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Treatment Strategy for Early Hepatocellular Carcinomas: Comparison of Radiofrequency Ablation With or Without Transcatheter Arterial Chemoembolization and Surgical Resection

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Background: The preferred choice between surgical treatment and radiofrequency ablation (RFA) for the treatment of small resectable hepatocelluar carcinoma (HCC) has become a subject for debate.
Methods: We compared the results of hepatic resection (n = 199) with those of RFA (n = 87), of which 69 patients were treated with transcatheter arterial chemoembolization followed by RFA, for 286 patients with 3 or fewer nodules, none of which exceeded 3 cm in diameter at Hiroshima University Hospital.
Results: In subgroup analysis of single HCC with tumor size exceeding 2 cm in Child-Pugh class A, the disease-free survival time was significantly longer in the surgical resection group than in the RFA group ($P = 0.048$). In the subgroups of a single and multiple HCC with tumor size $\leq$2 cm in Child-Pugh class A, the overall and disease-free survival rates were almost the same for the surgical resection and RFA groups ($P = 0.46$ and 0.58, respectively, in single HCC, and $P = 0.98$ and 0.98, respectively, in multiple HCC).
Conclusion: Surgical resection may provide better long-term disease-free survival than RFA in the subgroup of a single HCC exceeding 2 cm of Child-Pugh class A.
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KEY WORDS: **early hepatocellular carcinoma; hepatectomy; radiofrequency ablation**

INTRODUCTION

Hepatocelluar carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer death worldwide [1]. Although the majority of cases are still found in Asia and Africa, recent studies have shown that the incidence and mortality rates of HCC are increasing in North America and Europe [2]. Over the past two decades, great progress has been made in the diagnosis of HCC using non-invasive diagnostic modalities, and it is feasible to make early detection of HCC. Current options for the treatment of the early HCC consist of surgical resection, liver transplantation, transcatheter arterial chemoemolization (TACE), and percutaneous tumor ablation. These modalities have all been used for HCC patients according to the clinical characteristics of their tumors and the hepatic functional reserve of the patients. Hepatic resection has been shown to be the most efficacious treatment for HCC [3]; however, hepatic resection is limited to patients with good hepatic functional reserve. Radiofrequency ablation (RFA) is a recently introduced technique that is rapidly being adopted worldwide because of its greater efficacy for local cure compared with ethanol injection [4,5]. RFA is usually indicated for patients with three or fewer nodules, none of which exceed 3 cm in diameter [6]. Livraghi et al. [7] showed that RFA is just as effective as surgery for the treatment of very early HCC (single HCC nodules measuring 2.0 cm or less) in terms of sustained local disease control and survival. They advocated that RFA can be considered as the preferred treatment for patients with single HCC of 2.0 cm or less, even when surgical resection is possible. Recent, studies compared local ablation therapies with surgical resection [8–14]. However, few studies have evaluated the results of RFA in comparison with surgical resection within a subgroup (e.g., nodules $\leq$2.0 cm vs. >2.0 cm, and single vs. multiple HCCs) analysis of patients with early HCC (three or fewer nodules that are $\leq$3 cm in diameter). The aim of this retrospective study is to compare the patients with early HCC who were submitted to surgical resection and RFA from these points of view.

PATIENTS AND METHODS

From 2001 to 2007, 286 patients underwent liver resection, or RFA for single or multiple (less than 3) HCC measuring $\leq$3 cm as an initial treatment at Hiroshima University Hospital.

The diagnosis of HCC was based on routine imaging modalities including ultrasonography (US), computed tomography (CT) during hepatic angiography, and magnetic resonance imaging (MRI). HCC was diagnosed based on the following classic imaging manifestations: hyperattenuation on CT during hepatic arteriography and hypoattenuation on CT during arterial portography [15]. In case of hypovascular lesion, fine-needle biopsy was performed to obtain histological confirmation in patients who underwent RFA. Before treatment, all patients underwent liver function tests including bilirubin, albumin,

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prothrombin time, and indocyanine green retention rate at 15 min (ICGR 15) tests.

Hepatic Resection

In the current study, 199 patients were subjected to surgical resection of early HCC. The surgical procedure was determined according to the extent of the tumor, hepatic reserve function, and the patients' wishes. Liver function was assessed by Child-Pugh classification and ICGR 15. If liver function would allow, anatomic resection (segmentectomy (n = 76), sectionectomy (n = 11), or hemihepatectomy (n = 5)) was performed. In patients with insufficient hepatic reserve, limited resection (n = 107) was performed. For example, right hemihepatectomy could be tolerated if ICGR 15 was in the normal range. One-third of the liver parenchyma could be resected for patients with ICGR 15 of 10–19%, segmentectomy was possible with ICGR 15 of 20–29%, and limited resection was possible with ICGR 15 of 30% and more [16]. The procedures of hepatectomy were the same as those described previously [17,18].

RFA

Eighty-seven patients were subjected to RFA. Patients requesting not to undergo hepatectomy underwent RFA. Among the 87 patients, 69 patients were diagnosed as HCC based on CT imaging. The remaining 18 patients were diagnosed as HCC by histopathological methods. Patients were treated with RFA following TACE, if HCC nodules had hypervascularity. TACE was performed an average 3 days before RFA TACE was performed through the femoral artery using the technique of Seldinger under local anesthesia. An angiographic catheter was inserted selectively into the hepatic feeding artery of a segment or subsegments containing the target tumor. We used cisplatinum (Randa; Nippon Kayaku, Tokyo, Japan) as an anticancer drug mixed with iodized oil (Lipiodol; Nihon Schering, Tokyo, Japan) at a concentration of 10 mg/ml and injected at a dose of 10–40 mg/person. The selected dose was based on tumor size. Injection was discontinued upon full accumulation of iodized oil in the tumor vessels. No gelatin sponge or coil embolization was used after TACE in the present study.

RFA was conducted using a commercially available system (Cool-tip RF system; Radionics, Burlington, MA) and electrode that was 17-gauge. Sixty-nine patients whose tumor had hypervascularity were treated with a combination of TACE with RFA. The remaining 18 patients were treated by RFA alone. All patients underwent RFA with a percutaneous approach under real-time ultrasonographic guidance in a ward setting under local anesthesia and conscious sedation [19]. The treatment response was evaluated using CT image. When the diameter of the non-enhancing area was greater than that of the ablated nodule, RFA was considered to have produced a complete effect. HCCs with incomplete response were reevaluated for a new session.

Follow-up

Follow-up evaluation after the surgery or RFA consisted of blood chemistry tests and measurements of tumor markers including α-fetoprotein (AFP) and Des-γ-carboxy prothrombin (DCP), every month. Patients were examined by ultrasound every 3 months and by computed tomographic (CT) scan every 6 months. When recurrence was indicated by any of these examinations, patients underwent CT during arterial portography and arteriography.

Complications were stratified according to the Clavien classification of postoperative surgical complications [20] and imaging-guided tumor ablation: standardization of terminology and reporting [21]. Major complications were defined as those which required treatment or additional hospitalization, or which resulted in permanent adverse sequelae (Clavien classification grade II or higher). This includes any case in which a blood transfusion or interventional drainage procedure is required.

Treatment for Recurrence

All patients with intrahepatic recurrence were managed with ablative therapies (RFA or ethanol injection), TACE, or surgery including liver transplantation according to the same criteria used at the time of initial resection.

Histopathological Examination

The resected specimens were serially sectioned at 10-mm intervals and examined macroscopically. The criteria used to identify intrahepatic micrometastasis were essentially those proposed by the Liver Cancer Study Group of Japan; that is, tumors surrounding the main tumor with multiple other satellite nodules or small solitary tumors located near the main tumor that are histologically similar or less differentiated than the main tumor [22].

Statistical Analyses

Values for continuous variables are presented as means ± SD. Categorical variables were compared using the chi-square test and continuous variables using Student's *t*-test. Overall survival and disease-free survival analyses were carried out using the Kaplan–Meier methods; comparisons between different groups were carried out using the log rank test. The following variables were examined: age (≥70 vs. <70), sex, positivity for hepatitis C virus (HCV) antibody, ICGR 15 (≥15 vs. <15), Child-Pugh class (A vs. B), main tumor size (>20 mm vs. ≤20 mm), tumor number (single vs. multiple), plasma DCP level (≥100 AU/ml vs. <100 AU/ml), and plasma AFP level (≥100 ng/ml vs. <100 ng/ml). Multivariate analyses for survival and disease-free survival were carried out using the Cox's regression model. The regression model was used to evaluate variables found to be associated with infection by univariate analysis ($P < 0.1$). A *P*-value of less than 0.05 was considered significant. Calculations were performed using SPSS software (version 16; SPSS, Inc., Chicago, IL).

RESULTS

There were no differences in age and gender between the surgical resection and RFA groups. However, the hepatic resection group included more patients with hepatitis B virus (HBV) ($P = 0.049$). With regard to hepatic reserve function, ICGR 15 was significantly better in the surgical resection group than in the RFA group ($P = 0.004$); the ICGR 15 was 19.5 ± 9 in the surgical resection group and 23.7 ± 12 in the RFA group. The surgical resection group included more patients with well-preserved liver function (Child-Pugh class A) without statistical significance ($P = 0.06$). On the other hand, regarding with tumor-related factors, the tumor size and DCP level were significantly greater in the surgical resection group than in the RFA group ($P = 0.001$ and 0.03, respectively), and the tumor number was also greater in the surgical resection group than in the RFA group with statistical significance ($P = 0.023$). The mean follow-up of surgical resection and RFA groups were 35 ± 2.5 and 32 ± 2.5 months, respectively. There was no significant difference in overall survival between two groups ($P = 0.11$); the 3-year overall survival rates were 91% in the surgical resection group and 81% in the RFA group (Fig. 1). There was also no significant difference in disease-free survival between two groups ($P = 0.88$); the 3-year disease-free survival rates were 41% in the surgical resection group and 34% in the RFA group

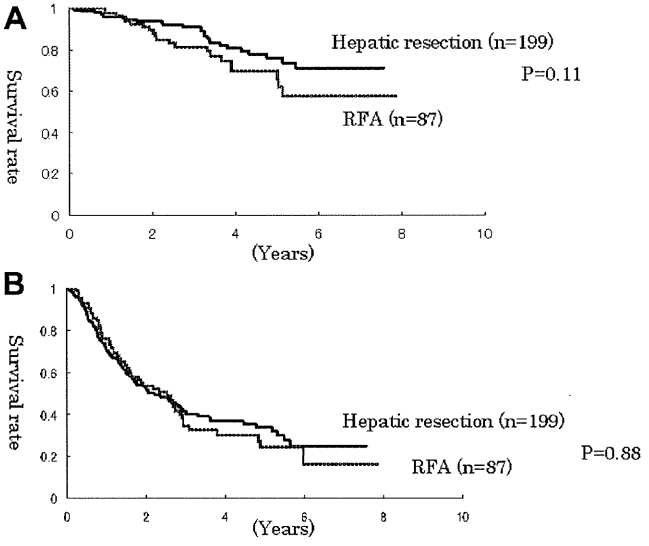

Fig. 1. **A**: Comparison of overall survival rates between patients in the surgical resection and the RFA groups. **B**: Comparison of disease-free survival rates between patients in the surgical resection and RFA groups.

(Fig. 1). Each clinical characteristic including complication was compared between the hepatic resection and RFA groups by univariate analysis, as presented in Table I. There was no mortality during initial hospital stays in both hepatic resection and RFA groups except for one patient who died as a result of suicide within 1 month of hepatic resection. There were no major complications after RFA, whereas major complications occurred in seven patients after hepatectomy. The rate of morbidity after hepatectomy tended to be higher than that after RFA ($P = 0.076$). The hospital stay of hepatectomized patients was significantly longer as compared to that of patients who had underwent RFA ($P = 0.0001$). There was recurrence at the site of the treated tumor in 4 patients who underwent RFA. Complete necrosis was confirmed by imaging in 92% of patients with RFA. Among the four patients who showed recurrence at the site of the treated tumor, three patients had HCC with tumor size exceeding 2 cm (tumor size: 2.0, 2.5, and 3.0 cm), and one patient had HCC with tumor size of 1.5 cm which was located near the liver surface.

Next, subgroup comparisons of overall and disease-free survivals were made between surgical resection and RFA groups (Table II). In subgroup analysis for a single HCC with tumor size exceeding 2 cm in Child-Pugh class A, the disease-free survival was longer in the surgical resection group (n = 72) than in the RFA group (n = 15) with statistical significance ($P = 0.048$); the 3-year disease-free survival rates were 43% in the surgical resection group and 27% in the RFA group (Fig. 2B). In the same subgroup, however, the overall survival was longer in the surgical resection group than in the RFA group without statistical significance ($P = 0.57$); the 3-year overall survival rates were 88% in the surgical resection group and 74% in the RFA group. For multiple HCCs with tumor size exceeding 2 cm in Child-Pugh class A, the overall and disease-free survival rates were longer in the surgical resection (n = 27) than RFA groups (n = 5) without statistical significance. On the other hand, in the subgroup of a single HCC with tumor size $\leq$2 cm in Child-Pugh class A, the overall and disease-free survival rates were almost the same for the surgical resection (n = 53) and RFA (n = 41) groups; the 3-year overall and disease-free survival rates were 95% and 59%, respectively, in the surgical resection group and 94% and 48%, respectively, in the RFA group (Fig. 2A). Moreover, for multiple HCCs with tumor size $\leq$2 cm in Child-Pugh class A, the overall and disease-free survival rates were also almost the same for the surgical resection (n = 30) and RFA groups (n = 11). The subgroup analyses of patients with Child-Pugh class B could not be precisely evaluated due to the small number (less than 4) of cases in each subgroup.

Table III summarizes the results of univariate analyses for all patients according to the clinical characteristics. The Child-Pugh class B ($P = 0.001$) and the tumor number ($P = 0.025$) were significant adverse prognostic factors for overall survival. Similarly, HCV positivity ($P = 0.02$), ICGR 15 $\geq$ 15% ($P = 0.043$), and the tumor number (2 or 3) ($P = 0.0002$) were significant adverse prognostic factors for disease-free survival. In multivariate analyses, Child-Pugh class B ($P = 0.043$) was an independent variable related to adverse overall survival (Table IV). The overall survival rates at 1, 3, and 5 years of 254 patients of Child-Pugh class A were 97%, 91%, and 77%, respectively. The corresponding survival rates of 32 patients of Child-Pugh class B were 94%, 67%, and 56%, respectively.

Table V presents the pathological findings. The incidence of regional cancer spread was significantly lower for HCCs smaller than

TABLE I. Background Characteristics of Patients With Resection or RFA

Variables	Hepatic resection (n = 199)	RFA (n = 87)	*P*-value
Gender (male/female)	137 (68%)/62 (31%)	53 (61%)/34 (39%)	0.19
Age (year)	65.7 ± 9.0	66.3 ± 8.2	0.6
Virus (B/C/others)	38 (19%)/145 (73%)/16 (8%)	9 (10%)/73 (84%)/5 (5%)	0.049
Total bilirubin (mg/dl)	0.86 ± 0.34	0.93 ± 0.36	0.1
Prothrombin time (%)	86.6 ± 14.9	83.7 ± 15.4	0.139
Serum albumin (g/dl)	3.82 ± 0.47	3.78 ± 0.50	0.537
Platelet count (10^4/mm^3)	11.8 ± 7.1	10.5 ± 8.6	0.21
ICG R 15 (%)	19.5 ± 9.0	23.7 ± 12	0.004
Child-Pugh classification (A/B)	182 (91%)/17 (9%)	72 (83%)/15 (17%)	0.06
Tumor size (mm)	2.1 ± 0.63	1.8 ± 0.52	0.001
Tumor number (single/multiple)	132 (66%)/67 (34%)	67 (77%)/20 (23%)	0.023
DCP (AU/ml)	197 ± 756	72 ± 223	0.03
AFP (ng/ml)	310 ± 1322	85 ± 166	0.11
Hospital stay (day)	15 ± 8	8 ± 3	0.0001
Major complications			0.076
Ascites or pleural effusion	2 (1%)	0	
Rupture of esophageal varices	2 (1%)	0	
Biliary leakage	3 (2%)	0	

RFA, radiofrequency ablation; B, hepatitis B virus; C, hepatitis C virus; ICGR 15, indocyanine green retention rate at 15 min; DCP, Des-γ-carboxy prothrombin; AFP, alpha-fetoprotein.

TABLE II. Overall Survival and Disease-Free Survival Rate for Patients of Child-Pugh Class A

	Overall survival rate (%)				Disease-free survival rate (%)			
Factor	1 year	3 years	5 years	*P*-value	1 year	3 years	5 years	*P*-value
Single HCC >2 cm				0.57				0.048
Resection (n = 72)	94	88	83		71	43	36	
RFA (n = 15)	100	74	74		44	27	9	
Multiple HCC >2 cm				0.18				0.98
Resection (n = 27)	96	96	43		60	28	22	
RFA (n = 5)	100	75	38		80	0	0	
Single HCC ≤2 cm				0.46				0.58
Resection (n = 53)	100	95	88		90	59	53	
RFA (n = 41)	97	94	83		81	48	43	
Multiple HCC ≤2 cm				0.98				0.98
Resection (n = 30)	92	92	69		61	22	22	
RFA (n = 11)	100	92	73		80	18	18	

RFA, radiofrequency ablation.

2 cm than for HCC >2 cm; with microvascular invasion (22% vs. 4.3%) and micrometastasis (20.3% vs. 8.3%) being more common in HCCs >2 cm compared to those ≤2 cm.

Ninety-seven (49%) of the patients in the hepatic resection group and 43 (49%) of the patients in the RFA group showed HCC recurrences. The pattern of recurrence and the details of treatments for the recurrences in both groups are shown in Table VI. The main treatment for recurrence was TACE (34%), followed by RFA (28%) and repeat hepatectomy (23%) in the surgical resection group, whereas the main treatment for recurrence was RFA (37%), and followed by TACE (35%) and hepatectomy (16%) in the RFA group. One patient underwent salvage liver transplantation for recurrence in the surgical resection group. The proportion of patients with extrahepatic recurrence tended to be higher in the hepatic resection group ($P = 0.07$), but the modalities of the treatments used for recurrence did not differ among the two groups.

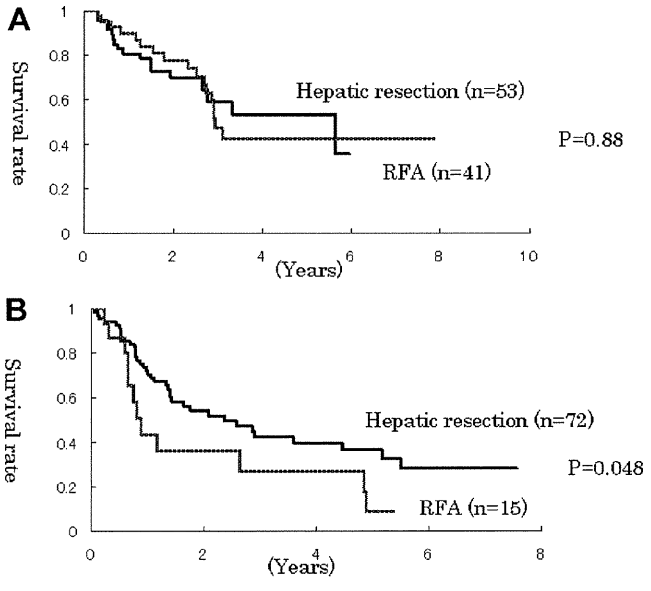

Fig. 2. **A**: Comparison of disease-free survival rates in patients with a single HCC with tumor size less than 2 cm in Child-Pugh A between patients in the surgical resection and RFA groups. **B**: Comparison of disease-free survival rates in patients with a single HCC with tumor size >2 cm in Child-Pugh A between patients in the surgical resection and RFA groups.

DISCUSSION

Livraghi et al. [7] recently demonstrated that the estimated 3- and 5-year survival rates for the potentially operable subgroup (100 patients with a single HCC ≤2 cm) were 89% and 68%, respectively. They showed that the 5-year survival rate in their study was comparable to that reported by the Liver Cancer Study Group of Japan, which revealed a 5-year survival rate of 70% for 2078 patients. They concluded that RFA can be considered the preferred treatment for patients with single HCC ≤2 cm, even when surgical resection is possible, since RFA is much less invasive and has a lower complication rate, and other approaches can be used as salvage therapy for the few cases in which RFA is unsuccessful or unfeasible. In our study, a combination of TACE and RFA was performed in the majority of patients with hypervascular HCC nodules who did not undergo surgical resection. The diagnosis by CT imaging has the possibility of misdiagnosis of HCC for nodule measuring 2.0 cm or less. Vascular occlusion by TACE permits the formation of larger thermal lesions by reducing heat loss [23,24]. In addition, the accumulation of lipiodol might be useful for obtaining the border of the tumors at CT scan after RFA [19]. In our current study, RFA for HCC smaller than 2 cm has overall and disease-free survival rates similar to those for the resection group. The previous histopathologic studies have shown that, although HCC nodules measuring 1.5 cm or less are uniformly well differentiated, those between 1.5 and 2.0 cm in diameter often contain zones of less differentiated tissue with more intense proliferative activity [25]. Takayama et al. [26] also found that among 70 patients with an early single HCC of 2 cm or less in diameter, only 15 HCC lesions were well-differentiated, and microscopic regional spread (vascular invasion and micrometastasis) was identified in 23 (33%) patients. On the other hand, Wakai et al. [27] shown that vascular invasion was more frequent in patients with HCC >2 cm (16/62, 26%) than in patients with HCC ≤2 cm (1/23, 4%, $P = 0.033$). In the current study, the incidence of micrometastasis and microvascular invasion was significantly lower among patients with HCCs 2 cm or less in diameter than among patients with HCCs larger than 2.0 cm in diameter. Recently, Shi et al. [28] found that among patients with HCCs ≤3 cm, 38 (86%) out of 44 identified micrometastases were located within 1 cm of tumor in the same direction of portal venous, and a resection margin of 1.0 cm is recommended for HCCs ≤3 cm. Theoretically, a single electrode insertion can produce a necrotic area of up to 3.0 cm in diameter, thus allowing full ablation of a 2-cm tumor plus a 0.5–1.0 cm safety margin. Our studies have suggested that the choice of RFA does not matter for patients with single or multiple (less than 3) HCC ≤2 cm, if HCC lesions cannot be visualized by US or are close to anatomic structures that might be damaged by RFA.

TABLE III. Univariate Analysis of Clinical Factors for Overall Survival and Disease-Free Survival Rate

	Overall survival rate (%)				Disease-free survival rate (%)			
Factor	1 year	3 years	5 years	*P*-value	1 year	3 years	5 years	*P*-value
Age (year)				0.53				0.7
≤70 (n = 175)	97.6	89.1	76.1		73.1	38.4	31.7	
>70 (n = 111)	94.1	85.5	71.1		72.9	39.4	27.5	
Gender				0.15				0.8
Male (n = 190)	96.6	86.4	72.2		72.4	40.4	34.4	
Female (n = 96)	95.6	89.9	77.3		74.4	35.2	20.2	
Virus				0.09				0.02
HCV (n = 218)	96	86.3	70.9		71.8	31.9	34.4	
Non-HCV (n = 68)	96.8	89.9	84.6		74.4	54	47.6	
Child-Pugh classification				0.001				0.227
A (n = 254)	96.6	90.7	76.6		72.5	39.2	32.6	
B (n = 32)	93.5	67	56.2		74.3	34.3	10.7	
ICGR 15 (%)				0.6				0.043
<15 (n = 148)	96.6	85.1	79		75.1	46.9	36.9	
≥15 (n = 137)	95.6	90.4	68.9		70.2	28.6	22.6	
Tumor size (mm)				0.26				0.07
≤2.0 (n = 157)	96.4	89.8	78.7		79.2	40.2	33.3	
>2.0 (n = 129)	96	85.6	69.8		65.1	36.2	27	
Tumor number				0.025				0.0002
Single (n = 199)	96.8	87.2	80.4		75.9	45.3	36.7	
2 or 3 (n = 87)	95	88.9	59.6		65.1	23.9	16.6	
DCP (AU/ml)				0.11				0.46
<100 (n = 227)	97.1	89.5	76.1		73.1	38.7	30.5	
≥100 (n = 59)	92.8	85.6	69.3		71.2	39.3	30.6	
AFP (ng/ml)				0.65				0.64
<100 (n = 215)	96.5	90.2	74.8		73	38.1	30	
≥100 (n = 71)	95.5	89.4	74.9		73.2	42.1	33.8	
Treatment				0.11				0.88
Resection (n = 199)	95.6	90.9	76		71.4	41.2	33.7	
RFA (n = 87)	97.6	81.4	71		76.5	34.3	24.7	

HCV, hepatitis C virus; ICGR 15, indocyanine green retention rate at 15 min; DCP, Des-γ-carboxy prothrombin; AFP, alpha-fetoprotein.

A preliminary report of the Japanese nationwide survey has shown that surgical resection provides a lower time-to-recurrence rate than RFA does among patients with HCCs no more than three tumors (≤3 cm) [12]. In the current study, we have also shown that in subgroup analysis of a single HCC with tumor size >2 cm in Child-Pugh class A, the disease-free survival was longer in the surgical resection group than in the RFA group with significance. The overall survival was longer in the surgical resection group than in the RFA group, although the result was not significant. Our histopathological study has shown that the incidence of micrometastasis was significantly higher among patients with HCCs exceeding 2 cm in diameter (20%) than among patients with HCCs 2.0 cm or less in diameter (8.3%). These findings have suggested that RFA is less effective than hepatic resection to eradicate venous tumor thrombi and micrometastasis in the adjacent liver in addition to the complete removal of the primary HCC with tumor size >2 cm [29,30]. Surgical resection may provide better long-term disease-free survival than RFA in the subgroup of a single HCC exceeding 2 cm of Child-Pugh class A.

We have shown that in subgroup analysis of multiple HCCs exceeding 2 cm in Child-Pugh class A, the overall survival and the disease-free survival in the surgical resection group was not significant different from that in the RFA group. The strategy for multiple HCCs larger than 2 cm in Child-Pugh class A remains unclear because of small sample number in RFA group.

TABLE IV. Multivariate Analysis of Overall Survival

Variable	HR	95% CI	*P*-value
Child-Pugh class (B vs. A)	1.669	1.016–2.741	0.043

In our study, Child-Pugh class A and a single tumor were significant favorable prognostic factors for overall survival, and HCV negativity, lower ICGR 15, and a single tumor were significant favorable prognostic factors for disease-free survival in univariate analysis, although in a multivariate study only Child-Pugh class A was an independent favorable factor for overall survival. The preliminary report of the Japanese nationwide survey has shown that in multivariate analysis, low tumor marker, tumor size <2 cm, better liver function (Child-Pugh class A), and the presence of HCV infection were favorable factors for overall survival, and a single tumor, low tumor marker levels, small tumor size, the absence of HCV, and younger age were negative factors for recurrence [12]. These results are similar to trends found in the nationwide study.

Radiofrequency is much less invasive, involves a short hospital stay, and has low mortality associated with the procedure. With the intention of avoiding the risk of hepatic failure that can follow hepatic resection, percutaenous ablation treatments have been proposed due to the efficacy, tolerability, and low risk of the procedure. However, in the

TABLE V. Pathological Characteristics of HCC of Patients With Hepatic Resection

Characteristics	HCC >2 cm (n = 103)	HCC ≤2 cm (n = 96)	*P*-value
Histological type			
Well/moderate/poor/unknown	11/75/11/6	22/65/4/5	0.023
Regional cancer spread			
Microvascular invasion	23 (22%)	4 (4.3%)	0.0001
Intrahepatic micrometastasis	21 (20.3%)	8 (8.3%)	0.027

TABLE VI. Recurrence and Treatments for Recurrence After Hepatic Resection or RFA

	Hepatic resection (n = 199)	RFA (n = 87)	*P*-value
HCC recurrence: yes[a]	97 (49%)	43 (49%)	0.84
Pattern of recurrence[b]			0.07
Intrahepatic	86 (89%)	43 (100%)	
Intahepatic + extrahepatic	7 (7%)	0 (0%)	
Extrahepatic	4 (4%)	0 (0%)	
Treatment: yes[b]	93 (96%)	41 (95%)	0.5
Main modalities[b]			0.67
Hepatectomy	23 (24%)	7 (16%)	
RFA	27 (28%)	16 (37%)	
PEI	3 (3%)	2 (5%)	
TACE	33 (34%)	15 (35%)	
Liver transplantation	1 (1%)	0 (0%)	
Others	6 (6%)	1 (2%)	

RFA, radiofrequency ablation; PEI, percutaneous ethanol injection; TACE, transcatheter arterial chemoembolization

[a]Data are expressed as the number of patients (percentage of total patients).

[b]Data are expressed as the number of patients (percentage of patients who had a recurrence).

current study, hepatic resection has been considered as an acceptable treatment, because the procedure-related mortality was zero after hepatectomy, and there was no significant difference in the incidence of morbidity between the two groups, regardless of the high tendency of the incidence of morbidity after hepatic resection.

Our retrospective study had some drawbacks. Clinical characteristics that can strongly influence outcomes differed significantly between the surgical resection group and the RFA group, as shown in other studies. In the current study, the proportion of the multinodular HCC patients and the levels of DCP were higher in the resection group than those in the RFA group, whereas the proportion of poor function liver reserve was lower in the resection group than that in the RFA group. Because multiple nodules and poor function liver reserve are major risks of recurrence, we conducted subgroup analysis according to the tumor size, tumor number, and Child-Pugh class. Ultimately, a randomized controlled trial would be necessary to prospectively determine if RFA and surgery are comparable therapies for early stage HCC.

In conclusion, RFA can be considered the preferred treatment for patients with single or multiple HCC $\leq$2 cm of Child-Pugh class A. Our results suggest that surgical resection may provide better long-term disease-free survival than RFA does in the subgroup of a single HCC exceeding 2 cm of Child-Pugh class A. A large prospective trial comparing surgical resection with RFA is on-going in the Japanese nationwide study, and thus, clear-cut guidelines are expected to be established in the near future.

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ORIGINAL ARTICLE

Significance of Platelet Count in the Outcomes of Hepatectomized Patients with Hepatocellular Carcinoma Exceeding the Milan Criteria

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Abstract
Background The appropriate treatment strategy for advanced hepatocellular carcinoma (HCC) that does not meet the Milan criteria (MC) is unclear. The aim of this study was to determine the significance of surgical treatment for such patients.
Study design From January 1990 to December 2007, 151 patients with HCC exceeding MC who underwent curative surgical treatment were enrolled. Survival and recurrence data and clinicopathological factors were examined. Prognostic factors were analyzed to identify those that contributed to improved surgical outcomes retrospectively.
Results After the initial hepatectomy, the overall 3-, 5-, and 10-year survival rates were 73%, 55%, and 33%, respectively, for the 151 patients in this study; the corresponding disease-free survival rates were 36%, 30%, and 17%, respectively. A platelet count under $10^5/mm^3$, multiple tumors, and liver cirrhosis of noncancerous tissue were adverse survival and disease-free survival factors by univariate analysis. Platelet count was an independent prognostic factor by multivariate analysis. The 3-, 5-, and 10-year overall survival rates of HCC exceeding MC in patients whose platelet count was $10^5/mm^3$ or greater reached 76%, 65%, and 44%, respectively, and were comparable with those that met MC (86%, 68%, and 37%, respectively).
Conclusions Hepatectomy for patients with advanced HCC exceeding MC improves survival, especially for patients with a sufficiently high platelet count, although recurrence rates after initial hepatectomy are high.

Keywords Hepatocellular carcinoma · Milan criteria · Platelet count · Advanced · Hepatectomy · Prognosis

Abbreviations

HCC	Hepatocellular carcinoma
TACE	Transarterial chemoembolization
OLT	Orthotopic liver transplantation
MC	Milan criteria
ICGR15	Indocianine green retention rate at 15 min
RFA	Radiofrequency ablation
LDLT	Living donor liver transplantation

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. There are various options to treat HCC, including partial hepatectomy, percutaneous ablation therapy, and transarterial chemoembolization (TACE). However, the resulting prognosis of HCC remains inadequate, despite technical refinements in these treatments, due to the high incidence of recurrence of HCC.[1,2]

Orthotopic liver transplantation (OLT) is the preferred treatment for patients with cirrhosis and early HCC per the

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Milan criteria (MC: defined as a solitary HCC of a size <5 cm or 2 or 3 tumors <3 cm with no gross vascular invasion).[3] In patients with early HCC, such as within MC, as long as liver function is preserved, liver resection effects an overall 5-year survival rate that is comparable with that of liver transplantation, with minimal morbidity and mortality.[4–6]

The treatment strategy for advanced HCC exceeding MC has not been discussed sufficiently. Due to advanced tumor status, ablation therapy cannot be the first treatment, nor can OLT. Although hepatectomy or TACE is used to treat advanced HCC patients, the 5-year overall survival rate after curative hepatectomy for advanced HCC (tumor size, >5 cm) is 30% to 35%, and its recurrence after hepatectomy is unavoidable.[7,8]

We retrospectively analyzed the impact of hepatectomy on tumor control in patients with HCC exceeding MC. In this study, we examine the rationale for partial hepatectomy as an initial treatment and discuss the development of other strategies for recurrent HCC.

Methods

Patient data began to be collected prospectively by our program in 1986. Between January 1990 and December 2007, 781 consecutive adult patients underwent hepatectomy for HCC at Hiroshima University Hospital. A total of 651 consecutive HCC patients underwent curative intent hepatectomy in our hospital. Curative intent hepatectomy was defined as the removal of all recognizable tumors; patients with macroscopic vascular invasion in the first portal branch, portal vein trunk or hepatic vein trunk, and/or extrahepatic metastasis were excluded due to their poor prognosis.

Data for the remaining 622 HCC patients were included in the analysis. We divided the remaining patients into two groups: transplantable (meeting MC: single lesion with a maximum diameter <5 cm or three lesions with a maximum diameter <3 cm) and advanced (exceeding MC). We focused on the advanced group.

The indications and procedure for hepatectomy have been described.[9,10] Briefly, Child–Pugh class C was regarded as a contraindication for hepatectomy. The decision to perform hepatectomy was made based on liver function and extent of tumor. Liver function was assessed according to Child–Pugh classification and indocianine green retention rate at 15 min (ICGR15). In patients who lacked ascites and had normal bilirubin levels, ICGR15 became the chief determinant of resectability. For example, right hemihepatectomy could be tolerated if ICGR15 was in the normal range. One third of the liver parenchyma could be resected for patients with ICGR15 of 10–19%; segmentectomy was possible for patients with ICGR15 of 20–29%; and limited resection was possible for patients with ICGR15 of ≥30% (9.10).

Hepatectomy was indicated when all tumors could be resected with sufficient hepatic functional reserve, as determined by preoperative imaging. However, when the HCC tumors were hypovascular—suggesting that the tumor was well-differentiated HCC—and ≤2 cm and when the number of tumors ≤3, percutaneous ablation therapies were preferable despite hepatectomy being feasible, depending on the tumor location in the liver. Clinicopathological findings were recorded according to the criteria of the Liver Cancer Study Group in Japan.[11] Liver cirrhosis was confirmed by histological examination of the resected specimen.

A modified Clavien classification was used to grade the severity of postoperative complications.[12] Grade I complications were defined as deviations from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic, or radiological intervention. Grade I complications also included wound infections that opened at the bedside.

Grade II complications were defined as those that required pharmacological treatment; blood transfusion and total parenteral nutrition were also included. Grade III complications were those that required surgical, endoscopic, or radiological intervention. Grade IV complications were life-threatening complications that required intermediate care/intensive care unit management. Grade V complications resulted in death. Operative mortality was defined as death within 30 days after surgery. In-hospital mortality was defined as death within the hospitalization period.

Postoperative follow-up evaluations consisted of a clinical physical examination, blood chemistry tests, and measurements of tumor marker levels, including alpha-fetoprotein and des-gamma-carboxy prothrombin, every month for 2 years. After 2 years, patients were assessed every 3 months. Patients were examined by abdominal ultrasonography every 3 months and by computed tomography every 6 months during the follow-up periods.

Our follow-up protocol included an evaluation by hepatologists to monitor cancer recurrence and the progress of chronic hepatitis or liver cirrhosis. When recurrence was noted in any of these examinations, patients underwent hepatic angiography. The patents were followed up regularly until December 31, 2008, and every patient was followed up for at least 6 months. All patients who experienced intrahepatic recurrence were managed with ablative therapy (radiofrequency ablation (RFA) or ethanol injection), TACE, or surgery, including liver transplantation, according to the same criteria as for the initial resection.

Statistical analyses were performed using unpaired Student's *t* test and chi-square test with Fisher's exact test. Overall survival and disease-free survival rates were calculated using the Kaplan–Meier method and compared using log-rank test. Disease-free survival was calculated, considering any death or recurrence as an event. A *P* value <0.05 was considered to be statistically significant. Statistical analysis was performed using StatView for Windows (Version 5.0; SAS Institute, Cary, NC, USA).

Results

As shown in Fig. 1, there were 151 patients with initially resectable advanced HCC who did not fulfill MC (i.e., exceeding MC) and 471 patients who met MC.

In the exceeding-MC group, the mean follow-up period for all survivors was 4.1±3.1 years (range, 0.5 to 14.5 years). Table 1 shows the patients' backgrounds. Overall operative mortality and in-hospital mortality rates were the same, i.e., 0.7% (n=1) in both conditions. The incidence of complications that developed after hepatectomy is also shown in Table 1. Thirty of the 151 patients (20%) had postoperative complications (Table 1). Nineteen of the 151 patients (13%) were grade III or more.

Figure 2a shows the survival rates of patients who underwent curative resection of HCC (meeting MC and exceeding MC). The survival rate of the exceeding-MC group was significantly lower than that of the group that met MC (P=0.030). The 3-, 5-, and 10-year survival rates

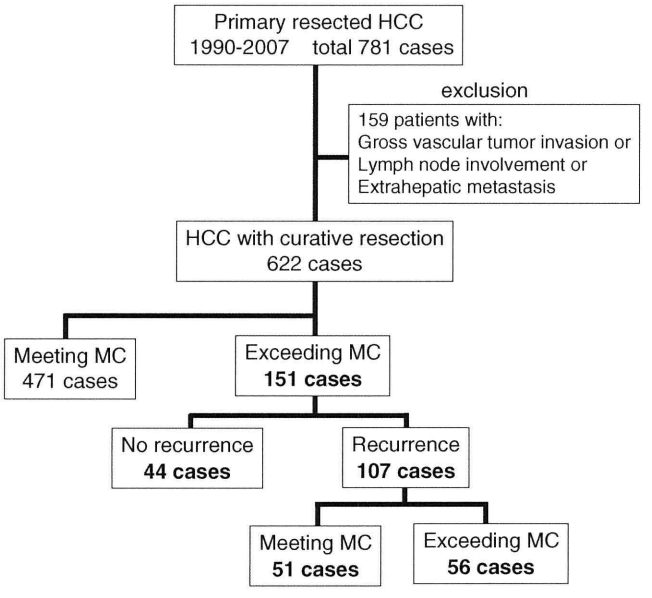

Fig. 1 Overview of outcomes of patients with primary resected hepatocellular carcinoma (HCC). The number of HCC patients who underwent curative resection was 622, subdivided by the Milan criteria (MC)

Table 1 Patients' backgroud

	Number of patients	Percent
Age (year)		
≤60	57	38.4
>60	94	61.6
Gender		
Male	127	84.1
Female	24	15.9
Type of hepatitis virus		
Non-HCV	61	40.4
HCV	90	59.6
Child–Pugh grade		
A	129	85.4
B	22	14.6
Type of hepatectomy		
Limited resection	82	54.3
Segmentectomy or more	69	45.7
Operative mortality: yes	1	0.7
In-hospital mortality: yes	1	0.7
Postoperative complications[a]: yes	30	19.9
Grade I, II	11	7.3
Grade III or more	19	12.6

[a] Postoperative complications was defined as any event satisfying the criteria advocated by Dindo et al.[12]

of the exceeding-MC group were 77%, 55%, and 33% and 86%, 68%, and 37% in those that met MC, respectively. The 3-, 5-, and 10-year disease-free survival rates of the exceeding-MC group were 36%, 30%, and 17% and 47%, 30%, and 13% in those that met MC, respectively (Fig. 2b).

Table 2 summarizes the results of the univariate analysis according to clinicopathological factors. A platelet count $<10^5/mm^3$ (P<0.001), multiple tumors (P=0.012), and cirrhosis of noncancerous tissue (P=0.035) were significant adverse prognostic factors for overall survival. Similarly, a platelet count $<10^5/mm^3$ (P=0.001), multiple tumors (P=0.005), and cirrhosis of noncancerous tissue (P=0.020) were significant adverse prognostic factors for disease-free survival.

By multivariate analysis, a platelet count $<10^5/mm^3$ (P=0.007) was found to be an independent adverse prognostic factor for overall survival (Table 3), and the 3-, 5-, and 10-year overall survival rates of patients with HCC exceeding MC whose platelet count was $\geq 10^5/mm^3$ were 76%, 65%, and 44%, respectively, comparable with the group that met MC (86%, 68%, and 37%, respectively; Fig. 2a). A platelet count $<10^5/mm^3$ (P=0.039) was also an independent adverse factor for disease-free survival (Table 3).

Out of 151, a total of 107 (71%) patients with HCC exceeding MC experienced a recurrence after the initial hepatectomy. Table 4 shows the patterns of cancer recurrence

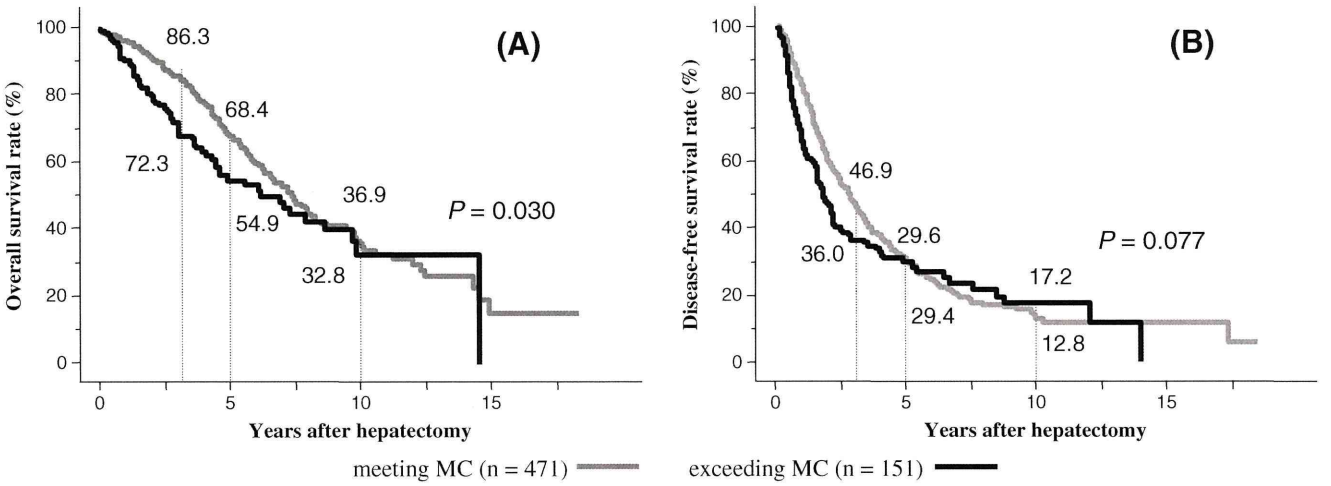

Fig. 2 Survival and disease-free survival curves of patients who received curative resection of HCC that met (471 patients) or were exceeding (151 patients) MC. (A) The 3-, 5-, and 10-y survival rates of patients exceeding MC were 72.3%, 54.9%, and 32.8%, respectively, and 86.3%, 68.4%, and 36.9%, respectively, in those who met MC. (B) The 3-, 5-, and 10-y disease-free survival rates of patients exceeding MC were 36.0%, 29.4%, and 17.2%, respectively, and 46.9%, 29.6%, and 12.8%, respectively, in those who met MC

and compares the consequent treatment details between patients whose platelet counts were $\geq 10^5/mm^3$ and $<10^5/mm^3$. The rate of HCC recurrence was significantly lower in patients whose platelet count was $\geq 10^5/mm^3$; 76 (66%) of such patients experienced a recurrence of HCC after hepatectomy, as compared to 31 (89%) patients whose platelet count was $<10^5/mm^3$ (P=0.009). Further, regarding the pattern of recurrence, the proportion of patients who had a recurrence of HCC that met MC was significantly higher in patients with a platelet count$\geq 10^5/mm^3$ than those with a platelet count of $<10^5/mm^3$ (51% vs. 39%; P<0.001).

The proportion of patients who received curative treatment for the first recurrence, such as repeat hepatectomy and local ablation therapy, had significantly higher platelet counts, i.e., $\geq 10^5/mm^3$ (44% vs. 23%; P=0.047).

Of the 107 patients who experienced a recurrence, 51 (48%) met MC and 56 (52%) were exceeding MC, including extrahepatic recurrence (Fig. 1). The 3- and 5-year survival rates after recurrence were significantly superior in patients with a recurrence that met MC (71% and 40%, respectively) than those exceeding MC (17% and 9%) (P<0.001; Fig. 3).

Table 5 shows the details of the treatments for recurrences after hepatectomy. The proportions of patients who received ablation therapy or repeat hepatectomy after recurrence was higher in patients with a recurrence that met MC than those exceeding MC (P=0.001). Two patients with a recurrence that met MC, who underwent salvage living donor liver transplantation (LDLT), did not have a recurrence after liver transplantation at the 2- and 3-year follow-up, respectively. One patient with a recurrence that was exceeding MC, and who underwent salvage LDLT, experienced a recurrence of HCC within 1.5 years.

Discussion

The ultimate goal of a treatment for HCC is to prolong survival by eradicating malignant legions while preserving hepatic function. Surgical resection, by partial hepatectomy or total hepatectomy followed by OLT, is the standard treatment with a curative intent.[13] The resectability and choice of procedure depend on many factors, including baseline liver function, absence of extrahepatic metastasis, size of residual liver, availability of resources (including liver grafts), and expertise of the surgical team.

Although hepatic resection, ablation therapy, and liver transplantation are accepted, effective treatments for patients with cirrhosis and early HCC, the proper strategy for advanced HCC has not been established. Therefore, we studied HCC patients who were exceeding MC—who are not eligible for OLT as the initial treatment. We investigated the impact of hepatectomy on outcomes of HCC that exceeded MC and examined the rationale of hepatectomy as an initial treatment for HCC exceeding MC.

In our series, the 5- and 10-year survival rates of patients with HCC exceeding MC were 55% and 33%, respectively, comparable with Kamiyama et al.[14] We also identified significant prognostic factors of patients with HCC exceeding MC who underwent hepatectomy: platelet count, tumor number, and cirrhosis. Moreover, our multivariate analysis revealed that platelet count was the sole independent prognostic factor in these HCC patients.

The prognosis of such patients after hepatectomy was clearly stratified by platelet count, which is typically predictable by preoperative laboratory tests. The 3-, 5-, and 10-year overall survival rates of patients with HCC exceeding MC, whose platelet count was $\geq 10^5/mm^3$, were

Table 2 Overall and disease-free survival rates of patients with HCC exceeding MC according to clinicopathological factor

		Overall survival (%)				Disease-free survival (%)			
		3-year	5-year	10-year	*P* value	3-year	5-year	10-year	*P* value
All cases (n=151)		73	55	33		36	30	17	
Age (year)	≤60 (n=57)	69	58	38	0.873	35	28	17	0.977
	>60 (n=94)	74	53	30		37	30	18	
Gender	Male (n=127)	75	55	34	0.647	34	27	15	0.247
	Female (n=24)	61	56			45	45		
Type of hepatitis virus	Non-HCV (n=61)	71	65	36	0.498	46	39	25	0.054
	HCV (n=90)	73	50	32		29	22	12	
Total bilirubin (/mm^3)	<1.0 (n=125)	71	52	32	0.151	37	32	19	0.515
	≥1.0 (n=26)	78	72	36		30	19	9	
Platelet counts (/mm^3)	<10^5 (n=35)	61	27		< 0.001	16	8		0.001
	≥10^5 (n=116)	76	65	44		42	36	21	
ALT (IU/l)	<60 (n=106)	71	49	29	0.08	36	30	15	0.707
	≥60 (n=45)	77	70	45		36	27	18	
Alb (g/dL)	<3.5 (n=37)	73	52	41	0.995	42	31	23	0.55
	≥3.5 (n=114)	73	58	32		35	29	15	
ICG-R15 (%)	<20 (n=111)	72	57	43	0.303	40	32	20	0.467
	≥20 (n=39)	75	52			26	22		
Child–Pugh grade	A (n=129)	73	56	30	0.643	35	29	18	0.645
	B (n=22)	72	54	46		43	32	17	
AFP (ng/mL)	<400 (n=101)	77	56	31	0.905	33	26	16	0.495
	≥400 (n=48)	65	55	41		45	39	22	
Number of tumors	Single (n=60)	79	71	52	0.012	52	41	28	0.005
	Mutiple (n=91)	68	45	23		26	22	12	
Tumor distribution	One section (n=77)	81	56	43	0.083	42	33	28	0.091
	more (n=74)	61	55	25		32	23	8	
Non-cancer tissue	Cirrhosis (n=52)	67	39	29	0.035	23	15	8	0.02
	Others (n=99)	75	65	38		42	36	23	
Preoperative TAE	Yes (n=102)	72	55	30	0.91	35	28	15	0.366
	No (n=45)	73	56	50		40	33	25	
Type of hepatectomy	Limited resection (n=82)	73	51	29	0.743	34	27	8	0.472
	Segmentectomy or more (n=69)	71	60	39		39	33	33	
Transfusion	Yes (n=20)	64	46	0	0.071	25	17	0	0.103
	No (n=131)	74	57	37		38	31	21	
Microscopic vascular invasion	Yes (n=74)	60	48	30	0.089	30	28	17	0.144
	No (n=77)	84	61	35		42	31	18	
Histologic grading	Well or moderate (n=122)	71	55	30	0.718	34	28	18	0.777
	poor (n=26)	74	52	43		42	31	12	
Diabetes mellitus	Yes (n=53)	73	58	34	0.929	39	30	17	0.493
	No (n=95)	72	52	31		33	29	17	
SF criteria	Meeting SF (n=59)	74	52	23	0.704	30	28	15	0.734
	Exceeding SF (n=92)	71	57	38		40	32	19	

HCC hepatocellular carcinoma, *MC* Milan criteria, *ALT* alanine aminotransferase, *ICG-R15* indocianine green retension rate at 15 min, *AFP* alpha-fetoprotein, *SF* San Francisco criteria (1 lesion <6.5 cm, 2–3 lesions each <4.5 cm with total diameter <8 cm)

76%, 65%, and 44%, respectively, comparable with those that met MC (86%, 68%, and 37%, respectively).

Hepatectomy should be the first-line treatment in patients with HCC exceeding MC whose platelet count is >10^5/mm^3.

Table 3 Results of Cox's proportional hazards analysis for overall and disease-free survival after hepatectomy

Variables	*P* value	Relative risk	95% CI
Overall survival			
Plt. Count: $<10^5/mm^3$	0.007	2.155	1.232–3.774
Number of tumors: multiple	0.103	1.65	0.903–3.021
Tumor distribution: more than one section	0.168	1.439	0.858–2.410
Transfusion: Yes	0.13	1.667	0.861–3.228
Microscopic vascular invasion: Yes	0.067	1.596	0.969–2.629
Non-cancer tissue: cirrohsis	0.488	1.207	0.709–2.058
Disease-free survival			
HCV infection: Yes	0.585	1.148	0.699–1.887
Plt. Count: $<10^5/mm^3$	0.039	1.653	1.025–2.667
Number of tumors: multiple	0.202	1.368	0.845–2.221
Tumor distribution: more than one section	0.098	1.412	0.939–2.123
Non cancer tissue: cirrohsis	0.274	1.277	0.824–1.979

In general, platelet count, which reflects the severity of portal hypertension, is a significant predictor of survival. Several studies have shown that platelet count is a risk factor for carcinogenesis from chronic hepatitis and for survival and recurrence of HCC after treatment, including liver resection.[15–18] In fact, we observed that recurrence of HCC after hepatectomy decreased in patients whose platelet count was $\geq 10^5/mm^3$ and that the proportion of patients who experienced a recurrence of HCC that met MC was significantly higher in patients with a platelet count $<10^5/mm^3$. Further, the proportion of patients who underwent repeat hepatectomy or RFA as a curative treatment for a recurrence of HCC was significantly higher in patients whose platelet count was $\geq 10^5/mm^3$.

After resection with curative intent, many patients experience a recurrence, which is a significant cause of late death. In this study, the recurrence rate was high: 70.9% of patients with HCC exceeding MC were diagnosed as having had a recurrence (mean follow-up, 4.1 years). Tumor number was an independent factor of disease-free survival, and the 3-, 5-, and 10-year disease-free survival rates were 51%, 41%, and 28%, respectively, even in patients with a single tumor.

The reported cumulative 5-year recurrence rates range from 50% to 100%.[19–22] In our series, 107 (71%) of 151 patients with HCC exceeding MC experienced a recurrence of HCC, 51 (48%) of whom met MC. These results demonstrate that downstaging a recurrence to within MC was achieved by hepatectomy as an initial treatment for HCC exceeding MC. The proportion of patients who underwent repeat hepatectomy or local ablation therapy as a curative treatment for HCC recurrence was significantly higher in patients with a recurrence of HCC within MC versus exceeding MC. The outcomes after recurrence were significantly better in patients whose recurrence was downstaged to within MC compared with those who did

Table 4 Recurrent pattern and treatment of recurrent HCC after hepatectomy (comparison with platelet counts)

	Platelet counts $>10^5$ (n=116)	Platelet counts $<10^5$ (n=35)	*P* value
Cancer recurrence[a]: yes	76 (66)	31 (89)	0.009[c]
Recurrent pattern[b]			<0.001[c]
Meeting MC	39 (51)	12 (39)	
Exceeding MC or extrahepatic recurrence	37 (49)	19 (61)	
Treatments for recurrence[b]			0.047[c]
Curative treatment	34 (44)	7 (23)	
Non-curative treatment	41 (55)	22 (70)	
Salvage liver transplantation	1 (1)	2 (6)	

Curative treatment included partial hepatectomy, local ablation therapy; non-curative treatment included transarterial chemoembolization, systemic chemotherapy, radiation therapy and conservative

HCC hepatocellular carcinoma, *MC* Milan criteria

[a] Data are expressed as the number of patients (percentage of total patients)

[b] Data are expressed as the number of patients (percentage of patients who had a recurrence)

[c] Statistically significant difference

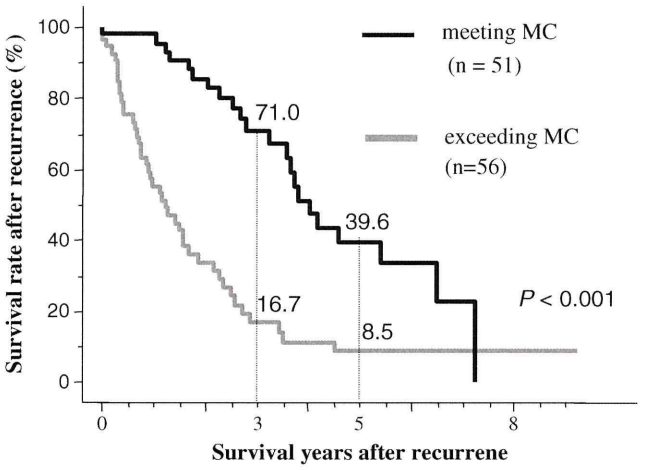

Fig. 3 Comparison of survival curves after recurrence of HCC according to recurrent pattern. The 3-and 5-year survival rates of patients with a recurrence that met MC were 71.0% and 39.6%, respectively, and 16.7% and 8.5%, respectively, in those who exceeding MC including extrahepatic recurrence

not achieve such downstaging. These results indicate that hepatectomy as an initial treatment is an important component of the treatment strategy for HCC exceeding MC.[23]

With regard to the treatment of recurrent HCC patients, we reported that the more hepatectomy was repeated, the shorter the recurrence-free interval became, suggesting a limitation of repeat hepatectomy in curing recurrent HCC.[24] Liver transplantation has been discussed as the next strategy to treat tumor recurrences after initial hepatectomy in patients with advanced HCC. Several studies have reported salvage transplantation for recurrence after hepatectomy,[6,25–27] suggesting that primary hepatectomy and salvage liver transplantation is a feasible and rational strategy for patients with small HCC that preserves liver function. In this series, of the patients who had recurrence after resection for tumors exceeding MC, approximately 48% had recurrent tumors that were within MC. This result also indicates that approximately half of the patients with recurrence would be candidates for salvage liver transplantation after partial hepatectomy performed for downstaging to within MC. Salvage LDLTs were adopted for three patients, two of whom, who had a recurrence that met MC, did not experience a recurrence after salvage LDLT at the 2- and 3-year follow-up, respectively. Yao et al. and Ravaioli et al. reported that locoregional treatments, including RFA, were effective for downstaging prior to liver transplantation.[23,28] In general, RFA was indicated for HCCs with diameters less than 3 cm. Although RFA may be effective for downstaging multiple small HCCs, its effectiveness may be limited in the case of downstaging large HCCs with diameters greater than 3 cm. Further studies are required to clarify the indications for the use of RFA and hepatectomy as downstaging modalities prior to liver transplantation.

A significant proportion of patients with HCC exceeding MC might benefit from liver transplantation. Mazzaferro et al. proposed an expansion of the indications for liver transplantation, using up to seven criteria.[29] Takada et al. demonstrated that LDLT could be safely extended to ≤10 tumors (all ≤5 cm in diameter and PIVA-II≤400 mAU/mL) with acceptable outcomes.[30] Liver transplantation has been proposed as an initial treatment for patients with HCC exceeding MC whose platelet count is $<10^5/mm^3$, although the extension of the indications of liver transplantation is restricted.

Conclusion

Hepatectomy for patients with HCC exceeding MC increases survival rates, especially for patients with sufficiently high platelet counts, although their recurrence rates

Table 5 Treatments for recurrent HCC after initial hepatectomy

Modalities	Recurrent pattern *N* (%)[a]		*P* value
	Meeting MC (*n*=51)	Exceeding MC or extrahepatic (*n*=56)	
Partial hepatectomy	10 (20)	5 (9)	<0.001[b]
Salvage liver transplantation	2 (4)	1 (2)	
Resection of distant metastasis	0	3 (5)	
Percutaneous ablation therapy	18 (35)	8 (14)	
TACE	21 (41)	23 (41)	
Chemotheraphy and/or radiation	0	10 (18)	
Non-treatment	0	6 (11)	

MC Milan criteria, *TACE* transarterial chemoembolization

[a] Data are expressed as the number of patients (percentage of patients who had a recurrence of each group)

[b] Statistically significant difference

after initial hepatectomy are high. Hepatectomy as an initial treatment is an important component of the treatment for HCC exceeding MC to downstage the recurrence to within MC.

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Research Article

Evidence for the Immunosuppressive Potential of Calcineurin Inhibitor-Sparing Regimens in Liver Transplant Recipients with Impaired Renal Function

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Patients requiring liver transplantation (LT) frequently experience renal insufficiency (RI), which affects their survival. Although calcineurin inhibitor-sparing immunosuppressive regimens (CSRs) are well known to prevent RI, the immune state in recipients receiving CSR remains to be intensively investigated. Among 60 cases of living-donor LT at our institute, 68% of the patients had none to mild RI (non-RI group) and 32% of the patients had moderate to severe RI (RI group). The RI group received a CSR comprising reduced dose of tacrolimus, methylprednisolone, and mycophenolate mofetil, while the non-RI group received a regimen comprising conventional dose of tacrolimus and methylprednisolone. One year after LT, the mean estimated glomerular filtration rate (eGFR) in the RI group had significantly improved, although it was still lower than that of the non-RI group. Serial mixed lymphocyte reaction assays revealed that antidonor T-cell responses were adequately suppressed in both groups. Thus, we provide evidence that CSR leads to improvement of eGFR after LT in patients with RI, while maintaining an appropriate immunosuppressive state.

1. Introduction

Renal insufficiency (RI) has been widely recognized as a serious complication of liver transplantation that significantly compromises patient outcome [1–4]. Since a number of patients already have varying degrees of RI, including hepatorenal syndrome, before undergoing liver transplantation, and since postoperative standard immunosuppression protocols based on calcineurin inhibitors (CNIs) can lead to severe tubular atrophy, interstitial fibrosis, and focal hyalinosis of the small renal arteries and arterioles, a majority of liver recipients develop some degree of RI [5–7]. An analysis of data from the Scientific Registry of Transplant Recipients indicates that the cumulative incidence of stage 4 [estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2] or stage 5 chronic kidney disease (eGFR < 15 mL/min/1.73 m^2 or need for renal replacement therapy) after liver transplantation is 18% at 5 years [8].

Late renal failure is associated with both pre- and posttransplant factors, including higher concentrations of CNIs both early and late posttransplant and can be predicted by creatinine levels in the first year posttransplant [9, 10]. The recognition of these effects induced interest in strategies using a CNI-sparing immunosuppressive regimen (CSR). Current strategies to overcome CNI toxicity include reduction or withdrawal of CNIs concurrent with switching over to less nephrotoxic drugs like the mammalian target of rapamycin (mTOR) inhibitor or mycophenolate mofetil (MMF) [11–17]. Although these strategies have clearly demonstrated the ability to reduce the incidence of nephrotoxicity in various studies, CSR may result in an increased risk for acute rejection episodes in a subset of patients.

In the present study, we investigated the immune state in liver transplant patients suffering from RI who received a CSR comprising a reduced dose of CNI, methylprednisolone, and MMF. For monitoring the immune-state response to

antidonor allostimulation in these patients, we employed a mixed lymphocyte reaction (MLR) assay using an intracellular carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeling technique. By applying the CFSE-based method, the proliferation of viable $CD4^+$ and $CD8^+$ responder T-cells in response to allostimulation could be separately quantified using multiparameter flow cytometry [18]. The technique allowed us to find that antidonor T-cell responses were adequately suppressed in patients with RI who received the CSR and in patients without RI who received a conventional immunosuppressive regimen.

2. Patients and Methods

2.1. Patients. Between January 2003 and December 2009, 122 patients underwent living-donor LTs at Hiroshima University Hospital. Of these, 50 patients infected with hepatitis C virus (HCV) and 12 patients who received liver allografts from ABO-blood group incompatible donors were excluded from the study, because they were treated with the diverse immunosuppressive protocols. For the remaining 60 patients, the relationship between RI prior to LT and the clinical/immunological state after LT was investigated. The following information was collected at the time of the transplant: age, sex, etiology of liver disease, model for end-stage liver disease (MELD) score, and diagnosis of hepatocellular carcinoma (HCC) prior to LT. Renal function was evaluated in each participant by determining eGFR. The eGFR of each participant was calculated from their serum creatinine value (SCr) and their age by using the new Japanese equation [19] as follows:

$$\begin{aligned} &\text{eGFR } (\text{mL/min/1.73 m}^2) \\ &\quad = 194 \times \text{Age} - 0.287 \\ &\qquad \times \text{S} - \text{Cr} - 1.094 \text{ (if female} \times 0.739). \end{aligned} \tag{1}$$

In this study, RI was defined as none to mild (eGFR ≥ 60 mL/min/1.73 m^2) and moderate (30–59 mL/min/1.73 m^2) to severe (< 30 mL/min/1.73 m^2). The MELD score was calculated for each patient using the United Network for Organ Sharing (UNOS) formula based on the laboratory values obtained just prior to LT. Patients were monitored for renal function using serum creatinine level and eGFR at 1, 3, 6, and 12 months after LT.

2.2. Immunosuppressive Protocol. The basic immunosuppressive regimen after LT for the non-RI group comprised tacrolimus (TAC) and methylprednisolone, with gradual tapering of doses. Patients with RI received a CSR comprising a reduced dose of TAC, methylprednisolone, and MMF (Figure 1). In the conventional regimen, the trough whole blood levels of TAC were maintained between 8 and 15 ng/mL in the first few postoperative weeks and between 5 and 10 ng/mL thereafter. In the CSR, the trough whole blood levels of TAC were maintained between 5 and 10 ng/mL in the first few postoperative weeks and between 3 and 5 ng/mL thereafter.

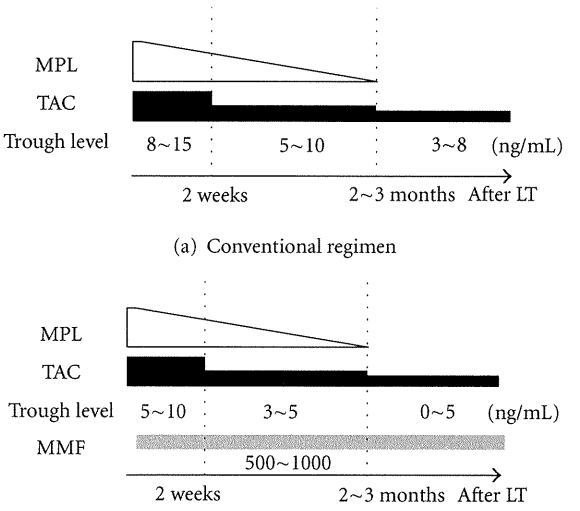

FIGURE 1: Immunosuppressive protocol after liver transplantation. The basic immunosuppressive regimen comprised tacrolimus (TAC) and methylprednisolone (MPL), with doses gradually being tapered off. The trough whole blood levels of TAC were maintained between 8 and 15 ng/mL in the first few postoperative weeks and between 5 and 10 ng/mL thereafter (a). Renal insufficiency (RI) group received CNI-sparing immunosuppressive regimen (CSR) consisting of TAC reduction and concomitant use of mycophenolat mofetil (MMF) (b).

2.3. Immune Monitoring by an In Vitro MLR Assay. For monitoring the immune state, an in vitro MLR assay was performed at 1, 3, 6, and 12 months after LT. Briefly, peripheral blood mononuclear cells prepared from the blood of the recipients, donors, and healthy volunteers with the same blood type as the donors (third-party control) for use as the stimulator cells were irradiated with 30 Gy, and those obtained from the recipients for use as responder cells were labeled with 5 lm CFSE (Molecular Probes Inc., Eugene, OR, USA), as described previously [18]. The stimulator and responder cells were incubated for 5 days. CFSE stably stains intracellular proteins without causing toxicity, and the fluorescence intensity of each stained cell segregates equally among daughter cells during cell division, resulting in sequential halving of the cellular fluorescence intensity with every successive generation. After culturing for MLR, the harvested cells were stained with either phycoerythrin- (PE-) conjugated antihuman CD4 or PE-conjugated antihuman CD8 monoclonal antibodies and subjected to analysis by flow cytometry. All analyses were performed on a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA). T-cell proliferation was visualized by the serial-halving of the fluorescence intensity of CFSE. $CD4^+$ and $CD8^+$ T-cell proliferation and stimulation index were quantified using a method described previously [18].

2.4. Statistical Analysis. Quantitative variables were expressed as mean ± standard deviation (SD) or median (range). Categorical variables were presented as values and

Table 1: Patient characteristics at living donor liver transplantation.

(eGFR (mL/min/1.73 m^2))	Non-RI group (n = 41) (94.8 ± 26.9)	RI group (n = 19) (42.5 ± 15.9)	P value
Age at LT (years)	49.2 ± 11.5	52.9 ± 9.0	0.23
Male sex—n (%)	21 (51.2)	13 (68.4)	0.21
Primary diagnosis—n (%)			0.63
HBV	15 (36.6)	9 (47.4)	
Alcoholic	8 (19.5)	5 (26.3)	
AIH	4 (9.8)	1 (5.3)	
Others	14 (34.1)	4 (21.1)	
MELD	16.5 ± 7.1	24.7 ± 10.7	< 0.01
eGFR at 1st year after LT (mL/min/1.73 m^2)	77.2 ± 28.2	60.1 ± 13.5	< 0.01
eGFR > 60 at 1st year after LT—n (%)	26 (72.2)	10 (58.8)	0.33
AR within 1st year—n (%)	10 (24.4)	5 (26.3)	0.87
Bacterial infections—n (%)	13 (31.7)	8 (42.1)	0.43
Fungal infections—n (%)	4 (9.8)	4 (21.1)	0.23
CMV infections—n (%)	10 (24.4)	7 (36.8)	0.32

RI, renal insufficiency; LT, liver transplantation; HBV, hepatitis B virus; AIH, Autoimmune hepatitis; eGFR, estimated glomerular filtration rate; MELD, model for end-stage liver disease; AR, acute rejection; CMV, cytomegalovirus. Data are expressed as means ± standard deviation. Difference with $P < 0.05$ was considered significant.

percentages. Student's t-test, Mann-Whitney test, chi-square test, and Fischer's exact test were used to compare variables between the two groups. Paired t-tests were performed to compare continuous variables throughout the study period. The Kaplan-Meier analyses were used to compare time-to-event variables. P Values < 0.05 were considered statistically significant.

3. Results

The 60 patients included 34 males and 26 females; their ages ranged from 20 to 69 (median 52) years. The primary diseases in these patients included hepatitis B virus-related cirrhosis in 24 patients (of these, 18 patients had HCC), alcoholic cirrhosis in 13 patients (of these, 6 patients had HCC), autoimmune hepatitis in 5 patients (of these, 1 patient had HCC), and other diseases in 18 patients.

Before the LTs, 68% of the patients had none to mild RI (non-RI group; mean eGFR, 94.8 ± 26.9 mL/min/1.73 m^2) and 32% of the patients had moderate to severe RI (RI group; mean eGFR, 42.5 ± 15.9 mL/min/1.73 m^2). The characteristics of these patients are listed in Table 1. There was a difference in MELD score between the groups. Mean TAC trough levels during the first year after LT in the non-RI and RI groups are shown in Figure 2(a). There were differences in mean TAC trough levels during 3 months after LT between the groups. One year after the LDLTs, the mean eGFR in the non-RI group had significantly deteriorated (from 94.8 ± 26.9 to 77.2 ± 28.2 mL/min/1.73 m^2, $P < 0.01$). In contrast, the mean eGFR in the RI group had significantly improved after LT (from 42.5 ± 15.9 to 60.1 ± 13.5 mL/min/1.73 m^2, $P < 0.01$), although it was still lower than that of the non-RI group (Figure 2(b)). Notably, 53% of the patients in the RI group were completely cured of RI by 1 year after LT. None of the patients had severe RI at 1 year after LT nor required chronic hemodialysis during the observation period.

To evaluate the immune status of these patients, we employed a serial MLR assay using a CFSE-labeling technique. Lack of proliferation of both $CD4^+$ and $CD8^+$ T-cells in the antidonor CFSE-MLR assay indicates suppression of the antidonor response, whereas a remarkable proliferation of these T-cells reflects a strong antidonor response. In both groups, limited $CD4^+$ and $CD8^+$ T-cell proliferation was observed in the antidonor responses as compared with the anti-third-party responses through the first year. At 1 month after LT, the average of stimulation index (SI) for $CD4^+$ T-cells in response to anti-third-party stimulation was >2 (the average value in healthy volunteers without any immunosuppressive treatment) that is, there was a normal response in the anti-third-party (Figures 3(a) and 3(b)). At 1 year after LT, the average of SIs for $CD4^+$ and $CD8^+$ T-cells in response to both antidonor and anti-third-party stimulation was <2 (Figures 3(c) and 3(d)). There were no significant differences in acute rejection rates, bacterial, fungal, or cytomegalovirus infection rates and patient survival between the groups (Table 1).

4. Discussion

Chronic RI is a serious complication in liver transplantation that significantly compromises patient survival and outcome. Depending on the criteria applied for a definition of chronic renal insufficiency and the duration of followup, the reported rate of chronic renal insufficiency after liver transplantation may vary from 10% to 80% [1, 20–22]. CNI toxicity has been defined as one of the possible risk factors for renal insufficiency in long-term liver transplant survivors. It has been shown that exposure to CNIs within the first 6 months

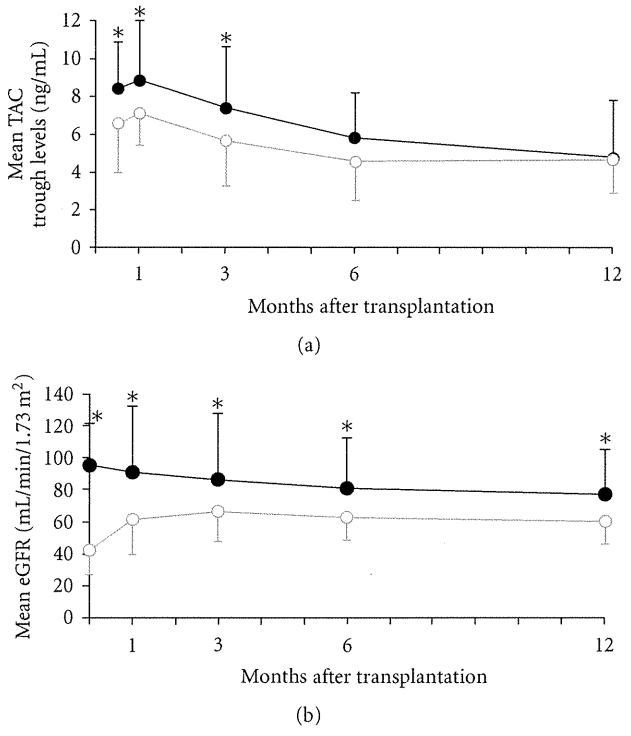

FIGURE 2: Kinetics of mean trough levels of tacrolimus and mean estimated glomerular filtration rate (eGFR) in the RI group and non-RI group during the first year after transplantation. (a) Mean trough levels of tacrolimus in the non-RI group (black line) and RI group (gray line). (b) Mean estimated glomerular filtration rate (eGFR) in the non-RI group (black line) and RI group (gray line). Data are median ± SD of values. $^*P < 0.05$.

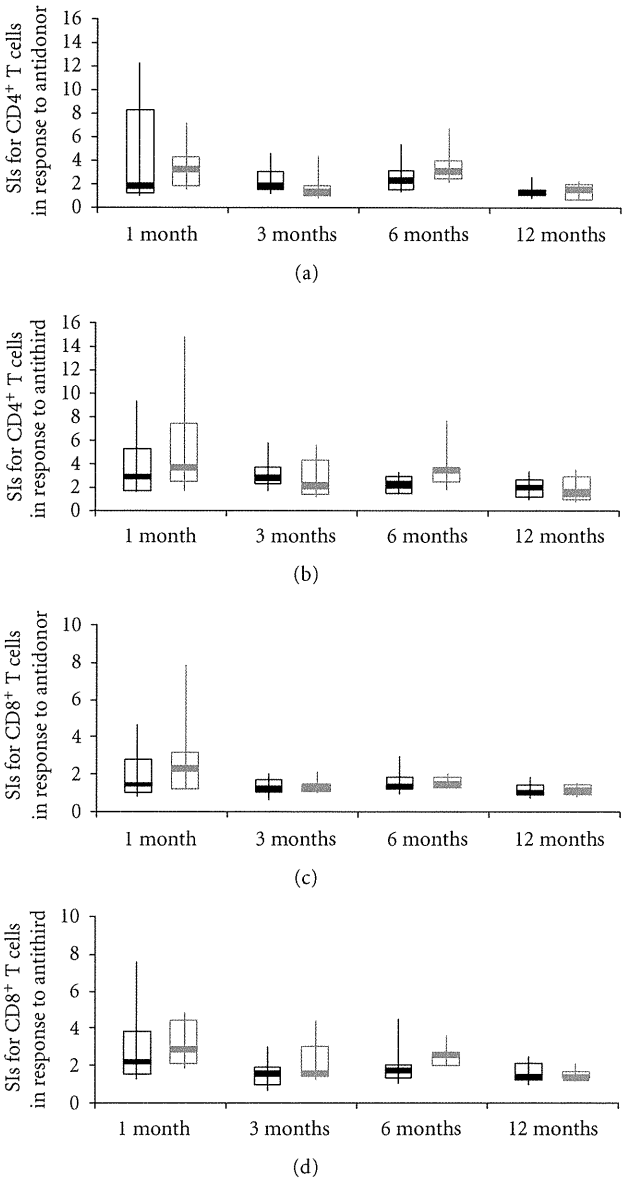

FIGURE 3: Kinetics of stimulation index in the RI group and non-RI group during the first year after transplantation. Stimulation index (SI) of each of the $CD4^+$ T-cell (a, b) and $CD8^+$ T-cell (c, d) subsets in the antidonor (a, c) and anti-third-party (b, d) MLR in patients in non-RI group (black line) and RI group (gray line). $CD4^+$ and $CD8^+$ T-cell proliferation and their SIs were quantified as follows. The number of division precursors was extrapolated from the number of daughter cells of each division, and the number of mitotic events in each of the $CD4^+$ and $CD8^+$ T-cell subsets was calculated. Using these values, the mitotic index was calculated by dividing the total number of mitotic events by the total number of precursors. The SIs of allogeneic combinations were calculated by dividing the mitotic index of a particular allogeneic combination by that of the self-control. The box plot represents the 25th to 75th percentile, the dark line is the median, and the extended bars represent the 10th to the 90th percentile.

after liver transplantation represents a risk factor for renal failure [23]. The GFR at 1 year had a better correlation with later renal function than the pretransplant GFR [24]. The recognition of these facts induced interest in preventing CNI toxicity. It has also reported that the use of adjunctive MMF immediately after LT might protect against CNI nephrotoxicity, potentially without the need for dose reduction or increased risk of adverse events [25]. Therefore, current strategies to overcome CNI toxicity include reduction or withdrawal of CNIs along with switching to mTOR inhibitor or MMF-based regimens [11, 12, 14, 15, 26–28]. These strategies have been documented in several recent and ongoing trials to achieve an improvement in renal function in a large proportion of liver transplant patients.

In our CSR using MMF, wherein our study results agree with the results from previous studies, patients with pre-transplant renal insufficiency were associated with less impairment of renal function without an increased frequency of rejection, infection, or patient survival. In addition to this clinical evidence for the usefulness of the CSR using MMF, the present study provides immunological evidence, by analyzing the data obtained from an MLR assay, that antidonor T-cell responses were adequately suppressed in patients who received the CSR and in patients who received the conventional immunosuppressive regimen. Notably, the individual variations of SIs of $CD4^+$ T-cell and $CD8^+$ T-cell subsets on antidonor T-cell responses in patients who received the CSR were smaller than those in patients who