で低かった．今後の検討課題である．

われわれは肝細胞癌患者の約半数が，このIFN併用5FU動注化学療法に感受性をもっていると考えているが，血小板数12万未満，門脈浸潤がVP3に留まるもの，総ビリルビンが正常の3要素を満たす症例では，奏功率が高かった．これらの理由に関しても，とくに血小板数が低いほうが奏功率が高い理由も今後解明していかなければならない．

本研究では幸いにも重篤な副作用はほとんどなく，すべての副作用は対症療法でコントロール可能であった．以上より，門脈腫瘍塞栓を伴った進行肝細胞癌に対して，腫瘍マーカーを含めた注意深い治療効果観察のもとで，このIFN併用5FU動注化学療法を行うことを推奨する．もし1クール終了後，治療効果が認められれば，併用療法を継続すべきだろう．

おわりに

われわれはIFNα併用の5FU動注化学療法が，門脈浸潤を合併した進行肝細胞癌患者に効果があることを示した．

文　献

1) Wadler S, Schwartz EL, Goldman M, et al. Fluorouracil and recombinant alfa-2a-interferon : an active regimen against advanced colorectal carcinoma. J Clin Oncol 1989 ; 7 : 1769-75.
2) Patt YZ, Yoffe B, Charnsangavej C, et al. Low serum alpha-fetoprotein level in patients with hepatocellular carcinoma as a predictor of response to 5-FU and interferon-alpha-2b. Cancer 1993 ; 72 : 2574-82.
3) Urabe T, Kaneko S, Matsushita E, et al. Clinical pilot study of intrahepatic arterial chemotherapy with methotrexate, 5-fluorourasil, cisplatin, and subcutaneous interferon-alpha-2b for patients with locally advanced hepatocellular carcinoma. Oncology 1998 ; 55 : 39-47.
4) Sakon M, Nagano H, Dono K, et al. Combined Intraarterial 5-Fluorouracil and Subcutaneous Interferon-alpha therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. Cancer 2002 ; 94 : 435-42.
5) Obi S, Yoshida H, Toune R, et al. Combination therapy of intraarterial 5-fluorouracil and systemic interferon-alpha for advanced hepatocellular carcinoma with portal venous invasion. Cancer 2006 ; 106 : 1990-7.
6) Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982 ; 5 : 649-55.
7) Llovet JM, Sala M, Castells L, et al. Randomized controlled trial of interferon treatment for advanced hepatocellular carcinoma. Hepatology 2000 ; 31 : 54-8.
8) Takaoka A, Hayakawa S, Yanai H, et al. Integration of interferon-alpha/beta signally to P53 responsis in tumor suppression and antiviral defense. Nature 2003 ; 424 : 516-23.
9) Moriyama M, Hoshida Y, Kato N, et al. Genes associated with human hepatocellular carcinoma cell chemosensitivity to 5-fluorouracil plus interferon-alpha combination chemotherapy. Int J Oncol 2004 ; 25 : 1279-87.

*　　*　　*

Journal of Surgical Oncology 2007;95:311–316

Clinicopathologic Features of Poorly Differentiated Hepatocellular Carcinoma

KOICHI OISHI, MD,[1]* TOSHIYUKI ITAMOTO, MD,[1] HIRONOBU AMANO, MD,[1] SABURO FUKUDA, MD,[1] HIDEKI OHDAN, MD,[1] HIROTAKA TASHIRO, MD,[1] FUMIO SHIMAMOTO, MD,[2] AND TOSHIMASA ASAHARA, MD[1]

[1]*Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan*

[2]*Department of Health Sciences, Faculty of Human Culture and Science, Prefectural University of Hiroshima*

Background and Objectives: Clinicopathologic features of poorly differentiated hepatocellular carcinoma (HCC) have not been elucidated. The purpose of this study was to clarify the characteristics of poorly differentiated HCC in hepatectomized patients.

Methods: From 1986 to 2001, 354 HCC patients underwent curative hepatectomy in our institution and were prospectively followed. Histological examinations revealed 43 well-differentiated HCC tumors, 273 moderately differentiated HCC tumors, and 38 poorly differentiated HCC tumors. Clinicopathologic factors and outcomes after hepatectomy were compared statistically.

Results: Only serum alpha-fetoprotein level was significantly different in the poorly differentiated HCC group from that in the moderately differentiated HCC group preoperatively ($P = 0.0001$). Although there were no significant differences between overall survival rates or between disease-free survival rates in the three groups, distant metastasis within 2 years after hepatectomy occurred more frequently in the poorly differentiated HCC group (21%) than in the well-differentiated HCC group (2%) ($P = 0.011$) or moderately differentiated HCC group (8%) ($P = 0.018$). Distant metastasis occurred in about 40% of patients in the poorly differentiated HCC group with tumor size greater than 3 cm.

Conclusions: Poorly differentiated HCC tumors larger than 3 cm are already of advanced stage representing distant metastasis in the early period after hepatectomy.

J. Surg. Oncol. 2007;95:311–316. © 2007 Wiley-Liss, Inc.

KEY WORDS: **hepatocellular carcinoma; hepatectomy; prognostic factor; tumor differentiation; liver transplantation**

INTRODUCTION

Hepatocellular carcinoma (HCC) is the one of most common malignancies worldwide. There are various options for treatment for HCC, including partial hepatectomy, percutaneous ablation therapy, and transcatheter arterial chemoembolization (TACE). However, the prognosis of HCC remains unsatisfactory despite technical refinements in these treatments [1–3]. Recently, orthotopic liver transplantation (OLT) has been established as an alternative therapy for unresectable small HCC tumors (5 cm) in patients with decompensated cirrhosis, with survival rates similar to those of patients receiving OLT for non-malignant disease [4,5]. It has been proposed that there should be an expansion of the selection criteria for HCC patients [6–8]. Some authors have emphasized that poorly differentiated HCC should be a contraindication

No competing interests was declared.

*Correspondence to: Koichi Oishi, MD, Department of Surgery, Division of Frontier Medical Science, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Fax: +81-82-257-5224. E-mail: koishi@hiroshima-u.ac.jp

Received 22 March 2006; Accepted 27 July 2006

DOI 10.1002/jso.20661

Published online in Wiley InterScience (www.interscience.wiley.com).

of OLT because of the high recurrence rate after OLT [9–14]. However, it is impossible to discriminate poorly differentiated HCC from well-differentiated or moderately differentiated HCC before treatment, and there have been only a few reports on the behavior and impact on survival of poorly differentiated HCC without surgical treatment [15]. To clarify the clinicopathologic characteristics of poorly differentiated HCC, we investigated the prognostic factors of patients with poorly differentiated HCC who underwent partial hepatectomy in comparison with those of patients with well-differentiated or moderately differentiated HCC.

METHODS

Between April 1986 and December 2001, 519 consecutive HCC patients underwent partial hepatectomy in our institution. Of those 519 patients, 405 patients underwent a curative hepatectomy, defined as removal of all recognizable HCC tumors. Twenty-four patients were excluded from this study because of uncertain pathological diagnosis of tumor differentiation due to entire necrosis of the tumor induced by TACE. Twelve patients who died of illness unrelated to liver disease within 2 years after hepatectomy and 14 patients whose outcomes during the follow-up period were uncertain were also excluded from this study. One patient was diagnosed as having undifferentiated HCC and was excluded from this study. The remaining 354 HCC patients were enrolled in this study. The patients included 273 men (77%) and 81 women (23%). The median age at operation was 61.9 ± 9.0 years (range, 31 to 83 years). Clinicopathologic findings were recorded according to criteria of the Liver Cancer Study Group in Japan [16]. Histological examinations of resected specimens sampled from the entire tumor were performed by an experienced pathologist who did not know the outcomes of patients. Histological grading of HCC was classified into three categories, well-, moderately, and poorly differentiated HCC, according to the classification of the Liver Cancer Study of Japan [16]. Well-differentiated HCC corresponds to grade I or to grade II with a thin trabecular pattern of Edmondson's classification [17]. Moderately differentiated HCC corresponds to grade II or to grade III with a clear trabecular pattern of Edmondson's classification. Poorly differentiated HCC corresponds to grade III with an indistinct trabecular pattern or to grade IV of Edmondson's classification. In the case of a mixed pattern of various grades of differentiation, the dominant grade was noted. Forty-three patients were diagnosed as having well-differentiated HCC (W group), 273 patients were diagnosed as having moderately differentiated HCC (M group), and 38 patients were diagnosed as having poorly differentiated HCC (P group). Hepatectomies were considered major when they involved two or more Couinaus' segments [18].

Follow-up evaluation after the operation consisted of clinical physical examinations, blood chemistry tests, and measurements of levels of tumor markers, including alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin, every month for 2 years. After 2 years, the patients were assessed every 3 months. Patients were examined by abdominal ultrasonography every 3 months and by computed tomographic scan every 6 months during the follow-up periods. When recurrence was indicated by any of these examinations, patients underwent hepatic angiography.

Parametric analyses were performed using Student's *t*-test, and non-parametric analyses were performed using the Fisher's exact test. Survival and disease-free survival rates were calculated using the Kaplan–Meier method and compared using the log-rank test. Disease-free survival was calculated by considering any death or recurrence as an event. A *P*-value of less than 0.05 was considered to be statistically significant. Statistical analysis was carried out using the software of StatView for Windows (Version 5.0; SAS Institute, Inc., Cary, NC).

RESULTS

The median follow-up period for survivors was 63.1 ± 35.6 months (range 24.0–204.0 months). Operative mortality and in-hospital mortality rates were 0.8% and 1.4%, respectively. Patient, tumor, and treatment characteristics in the three groups are shown in Table I. There were no significant differences between the M group and P group except for serum AFP levels. Twenty (53%) of the 38 patients in the P group had preoperative serum AFP levels of more than 400 ng/ml, whereas only 59 (22%) of the 273 patients in the M group had preoperative serum AFP levels of more than 400 ng/ml. The difference was significant ($P = 0.0001$). The P group had significantly better liver function than the W group did (Child-Pugh grade A: 92% vs. 67%) ($P = 0.0071$). Mean tumor size in the P group was significantly larger than that in the W group ($P < 0.0001$), and the frequencies of capsule formation and microscopic vascular invasion in the P group were significantly higher than those in the W group ($P = 0.0145$ and $P = 0.0011$, respectively). The percentage of patients in the P group with serum AFP levels over 400 ng/ml was significantly higher than that in the W group ($P < 0.0001$). Major hepatectomy was performed more frequently in the P group than in the W group ($P < 0.0001$). The numbers of HCC recurrences or new HCC appearance in the liver in patients in the W group, M group, and P group were 27 (62.8%), 171 (62.6%), and 24 (63.2%), respectively. Repeated surgery, transcatheter hepatic artery treatment,

TABLE I. Comparison of Backgrounds of HCC Patients Who Underwent Partial Hepatectomy in Three Groups Based on Degree of Tumor Differentiation

Characteristics	Group			P-value	
	W (n = 43)	M (n = 273)	P (n = 38)	W versus P	M versus P
Sex (% Male)	30/43 (70)	214/273 (78)	29/38 (76)	N.S.	N.S.
Age (years)[a]	61.7 ± 8.8	61.7 ± 9.3	63.7 ± 7.6	N.S.	N.S.
HBsAg-positive (%)	10/42 (24)	68/268 (25)	6/38 (16)	N.S.	N.S.
Anti-HCV-positive (%)	28/37 (76)	160/245 (65)	25/34 (74)	N.S.	N.S.
Live cirrhosis (%)	32/43 (74)	161/273 (59)	22/38 (58)	N.S.	N.S.
Child-Pugh grade A (%)	29/43 (67)	228/273 (84)	35/38 (92)	0.0071	N.S.
Mean tumor size (cm)[a]	1.83 ± 0.64	3.98 ± 2.71	4.20 ± 3.18	<0.0001	N.S.
Number of tumors (% multiple)	9/43 (21)	72/273 (23)	14/38 (37)	N.S.	N.S.
Distribution of tumors (% unilobar)	37/43 (86)	253/273 (93)	32/38 (84)	N.S.	N.S.
Capsule formation (% positive)	25/43 (58)	232/273 (85)	32/38 (84)	0.0145	N.S.
Microsocopic vascular invasion (% positive)	5/43 (12)	119/273 (44)	17/38 (45)	0.0011	N.S.
Serum AFP (ng/mL) (>400)	3/43 (7)	59/273 (22)	20/38 (53)	<0.0001	0.0001
Preoperative TAE (% yes)	27/43 (63)	206/273 (75)	31/38 (82)	N.S.	N.S.
Operative procedure (% major hepatectomy)	0/43 (0)	59/273 (22)	11/38 (29)	<0.0001	N.S.

[a]Values are means ± standard deviations.
HBsAg, hepatitis B surface antigen; anti-HCV, anti-hepatitis C virus antibody; AFP, alpha-fetoprotein; TAE, transcatheter arterial embolization; W, well-differentiated hepatocellular carcinoma; M, moderately differentiated hepatocellular carcinoma; P, poorly differentiated hepatocellular carcinoma; N.S., not significant.

and percutaneous ablation therapy were required for 7, 7, and 4 patients, respectively, in the P group, for 7, 7, and 4 patients, respectively, in the W group and for 30, 56, and 38 patients, respectively, in the M group. Two patients in the M group required living donor liver transplantation.

Survival rates of patients in the P group at 2, 5, and 10 years were 71%, 46%, and 39%, respectively, whereas those in the W group were 84%, 69%, and 42%, respectively, and those in the M group were 82%, 54%, and 27%, respectively (Fig. 1). Disease-free survival rates in the P group at 2, 5, and 10 years were 50%, 25%, and 17%, respectively, whereas those in the W group were 70%, 27%, and 0%, respectively, and those in the M group were 52%, 23% and 13%, respectively (Fig. 2). There were no significant differences among these three groups in survival rates or disease-free survival rates.

Cancer death within 2 years after hepatectomy occurred more frequently in the P group than in the W group ($P = 0.1916$) or M group ($P = 0.1165$), but the differences were not significant. Distant metastases within 2 years after hepatectomy occurred in 21% of the patients in the P group but in only 2% of the patients in the W group and in only 8% of the patients in the M

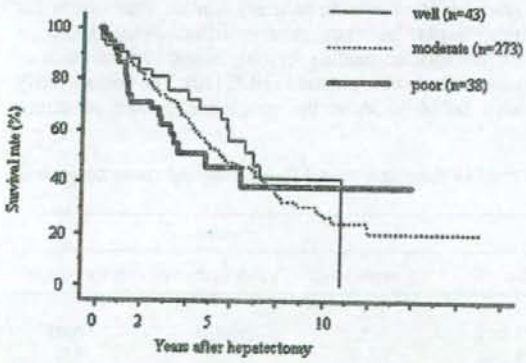

Fig. 1. Survival rates stratified by tumor differentiation.

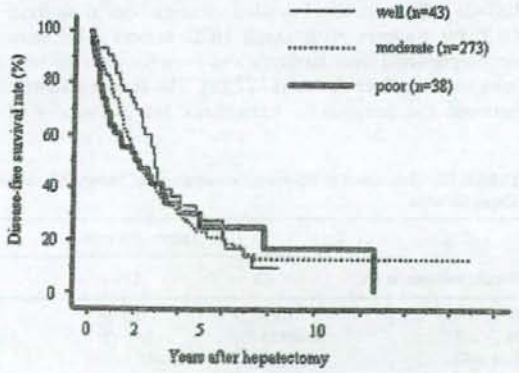

Fig. 2. Disease-free survival rates stratified by tumor differentiation.

TABLE II. Comparison of the Incidences of Cancer Death and Distant Metastasis Within 2 Years After Partial Hepatectomy in Three Groups Based on Degree of Tumor Differentiation

Events that occurred within 2 years after partial hepatectomy	Group			P-value	
	W (n = 43)	M (n = 273)	P (n = 38)	W versus P	M versus P
Cancer death (%)	7/43 (16)	47/273 (17)	11/38 (29)	N.S.	N.S.
Distant metastasis (%)	1/43 (2)	22/273 (8)	8/38 (21)	0.0108	0.0184

W, well-diferentiated hepatocellular carcinoma; M, moderately differentiated hepatocellular carcinoma; P, poorly differentiated hepatocellular carcinoma; N.S., not significant.

group. The differences between the P group and W group ($P = 0.0108$) and between the P group and M group ($P = 0.0184$) were significant (Table II).

The most frequent site of distant metastasis that occurred within 2 years after partial hepatectomy was the lung in both the M group and P group. However, there was no specific site of distant metastasis according to degree of tumor differentiation.

Analysis of the 14 variables shown in Table I showed tumor diameter to be the only significant factor in the rate of distant metastasis within 2 years after hepatectomy in the P group. Distant metastasis was rare in the W group and M group when tumor diameter was smaller than 5 cm, whereas 36% of the patients in the P group with tumor size between 3.1 cm and 5 cm and 38% of the patients in the P group with tumor size larger than 5 cm had distant metastasis (Table III).

DISCUSSION

Although advances in surgical techniques and perioperative management have made hepatic resection a safe procedure [19] and have made it possible to improve the prognosis of resectable HCC cases, the incidence of recurrence of HCC has remained high and continues to be the main cause of late death. Cumulative recurrence rates in the liver remnant have been reported to be over 75% [20–26]. These results have led some authors to perform OLT for patients with small HCC tumors who have decompensated liver cirrhosis and even for patients with compensated liver cirrhosis [27,28]. The severe disparity between the demand for transplants and the supply of organs from deceased donors has precluded an expansion of the selection criteria to include HCC patients with preserved liver function [29]. In Japan, use of deceased donor organs is rare, and living donor LT has become a common practice in the treatment of HCC patients with decompensated liver cirrhosis [14]. Therefore, the optimal surgical treatment for HCC patients with preserved liver function, hepatic resection or OLT, is controversial.

Heterogeneity of differentiation is a well-known phenomenon in HCC. Some authors believe that the worst area of differentiation in the tumor determines the prognosis. In this study, the dominant tumor differentiation was used in the grading, which is similar to the Gleason scoring system in prostate carcinoma [30]. Recent reports have emphasized that poorly differentiated HCC is a prognostic factor after OLT [9–14,31,32]. However, there have been few reports of poorly differentiated HCC being a crucial adverse prognostic factor in hepatectomized patients [21], despite the fact that the prognostic impacts of clinicopathologic factors on the risk of recurrence after hepatic resection have been extensively studied. Moreover, there have been no reports in which characteristics of poorly differentiated HCC after hepatic resection are described. The degree of tumor differentiation has been shown to have no significant impact on overall survival or recurrence-free survival after hepatic resection in many studies. One reason for this might be that poorly differentiated HCC is uncommon, accounting for only about 10% of cases of resectable or transplantable HCC [10]. The present study also failed to show the prognostic impact of tumor

TABLE III. Relationship Between Occurrences of Distant Metastasis Within 2 Years After Partial Hepatectomy and Tumor Diameter in Three Groups

Distant metastasis (%)	Tumor diameter (cm)			P-value		
	≤3	3.1–5	5<	≤3 versus 3.1–5	3.1–5 versus 5<	≤3 versus 5<
W (n = 43)	1/41 (2)	0/2 (0)		N.S.		
M (n = 273)	4/123 (3)	7/96 (7)	11/54 (20)	N.S.	0.0334	0.0005
P (n = 38)	1/19 (5)	4/11 (36)	3/8 (38)	0.047	N.S.	N.S.

W, well-diferentiated hepatocellular carcinoma; M, moderately differentiated hepatocellular carcinoma; P, poorly differentiated hepatocellular carcinoma; N.S., not significant.

differentiation on either overall survival or recurrence-free survival after hepatic resection. However, it was clarified in the present study that early metastases to distant organs occurred more frequently after curative hepatic resection in patients with poorly differentiated HCC than in patients with well-differentiated or moderately differentiated HCC. In patients with poorly differentiated HCC, about 40% of tumors larger than 3 cm showed aggressive behavior, and the only prognostic factor influencing distant metastasis within 2 years after hepatectomy was tumor size. Moreover, it has become clear that the risk of early metastases to distant organs in patient with poorly differentiated HCC tumors of 3 to 5 cm in diameter is equivalent to that in patients with poorly differentiated HCC tumors of more than 5 cm in diameter. Accordingly, cancer recurrence can occur in the early period after OLT even for patients with HCC meeting the Milan criteria. We should take degree of tumor differentiation into account for deciding the indication of OLT for HCC, even for small HCC meeting the Milan criteria.

Although the precise mechanism of the aggressive behavior of poorly differentiated HCC has not been clarified, one study has shown that reduced expression of E-cadherin is correlated with tumor progression and with advanced histological grade and that the ability of invasion and metastasis is acquired by reduction in cell–cell contact [33]. Various techniques to detect HCC cells in circulating peripheral blood and to predict recurrence after surgical treatment have been developed, and it has been shown that there is a correlation between the presence of cancer cells in peripheral blood and cancer recurrence. A representative method is detection of AFP-mRNA using the reverse transcriptase polymerase chain reaction technique [34,35]. However, this method has problems of low sensitivity and specificity and is considered not to be useful in a clinical setting [36,37]. A new system for the detection of HCC cells with high sensitivity and specificity has recently been reported [38]. This method might enable reliable evaluation of the aggressive behavior of poorly differentiated HCC before surgery.

In the present study, we could not discriminate the clinicopathologic characteristics of poorly differentiated HCC and those of moderately differentiated HCC except for preoperative serum AFP level. In half of the patients with poorly differentiated HCC, serum AFP values were over 400 ng/ml. Although it is impossible to make a strict discrimination of degrees of differentiation before surgery by the use of imaging modalities, a serum AFP value of more than 400 ng/ml might be a useful criterion for predicting histological tumor grade. For accurate histological tumor grading before surgery, it might be necessary to perform a needle biopsy [9]. However, we have not routinely performed needle biopsy because of the risk of cancer seeding [39], especially in cases of poorly differentiated HCC, and because of the probability of an erroneous diagnosis caused by heterogeneity in an HCC tumor.

CONCLUSIONS

Poorly differentiated HCCs, when they reach a size of more than 3 cm, are usually of advanced stage evidenced by frequent distant metastasis in the early period after curative hepatectomy. Tumor differentiation should be taken into account in selecting HCC patients for OLT, even for small HCCs meeting Milan criteria.

REFERENCES

1. Castells A, Bruix J, Bru C, et al.: Treatment of small hepatocellular carcinoma in cirrhotic patients: A cohort study comparing surgical resection and percutaneous ethanol injection. Hepatology 1993;18:1121–1126.
2. Groupe d'Etude et de Traitement du Carcinome Hepatocellulaire: A comparison of lipiodol chemoembolization and conservative treatment for unresectable hepatocellular carcinoma. N Engl J Med 1995;332:1256–1261.
3. Arii S, Yamaoka Y, Futagawa S, et al.: Results of surgical and nonsurgical treatment for small-sized hepatocellular carcinomas: A retrospective and nationwide survey in Japan. The Liver Cancer Study Group of Japan Hepatology 2000;32:1224–1229.
4. 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;334:693–699.
5. 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: 1485–1489.
6. Marsh JW, Dvorchik I, Bonham CA, et al.: Is the pathologic TNM staging system for patients with hepatoma predictive of outcome? Cancer 2000;88:538–543.
7. Yao FY, Ferrell L, Bass NM, et al.: Liver transplantation for hepatocellular carcinoma: Expansion of the tumor size limits does not adversely impact survival. Hepatology 2001;33:1394–1403.
8. Yao FY, Ferrell L, Bass NM, et al.: Liver transplantation for hepatocellular carcinoma: Comparison of the proposed UCSF criteria with the Milan criteria and the Pittsburgh modified TNM criteria. Liver Transpl 2002;8:765–774.
9. Tamura S, Kato T, Berho M, et al.: Impact of histological grade of hepatocellular carcinoma on the outcome of liver transplantation. Arch Surg 2001;136:25–30; discussion 31.
10. De Carlis L, Giacomoni A, Lauterio A, et al.: Liver transplantation for hepatocellular cancer: Should the current indication criteria be changed? Transpl Int 2003;16:115–122.
11. Hemming AW, Cattral MS, Reed AI, et al.: Liver transplantation for hepatocellular carcinoma. Ann Surg 2001;233:652–659.
12. 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;6:224–232; discussion 232.
13. Zavaglia C, De Carlis L, Alberti AB, et al.: Predictors of long-term survival after liver transplantation for hepatocellular carcinoma. Am J Gastroenterol 2005;100:2708–2716.
14. Todo S, Furukawa H: Living donor liver transplantation for adult patients with hepatocellular carcinoma: Experience in Japan. Ann Surg 2004;240:451–459; discussion 459–461.

15. Akamatsu M, Ishikawa T, Shiratori Y, et al.: Factors predisposing to poorly differentiated hepatocellular carcinoma and its recurrence. Hepatogastroenterology 2005;52:391–397.
16. Liver Cancer Study Group of Japan. "Classification of Primary Liver Cancer". Tokyo: Kanehara & Co., 1997.
17. Edmondson HA, Steiner PE: Primary carcinoma of the liver: A study of 100 cases among 48,900 necropsies. Cancer 1954;7: 462–503.
18. Couinaud C: "Le foie: etudes anatomiques et chirurgicales". Paris: Masson, 1957.
19. Itamoto T, Katayama K, Nakahara H, et al.: Autologous blood storage before hepatectomy for hepatocellular carcinoma with underlying liver disease. Br J Surg 2003;90:23–28.
20. Arii S, Tanaka J, Yamazoe Y, et al.: Predictive factors for intrahepatic recurrence of hepatocellular carcinoma after partial hepatectomy. Cancer 1992;69:913–919.
21. Ikeda K, Saitoh S, Tsubota A, et al.: Risk factors for tumor recurrence and prognosis after curative resection of hepatocellular carcinoma. Cancer 1993;71:19–25.
22. Imamura H, Matsuyama Y, Tanaka E, et al.: Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. J Hepatol 2003;38: 200–207.
23. Belghiti J, Panis Y, Farges O, et al.: Intrahepatic recurrence after resection of hepatocellular carcinoma complicating cirrhosis. Ann Surg 1991;214:114–117.
24. Nagasue N, Uchida M, Makino Y, et al.: Incidence and factors associated with intrahepatic recurrence following resection of hepatocellular carcinoma. Gastroenterology 1993;105:488–494.
25. Chen MF, Hwang TL, Jeng LB, et al.: Postoperative recurrence of hepatocellular carcinoma. Two hundred five consecutive patients who underwent hepatic resection in 15 years. Arch Surg 1994; 129:738–742.
26. Balsells J, Charco R, Lazaro JL, et al.: Resection of hepatocellular carcinoma in patients with cirrhosis. Br J Surg 1996;83:758–761.
27. Michel J, Suc B, Montpeyroux F, et al.: Liver resection or transplantation for hepatocellular carcinoma? Retrospective analysis of 215 patients with cirrhosis. J Hepatol 1997;26:1274–1280.
28. Poon RT, Fan ST, Lo CM, et al.: Long-term survival and pattern of recurrence after resection of small hepatocellular carcinoma in patients with preserved liver function: Implications for a strategy of salvage transplantation. Ann Surg 2002;235:373–382.
29. Llovet JM, Fuster J, Bruix J: Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: Resection versus transplantation. Hepatology 1999;30:1434–1440.
30. Gleason DF, Mellinger GT: Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol 1974;111:58–64.
31. Colella G, Rondinara GF, De Carlis L, et al.: Liver transplantation for hepatocellular carcinoma: Prognostic factors associated long-term survival. Transpl Int 1996;9:S109–111.
32. 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;33:1080–1086.
33. Endo K, Ueda T, Ueyama J, et al.: Immunoreactive E-cadherin, alpha-catenin, beta-catenin, and gamma-catenin proteins in hepatocellular carcinoma: Relationships with tumor grade, clinicopathologic parameters, and patients' survival. Hum Pathol 2000;31:558–565.
34. Matsumura M, Niwa Y, Kato N, et al.: Detection of alpha-fetoprotein mRNA, an indicator of hematogenous spreading hepatocellular carcinoma, in the circulation: A possible predictor of metastatic hepatocellular carcinoma. Hepatology 1994;20: 1418–1425.
35. Funaki N, Tanaka J, Seto S, et al.: Highly-sensitive identification of alpha-fetoprotein mRNA in circulating peripheral blood of hepatocellular carcinoma patients. Life Sci 1995;57:1621–1631.
36. Lemoine A, Le Bricon T, Salvucci M, et al.: Prospective evaluation of circulating hepatocytes by alpha-fetoprotein mRNA in humans during liver surgery. Ann Surg 1997;226:43–50.
37. Kienle P, Weitz J, Klaes R, et al.: Detection of isolated disseminated tumor cells in bone marrow and blood samples of patients with hepatocellular carcinoma. Arch Surg 2000;135: 213–218.
38. Waguri N, Suda T, Nomoto M, et al.: Sensitive and specific detection of circulating cancer cells in patients with hepatocellular carcinoma; detection of human telomerase reverse transcriptase messenger RNA after immunomagnetic separation. Clin Cancer Res 2003;9:3004–3011.
39. Takamori R, Wong LL, Dang C, Wong L: Needle-tract implantation from hepatocellular cancer: Is needle biopsy of the liver always necessary? Liver Transpl 2000;6:67–72.

World Journal of Surgery

Published Online: 5 January 2007

World J Surg (2007) 31: 345–352
DOI: 10.1007/s00268-006-0513-7

Clinicopathologic Features of Hepatocellular Carcinoma Patients with Compensated Cirrhosis Surviving More than 10 Years after Curative Hepatectomy

Saburo Fukuda, MD, Toshiyuki Itamoto, MD, Hironobu Amano, MD, Toshihiko Kohashi, MD, Hideki Ohdan, MD, Hirotaka Tashiro, MD, Toshimasa Asahara, MD

Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, 1-2-3, Kasumi, Minami-Ku, Hiroshima, 734-8551, Japan

Abstract

Background: The aim of this study was to clarify the clinicopathologic features of hepatocellular carcinoma (HCC) patients with compensated cirrhosis surviving more than 10 years after initial hepatectomy.

Study Design: Among 250 patients who underwent hepatectomy for HCC between 1987 and 1994 at our institute, 145 patients who had Child-Pugh class A liver function and who underwent curative resection were included in this study. Clinicopathologic factors in 10-year survivors and patients who died within 10 years (nonsurvivors) were compared, and the prognostic factors affecting survival were identified.

Results: There were 29 patients who survived for more than 10 years after initial hepatectomy, and 9 of those patients survived without cancer recurrence. The 3-, 5-, and 10-year survival rates were 76.2%, 53.0%, and 26.9% respectively. The corresponding disease-free survival rates were 43.1%, 25.7%, and 9.9% respectively. In multivariate analysis, liver fibrosis grade F0–2, female gender, ICG-R15 value of less than 15%, and absence of microscopic vascular invasion were favorable independent factors associated with 10-year survival. Disease-free interval after initial hepatectomy in 10-year survivors with recurrence was significantly longer than that in nonsurvivors with recurrence, 5.1 and 1.9 years respectively ($P = 0.0004$). The number of intrahepatic recurrent nodules in 10-year survivors tended to be fewer than that in nonsurvivors.

Conclusions: Based on the results of our study, liver fibrosis grade F0–2, female gender, ICG-R15 value of less than 15% and absence of microscopic vascular invasion at initial hepatectomy might be biologically favorable conditions for long-term survival. Close follow-up as well as multimodal treatment could contribute to prolongation of survival in such patients, even if HCC recurrence develops.

Hepatectomy for hepatocellular carcinoma (HCC) has become a safe procedure in appropriately selected patients with a low operative mortality rate due to improvements in intraoperative techniques and postoperative care.[1] As a result, the short-term prognosis of patients with HCC has improved. However, the long-term prognosis still remains unsatisfactory because of the high incidence of postoperative recurrence.[2,3] Therefore, there

Correspondence to: Saburo Fukuda, MD, e-mail: safukuda@aol.com

are only a few reports on the clinicopathologic features of HCC patients who survived more than 10 years after hepatectomy.[4,5] Elucidation of the predictive factors for long-term survival after curative hepatectomy will be helpful in the choice of optimal management for patients with postoperative HCC recurrence.

In this study, we clarified the clinicopathologic features of HCC patients who survived more than 10 years after initial hepatectomy and identified the prognostic factors affecting survival.

MATERIALS AND METHODS

A total of 250 patients underwent initial hepatectomy for HCC between 1987 and 1994 at our institution. Ninety-one of those patients with Child-Pugh class B liver function or who underwent noncurative resection and 14 patients who were lost to follow-up were excluded. The remaining 145 patients with Child-Pugh class A liver function who underwent curative hepatectomy and were followed for more than 10 years after initial hepatectomy were included in this study. The median duration of follow-up was 4.5 years (range: 0.04–18.2 years). The median age of the patients at the initial hepatectomy was 61 years (range: 36–79 years). Curative resection was defined as complete resection of all macroscopically detectable tumors with histological tumor clearance along the parenchymal transection line.

The clinicopathologic factors were compared in the patients who survived for more than 10 years (10-year survivors, n = 29) and those who died within 10 years (nonsurvivors, n = 116). Host factors included age, gender, status of hepatitis virus infection, serum total bilirubin (T. Bil), albumin (Alb), prothrombin time (PT) activity, platelet count, indocyanine green retention rate at 15 minutes (ICG-R15), histological liver fibrosis, and hepatitis activity of noncancerous liver parenchyma. Hepatitis C virus antibody (HCV Ab) could not be determined because some of the patients underwent operation before hepatitis C serology tests became available. The degree of liver fibrosis and the degree of hepatitis activity were determined using the New Inuyama classification, which is widely used in Japan.[6] Briefly, the fibrosis was graded as follows: F0, no fibrosis; F1, portal fibrosis widening; F2, portal fibrosis widening with bridging fibrosis; F3, bridging fibrosis plus lobular distortion; F4, liver cirrhosis. Hepatitis activity was graded as follows: A1, mild; A2, moderate; A3, severe. Tumor factors included the number of tumors, tumor size in diameter, tumor differentiation, presence of histological vascular invasion (vp), intrahepatic metastasis (im) and preoperative serum level of alpha-fetoprotein (AFP). Treatment factors included preoperative transcatheter arterial chemoembolization (TACE), type of hepatectomy (limited or anatomical resection), status of surgical margin (with less than 5 mm defined histologically as a positive surgical margin), and blood transfusion during the perioperative period.

Follow-up evaluation after hepatectomy consisted of clinical physical examinations, blood chemistry tests, and measurements of levels of tumor markers, including alpha-fetoprotein and des-gamma-carboxy prothrombin, every month for 2 years. After 2 years, the patients were assessed every 3 months. Patients were examined by ultrasonography (US) every 3 months and computed tomography (CT) every 6 months. When recurrence was suspected on the basis of tumor marker elevation, and US and CT findings, angiography was performed for diagnosis of recurrence.

The survival rate and disease-free survival rate were calculated using the Kaplan–Meier method. Comparison was performed using the Chi-squared test with Yates' correction or Fisher's exact test when appropriate in univariate analysis, and all significant factors identified by the univariate analysis were entered into multivariate logistic regression analysis to identify independent factors. All statistical analyses were carried out using the computer software package Statview (SAS Institute, Cary, NC, USA). Differences were considered to be statistically significant if the P value was less than 0.05.

RESULTS

Twenty-nine of the 145 patients who had Child-Pugh class A liver function and had undergone curative resection survived for more than 10 years after initial hepatectomy (survival range: 10.1–18.2 years at the time of last follow-up), and 9 of those 29 survived without cancer recurrence. The other 116 patients died within 10 years. The 3-, 5-, and 10-year survival rates were 76.2%, 53.0%, and 26.9% respectively. The corresponding disease-free survival rates were 43.1%, 25.7%, and 9.9% respectively (Fig. 1). The cause of death was cancer recurrence in 84 patients, hepatic failure in 11 patients, gastrointestinal tract bleeding in 4 patients, other diseases in 15 patients (including 7 patients with other malignant diseases), and unknown in 10 patients. HCC recurrence developed in 111 patients, including 103 patients with intrahepatic recurrence and 8 patients with extrahepatic recurrence (bone metastasis in 3 patients and lung metastasis in 5 patients).

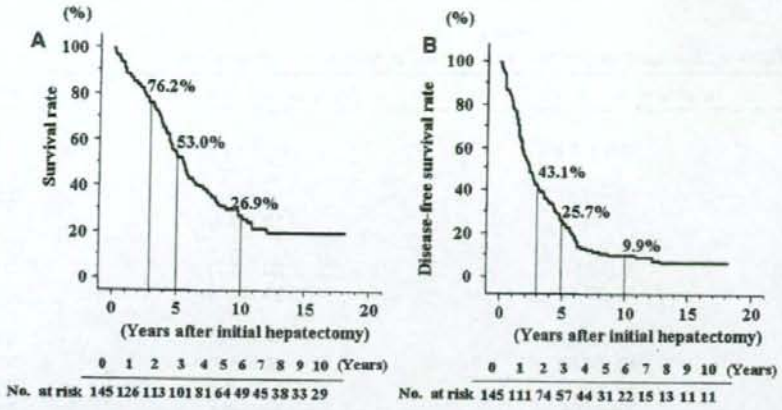

Figure 1. A Overall survival of 145 patients who had Child–Pugh class A liver function and who underwent curative resection. **B** Disease-free survival of 145 patients who had Child–Pugh class A liver function and who underwent curative resection.

Table 1 shows a comparison of the clinicopathologic factors in 10-year survivors and nonsurvivors by univariate analysis. Gender (female), ICG-R15 value (less than 15%), and liver fibrosis of noncancerous liver parenchyma (F0–2) were identified as significant favorable host factors. The incidence of HBV-related HCC tended to be high in 10-year survivors, whereas the incidence of HCV-related HCC tended to be high in nonsurvivors, but these did not reach significance. Microscopic vascular invasion (vp negative) and intrahepatic metastasis (im negative) were identified as significant favorable tumor factors. The tumor size tended to be larger and the number of tumors tended to be greater in nonsurvivors than in 10-year survivors, but these did not reach significance. No significant difference was found in treatment factors. In multivariate logistic regression analysis, liver fibrosis, gender, ICG-R15, and vp were identified as independent factors associated with 10-year survival (Table 2).

Table 3 shows a comparison of clinical data and management of HCC recurrence in 10-year survivors and nonsurvivors. Half of the 10-year survivors had recurrence more than 5 years after initial hepatectomy, and no patients had recurrence within 1 year. In contrast, 64 (70.3%) of the nonsurvivors had recurrence within 3 years of the initial hepatectomy, and 23 patients (25.3%) had recurrence within 1 year. The median disease-free intervals of 10-year survivors and nonsurvivors were 5.1 years (range: 1.2–12.8 years) and 1.9 years (range: 0.06–8.7 years) respectively, and the difference was statistically significant ($P = 0.0004$). The number of intrahepatic recurrent nodules in 10-year survivors tended to be fewer than that in nonsurvivors. Twelve out of 19 patients (63.2%) with intrahepatic recurrence among the 10-year survivors had a single recurrent nodule, whereas 55 out of the 84 patients among the nonsurvivors had multiple recurrent nodules. However, the difference was not significant ($P = 0.0692$). Treatment strategies for intrahepatic recurrent tumors in the 10-year survivors and nonsurvivors were similar.

Table 4 shows the clinicopathologic features of 10-year disease-free survivors (cases 1–9). Eight patients were alive at the time of last follow-up and 1 patient died of another disease. Six of the patients were females. HBV-related HCC was found in 4 patients, HCV-related HCC was found in 3 patients, and there was no positivity for HBsAg or HCVAb in 2 patients. Eight patients had an ICG-R15 value of less than 15% and 5 patients had liver fibrosis grade F0–2. Microscopic vascular invasion (vp) was present in only 2 patients.

Figure 2 shows the clinical course of 20 patients with HCC recurrence who survived for more than 10 years (cases 10–29). Therapeutic modalities used for recurrence included repeat hepatectomy in 5 patients, local ablation in 5 patients, TACE in 7 patients, chemotherapy in 1 patient, pulmonary resection in 1 patient who had lung metastasis (case 13), and no treatment in 1 patient who refused further therapy (case 11). At the last follow-up, 6 of the patients had no evidence of recurrence after treatment for recurrence. The other patients had further recurrence and underwent multimodal treatments combined with repeat hepatectomy and nonsurgical treatments.

DISCUSSION

Our previous studies showed that Child–Pugh class B and noncurative resection are adverse prognostic factors for long-term survival.[3,7] Thus, the current study focused on patients who had Child–Pugh class A liver function and who had undergone curative resection with the aim of elucidating the prognostic factors affecting survival after

Table 1.
Comparison of clinicopathologic factors in 10-year survivors and nonsurvivors

Variables	10-year survivors, $n = 29$ (%)	Nonsurvivors, $n = 116$ (%)	P value
Host factors			
Age[a]	59.6 ± 7.9	60.4 ± 8.8	0.6816
Gender			
Male	19 (65.5)	97 (83.6)	0.0293*
Female	10 (34.5)	19 (16.4)	
HBs Ag			
Positive	9 (32.1)	20 (17.7)	0.0905
Negative	19 (67.9)	93 (82.3)	
HCV Ab			
Positive	10 (45.4)	58 (66.7)	0.0665
Negative	12 (54.6)	29 (33.3)	
T. Bil (mg/dl)[a]	0.7 ± 0.3	0.7 ± 0.3	0.4036
Alb (g/dl)[a]	3.8 ± 0.4	3.8 ± 0.4	0.8619
PT activity (%)[a]	105 ± 24	99 ± 25	0.2863
Platelet ($\times 10^3/mm^3$)[a]	149 ± 57	134 ± 55	0.5992
ICG-R 15 (%)			
<15	22 (75.9)	60 (51.7)	0.0190*
≥15	7 (24.1)	56 (48.3)	
Liver fibrosis			
F0–2	18 (62.1)	45 (39.1)	0.0261
F3–4	11 (37.9)	70 (60.9)	
Hepatitis activity			
A1–2	25 (86.2)	90 (78.3)	0.3403
A3	4 (13.8)	25 (21.7)	
Tumor factors			
Number of tumors			
Solitary	26 (89.7)	84 (72.4)	0.0524
Multiple	3 (10.3)	32 (27.6)	
Tumor size (mm)			
<30	18 (62.1)	54 (46.6)	0.1350
≥30	11 (37.9)	62 (53.4)	
Differentiation			
Well or Moderate	25 (89.3)	94 (87.9)	0.8343
Poor	3 (10.7)	13 (12.1)	
Vp			
Positive	5 (17.2)	45 (39.1)	0.0269*
Negative	24 (82.8)	70 (60.9)	
Im			
Positive	5 (17.2)	43 (37.7)	0.0371
Negative	24 (82.8)	71 (62.3)	
AFP			
<200	21 (72.4)	80 (70.2)	0.8132
≥200	8 (27.6)	34 (29.8)	
Treatment factors			
Preoperative TACE			
Yes	23 (79.3)	93 (80.2)	0.8346
No	6 (20.7)	23 (19.8)	
Type of hepatectomy			
Limited	12 (41.4)	61 (52.6)	0.2803
Anatomical	17 (58.6)	55 (47.4)	
Surgical margin (mm)			
<5	20 (69.0)	77 (66.4)	0.7912
≥5	9 (31.0)	39 (33.6)	

Table 1.
Continued

Variables	10-year survivors, n = 29 (%)	Nonsurvivors, n = 116 (%)	P value
Blood transfusion			
Yes	2 (6.9)	18 (15.5)	0.2285
No	27 (93.1)	98 (84.5)	

HBs Ag: hepatitis B surface antigen; HCV Ab: hepatitis C virus antibody; T. Bil: total bilirubin; Alb: albumin; PT: prothrombin; ICG-R15: indocyanine green retention rate at 15 minutes; vp: histological vascular invasion; im: intrahepatic metastasis; AFP: alpha-fetoprotein; TACE: transcatheter arterial chemoembolization.
*$P < 0.05$.
[a]Mean ± standard deviation.

Table 2.
Independent factors associated with 10-year survival by multivariate logistic regression analysis

Variables	Relative risk	95% CI	P value
Liver fibrosis: F0–2	3.118	1.184–8.214	0.0213*
Gender: female	3.146	1.126–8.788	0.0287*
ICGR15: < 15%	2.910	1.056–8.018	0.0389*
vp : negative	3.231	1.022–10.212	0.0457*
Im: negative	2.053	0.652–6.462	0.2190

ICG-R15: indocyanine green retention rate at 15 minutes; vp: histological vascular invasion; im: intrahepatic metastasis; 95% CI: 95% confidence interval.
*$P < 0.05$.

Table 3.
Comparison of clinical data and management of HCC recurrence in 10-year survivors and nonsurvivors

	10-year survivors, n = 20[a] (%)	Nonsurvivors, n = 91[b] (%)	P value
Disease-free interval after initial hepatectomy			
Within 1 year	0	23 (25.3)	0.0004*
1 to 3 years	6 (30.0)	41 (45.0)	
3 to 5 years	4 (20.0)	16 (17.6)	
More than 5 years	10 (50.0)	11 (12.1)	
Number of intrahepatic recurrent tumor nodules			
Single	12 (63.2)	29 (34.5)	0.0692
2 to 3	4 (21.0)	34 (40.5)	
More than 4	3 (15.8)	21 (25.0)	
Treatment for intrahepatic recurrent tumor			
Re-hepatectomy	5 (26.3)	15 (17.9)	0.314
Local ablation	5 (26.3)	22 (26.2)	
TACE	7 (36.8)	20 (23.8)	
Chemotherapy	1 (5.3)	4 (4.8)	
Conservative	1 (5.3)	23 (27.3)	

TACE: transcatheter arterial chemoembolization.
* $P < 0.05$.
[a]Including 1 patient with lung metastasis.
[b]Including 4 patients with lung metastasis and 3 patients with bone metastasis.

curative hepatectomy. In multivariate analysis, liver fibrosis grade F0–2, female gender, ICG-R15 value of less than 15%, and absence of microscopic vascular invasion were identified as favorable independent factors for 10-year survival.

Microscopic vascular invasion is a well-known risk factor for poor prognosis after hepatectomy, because the presence of vascular invasion is considered to be one of the strongest predictors of intrahepatic metastasis caused by spread of cancer cells via the portal venous

Table 4.
Clinicopathologic features of 10-year disease-free survivors after hepatectomy

Case	Age	Gender	Virus status	ICGR15 (%)	Liver fibrosis	Liver hepatitis	Number of tumors	Tumor size (cm)	Differentiation	vp	Disease-free duration (years)	Current status
1	41	Female	nBnC	10.4	F1	A1	1	5.0	Moderate	+	18.2	Alive
2	45	Female	B	8.8	F2	A1	1	2.5	Moderate	–	16.9	Alive
3	64	Female	nBnC	5.3	F1	A1	1	3.2	Moderate	–	14.6	Alive
4	67	Female	B	12.8	F4	A1	1	1.3	Well	–	14.1	Alive
5	51	Male	B	5.3	F3	A3	2	2.0	Poor	–	14.1	Alive
6	69	Female	B	7.2	F3	A1	1	1.1	Moderate	–	11.8	Alive
7	63	Female	C	19.7	F4	A2	1	3.3	ND*	–	10.9	Dead
8	62	Male	C	13.5	F2	A2	1	3.6	Moderate	–	10.1	Alive
9	65	Male	C	14.5	F2	A2	2	4.2	Moderate	+	10.0	Alive

ND: not determined B: Hepatitis B virus; C: Hepatitis C virus; nBnC: nonB nonC hepatitis; ICG-R15: indocyanine green retention rate at 15 minutes; vp: histological vascular invasion; –: negative; + : positive.

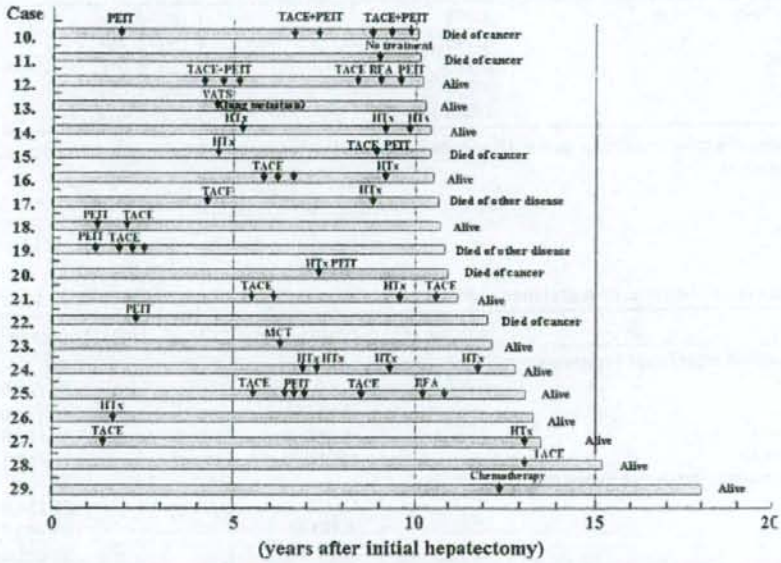

Figure 2. Clinical course of 20 patients with HCC recurrence who survived for more than 10 years (cases 10 to 29). *PEIT*: percutaneous ethanol injection therapy; *MCT*: microwave coagulation therapy; *RFA*: radiofrequency ablation; *TACE*: transcatheter arterial chemoembolization; *HTx*: hepatectomy; *VATS*: video-assisted thoracoscopic surgery.

system.[8–10] Female patients were predominant among the 10-year survivors in our study. As a possible reason for the better prognosis in females, several investigators have demonstrated that a potential role of androgen is associated with liver carcinogenesis.[11,12] The ICG-R15 value has been reported to be one of the most significant factors for long-term survival.[13] Even among patients with Child–Pugh class A, the ICG-R15 value varied greatly. In our study, the ICG-R15 value ranged from 2.6% to 41.5% (data not shown). Therefore, the ICG-R15 value is thought to be a better criterion than the Child–Pugh classification for evaluation of liver function. Previous studies have demonstrated that HCC patients with advanced liver fibrosis or cirrhosis had a poor prognosis even after curative resection.[14,15] However, there have been only a few studies based on histological analysis of liver fibrosis status. In our study, the degree of liver fibrosis was divided into no or mild fibrosis (F0–F2) and severe fibrosis or cirrhosis (F3–F4) according to New Inuyama classification, which is widely used in Japan.[6] The current study revealed that the degree of liver fibrosis (F0–F2) was the strongest prognostic factor associated

with 10-year survival. Several studies have suggested that fibrosis or cirrhosis of the underlying liver might predispose patients to multicentric hepatocarcinogenesis.[8,16] Yoshida *et al.* demonstrated that the risk of HCC was 24 times higher in patients with liver cirrhosis than in patients with normal liver or mild fibrosis.[17]

These favorable factors described above were represented clearly in 9 patients who had survived disease-free for 10 years (Table 4). Among them, 67% were females, 89% had an ICG-R15 of less than 15%, 56% had liver fibrosis grade F0-2, and 78% had no microscopic vascular invasion. The incidence of HCV-related HCC was low (3 out of 9 patients) and the incidence of HBV-related HCC was high (4 out of 9 patients) in the 10-year disease-free survivors compared with the incidence in the nonsurvivors. Although several investigators have suggested that absence of HCV Ab or presence of HBs Ag is potentially a good predictor of long-term survival,[4,18,19] viral status was not identified as a significant prognostic factor associated with 10-year survival in this study. Recently, postoperative interferon therapy has been reported to decrease the incidence of recurrence after resection of HCV-related HCC.[20,21] However, it was not common to receive interferon therapy postoperatively 10 years ago. Thus, whether patients received postoperative interferon therapy or not is not discussed in the current study.

Hepatocellular carcinoma recurrence is frequent even after curative resection.[22–24] In our study, 111 (76.6%) of the 145 patients had HCC recurrence after curative resection. Even among 10-year survivors, 20 (69%) had HCC recurrence. However, there was a significant difference with regard to the time interval before recurrence. The median disease-free intervals of 10-year survivors and nonsurvivors were 5.1 and 1.9 years respectively. Intrahepatic recurrence includes intrahepatic metastasis from the primary tumor and a metachronous second primary tumor, but it is difficult to distinguish them clearly. Poon *et al.* suggested that early recurrence (within 1 year) arises mainly from intrahepatic metastasis, which tends to be multifocal, and that late recurrence (after 1 year) is more likely to be related to multicentric occurrence in origin.[25] From this point of view, intrahepatic recurrence in nonsurvivors might originate from not only multicentric occurrence, but also intrahepatic metastasis. In addition, more advanced status of liver fibrosis in nonsurvivors might accelerate multicentric hepatocarcinogenesis in the liver remnant. Presumably, these differences resulted in the different outcomes despite similar treatments after recurrence.

Our first choice of treatment for a recurrent tumor is principally repeat hepatectomy because this procedure has been accepted as the most effective treatment.[26] However, repeat hepatectomy is limited to patients with resectable intrahepatic recurrence and well-preserved liver function. Our study indicated that nonsurgical treatment such as percutaneous ethanol injection therapy, radiofrequency ablation therapy or TACE, could contribute to prolongation of survival when repeat hepatectomy is not indicated. Therefore, it is important to select appropriate treatment according to the pattern of recurrence, location of the tumor, and preserved liver function. Recent studies have supported an aggressive treatment strategy combined with hepatectomy and nonsurgical treatment.[27,28]

Recent studies have demonstrated that liver transplantation (LT) is a good alternative to hepatectomy, providing excellent survival and disease-free survival rates.[29–31] However, there is controversy as to whether hepatectomy or LT should be offered to patients with resectable HCC and Child–Pugh class A. Bigourdan *et al.* reported that LT provided better survival and recurrence-free survival even in patients with Child–Pugh class A and small HCC, compared with hepatectomy.[32] However, in view of the shortage of organ donors, it seems reasonable to consider hepatectomy as an initial treatment, especially in Japan, where use of a deceased donor liver has been limited. Our current study has provided some insights into the optimal management of these patients. For patients with unfavorable prognostic factors at initial hepatectomy such as liver fibrosis grade F3–4, ICG-R15 value of more than 15%, presence of microscopic vascular invasion, and male gender, treatment might not be successful due to progressive development of HCC or liver function deterioration. In cases of early HCC recurrence with development of multifocal nodules, it might be better to consider LT as a salvage treatment.

In conclusion, liver fibrosis grade F0–2, female gender, ICG-R15 value of less than 15%, and absence of microscopic vascular invasion at initial hepatectomy might be biologically favorable conditions for long-term survival. Close follow-up as well as multimodal treatment could contribute to prolongation of survival in such patients, even if HCC recurrence develops.

REFERENCES

1. Itamoto T, Katayama K, Nakahara H, *et al.* Autologous blood storage before hepatectomy for hepatocellular carcinoma with underlying liver disease. Br J Surg 2003;90:23–28.
2. Itamoto T, Nakahara H, Tashiro H, *et al.* Indications of partial hepatectomy for transplantable hepatocellular carci-

noma with compensated cirrhosis. Am J Surg 2005;189: 167–172.
3. Nakahara H, Itamoto T, Katayama K, *et al.* Indication of hepatectomy for cirrhotic patients with hepatocellular carcinoma classified as Child-Pugh class B. World J Surg 2005;29:734–738.
4. Shirabe K, Shimada M, Kajiyama K, *et al.* Clinicopathologic features of patients with hepatocellular carcinoma surviving >10 years after hepatic resection. Cancer 1998;83:2312–3216.
5. Shimada K, Sano T, Sakamoto Y, *et al.* A long-term follow-up and management study of hepatocellular carcinoma patients surviving for 10 years or longer after curative hepatectomy. Cancer 2005;104:1939–1947.
6. Ichida F, Tsuji T, Omata M, *et al.* New Inuyama classification: new criteria for histological assessment of chronic hepatitis. Int Hepatol Commun 1996;6:112–119.
7. Fukuda S, Itamoto T, Nakahara H, *et al.* Clinicopathologic features and prognostic factors of resected solitary small-sized hepatocellular carcinoma. Hepatogastroenterology 2005;52:1163–1167.
8. Yamamoto J, Kosuge T, Takayama T, *et al.* Recurrence of hepatocellular carcinoma after surgery. Br J Surg 1996; 83:1219–1222.
9. Nagao T, Inoue S, Yoshimi F, *et al.* Postoperative recurrence of hepatocellular carcinoma. Ann Surg 1990;211:28–33.
10. Shimada M, Takenaka K, Taguchi K, *et al.* Prognostic factors after repeat hepatectomy for recurrent hepatocellular carcinoma. Ann Surg 1998;227:80–95.
11. Nagasue N, Yu L, Yukaya H, *et al.* Androgen and oestrogen receptors in hepatocellular carcinoma and surrounding liver parenchyma: impact on intrahepatic recurrence after hepatic resection. Br J Surg 1995;82:542–547.
12. De Maria N, Manno M, Villa E. Sex hormones and liver cancer. Mol Cell Endocrinol 2002;193:59–63.
13. Imamura H, Sano K, Sugawara Y, *et al.* Assessment of hepatic reserve for indication of hepatic resection: decision tree incorporating indocyanine green test. J Hepatobiliary Pancreat Surg 2005;12:16–22.
14. Bilimoria MM, Lauwers GY, Doherty DA, *et al.* Underlying liver disease, not tumor factors, predicts long-term survival after resection of hepatocellular carcinoma. Arch Surg 2001;136:528–535.
15. Wayne JD, Lauwers GY, Ikai I, *et al.* Preoperative predictors of survival after resection of small hepatocellular carcinomas. Ann Surg 2002;235:722–730.
16. Ko S, Kanehiro H, Hisanaga M, *et al.* Liver fibrosis increases the risk of intrahepatic recurrence after hepatectomy for hepatocellular carcinoma. Br J Surg 2002;89:57–62.
17. Yoshida H, Shiratori Y, Moriyama M, *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. Ann Intern Med 1999;131:174–181.
18. Takenaka K, Yamamoto K, Taketomi A, *et al.* A comparison of the surgical results in patients with hepatitis B versus hepatitis C-related hepatocellular carcinoma. Hepatology 1995;22:20–24.
19. Miyagawa S, Kawasaki S, Makuuchi M. Comparison of the characteristics of hepatocellular carcinoma between hepatitis B, C viral infection: tumor multicentricity in cirrhotic liver with hepatitis C. Hepatology 1996;24:307–310.
20. Ikeda K, Arase Y, Saitoh S, *et al.* Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor—a prospective randomized study of hepatitis C virus-related liver cancer. Hepatology 2000;32:228–232.
21. Kubo S, Nishiguchi S, Hirohashi K, *et al.* Randomized clinical trial of long-term outcome after resection of hepatitis C virus-related hepatocellular carcinoma by postoperative interferon therapy. Br J Surg 2002;89:418–422.
22. Nagasue N, Ono T, Yamanoi A, *et al.* Prognostic factors and survival after hepatic resection for hepatocellular carcinoma without cirrhosis. Br J Surg 2001;88:515–522.
23. Nagashima I, Hamada C, Naruse K, *et al.* Surgical resection for small hepatocellular carcinoma. Surgery 1996; 119:40–45.
24. Lise M, Bacchetti S, Da Pian P, *et al.* Prognostic factors affecting long term outcome after liver resection for hepatocellular carcinoma: results in a series of 100 Italian patients. Cancer 1998;82:1028–1036.
25. Poon RT, Fan ST, Ng IO, *et al.* Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. Cancer 2000; 89:500–507.
26. Minagawa M, Makuuchi M, Takayama T, *et al.* Selection criteria for repeat hepatectomy in patients with recurrent hepatocellular carcinoma. Ann Surg 2003;238:703–710.
27. Poon RT, Fan ST, Lo CM, *et al.* Intrahepatic recurrence after curative resection of hepatocellular carcinoma: long-term results of treatment and prognostic factors. Ann Surg 1999;229:216–222.
28. Shimozawa N, Hanazaki K. Longterm prognosis after hepatic resection for small hepatocellular carcinoma. J Am Coll Surg 2004;198:356–365.
29. 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;334:693–699.
30. Llovet JM, Bruix J, Fuster J, *et al.* Liver transplantation for small hepatocellular carcinoma: the tumor-node-metastasis classification does not have prognostic power. Hepatology 1998;27:1572–1577.
31. Bismuth H, Majno PE, Adam R. Liver transplantation for hepatocellular carcinoma. Semin Liver Dis 1999;19:311–322.
32. Bigourdan JM, Jaeck D, Meyer N, *et al.* Small hepatocellular carcinoma in Child A cirrhotic patients: hepatic resection versus transplantation. Liver Transpl 2003; 9:513–520.

American Journal of Transplantation 2006; 6: 797–805
Blackwell Munksgaard

doi: 10.1111/j.1600-6143.2006.01247.x

Near-Infrared Spectroscopic Analysis of Hemodynamics and Mitochondrial Redox in Right Lobe Grafts in Living-Donor Liver Transplantation

H. Mitsuta, H. Ohdan*, Y. Fudaba, T. Irei, H. Tashiro, T. Itamoto and T. Asahara

Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Japan
**Corresponding author: Hideki Ohdan, hohdan@hiroshima-u.ac.jp*

Near-infrared spectroscopy (NIRS), which enables non-destructive evaluation of hemoglobin (Hb) oxygenation and the redox state of cytochromeoxidase (Cyt.aa$_3$) in living tissues, has been employed during surgery to detect possible impairment of hemodynamics and mitochondrial respiration in the anterior segment of a right lobe liver graft in living-donor liver transplantation (LDLT). Thirty-six patients undergoing LDLT using a right lobe graft without the middle hepatic vein (MHV) were enrolled in this study. During the course of harvesting and implantation, NIRS measurements were performed on the anterior segments of the liver grafts. In two recipients of liver grafts with Hb residue over 70% in the anterior segment after *ex vivo* flushing, the MHV tributary was reconstructed, while it was not reconstructed in the other 34 recipients. Of those 34 recipients, 16 recipients of liver graft with 40–70% Hb residue showed transient increase of transaminase levels after LDLT. Of those 16 recipients, six recipients who showed reduction in oxidized Cyt.aa$_3$ in the anterior segment suffered from persistent hyperbilirubinemia after LDLT. In patients showing impairment of mitochondrial redox associated with congestion caused by deprivation of the MHV tributaries, reconstruction of the MHV tributaries might have a beneficial effect.

Key words: Congestion, living-donor transplantation, middle hepatic vein, mitochondrial redox, near-infrared spectroscopy, reconstruction

Received 7 October 2005, revised 3 December 2005 and accepted for publication 7 December 2005

Introduction

Living-donor liver transplantation (LDLT) is currently accepted as an important potential source of organs for treatment of children and even adults with end-stage liver disease. In adult-to-adult LDLT using a left lobe graft, however, small-for-size grafts are sometimes insufficient to meet the metabolic demands, resulting in a lower chance of survival. One solution to this problem is to use a right liver graft. Two surgical procedures for harvesting a right lobe have been reported: a right lobectomy with drainage of the right hepatic vein (RHV) alone (1–3) and an extended right lobectomy with both RHV and middle hepatic vein (MHV) drainage (4–6). In the case of the former procedure, which is widely used because it is less invasive for donors, the need for drainage from the MHV tributaries (anterior segment branches) has not yet been established. Venous outflow problems associated with deprivation of the MHV tributaries in LDLT using right lobes are common and are sometimes devastating (7–11). To prevent such venous outflow problems, a reliable method for evaluating the need for drainage from the MHV tributaries should be established.

We previously reported the usefulness of intra-operative near-infrared spectroscopy (NIRS), which enables non-destructive evaluation of hemoglobin (Hb) oxygenation and the redox state of cytochromeoxidase (Cyt.aa$_3$) in living organ tissues (12–17), for evaluating the extent of congestion in the anterior segment of the graft after LDLT using right lobe grafts that do not have the MHV (18). By determining the kinetics of Hb washout of the right hepatic lobe graft with clamping of the MHV tributary during *ex vivo* perfusion, NIRS enabled prediction of venous outflow problems caused by deprivation of the MHV tributaries before implantation, indicating that this method is useful for determining whether MHV tributaries should be reconstructed. When the graft suffered from severe outflow disturbance associated with deprivation of the MHV tributaries, the region where Hb remained after *ex vivo* flushing was visible even to the naked eye. However, the difference in the degrees of Hb residue could not be detected by the naked eye. When over 60% of Hb was released from the anterior segment within 180 s after initial flushing, temporary mild or even no remarkable congestion in the anterior segment occurred after surgery. In contrast, when over 70% of Hb remained after *ex vivo* perfusion in the anterior segment, persistent circulatory impediment causing atrophy of the anterior segment inevitably occurred. Hence, we have decided on our policy that the MHV tributary should be reconstructed in recipients of liver graft with high Hb

residue in the anterior segment after *ex vivo* flushing (over 70%) and have applied this criterion to 36 subsequent patients who underwent LDLT using a right lobe graft without the MHV. Through analysis of those cases, we verified the usefulness of intra-operative NIRS for prediction of venous outflow problems associated with deprivation of the MHV tributaries in LDLT using right lobes.

Patients and Methods

Patient population

Thirty-six patients who underwent adult-to-adult LDLT using a right lobe graft without the MHV at Hiroshima University Hospital were enrolled in this study (Table 1). Patients in whom serious complications other than venous outflow problems (e.g. sepsis, acute rejection and bile leakage) occurred within 1 month after LDLT were excluded. The 36 patients included 23 males and 13 females ranging in age from 27 to 68 years (mean ± SD age, 50.3 ± 10.3 years). Graft weight and graft-to-recipient body weight ratio (GRWR) ranged from 482 to 940 g (mean weight, 664.7 ± 130.1 g) and from 0.68% to 1.92% (mean ratio, 1.04 ± 0.26%), respectively. Original diseases of the patients were hepatitis C virus-related cirrhosis in 15 patients (associated with hepatocellular carcinoma in 10 patients), hepatitis B virus-related cirrhosis in 8 patients (associated with hepatocellular carcinoma in 5 patients), fluminant hepatic failure in 5 patients, autoimmune hepatitis in 4 patients, primary biliary cirrhosis in 1 patient and metastatic liver tumor in 1 patient. The graft donors were 22 offsprings, 6 siblings, 2 parents and 1 spouse, with ages ranging from 18 to 61 years (mean age, 33.6 ± 12.7 years). Informed consent for participation in the study was obtained from patients. The study protocol was approved by the Medical Ethics Committee of Hiroshima University.

Surgical technique

Donor hepatectomy and the recipient transplantation procedure were performed as described previously (18). In brief, the right lobe, without the

Table 1: Patient characteristics

	Recipient				Donor						
Case	Age	Gender	Diagnosis	MELD	Age	Gender	Relationship	Graft weight (mL)	GRWR (%)	Size of MHV tributaries (mm)	Residual Hb content (%)
1	58	M	LC(HC)	27.7	27	F	Offspring	556	0.9	<4	52.1
2	37	F	PBC	5.8	36	F	Sibling	566	0.9	<4	6.5
3	63	M	LC(HC), HCC	13.3	29	M	Offspring	762	1.2	<4	24.1
4	50	M	LC(HC), HCC	14.8	54	F	Spouse	604	0.8	<4	22.5
5	60	F	LC(HC)	20.3	33	M	Offspring	580	0.9	<4	48.7
6	66	F	LC(HC), HCC	16.7	37	F	Offspring	672	0.9	5	2.8
7	57	M	LC(HC), HCC	7.0	29	M	Offspring	602	1.1	7	59.2
8	27	M	AIH	35.7	28	M	Sibling	708	1.1	7	65.7
9	62	M	LC(HB), HCC	12.0	28	M	Offspring	856	1.0	>7	82.9
10	40	M	FH	24.5	42	F	Sibling	696	0.8	6	26.5
11	59	F	FH	30.1	29	M	Offspring	940	1.5	4	36.2
12	48	F	AIH	30.4	53	M	Sibling	902	1.7	5	61.4
13	52	M	LC(HC)	11.8	22	M	Offspring	742	0.9	5	63.7
14	57	M	LC(HB), HCC	9.2	32	F	Offspring	566	0.7	6	66.1
15	58	M	LC(alcholic)	16.7	26	M	Offspring	710	1.0	4	29.7
16	56	M	FH	34.6	28	F	Offspring	548	0.9	6	0.3
17	49	M	LC(HC), HCC	18.9	21	M	Offspring	550	0.8	4	49.3
18	60	M	LC(HC), HCC	10.4	24	M	Offspring	564	1.0	4	26.2
19	54	M	LC(HB), HCC	13.3	20	M	Offspring	896	1.4	4	22.3
20	55	F	LC(HC), HCC	10.9	60	M	Sibling	526	0.9	5	41.7
21	50	M	LC(HB), HCC	27.0	20	M	Offspring	884	1.2	6	31.5
22	29	M	LC(HC)	20.1	61	M	Parent	536	1.4	5	19.0
23	47	M	LC(HC), HCC	10.6	20	M	Offspring	632	1.0	3	21.9
24	43	M	LC(HB), HCC	35.2	57	F	Spouse	678	1.0	2	36.9
25	28	M	Liver metastasis	6.0	57	F	Parent	494	0.8	<4	54.3
26	56	M	FH	43.9	26	M	Offspring	614	0.9	<4	16.5
27	58	M	LC(HB)	29.4	30	M	Offspring	550	0.9	6	0.0
28	44	F	AIH	18.1	49	F	Sibling	660	1.2	5	39.4
29	46	F	LC(HB)	23.4	18	F	Offspring	482	0.8	<4	25.7
30	68	F	LC(HC)	20.7	43	F	Offspring	618	0.9	<4	51.0
31	57	M	LC(HC), HCC	19.1	30	M	Offspring	630	0.9	5	59.5
32	50	F	AIH	44.1	29	M	Offspring	714	0.9	4.2	6.4
33	20	F	FH	29.0	43	F	Parent	538	0.9	4	62.6
34	58	M	LC(HC), HCC	16.9	29	M	Offspring	900	1.1	>7	55.3
35	57	F	LC(HB), HCC	3.1	18	M	Offspring	630	1.2	5.5	58.1
36	50	F	LC(alcholic)	20.7	22	M	Offspring	824	1.9	>7	73.8

M = male; F = female; MELD = model for end-stage liver disease; MHV = middle hepatic vein; PBC = primary biliary cirrhosis; LC = liver cirrhosis; HC = hepatitis C; HB = hepatitis B; AIH = autoimmune hepatitis; HCC = hepatocellular carcinoma; FH = fulminant hepatic failure.

MHV, was harvested from the donor as follows. An intra-operative ultrasonographic (US) examination was performed for final identification of the anatomy of the hepatic veins and portal veins (PV). Parenchymal transection was performed on the right side of the gallbladder fossa. During parenchymal transection, major right tributaries of the MHV draining from the anterior segment were clamped using a vascular clip and transected. After hepatectomy, *ex vivo* perfusion of the right lobe graft was performed through the PV. The initial perfusate was saline solution (500 mL) followed by the University of Wisconsin solution (1000 mL). During initial perfusion, real-time and continuous NIRS measurement was performed on the anterior segment of the right lobe graft to determine the kinetics of Hb washout from the hepatic tissue as described later.

For the recipient, the implantation was performed after total hepatectomy. The middle and left hepatic veins were closed, and then the graft RHV was anastomosed to the RHV of the recipient in an end-to-end fashion. When indicated, the MHV tributary was reconstructed by use of the recipient's external iliac vein as an interposition or by directly anastomosing to the recipient's MHV trunk. After end-to-end anastomosis of the graft right PV to the PV of the recipient, the graft was reperfused before microsurgical reconstruction of the hepatic artery (HA) (end-to-end anastomosis of the HA of the graft to the right or left HA of the recipient). The bile duct of the graft liver was anastomosed in an end-to-end fashion to the common or right hepatic bile duct of the recipient. All recipients continuously received i.v. injection of prostaglandin E_1 (PGE_1) (0.01 μg/kg/min) during surgery and for 24 h after surgery. Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and serum bilirubin were measured as indexes of liver function. The initial immunosuppressive regimen consisted of tacrolimus and steroids. Computed tomography (CT) scans and Doppler US were routinely performed.

Intra-operative NIRS

During the course of harvesting and implantation, *in vivo* NIRS measurements were performed on the anterior segments of the liver grafts to determine Hb and Cyt.aa_3 contents in the hepatic tissues as indicators of congestion and redox state. For these measurements, multi-point (2–3 points) scanning was performed, and data were averaged to quantitatively evaluate the state of venous congestion in the whole anterior segment. In addition, during initial *ex vivo* perfusion, continuous NIRS measurement was performed on the anterior segment of the right lobe graft to determine the kinetics of Hb washout from the hepatic tissue. The center point of the right paramedian sector was serially scanned every 30 s for at least 180 s. Since tissue Hb contents incessantly altered during *ex vivo* perfusion, such measurements did not allow multiple-point scanning. For fear of a detached endothelium of the HA, *ex vivo* HA perfusion was not performed in any cases of the present series. Probably because of intra-hepatic communication between the PV and HA, most (but not all) of Hb was washed out by *ex vivo* perfusion via the PV if outflow disturbance did not occur. NIRS measurement was carried out as follows. NIR reflectance was measured with a multichannel photodetector (MCPD-2000, Otsuka Electronics, Osaka, Japan) connected to a personal computer (PC-9801FS, NEC, Tokyo, Japan). NIR light from a halogen lamp at 300 W was directed through a flexible bundle of quartz optical fibers to the liver, and the reflected light was conveyed through another bundle to a spectrophotometer. The tips of the two fiber bundles were fixed at a position approximately 1 cm above the liver, and the distance between the two probes was approximately 1 cm. The reflected light was measured sequentially in the range of 700–1000 nm with an interval of 2 nm. The sampling time for each scan was 4 s. The difference between the spectrum from the liver immediately after laparotomy of the donor and that from the liver shortly before closure of the recipient's abdomen and the difference between the spectrum from the liver just before initial *ex vivo* perfusion and that from the liver during *ex vivo* perfusion were calculated, i.e. the difference in the gross optical densities between them was calculated at 2 nm wavelength intervals to obtain the 'subtracted spectrum' (16,19). Following Beer-Lambert law (12), then multi-component analysis of the subtracted spectrum was performed by use of least-square curve-fitting on the basis of singular value decomposition to quantify the changes in each component (20). The five components were fitted with the following equation:

$$\begin{aligned} OD(\lambda) = {} & L(\lambda)[e_1(\lambda)\Delta[\text{oxy} - \text{Hb}] \\ & + e_2(\lambda)\Delta[\text{deoxy} - \text{Hb}] + e_3(\lambda)\Delta[\text{oxidized Cyt.aa}_3] \\ & + e4(\lambda)\Delta[\text{reduced Cyt.aa}_3] + e_5(\lambda)\Delta[\text{water}]) \end{aligned}$$

where $OD(\lambda)$, $L(\lambda)$ and $e_{1-5}(\lambda)$ are optical density, mean light path length and extinction coefficient of each component at a wavelength of λ, respectively. The changes in Hb contents were calculated by adding the changes in oxy-Hb and deoxy-Hb. Hb residue in the anterior segment of the liver graft after *ex vivo* perfusion was represented by percentage of total change in Hb content in the posterior segment (18). The whole process of this analysis took about 10 min.

Statistical analysis

Statistical analysis was performed using Student's *t*-test. Multivariate analysis was performed using factor analysis. Survival curves were estimated by the Kaplan-Meier method and compared with log-rank test. Differences at $p < 0.05$ were considered significant.

Results

Patients

In two of the 36 cases, over 70% of Hb remained in the anterior segment even after *ex vivo* perfusion. In those recipients of liver grafts with high Hb residue, the MHV tributary was reconstructed in accordance with our policy (high Hb residue group). In the other 34 recipients of liver grafts with Hb residue of less than 70% in the anterior segment after *ex vivo* flushing, the MHV tributary was not reconstructed. Those 34 recipients were divided into two groups according to the percentage of remaining Hb content in the anterior segment of the graft liver after *ex vivo* flushing: 16 recipients of liver grafts with 40–70% Hb residue (intermediate Hb residue group) and 18 recipients of liver grafts with Hb residue of less than 40% (low Hb residue group). We found in our previous study that Cyt.aa_3 redox was preserved in all recipients of liver grafts with Hb residue of less than 40%, whereas all recipients of liver grafts with Hb residue over 70% showed reduction in oxidized Cyt.aa_3 in the anterior segment (18).

Reconstruction of the MHV tributary prevented anterior segment congestion in recipients of liver grafts with high Hb residue after ex vivo flushing

In the two patients in the high Hb residue group, the MHV tributary was reconstructed by use of the recipient's external iliac vein as an interposition or by directly anastomosing to the recipient's MHV trunk. In this study, we demonstrated that the extent of post-operative congestion (ΔHb: change in tissue Hb before harvesting and after implantation) in the anterior segment was significantly correlated with the tissue content of remaining Hb in that segment after *ex vivo* perfusion (at 180 s after initial

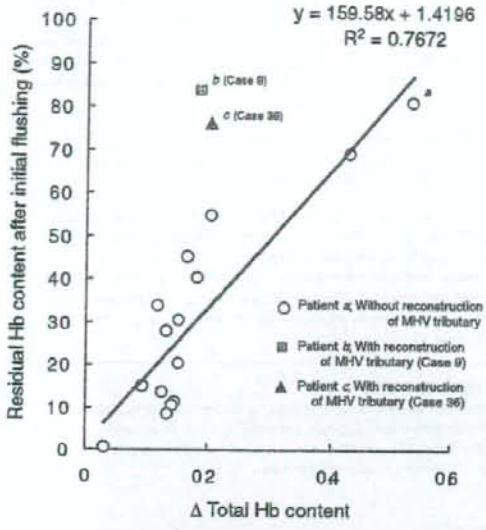

Figure 1: Relationship between the remaining Hb content after initial flushing and the extent of post-operative congestion in the anterior segment. During initial *ex vivo* perfusion, continuous NIRS measurement was performed on the anterior segment of the right lobe graft with clamping the MHV tributaries to determine the kinetics of Hb washout from the hepatic tissue. The extent of post-operative congestion (ΔHb) in the anterior segment was significantly correlated with the tissue content of remaining Hb in that segment after *ex vivo* perfusion (at 180 s after initial flushing). Open circles are data from a patient in our previous study. Closed squares and triangles are data from patients of liver grafts with high Hb residue in the present study. By reconstruction of the MHV tributaries, the extent of post-operative congestion was markedly relieved.

flushing). By reconstruction of the MHV tributary, the extent of post-operative congestion was markedly relieved in both patients in the high Hb residue group compared with the expected extent of post-operative congestion without reconstruction (Figure 1). Those two patients showed no remarkable elevation of liver function indexes, i.e. serum levels of AST, ALT and bilirubin, after LDLT (data not shown). Thus, reconstruction of the MHV tributary lessened anterior segment congestion in recipients in the high Hb residue group.

Mild congestion in the anterior segment caused by deprivation of the MHV tributaries led to transient increase of transaminase levels after LDLT

Figure 2 shows the kinetics of serum transaminase levels in the recipients of liver grafts with Hb residue of less than 70% in the anterior segment after *ex vivo* flushing, in whom the MHV tributaries were not reconstructed. Although, the peak values of transaminases did not differ among those patients during the initial phase, the levels of transaminases in intermediate Hb residue group recipients were transiently but significantly elevated at 2–3 weeks after transplantation. Spontaneous improvement of the transaminase levels might be due to growth of intrahepatic collaterals between the MHV tributaries and the RHV. In contrast, such an increase of transaminase levels was not observed in low Hb residue group recipients. We performed multivariate analyses to determine factors relevant to elevation of transaminases after LDLT. By factors analyses, no significant risk factors other than congestion in the anterior segment for elevation of serum transaminase levels were determined in the present series (data not shown). In addition, there was no difference between the intermediate Hb residue group and low Hb residue group in any of the pre-operative laboratory data, model for end-stage liver disease (MELD) score, graft volume and GRWR ratio (Table 2). These findings indicate that the differences in transaminases between groups are not due to either the small for size syndrome or pre-operative condition of recipients. Thus, mild congestion in the anterior segment caused by deprivation of the MHV tributaries leads to transient liver dysfunction.

Impairment of mitochondrial redox associated with congestion caused by deprivation of the MHV tributaries leads to the hyperbilirubinemia after LDLT

Figure 3A shows the kinetics of serum levels of total bilirubin in the recipients of liver graft with Hb residue of less than 70% in the anterior segment after *ex vivo* flushing. The intermediate Hb residue group included some patients showing persistent elevation of total bilirubin levels, whereas no patients in the low Hb residue group showed such persistent hyperbilirubinemia. We attempted to determine the NIRS parameters that can predict such persistent hyperbilirubinemia. Since the energy required for bile formation is provided by mitochondrial respiration in hepatocytes, impairment of mitochondrial redox may lead to hyperbilirubinemia. Since Cyt.aa_3 is the terminal member of the mitochondrial respiratory chain and its redox state changes in response to oxygen availability at the cellular level, a decrease in oxidized Cyt.aa_3 content in liver tissue should be an indicator of impairment of mitochondrial respiration. Hence, the 16 patients in the intermediate Hb residue group were further divided into two groups according to the change in oxidized Cyt.aa_3 content in the anterior segment liver tissues before harvesting and after implantation. We previously reported that mild congestion in the anterior segment of a right lobe liver graft reflected an increase in oxy-Hb rather than that in deoxy-Hb (18). In that series, no reduction of oxidized Cyt.aa_3 content was seen in the anterior segment in the majority of patients showing mild congestion in the anterior segment. In the present series, no patients in the low Hb residue group showed reduction in oxidized Cyt.aa_3 in the anterior segment, indicating a well-preserved mitochondrial redox state (data

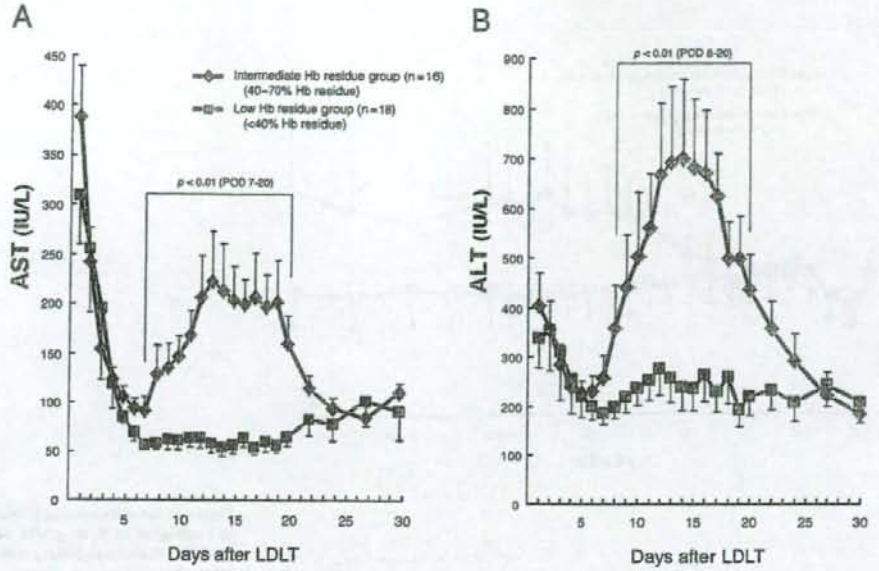

Figure 2: Kinetics of serum aspartate aminotransferase (AST) levels (A) and alanine aminotransferase (ALT) levels (B) in recipients of liver grafts with 40–70% Hb residue (intermediate Hb residue group, n = 16) and recipients of liver grafts with Hb residue of less than 40% (low Hb residue group, n = 18). Average +/− SD for the individual groups are shown. $p < 0.01$ between groups.

Table 2: Clinical parameters in the recipients of liver grafts with Hb residue of less than 70% in the anterior segment

	Low Hb residue group (n = 18)	Intermediate Hb residue group (n = 16)	p-Values
Recipient age (year)	51.4 ± 9.4 (29–66)	49.7 ± 13.8 (20–68)	0.61
Recipient gender (M/F)	12/6	10/6	
MELD	22.8 ± 11.7 (6–44)	18.2 ± 9.9 (3–36)	0.23
Pre-operative T-Bil	11.1 ±10.0 (1.3–28.8)	7.6 ± 9.0 (0.6–25.0)	0.48
Pre-operative AST	217.4 ± 620.4	357.1 ± 959.6	0.64
Pre-operative ALT	148.2 ± 410.6	264.9 ± 773.1	0.87
Donor age (year)	32.9 ± 13.5 (18–67)	35.4 ±12.9 (18–60)	0.56
Donor gender (M/F)	11/7	10/6	
Graft weight (g)	667.3 ± 131.8 (482–940)	639.9 ± 122.6 (494–902)	0.54
GRWR	1.05 ± 0.21 (0.82–1.42)	0.97 ± 0.23 (0.68–1.24)	0.34
Total ischemic time (min)	107.9 ± 31.6 (198–72)	91.9 ± 38.2 (193–47)	0.19

Average values ± SD (range) for the individual groups are shown.
M = male; F = female; MELD = model for end-stage liver disease; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GRWR = graft to recipient body weight ratio.

not shown). In contrast, 6 patients in the intermediate Hb residue group showed reduction in oxidized Cyt.aa_3 in the anterior segment of the liver graft after LDLT. In the patients showing reduced tissue content of oxidized Cyt.aa_3, serum levels of total bilirubin were significantly higher than those in patients in the intermediate Hb residue group who showed well-maintained tissue content of oxidized Cyt.aa_3 in the anterior segment (Figure 3B). Notably, the survival of grafts showing reduced tissue content of oxidized Cyt.aa_3 was significantly worse than those of grafts showing well-maintained oxidized Cyt.aa_3 content in the low and intermediate Hb residue groups, indicating the impact of mitochondrial redox impairment in the anterior segment on the graft outcome (Figure 4). In the present series, all donor livers were completely normal, ruling out the possibility of either fat infiltration or fibrosis, etc. There was no