP, alpha-feto protein; CI, confidential interval.

Of 14 cases with microvascular invasion in the explanted liver, 10 survived without recurrence for 8–45 months. Two other patients with macroscopic vascular invasion survived 19 and 60 months without recurrence.

In conclusion, the present analysis indicates that nodules newly found in the explant liver had little impact on recurrence-free survival. A decision for LDLT according to the Milan criteria based on preoperative evaluation is valuable for securing an excellent outcome.

Acknowledgements

This work was supported by a grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and grants-in-aid for Research on HIV/AIDS, a multicenter pilot clinical study to compare the safety and efficacy of a steroid-free immunosuppression protocol with monoclonal anti-IL2R antibody in HCV-positive living donor liver transplantation and Research on Measures for Intractable Diseases from the Ministry of Health, Labor and Welfare of Japan.

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References

1. Michael J, Suc B, Montpeyroux F, *et al.* Liver transplantation for hetatocellular carcinoma? Retrospecutive analysis of 215 patients with cirrhosis. *J Hepatol* 1997; **26**: 1274.
2. Figueras J, Jaurrieta E, Valls C, *et al.* Survival after liver transplantation in cirrhotic patients with and without hepatocellular carcinoma: a comparative study. *Hepatology* 1997; **25**: 1485.
3. Figueras J, Jaurrieta E, Valls C, *et al.* Resection or transplantation for hepatocellular carcinoma in cirrhotic patients: outcomes based on indicated treatment strategy. *J Am Coll Surg* 2000; **190**: 580.
4. 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.
5. Esnaola NF, Lauwers GY, Mirza NQ, *et al.* Predictors of microvascular invasion in patients with hepatocellular carcinoma who are candidates for orthotopic liver transplantation. *J Gastrointest Surg* 2002; **6**: 224.
6. Mazzaferro V, Regalia E, Doci R, *et al.* Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693.
7. Jonas S, Bechstein WO, Steinmuller T, *et al.* Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. *Hepatology* 2001; **33**: 1080.
8. Ravaioli M, Ercolani G, Cescon M, *et al.* Liver transplantation for hepatocellular carcinoma: further considerations on selection criteria. *Liver Transpl* 2004; **10**: 1195.
9. Klintmalm GB. Liver transplantation for hepatocellular carcinoma: a registry report of the impact of tumor characteristics on outcome. *Ann Surg* 1998; **228**: 479.
10. Adham M, Oussoultzoglou E, Ducerf C, *et al.* Results of orthotopic liver transplantation for liver cirrhosis in the presence of incidental and/or undetected hepatocellular carcinoma and tumour characteristics. *Transpl Int* 1998; **11**(Suppl. 1): S197.
11. 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.
12. Chui AK, Rao AR, McCaughan GW, *et al.* An active liver transplant programme for hepatocelluar carcinoma in cirrhotic patients: is it justified? *Clin Transplant* 1999; **13**: 531.
13. Hemming AW, Cattral MS, Reed AI, *et al.* Liver transplantation for hepatocellular carcinoma. *Ann Surg* 2001; **233**: 652.
14. 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.
15. Kaihara S, Kiuchi T, Ueda M, *et al.* Living-donor liver transplantation for hepatocellular carcinoma. *Transplantation* 2003; **75**: S37.
16. Fuster J, Charco R, Llovet JM, Bruix J, Garcia-Valdecasas JC. Liver transplantation in hepatocellular carcinoma. *Transpl Int* 2005; **18**: 278.
17. Bhattacharjya S, Bhattacharjya T, Quaglia A, *et al.* 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.
18. Ferrel L, Wright T, Lake J, Roberts J, Ascher N. Incidence and diagnostic features of macrogenerative nodules vs small hepatocellular carcinoma in cirrhotic livers. *Hepatology* 1992; **16**: 1372.
19. Terada T, Terasaki S, Nakamura Y. A clinicopathologic study of adenomatous hyperplasia of the liver in 209 consecutive cirrhotic livers examined by autopsy. *Cancer* 1993; **72**: 1551.
20. Lu DS, Yu NC, Raman SS, *et al.* Radiofrequency ablation of hepatocellular carcinoma: treatment success as defined by histologic examination of the explanted liver. *Radiology* 2005; **234**: 954.
21. Sotiropoulos GC, Malagó M, Molmenti E, *et al.* Liver transplantation for hepatocellular carcinoma in cirrhosis: is clinical tumor classification before transplantation realistic? *Transplantation* 2005; **79**: 483.
22. Zacherl J, Pokieser P, Wrba F, *et al.* Accuracy of multiphasic helical computed tomography and intraoperative

sonography in patients undergoing orthotopic liver transplantation for hepatoma: what is the truth? *Ann Surg* 2002; **235**: 528.
23. Figueras J, Jaurrieta E, Valls C, *et al.* Survival after liver transplantation with and without hepatocellular carcinoma: a comparative study. *Hepatology* 1997; **25**: 1485.
24. De Carlis L, Giacomoni A, Lauterio A, *et al.* Liver transplantation for hepatocellular cancer: should the current indication criteria be changed? *Transpl Int* 2003; **16**: 115.