Validation of Perioperative Steroids Administration in Liver Resection

A Randomized Controlled Trial

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Objective: We performed a randomized controlled trial to investigate the clinical benefits of perioperative treatment with steroids in patients undergoing liver resection.

Background: Perioperative steroids are considered to reduce surgical stress, but evidence supporting proposed clinical benefits is largely anecdotal.

Patients and Methods: The 210 patients scheduled to undergo liver resection were randomly assigned to a steroids group (n = 105) or a control group (n = 105). The steroids group received 500 mg hydrocortisone immediately before hepatic-pedicle clamping, followed by 300 mg hydrocortisone on postoperative day (POD) 1, 200 mg on POD 2, and 100 mg on POD 3. Serum levels of total bilirubin, aminotransferases coagulation factors, and inflammatory-related cytokines, and the clinical course were compared between the 2 groups. The primary end point was the postoperative bilirubin level.

Results: All 210 patients underwent radical liver resection with no operative mortality. The median bilirubin level on POD 2 was significantly lower in the steroids group [0.71 mg/dL (0.33–2.17)] than in the control group [1.03 mg/dl (0.39–3.57); $P = 0.01$]. The postoperative time courses of the bilirubin level ($P = 0.01$), the interleukin-6 level ($P = 0.01$) and the C-reactive protein level ($P = 0.01$) were significantly lower whereas the the prothrombin level ($P = 0.01$) and interleukin-10 level ($P = 0.01$) were significantly higher in the steroids group. There was no difference between the groups in the proportion of patients with complications (40% vs 43%; $P = 0.66$) or the length of the hospital stay (14 days vs 13 days; $P = 0.68$).

Conclusions: Perioperative treatment with steroids has a positive impact on the liver function of patients who undergo liver resection, without increasing the risk of complications.

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Pretreatment with steroids to reduce ischemia-reperfusion (I-R) injury is a well-known and widely used therapeutic option for liver surgery.[1,2] The production of free radicals from I-R injuries plays an important role in the development of surgical stress and the onset of inflammatory response and can induce cholestasis in liver parenchyma.[3–7] The roles of steroids as anti-inflammatory agents and in protecting against I-R injury have been demonstrated in several experimental models.[8–10] Previous clinical studies have found that indices of surgical stress correlate with postoperative morbidity and mortality.[11–15]

Conventionally, steroids treatment has been considered beneficial in patients undergoing liver surgery, but evidence supporting this claim is largely anecdotal. Several studies have shown that steroids have a positive effect on several clinical variables and reduce postoperative complications.[11,13] However, previous studies were small and could not clearly define the clinical impact of steroids treatment on outcomes. Inconsistent results have probably been caused by poor trial design, small sample size, or the enrollment of only patients who met strict eligibility criteria. Ideally, the design of a standard protocol for steroids treatment in liver resection should be based on the results of prospective studies enrolling patients who have various types of liver conditions and undergo different operative procedures.

To investigate the clinical benefits of perioperative treatment with steroids in patients undergoing liver resection, we performed a randomized controlled trial in which more than 200 patients minimizing the bias in patient selection.

PATIENTS AND METHODS

Inclusion and Exclusion Criteria

All patients scheduled to undergo liver resection for hepatic tumors at Nihon University Itabashi Hospital were considered as potential participants in this trial. The inclusion criteria were age between 15 and 85 years, and adequate organ functional reserves of important organ systems [hearts, lungs, kidneys, liver (Child-Pugh class A or B)]. Exclusion criteria are a recent history of treatment with steroids, unstable diabetes mellitus, or gastroduodenal ulcers. An English-language summary was available on: http://www.umin.ac.jp/ctr/index.htm at the Clinical Trials Registry managed by the University Hospital Medical Information Network in Japan.

Study Design

Randomization

Patients were randomly assigned to the steroids group or the control group by the minimization method, executed with the use of PC software. Randomization was done by a single investigator (Y.H.) who was not involved in subsequent surgical procedures or postoperative treatments and the results of assignment were blinded to the surgeons. Patients were preoperatively stratified according to 3 factors: Child-Pugh class (A or B), indocyanine green retention rate at 15 min (<20% or ≥20%), and age (<60 or ≥60 years).

All patients gave their informed consent before enrollment. The protocol was approved by the ethical committee of Nihon University Itabashi Hospital.

Determination of Sample Size

The sample size required to detect a difference in serum bilirubin levels of 0.7 mg/dL on postoperative day (POD) 2 by the Student

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t test was 144 patients, with 80% statistical power at the 5% significance level. The estimated relative risk between the steroids group and control group was 4.17 with upper and lower 95% confidence interval of 2.57 to 11.1. We anticipated analyzing the data using nonparametric tests and therefore increased the sample size.

End Points

The primary end point of this trial was the serum level of total bilirubin on POD 2. Historical data in 169 patients at our hospital indicated that levels of bilirubin and alanine aminotransferase (ALT) peaked on POD 2 and that the mean level of bilirubin in patients who received perioperative steroids was 0.9 mg/dL. Secondary end points were the results of liver-function tests, inflammatory cytokine levels, and postoperative complications. Intraoperative factors such as the duration of surgery, hepatic ischemia time, amount of blood loss, and resected liver volume were also compared.

Laboratory tests, including complete blood cell counts and levels of bilirubin, ALT, aspartate aminotransferase, albumin, prothrombin time, and C-reactive protein levels, were performed on the day before surgery (Pre), just after surgery (POD 0), and on POD 1, 2, 3, 5, and 7. Serum levels of interleukin-6 (IL-6) and interleukin-10 (IL-10) were measured as indicators of the surgical stress response on the day before surgery, just after surgery (POD 0), and on POD 1, 3, and 5. Infections were defined as positive cultures of blood, sputum, or other body fluids in association with clinical signs and symptoms of infection. Adverse effects of prolonged steroids use, such as abnormal glucose tolerance and delayed wound healing, were also evaluated.

Steroids Administration Protocol

In the steroids group, patients received 500 mg hydrocortisone immediately before hepatic pedicle clamping, 300 mg on POD 1, 200 mg on POD 2, and 100 mg on POD 3. The indications and procedures for liver resection were in accordance with Makuuchi's criteria[16] based on hepatic functional reserve. Nearly all liver transections were performed with intermittent clamping of the hepatoduodenal pedicle (Pringle's maneuver[17]) for 15 minutes, followed by release for 5 minutes. Before liver transection, intraoperative ultrasonography was performed in all patients to confirm suitable operative procedures. A central catheter was placed in the jugular vein, and central venous pressure was monitored and maintained below 5 cm H_2O throughout the operation. A closed irrigation drain was left in each liver stump. Standard systemic antibiotic therapy with cefazolin (Cefamezin α, Astellas, Japan), a first-generation cephalosporin, was routinely administered immediately before surgery and then given twice daily on POD 1 to 3. According to our policy, red blood cell transfusions were avoided, unless the hematocrit level fell to below 20.0%. Fresh frozen plasma transfusions were also avoided if the intraoperative blood loss was less than 1000 g or the albumin level was more than 2.6 g/dL on POD 2.

Statistical Analysis

Continuous variables were compared using the Student *t* or Mann-Whitney *U* test, and categorical variables were compared using the χ^2 or Fisher exact test, as appropriate. Multiple comparisons were made using repeated-measures analysis of variance. Significance was defined as $P < 0.05$. All analyses were performed using the statistical package SPSS 16.0 (SPSS, Chicago, IL).

RESULTS

Trial Profile

From 2006 through 2008, 241 patients were scheduled to undergo liver resections (Fig. 1). Before randomization, 31 patients were excluded because 11 had preoperative complications and 20 refused to participate in the study. The 210 patients were randomly assigned to either the steroids group (n = 105) or control group (n = 105). Ten patients (3 in the steroids group and 7 in the control group) were excluded after randomization, as they did not undergo liver resections because of peritoneal implantation (n = 8) or para-aortic lymph node metastasis (n = 2). Data from the 102 patients in the steroids group and 98 patients in the control group were analyzed.

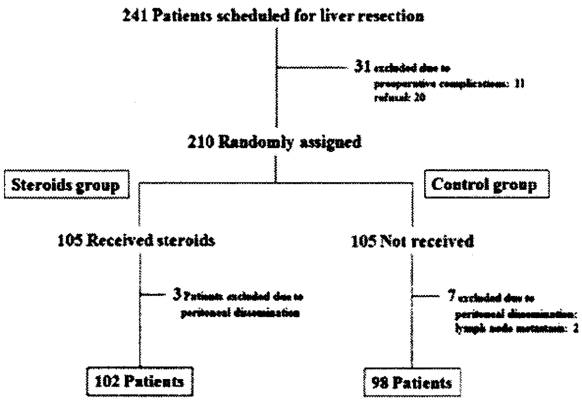

FIGURE 1. Allocation of patients.

Baseline Characteristics

The 2 groups were well matched for all baseline characteristics, including, age, underlying liver disease, diagnosis, hepatic function, and other preoperative laboratory data (Table 1). Operation was performed for hepatocellular carcinoma in 129 patients, liver metastases from colorectal cancer in 55, cholangiocellular carcinoma in 11, and other hepatic diseases (liver metastases from other organs in 2, gallbladder cancer in 1, or benign disease in 2).

There was no perioperative or surgery-related mortality and no significant difference in the surgical procedure, volume of blood loss, median operation time, ischemia time, resected liver volume, and need for blood transfusion between the 2 groups (Table 2). A total of 24 patients received blood transfusion. Among them, 23 patients (15 in steroids and 8 in control group) received fresh frozen plasma (FFP) transfusion, whereas red blood cell transfusions were required only for 7 patients (3.3%): 3 in the steroids group and 4 in the control group.

End Points

Hepatic Function and Biochemical Data

The bilirubin level reached the peak value on POD 2 in the control group (Fig. 2). The primary end point of bilirubin level on POD 2 was significantly lower in the steroids group than in the control group (0.71 mg/dL vs 1.03 mg/dL, $P = 0.01$; Table 3). There were also significant differences in the time course of the bilirubin level during the 7 days after operation between the groups ($P = 0.01$).

Peak transaminase levels were reached on POD 1 (Fig. 3). There were no significant differences between the groups in the time courses of ALT or aspartate aminotransferase levels ($P = 0.21$, 0.15, respectively). The time course of prothrombin time (%) was significantly higher ($P = 0.01$) and that of C-reactive protein levels was significantly lower ($P = 0.01$) in the steroid group than in the control group. Serum IL-6 levels were significantly lower ($P = 0.01$) and IL-10 levels were significantly higher in the steroids group, ($P = 0.01$).

TABLE 1. Baseline Characteristics

	Steroids Group (n = 102)	Control Group (n = 98)	p value
Age	69 (39–81)	70 (35–82)	0.58
Underlying liver disease*			
HBs antigen (+)	8	6	0.63
HCV antibody (+)	34	35	0.72
Alcohol abuse	16	22	0.22
Diagnosis*			0.22
Hepatocellular carcinoma	63	66	
Colorectal metastasis	32	23	
Cholangiocellular carcinoma	6	5	
Others	1	4	
Preoperative laboratory data			
Total bilirubin (mg/dL)	0.58 (0.19–2.51)	0.54 (0.29–1.86)	0.74
Alanine aminotransferase (IU/L)	28 (7–120)	29 (9–121)	0.83
Aspartate aminotransferase (IU/L)	28 (12–153)	34 (13–109)	0.10
Albumin (g/dL)	3.9 (2.4–5.1)	3.9 (2.6–4.8)	0.71
Prothrombin time (%)	100 (92–100)	100 (79–100)	0.49
ICG-R_{15}† (%)	9.4 (2.2–35.9)	9.9 (2.0–33.9)	0.57
Interleukin 6 (pg/mL)	2.6 (0.3–21.5)	2.5 (0.7–61.3)	0.42
Interleukin 10 (pg/mL)	2.0 (2.0–4.0)	2.0 (2.0–6.0)	0.62
ChildPugh class, A:B	99:03	94:04	0.66

Median with range, unless specified.
*Number of patients.
†Indocyanine green retention rate at 15 minutes.

TABLE 2. Operative Variables

	Steroids Group (n = 102)	Control Group (n = 98)	*P*
Hepatectomy procedure*			0.55
Hemihepatectomy	11 (11%)	15 (15%)	
Segmentectomy	59 (58%)	57 (58%)	
Limited resection	32 (31%)	26 (27%)	
Blood loss, g	324 (5–1577)	257 (10–1972)	0.06
Operation time, min.	330 (165–834)	316 (136–697)	0.08
Ischemia time, min.	72 (0–247)	60 (0–203)	0.06
Resected liver volume, g	77 (4–1930)	75 (6–1300)	0.75
Blood transfusion,*	15 (15%)	9 (9%)	0.23

Median with range, unless specified.
*Number of patients

Postoperative Outcomes and Complications

There was no postoperative mortality or adverse effects attributable to steroids treatment (Table 3). Complications were classified as major or minor.[18] There were no significant differences between the groups in overall (40% vs. 43%; $P = 0.66$), major (12% vs 12%; $P = 0.83$) and minor (37% vs 38%; $P = 0.92$) complications. Major complications included bile leakage and intra-abdominal abscess requiring percutaneous drainage, organ failure, and other complications requiring repeat operations. Minor complications included atelectasis, surgical-site infection, drainage-tube infection, pleural effusion, and ascites. One patient in the steroids group had intestinal perforation and underwent additional surgery. Three patients in the control group required repeat operations because of drainage-tube dislocation, bile leakage, or wound dehiscence. The median length of the hospital stay was 14 days in the steroids group and 13 days in the control group ($P = 0.68$).

DISCUSSION

Our study showed that perioperative steroids treatment contributed to stable bilirubin levels after surgery, without increasing morbidity or mortality. The positive impact of steroids treatment were validated on the basis of both clinical and biochemical variables. Our results suggest that steroids administration is of great value for postoperative management in patients who undergo liver resection.

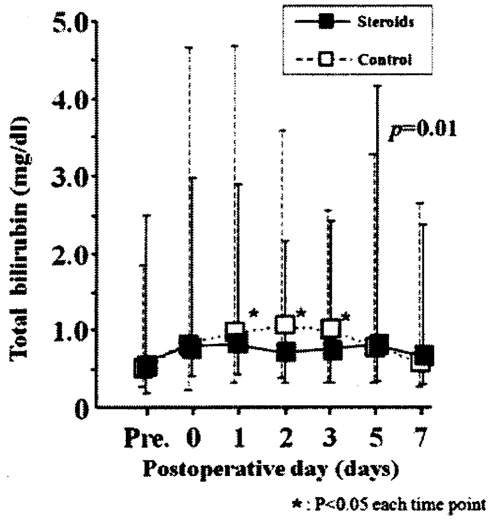

FIGURE 2. Comparison of serum total bilirubin levels between the steroids group (■) and control group (□). Data are expressed as median levels with ranges. Postoperative bilirubin levels were significantly lower in the steroids group than in the control group.

TABLE 3. Postoperative Outcomes and Complication

	Steroids Group (n = 102)	Control Group (n = 98)	P
Bilirubin level, mg/dL			
Just after operation	0.79 (0.40–2.98)	0.81 (0.23–4.66)	0.68
POD 1	0.81 (0.43–2.89)	0.95 (0.32–4.67)	0.01
POD 2	0.71 (0.33–2.17)	1.03 (0.39–3.57)	0.01
POD 3	0.74 (0.33–2.44)	0.99 (0.33–2.56)	0.01
Overall complication*	41 (40%)	42 (43%)	0.66
Major complication	12 (12%)	12 (12%)	0.83
Intra-abdominal infection	10 (10%)	4 (4%)	0.11
Bile leakage	3 (3%)	5 (5%)	0.49
Organ failure	1 (1%)	3 (3%)	0.36
Reoperation	1 (1%)	3 (3%)	0.36
Minor complication	38 (37%)	38 (38%)	0.92
Pleural effusion	14 (14%)	11 (11%)	0.59
Wound infection	10 (10%)	12 (12%)	0.58
Atelectasis	5 (5%)	8 (8%)	0.35
Drainage tube infection	6 (6%)	1 (1%)	0.07
Wound Dehiscence	3 (3%)	3 (3%)	1.00
Others	12 (12%)	14 (14%)	0.60
Hospital stay (days)	14 (8–79)	13 (7–40)	0.68

Median with range, unless specified.
*Number of patients.

Previous randomized controlled trials of steroids in liver resection had involved too small number of patients (20–76 patients)[11–13,15] to conclude the benefit of steroids or lacking of sample size calculations.[11,12,15] The Schmidt's study[13] was well designed and investigated the immunomodulating effect of preoperative pulse administration of high-dose steroids in liver resection. The steroids administrations produced positive effect on decreasing systemic inflamatory cytokine release and bilirubin concentration without adverse effect. However, this study includes only 10 patients in each arm and they suggested the necessity of more patients to elucidate the clinical impact. We designed this study of more than 200 patients along with CONSORT statement to obtain more reliable evidence.

In this study, the difference between groups of 0.3 mg/dL in postoperative bilirubin level seems small, but the bilirubin value is stable and reliable markers compared with other parameters. The finding that the control group contained 3 patients who had the bilirubin value beyond 3.0 mg/dL whereas there were no such patients in the steroids group indicates that the steroids will suppress the elevation of bilirubin level. Balzan[19] and Mullen[20] approved the prognostic value of bilirubin in their recent criteria to predict postoperative liver failure. In Balzan's work, the patients who met the prothrombin time less than 50% and serum bilirubin more than 50 μmol/L on POD 5 criterion had a 59% risk of early mortality. However, the mean value of postoperative bilirubin moved very narrow range of 25 to 40 μmol/L (within the range of 1.0 mg/dL). Once bilirubin will be elevated just after liver resection, it is hard to recover within normal range. Thus, we believe the difference of 0.3 mg/dL has a clinical impact on the patient outcome.

The median peak levels of ALT, aspartate aminotransferase, and lactate dehydrogenase (LDH) after surgery were less than 300 IU/L, suggesting that the clinical significance of these variables as end points was low. Consequently, in the present trial only 7 patients (3.3%) received red blood cell transfusions. This is one reason for the maintenance of low transaminase levels and is why we did not choose this variable as the primary end point. Strict perioperative management and policies on the use of blood transfusions contributed to the lack of mortality in our study. There was also no infection-related mortality, even though surgical-site infection is a known complication of steroids treatment.

Several experimental models have revealed the mechanism underlying I-R injury.[21,22] Interleukin-6 is considered as an integral mediator of the acute phase response.[23] Consequently, IL-6 levels are a simple measure of injury severity as well as a predictor of complications. In contrast, IL-10 inhibits the expression of a large number of proinflammatory cytokines.[22,24] Preoperative treatment with steroids is considered to reduce I-R injury by modulating IL-6 overproduction. In present trial, cytokinemia after liver resection was clearly attenuated and completely suppressed by perioperative steroids treatment, but the clinical value of this finding remains unclear. We simply used IL-6 and IL-10 levels as a pharmacokinetic marker of the response to steroids. We administered 500 mg hydrocortisone before clamping, followed by additional doses on POD 1 to 3 (300 mg, 200 mg, and 100 mg, respectively), because hydrocortisone has duration of biochemical action of about 12 hours, with a half-life of 1.5 hours. The dosage was sufficient to produce an observed biochemical effect, as reported previously.[11–15,25,26]

In conclusion, perioperative treatment with steroids significantly reduced postoperative bilirubin levels in patients undergoing liver resection. Our results suggest that steroids administration increases the margin of safety after liver resection. We propose that steroids administration should be included in standard protocols for patients undergoing elective liver resection.

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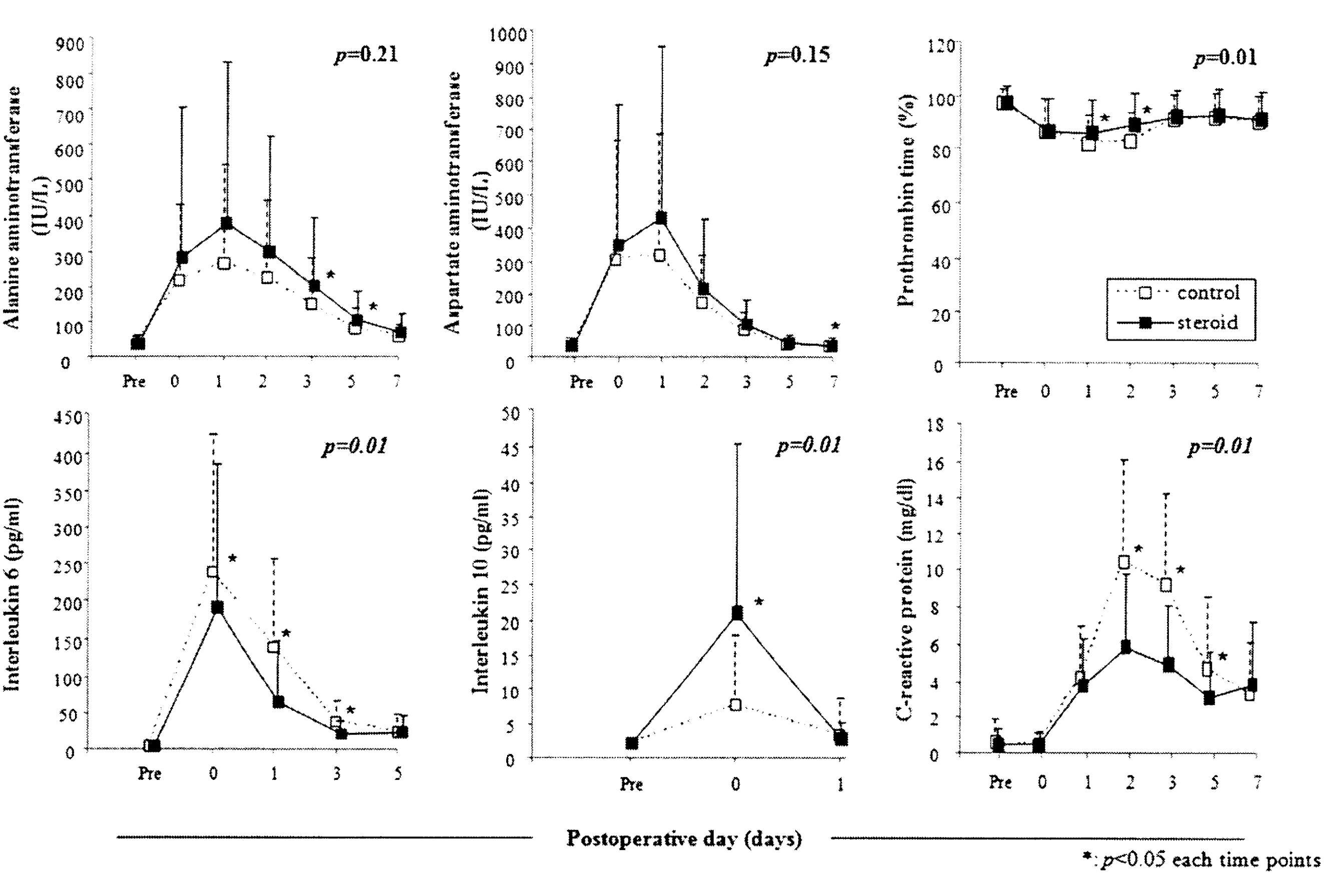

FIGURE 3. Comparison of serum levels of ALT, AST, PT%, CRP, IL-6 and IL-10 between the steroids group (■) and control group(□). Data are expressed as means +SD. Postoperative increases in the levels of IL-6 and CRP were significantly suppressed in the steroids group. PT (%) and IL-10 were higher in the steroids group than in the control group. AST, aspartate aminotransferase; PT, prothrombin time; and CRP, C-reactive protein levels.

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Hepatology Research

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Original Article

Postoperative surveillance with monthly serum tumor markers after living-donor liver transplantation for hepatocellular carcinoma

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Aim: Recurrence of hepatocellular carcinoma (HCC) after liver transplantation decreases patient survival. The usefulness of post-transplant surveillance with tumor markers, however, is not clear. We evaluated our cumulative experience with recurrent HCC detected during post-transplant surveillance.

Methods: We analyzed 100 patients with HCC detected in the explanted liver. Monthly to bimonthly measurement of tumor markers and yearly computed tomography were scheduled postoperatively.

Results: Preoperatively, 82 met the Milan criteria. The histological findings indicated that 61 fulfilled the Milan criteria. In nine patients, HCC recurred 10 months (2–29) after liver transplantation in the graft ($n = 1$), lung ($n = 2$), bone ($n = 3$) and multiple organs ($n = 3$). In all nine recipients, HCC was first suspected based on an increase in tumor marker levels. Recurrent HCC was confirmed by computed tomography ($n = 7$) or magnetic resonance imaging ($n = 2$) within 4 months (0–6) after first identifying an increase in the tumor marker levels. Six cases were treated surgically, two of which achieved prolonged survival of 16 and 38 months.

Conclusion: Frequent measurement of α-fetoprotein and des-γ carboxy prothrombin was useful for detecting recurrent HCC and may be useful long-term follow-up markers for post-transplant surveillance.

Key words: α-fetoprotein, des-γ carboxy prothrombin, hepatocellular carcinoma, liver transplantation, surveillance.

INTRODUCTION

LIVER TRANSPLANTATION IS an established therapeutic option for patients with small hepatocellular carcinoma (HCC) and advanced cirrhosis. Application of the well-established inclusion Milan criteria, namely, a single nodule of 5 cm or less or 2–3 nodules all 3 cm or less, has increased patient survival to 70% at 5 years with a less than 10% recurrence of HCC.[1,2] Reported risk factors for HCC recurrence include tumor size and number, bilobar spread of the tumor, elevated serum α-fetoprotein (AFP) and des-γ carboxy prothrombin (DCP) levels, poorly differentiated HCC, positive lymph nodes and vascular invasion.[3–9] Several centers have attempted to expand the Milan criteria based on the application of preoperatively-measureable risk factors.[7–10] Application of the University of California at San Francisco criteria successfully increased the indication for liver transplantation in patients with HCC, with a similar probability of HCC recurrence post-transplantation.[11,12]

Hepatocellular carcinoma recurs in 10–20% of transplant recipients, however, despite careful patient selection.[1,2,13,14] Once recurrence occurs, survival time is usually less than 1 year.[13,14] Surgical treatment of HCC

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recurrence improves the prognosis. For example, Regalia *et al.*[13] compared the prognosis between seven patients with resectable recurrent disease and 14 patients with unresectable disease and found significantly longer survival in the resected group. Roayaie *et al.*[14] reported that the absence of bone metastases, recurrence more than 12 months after transplantation and surgical treatment of the recurrence were independently associated with significantly longer survival from the time of recurrence. One of the aims of postoperative surveillance for HCC recurrence is to detect HCC recurrence at the resectable stage. Postoperative surveillance is now routinely performed in many centers, although the most effective modality for detecting HCC recurrence is not yet established. For postoperative surveillance, monthly to bimonthly testing for serum tumor markers with annual imaging studies are performed at our center. We herein report 100 HCC cases that we followed after transplantation, and report the efficiency of serum tumor marker measurements.

METHODS

Patient population

FROM JANUARY 1996 to March 2008, 330 adult patients underwent primary living donor liver transplantation (LDLT) in our program. Retrospective review of records of all liver transplant recipients at the University of Tokyo was approved by the University of Tokyo Institutional Review Board. Among these 330 recipients, HCC was histologically confirmed in the isolated livers of 100 recipients. HCC was preoperatively diagnosed in 95 recipients and incidentally identified in the explant in the remaining five. The recipients comprised 82 men and 18 women with a median (range) age of 56 years (40–67). Their underlying liver diseases were hepatitis C cirrhosis ($n = 59$), hepatitis B cirrhosis ($n = 30$), hepatitis B virus and hepatitis C virus dual infection ($n = 2$), alcoholic cirrhosis ($n = 2$), primary biliary cirrhosis ($n = 1$) and cryptogenic cirrhosis ($n = 6$). All received partial living donor grafts (right in 71, left in 26, and posterior in three).

Preoperative evaluation of HCC

The preoperative diagnosis of HCC was based on the findings of multi-phase dynamic helical computed tomography (CT) with contrast enhancement taken within 1 month prior to LDLT.[15] Images were reviewed by experienced radiologists, and the typical radiological characteristics of classical HCC, that is, lesions with enhancement in the arterial phase and low density during the portal phase, were diagnosed as HCC. Lesions not fulfilling these criteria, such as lesions that only showed early staining or low density in the portal phase were considered as regenerative or dysplastic nodules and were thus excluded from the staging established prior to transplantation. Chest CT scan and bone scintigraphy were routinely performed preoperatively to rule out metastatic lesions.

Based on the pre-transplantation imaging studies, 82 patients met the Milan criteria and 13 did not. Single nodules were present in 45 cases, two nodules in 27, and three or more nodules were detected in 23 cases. Mean (± standard deviation) maximum tumor size was 2.5 ± 1.3 cm (range, 0.7–8.0 cm). Vascular invasion and extrahepatic metastasis were ruled out.

Surgical procedure and immunosuppression

Our selection criteria for live liver donors, surgical techniques and use of immunosuppressants for LDLT are described elsewhere.[16–19] The post-transplant immunosuppression regimen consisted of steroid induction with tacrolimus or cyclosporin in case of tacrolimus intolerance and steroids for maintenance. The average hospitalization duration was 43 days after transplantation. Adjuvant chemotherapy was not performed in any of the cases.

Follow up after LDLT and recurrence

The postoperative surveillance schedule for patients with HCC was as follows. AFP and DCP were measured monthly for 2 years and every 2 months afterward. Abdominal helical CT with dynamic contrast was performed postoperatively at 1, 6 and 12 months, and yearly thereafter. In case of renal failure, superparamagnetic iron oxide-enhanced magnetic resonance imaging was performed. When an increase in the serum AFP (>10 ng/mL) or DCP (>40 mAU/mL) levels was noted, imaging studies were performed to rule out HCC recurrence; such imaging studies included abdominal ultrasonography, chest and abdominal CT scan with contrast, bone scintigraphy, head CT and positron emission tomography scan. Imaging studies were repeated until the recurrence sites were detected and in most of the cases two to three imaging studies were ordered monthly. Recurrence was defined as emergence of radiological findings compatible with HCC, or the appearance of new suspicious lesions. Recurrence-free survival was defined as the interval between LDLT and the date of diagnosis of recurrence or the latest follow up. All patients were followed until death or 31 May 2008.

Statistical analysis

Differences between groups were analyzed by the Mann–Whitney *U*-test for continuous variables and the χ^2-test for categorical variables. The overall and recurrence-free survival curves were generated by the Kaplan–Meyer method and compared with the log-rank test. Paired continuous data was evaluated by Wilcoxon signed-rank test. A *P*-value of less than 0.05 was considered to be statistically significant.

RESULTS

Pre- and postoperative characteristics and pathology findings

PATIENTS AND HISTOLOGICAL characteristics are summarized in Table 1. Preoperatively, AFP and DCP were measured in all cases and AFP-L3 was measured in 94. AFP (>10 ng/mL) and DCP (>40 mAU/mL) were positive in 61 cases (61%) and 40 cases (40%), respectively. AFP-L3 was positive (≥10%) in 21 cases (22%). After liver transplantation, serum AFP and DCP values promptly decreased. A detailed analysis of the serum tumor markers is described later.

After histopathological evaluation of the explanted liver, 61 remained within a size and number that fulfilled the Milan criteria, and 53 of them showed no evidence of vascular invasion, whereas eight had microvascular invasions. The other 39 had HCC with pathological findings that exceeded the Milan criteria. The histological grade of HCC was well-differentiated in 25, moderately differentiated in 60, poorly differentiated in six and necrotic tissue in nine.

Postoperative course and HCC recurrence

During the median follow up of 44 months (0.8–120), 19 subjects died. Overall, survival at 1, 3 and 5 years was 89%, 80% and 77%. The causes of death were recurrence of HCC in seven, and other in 12. Recurrence of HCC was diagnosed in nine patients. Recurrence-free survival at 1 and 3 years was 95% and 89%, respectively. By the time of HCC recurrence, seven were maintained on tacrolimus and steroid, and the other two were switched to cyclosporine and steroid.

Characteristics of HCC recurrence

Details of the patients with HCC recurrence and the tumor characteristics are shown in Tables 2 and 3. Recurrent HCC was first suspected based on increases in the tumor marker levels in all cases, and pain was

Table 1 Patient characteristics and histological findings

Factors	All patients (*n* = 100)	Recurrent (+) (*n* = 9)	Recurrent (-) (*n* = 91)	*P*
Preoperative findings				
Age (years)	56 (40–67)	54 (44–62)	56 (40–67)	0.48
Sex (M : F)	82:18	8:1	74:17	0.57
AFP (ng/mL)	20 (1–11999)	253 (9–6721)	17 (1–11999)	0.005
AFP-L3 (%)†	0 (0–77.8)	24 (0–77.8)	0 (0–56)	0.01
DCP (mAU/mL)	26 (10–10592)	134 (10–10592)	23 (10–876)	0.01
Number of tumor (single/multiple)	45/50	2/7	43/43	0.16
Max tumor size (cm)	2.5 ± 1.4	3.0 ± 1.5	2.3 ± 1.4	0.50
Milan criteria met	82	6	76	0.54
Pathological findings				
Number of tumor (single/multiple)	34/66	2/7	32/59	0.71
Max tumor size (cm)	2.6 ± 1.5	3.1 ± 1.9	2.5 ± 1.5	0.20
Vascular invasion positive	20	4	16	0.08
Histological grade				0.04
Well differentiated	25	0	26	
Moderately differentiated	60	7	52	
Poorly differentiated	6	2	4	
Necrosis	9	0	9	
Milan criteria met	61	4	57	0.31

†AFP-L3 was measured in 94 recipients.
AFP, α-fetoprotein; AFP-L3, L3 fraction of AFP; DCP, des-γ carboxy prothrombin; HCC, hepatocellular carcinoma.

Table 2 Characteristics of original hepatocellular carcinoma (HCC) in nine recurrent HCC cases

Case	Age/sex	Milan criteria†	Tumor marker before LT			Histological findings			Histological grade (vascular invasion)
			AFP (ng/mL)	AFP-L3 (%)	DCP (mAU/mL)	Max. tumor size (cm)	Number	Bilobar spread	
1	57/M	Met	55	13	22	5.0	1	No	Mod‡ (–)
2	54/M	Not met	96	<1	1994	7.0	14	No	Poor§ (+)
3	54/M	Not met	2526	3	10	3.7	16	Yes	Mod‡ (–)
4	53/M	Met	323	48	58	1.7	4	Yes	Mod‡ (–)
5	51/M	Met	9	<1	225	3.0	7	Yes	Mod‡ (–)
6	52/M	Met	253	ND	740	2.0	3	No	Mod‡ (+)
7	62/F	Not met	4517	52	161	1.0	19	Yes	Mod‡ (+)
8	44/M	Met	390	35	21	1.3	2	No	Mod‡ (–)
9	57/M	Met	2086	78	10 592	4.0	1	No	Poor§ (+)

†Based on preoperative imaging studies.
‡Mod, moderately differentiated.
§Poor, poorly differentiated.
AFP, α-fetoprotein; AFP-L3, L3 fraction of AFP; DCP, des-γ carboxy prothrombin; ND, not done; HCC, hepatocellular carcinoma; LT, liver transplantation.

Table 3 The mode and the timing of diagnosis of hepatocellular carcinoma (HCC) recurrence in nine recurrent HCC cases

Case	The first sign of recurrence (A)	Imaging test with negative result	Confirmatory test (B)	AFP/AFP-L3/ DCP†	Recurrent site	Days from A to B	Days from LT to B	Treatment	Outcome (days from LT)
1	DCP	CT ×2, BS ×1	CT	112/87/243 800	Liver	40	95	C, TACE	Dead (229)
2	AFP	CT ×3, BS ×1	CT	47/21/38	Lymph node	134	399	Ope, C, RTx	Dead (1017)
3	AFP	CT ×3, BS ×3, MRI ×1	PET, MRI	8830/<1/12	Bone	179	312	Ope, C, RTx	Dead (1329)
4	DCP, Pain	None	CT, BS	1/<1/342	Bone	17	308	RTx, C	Dead (371)
5	DCP	BS ×1	CT	3/<1/200	Lung	60	752	VATS	Alive (1963)
6	AFP, DCP	CT ×5, head CT ×1, AG ×1, PET ×1, BS ×1	CT	26 448/37/5354	Liver	153	574	Ope, C	Dead (766)
7	AFP	CT ×3, MRI ×1	CT	1311/68/297	Bone	208	229	None	Dead (312)
8	AFP	BS ×1	CT	16/<1/13	Lung	99	890	VATS	Alive (1431)
9	AFP, DCP	CT ×3, BS ×1, head CT ×1, head MRI ×1, PET ×1	CT	3602/71/2416	Liver	112	203	Ope, C	Dead (357)

†Tumor marker results obtained at the time of confirmatory tests.
AFP, α-fetoprotein; AFP-L3, L3 fraction of AFP; AG, angiography; BS, bone scintigraphy; C, chemotherapy; CT, computed tomography; DCP, des-γ carboxy prothrombin; HCC, hepatocellular carcinoma; LT, liver transplantation; MRI, magnetic resonance imaging; Ope, operation; PET, positron emission tomography scan; RTx, radiation therapy; TACE, trans-arterial chemoembolization; VATS, video-assisted thoracoscopic surgery.

associated with one case (case 4). In two cases (cases 1 and 4), the tumor marker increase at the time of tumor recurrence was different from that prior to LDLT. Once HCC recurrence was suspected, intensive radiographic examinations were performed. Most commonly, CT scans of the chest and abdomen and bone scintigraphy were included in the first set of imaging studies. This set of images successfully led to a diagnosis of HCC recurrence in three cases (cases 4, 5 and 8). In the other six cases with recurrence, however, repeated radiographic evaluations were necessary. In case 3, bone lesions were revealed by positron emission tomography scan, followed by magnetic resonance imaging 179 days after the first increase in AFP. In case 9, a sinus lesion detected by head CT scan was diagnosed as a postoperative fungal infection. The true recurrence site in this case was the graft, based on dynamic CT scan 112 days after the first increase in the serum tumor markers. The median (range) time from first sign of HCC recurrence to radiographic confirmation was 112 days (17–208). The median (range) number of patient visits for imaging studies was five times (2–10).

Hepatocellular carcinoma recurrence was diagnosed within 1 year in five recipients, between 1–2 years in two, and after 2 years in two. The median (95% confidence interval) survival after the diagnosis of HCC recurrence was 6.3 months (3.7–8.9). All but one subject were treated with following modalities; surgical tumor reduction in six, transarterial chemoembolization in one, systemic chemotherapy in six or regional irradiation in three. One (case 7) received symptom-relieving drugs. Two cases with lung lesion (cases 5 and 8) were surgically treated with video-assisted thoracoscopic surgery and both remain alive at 18 and 39 months after surgery and are free from other recurrence. Another two (cases 2 and 3) survived more than 1 year after surgery for recurrent HCC.

Sequential changes in AFP

The median (range) AFP value 2 months after LDLT was significantly lower than that pre-transplantation (2 ng/mL [1–182]; $P < 0.001$). Sequential changes in serum AFP levels among those without HCC recurrence are shown in Figure 1(a). AFP levels decreased immediately post-transplantation. In all cases without HCC recurrence, AFP levels decreased to lower than 20 ng/mL within 2 months post-transplantation. Serum AFP values at 1 year after LDLT were 2.8 ± 2.3 ng/mL, with

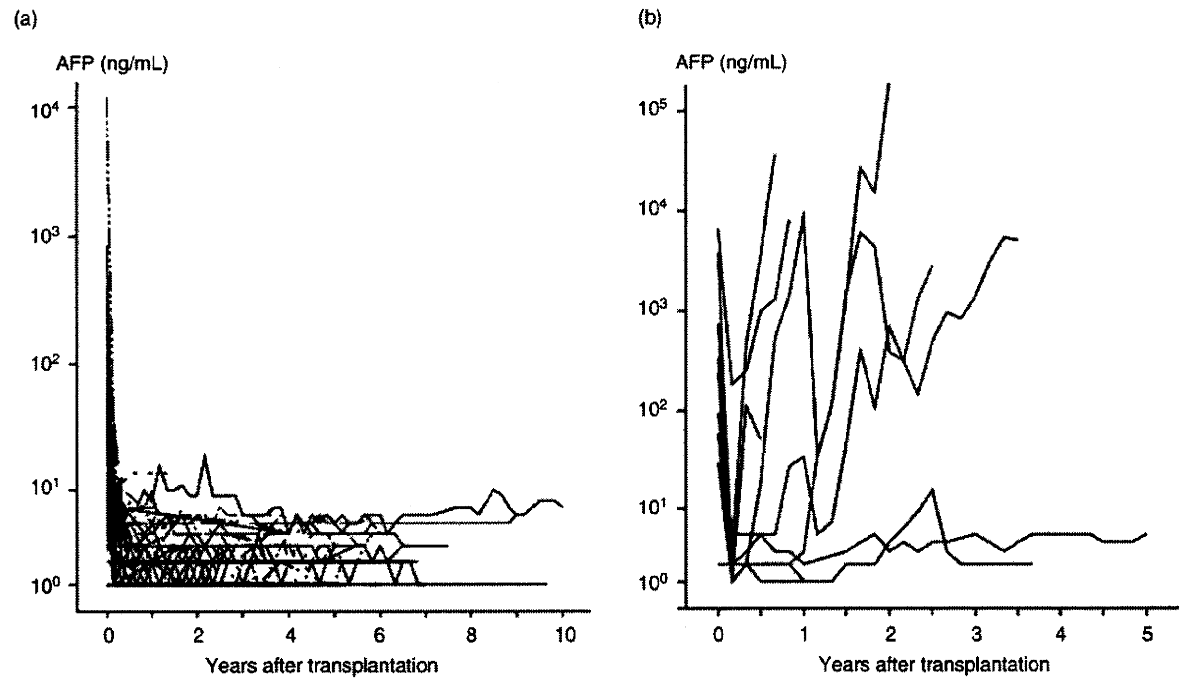

Figure 1 Sequential changes in serum α-fetoprotein (AFP) levels are shown. (a) Ninety-one recipients without hepatocellular carcinoma (HCC) recurrence. AFP levels decreased and remained below 20 ng/mL in call cases. (b) Nine cases with HCC recurrence. AFP levels increased to over 20 ng/mL in six cases and continuously increased thereafter.

the 90th percentile at less than 5 ng/mL. Figure 1(b) shows the changes in AFP levels among subjects with HCC recurrence. AFP levels increased to over 20 ng/mL in six cases and continuously increased thereafter. AFP-L3 was over 10% in five of six cases. An increase in serum AFP levels between 11 and 20 ng/mL was observed in four cases. In one case, HCC recurrence was diagnosed when the AFP values rose from 2 to 16 ng/mL (case 8). In the other two cases, AFP values fluctuated between 5 and 19 ng/mL without evidence of recurrent HCC. When cut-off levels of 10 and 20 ng/mL were used, the sensitivity and specificity for HCC recurrence after liver transplantation were 7/9 (78%) and 89/91 (98%), and 6/9 (67%) and 91/91 (100%), respectively.

Sequential changes in DCP

The median (range) DCP value 2 months after LDLT dropped significantly from the pre-transplantation value to 14 mAU/mL (10–93) ($P < 0.001$). Figure 2 shows the sequential changes in serum DCP levels among those with and without HCC recurrence. Figure 2(a) shows the decrease in the DCP values after surgery. The mean serum DCP value at 1 year was 27 ± 51 mAU/mL, with the 90th percentile at 27 mAU/mL. Unlike AFP, a transient increase in DCP levels without evidence of recurrence was observed postoperatively in five cases. The causes of a DCP increase to over 40 mAU/mL included the following: biliary duct obstructions with or without biliary lithiasis ($n = 3$), oral administration of warfarin for pulmonary embolism ($n = 1$) and unknown ($n = 1$) (Table 4). The DCP value of the latter case remained between 40 and 75 mAU/mL for 2 years until the end of the follow-up period. The DCP values in the earlier four cases promptly normalized after resolution of the medical conditions. Figure 2(b) shows the change in the DCP values among those with HCC recurrence. An increase in the DCP values to over 40 mAU/mL was observed in six patients with HCC recurrence, which was different from the five above-mentioned cases. In one case whose lung metastasis was surgically removed, DCP promptly normalized. In the other seven cases, the DCP values

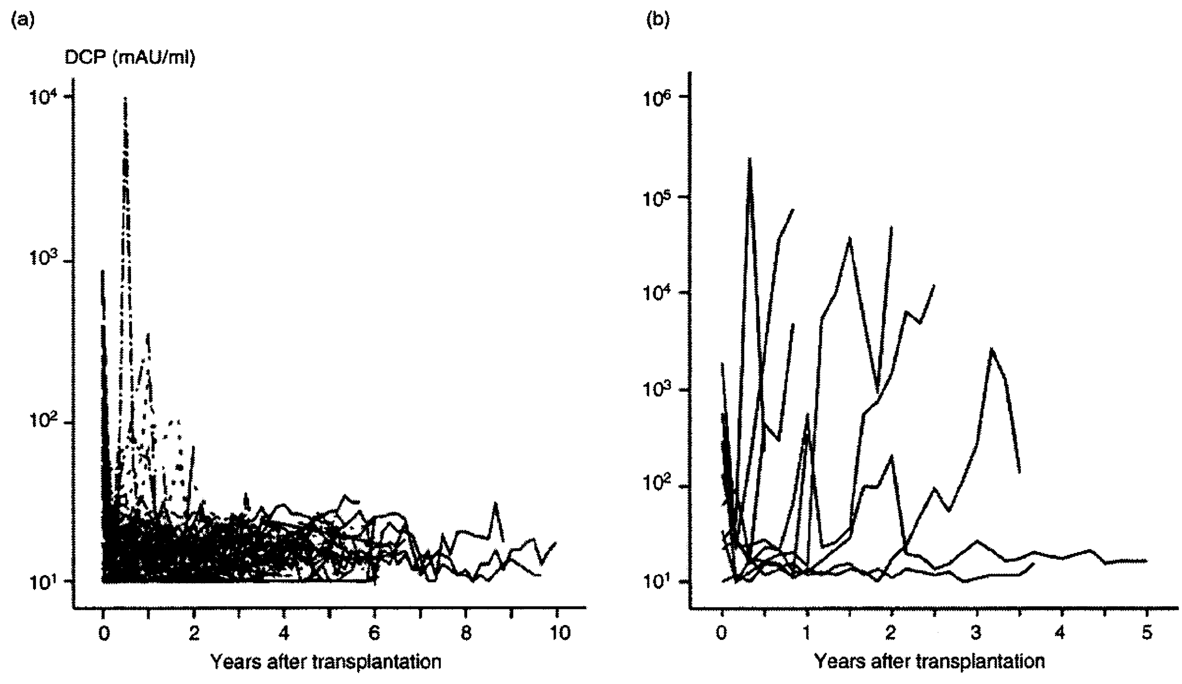

Figure 2 Sequential changes in serum des-γ carboxy prothrombin (DCP) levels are shown. (a) Ninety-one recipients without hepatocellular carcinoma (HCC) recurrence. DCP levels decreased to lower than 40 mAU/mL in most of the cases. A transient increase in DCP levels without evidence of recurrence was observed postoperatively in five cases. (b) Nine cases with HCC recurrence. An increase in the DCP values to over 40 mAU/mL was observed in six of the nine patients.

Table 4 Elevation of DCP value and outcome

Age/sex	Time from LT	Imaging study	Diagnosis	Duration of DCP >40 mAU/mL	Max. DCP value
67/M	1 year	US	Biliary stricture	3 months	56
56/M	10 months	US, CT, BS	Biliary stricture	3 months	361
63/M	4 months	None	Warfarin	3 months	9884
56/M	4 months	US, CT, BS	Unknown	2 years	75
60/M	8 months	CT	Biliary stricture	10 months	108

BS, bone scintigraphy; CT, computed tomography; DCP, des-γ carboxy prothrombin; LT, liver transplantation; US, ultrasonography.

never returned to normal. Using a cut-off level of 40 mAU/mL, sensitivity was 6/11 (54.5%) and specificity 86/89 (96.6%).

DISCUSSION

WE FOLLOWED 100 LDLT recipients with HCC for a median of 3.6 years, and diagnosed nine recurrent cases. Patients were monitored with frequent tumor marker measurements and annual dynamic CT scan of the liver, but chest CT scan was not routinely performed. Annual to biannual abdominal CT scans and monitoring of AFP levels seem to be standard at many centers,[5-8,13-15] but there is no established surveillance protocol for recurrent HCC following liver transplantation.

Regalia *et al.*[13] reported that 132 orthotopic liver transplantation recipients underwent post-transplant surveillance using serum AFP levels every 3 months and imaging studies (ultrasonography, CT, chest XP, bone scan) twice annually. In 21 patients, HCC recurred, 71% within 18 months. Survival after HCC recurrence was better among patients treated surgically than among those without surgery (2-year survival 57% vs 14%). The study from the Mount Sinai group of 57 HCC recurrent cases[14] indicated that median survival from recurrence was 8.9 months. Their HCC recurrence surveillance included CT chest and liver, and serum AFP levels were measured frequently. Recurrence was detected in 80% within 2 years, and 47/57 had multiple recurrences. Todo *et al.*[2] reported the results of LDLT for HCC patients in Japan. In his report, 40 of 316 patients had recurrent HCC. The recurrence sites in these three reports were similar: 15% to 19% in the graft; 42% to 53% in other organs, including lungs and bones; and 31% to 43% in multiple organs. In these reports, however, the mode of diagnosis and detection of the tumor as a result of periodical surveillance were not reported.

The Mount Sinai group[20] suggests the use of a different surveillance schedule for HCC recurrence in those at low or high risk. High-risk patients are those with HCC in which tumors exceed the Milan criteria, are poorly-differentiated or exhibit vascular invasion.[20] Others also report that it is more cost-effective when surveillance is performed only for patients with risk factors for HCC recurrence.[21,22] The risk factors determined by Chen include a tumor larger than 4.5 cm, tumors in both lobes of the liver, and macroinvasion. If patients have no risk, the odds for HCC recurrence are low, and the patient does not require postoperative surveillance for recurrence.[22] These cost-effectiveness analyses, however, were performed in a setting in which frequent imaging studies were considered mandatory. Because nearly half of the recurrences occur in multiple organs after liver transplantation, surveillance with imaging studies may not be sufficient.

In the present study, although the number of patients was small, the tumor marker levels were the first sign of recurrent HCC in all cases. When we focused on the two long-term survivors, recurrent HCC was found more than 2 years post-transplantation, and was detected as a solitary lung metastasis by chest CT scan. On the other hand, seven other patients with recurrent HCC died. To confirm the sites of recurrence in those cases, repeated imaging studies were required. As previously reported,[2,13,14] many cases experienced recurrence in multiple organs, and the tumors were often not amenable to resection. As a result, three cases received only palliative or symptom-relieving therapy. However, it would also be notable that two cases whose recurrence lesions were treated surgically survived more than 1 year. These results suggested that the surgical treatment for recurrent HCC, regardless of additional chemotherapy, may yield longer survival for patients with HCC recurrence.

α-Fetoprotein levels are reported to be elevated in cases with active hepatitis and thus the cut-off level to

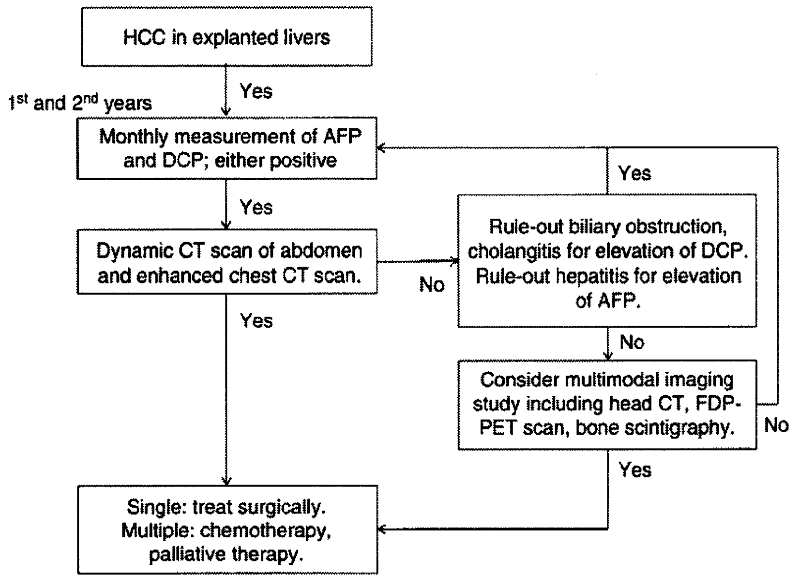

Figure 3 Flowchart outlining our surveillance system for the diagnosis of recurrent hepatocellular carcinoma (HCC) after liver transplantation. AFP, α-fetoprotein; CT, computed tomography; DCP, des-γ carboxy prothrombin; FDP-PET, ^{18}F-fluorodeoxyglucose positron emission tomography.

detect HCC among patients with chronic hepatitis or cirrhosis has been discussed.[23,24] To increase the specificity, the use of different cut-off levels for AFP (20, 100 or 200 ng/mL) are used. Measurement of the AFP-L3 fraction is useful for distinguishing the cause of the AFP elevation.[25] In the present study, AFP values among non-recurrent HCC recipients remained equal to or lower than 10 ng/mL. Further, we did not observe an AFP increase over 20 ng/mL among patients without HCC recurrence. Our findings indicated that an AFP increase to over 10 ng/mL may be an appropriate indicator for further evaluation for HCC recurrence. It should be noted, however, that recurrent hepatitis was not observed among our studied population and, accordingly, an increase in AFP levels due to chronic hepatitis was not observed.

In contrast, increased serum DCP levels were observed in five patients without recurrence. DCP levels are influenced by vitamin K metabolism, and thus by biliary obstruction.[26] Such a condition must be ruled out by taking a careful history or by performing liver imaging studies. Although the specificity of DCP in this study was lower than that of AFP, the combination of both markers may increase the sensitivity. When the cut-off values were 10 ng/mL for AFP and 40 mAU/mL for DCP, the sensitivity of the combination increased to 9/9 (100%) and specificity was 84/91 (91%).

Serum tumor marker measurement is easy to perform, and can be repeated for a long time. Based on the present study, we propose a surveillance protocol for recurrent HCC based on tumor marker measurement (Fig. 3). Although we reported the usefulness of monthly measurements of AFP and DCP, the optimal frequency is not clear. Because most of the recurrent cases were diagnosed within 2 years in our study, monthly measurement would be useful for the first 1–2 years after transplantation. For those surviving longer than 2 years, two to four times per year may be adequate for long-term follow up. Once recurrent HCC is suspected based on tumor marker levels or symptoms, then confirmatory imaging studies can be performed. If the recurrence comprises a solitary nodule, surgical resection should be planned. Because very late recurrence is also reported,[27] long-term measurement of serum tumor markers is justified in all HCC cases after liver transplantation.

In summary, the present findings indicate that tumor marker measurements were useful for early detection of recurrent HCC among 100 LDLT recipients with HCC. Tumor marker increases were followed by thorough imaging studies, and led to surgical treatment in six cases. Approximately 30–40% of recurrent HCC was detected in multiple sites, however, and thereby indication for surgery was limited. Alternative approaches must be considered for those with multiple site recurrence. Tumor marker increases may not lead to curative treatment in those cases, but may indicate a requirement for adjuvant chemotherapy or immunosuppressive therapy modifications to inhibit tumor growth. Further studies are necessary to determine the usefulness of monitoring AFP and DCP levels for HCC recurrence.

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Favorable Long-Term Surgical Outcomes of Hepatocellular Carcinoma in Patients With Hepatitis B Envelope Antibody

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Background: Surgical outcome of patients with hepatocellular carcinoma (HCC) in relation to the serum hepatitis B envelope (HBe) has not been clarified in detail.
Methods: We retrospectively studied 732 patients with HCC within the Milan criteria who underwent hepatectomy from 1991 through 2005. Serum hepatitis B surface antigen (HBs-Ag) and hepatitis C virus (HCV) antibody were routinely performed preoperatively, and 92 patients were only positive for HBs-Ag. Serum HBe antibody (HBe-Ab) was further examined, and surgical outcomes after hepatectomy were compared with those of 70 patients who were positive for HBe-Ab and 15 patients who were negative for HBe-Ab.
Results: The 5- and 10-year survival rates were significantly greater in patients who were positive for HBe-Ab (90% and 80%, respectively) than in patients who were negative for HBe-Ab (61% and 37%: $P = 0.0004$, respectively). The 5-year recurrence-free survival rate was significantly greater in patients who were positive for HBe-Ab (53%) than in patients who were negative for HBe-Ab (21%: $P = 0.0054$). Multivariate analysis showed that positive HBe-Ab was an independent prognostic factor for survival ($P = 0.0045$) and recurrence-free survival ($P = 0.004$).
Conclusions: Favorable long-term surgical outcomes were achieved in patients with HCC within the Milan criteria who were positive for HBe-Ab.
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KEY WORDS: **hepatocellular carcinoma, hepatitis B envelope, hepatoctomy**

INTRODUCTION

Worldwide, most hepatocellular carcinomas (HCC) develop in patients with chronic hepatitis or liver cirrhosis caused by hepatitis B virus (HBV) or hepatitis C virus (HCV) infection. Higher HBV viral loads are strongly associated with the development of HCC and recurrence of HCC after surgery, and serum hepatitis B envelope antigen (HBe-Ag) is a simple surrogate marker of HBV-DNA [1–5]. Moreover, in patients with HBV-related small HCC, higher rates of intrahepatic recurrence of HCC and poorer survival in patients who were positive for HBe-Ag than in those who were negative for HBe-Ag have also been reported [6,7]. However, there have been no previous reports of surgical outcomes of HCC within Milan criteria in relation to the HB envelope. Therefore, there might be a difference in outcome after hepatectomy for HCC within the Milan criteria in relation to the HB envelope.

MATERIALS AND METHODS

Between 1991 and 2005, 1,188 patients with HCC underwent initial curative hepatectomy for HCC at our institute. Of these, 732 patients were given diagnoses of HCC within the Milan criteria (550 patients were solitary HCC of 5 cm or less and 182 patients were 2 or 3 HCCs of 3 cm or less) on histopathological examination. Laboratory tests, serum hepatitis B surface antigen (HBs-Ag), and HCV-antibody were routinely performed preoperatively in all patients, and 92 patients (13%) were found to be positive for HBs-Ag, 522 (71%) positive for HCV, 7 (1%) positive for both HBs-Ag and HCV, and 111 (15%) negative for either HBs-Ag or HCV. Serum HBe-Ag and HBe-Ab were further examined preoperatively in 85 of 92 patients with HBs-Ag, showing 70 patients to be positive for HBe-Ab and 15 patients to be negative for HBe-Ab. The remaining seven patients were excluded from this study because HBe-Ag or HBe-Ab was not examined. We retrospectively compared the results with the surgical outcomes of 70 patients who were positive for HBe-Ab and 15 patients who were negative for HBe-Ab. Five hundred twenty-two patients with HCV, 7 patients who were positive for both HBs-Ag and HCV, and 111 patients who were negative for either HBs-Ag or HCV were excluded from this study.

There were 67 men (79%) and 18 women (21%), and the mean age was 55 years (range, 22–76 years). Serum aspartate aminotransferase (AST), Child–Pugh class, indocyanine green retention rate at 15 min ($ICGR_{15}$), Platelet count, and serum level of alpha-fetoprotein (AFP) were examined preoperatively. Serum hepatitis B viral load was examined in our hospital from 1997. However, serum hepatitis B viral load was not examined routinely, therefore hepatitis B viral load was assessed in 31 of 85 patients with positive HBs-Ag. All patients underwent hepatectomy, and all surgical procedures were systematized hepatectomy with the Glissonean pedicle transection method [8,9]. The choice of resection was made on the basis of the tumor size, tumor type, and liver function ($ICGR_{15}$). In most of the patients with small and simple nodular HCC without daughter lesions, segmentectomy or smaller resection was performed. However, in patients with large and simple nodular HCC, sectionectomy or larger resection was performed considering functional liver reserve. In patients with HCC with daughter lesions within the section,

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sectionectomy or larger resection was performed. However, in patients with HCC with daughter lesions and poor functional liver reserve, segmentectomy or smaller resection was performed. Pathologically, macroscopic tumor type, portal or hepatic vein invasion, intrahepatic metastasis, and multicentric occurrence were examined. The terminology of liver resection was determined based on the Terminology Committee of the International Hepato-Pancreato-Biliary Association in 2000 [10]. Pathological findings were evaluated according to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer of the Liver Cancer Study Group of Japan [11].

Follow-up

After surgery, patients were followed up every 4–12 weeks at the outpatient department of our institution. Ultrasonography or computed tomography was performed once every 3–4 months. Intrahepatic recurrence was defined clinically as the appearance of a new lesion with radiological features typical of HCC, as confirmed by the above two imaging methods or biopsy specimens. Survival duration was defined as the time from hepatic resection to the date of death or last contact.

Statistical Analysis

Because of sample size limitations, patients with small nodular type with indistinct margins were combined with those with simple nodular type. Patients with simple nodular with extranodular growth type, confluent multinodular type, and massive type were combined into a single group. Categorical variables were assessed using the chi-square test and continuous variables were assessed using the unpaired t-test or Fisher's test. The overall survival and recurrence-free survival rates among the patients were calculated by the Kaplan–Meier method and compared with the log-rank test. Univariate prognostic factors were entered into multivariate analysis (Cox's proportional hazard model) to identify independent predictors of survival or recurrence. P-values <0.05 were taken to indicate the statistical significance. We used Stat View (version 4.5 Hulinks, Tokyo, Japan) for statistical analysis.

RESULTS

Patient characteristics in relation to HB envelope are shown in Table I. The mean AST, $ICGR_{15}$, platelet count, and AFP did not differ between patients who were positive for HBe-Ab and those who were negative for HBe-Ab. Serum hepatitis B viral loads were assessed in 31 of 85 patients with positive HBs-Ag, and there was no significant difference between patients who were positive for HBe-Ab and those who were negative for HBe-Ab. The number of cases of Child–Pugh class did not differ between groups, nor did the number of cases of cirrhosis, macroscopic tumor type, portal invasion, intrahepatic metastasis, multicentric occurrence, or surgical procedure.

The median patient follow-up was 72 months (ranging from 0.6 to 209 months). No patient died after surgery in hospital. The overall survival rate and recurrence-free survival rate for 85 patients with HCC within the Milan criteria and HBs-Ag were 86% and 48%, at 5 years, respectively. The overall survival curves and recurrence-free survival curves in relation to the HBe-Ab are shown in Figures 1 and 2. The 5- and 10-year survival rates were significantly greater in patients who were positive for HBe-Ab (90% and 80%, respectively) than in patients who were negative for HBe-Ab (61% and 37%: $P = 0.0004$, respectively). The 5-year recurrence-free survival rate was significantly greater in patients who were positive for HBe-Ab (53%) than in patients who were negative for HBe-Ab (21%: $P = 0.0054$).

Outcomes were compared considering subgroups of the Milan criteria. In patients with a single HCC < 5 cm or less, the 5-year survival rate was significantly greater in patients who were positive for HBe-Ab (90%) than in patients who were negative for HBe-Ab (64%: $P = 0.0008$), and the 5-year recurrence-free survival rate was tended to be greater in patients who were positive for HBe-Ab (58%) than in patients who were negative for HBe-Ab (31%: $P = 0.09$). In patients with 2 or 3 HCCs <3 cm or less, the 5-year survival rate was tended to be greater in patients who were positive for HBe-Ab (92%) than in patients who were negative for HBe-Ab (50%: $P = 0.37$), and the 5-year recurrence-free survival rate was significantly greater in patients who were positive for HBe-Ab (29%) than in patients with negative for HBe-Ab (14%: $P = 0.0178$).

Outcomes were compared among groups with Child–Pugh class A. In patients with Child–Pugh class A, the 5-year survival rate and recurrence-free survival rate were significantly greater in patients who were positive for HBe-Ab (93% and 55%, respectively) than in patients with negative for HBe-Ab (57%: $P < 0.0001$ and 23%: $P = 0.0054$, respectively). However, in patients with Child–Pugh class B, assessment was not possible because of the limited sample size of patients who were positive for HBe-Ab (n = 3) and negative for HBe-Ab (n = 2).

The univariate analysis of prognostic factors and multivariate analysis by Cox's proportional hazard model are summarized in Tables II and III. The univariate prognostic factors were entered into a multivariate model to identify independent predictors of survival and recurrence-free survival. Multivariate analysis showed that positive

TABLE I. Patients Characteristics in Relation to the HB Envelope

		Positive for HBe-Ab (n = 70)	Negative for HBe-Ab (n = 15)	P-value
Serum hepatitis B viral load	Present	15 of 28 patients assessed	3 of 3 patients assessed	0.10
Sex	Male	57 (81%)	10 (67%)	0.20
Age (year)	Mean ± SD	56 ± 10	52 ± 11	0.06
AST (U/l)	Mean ± SD	39 ± 18	41 ± 16	0.82
$ICGR_{15}$ (%)	Mean ± SD	12 ± 8	14 ± 14	0.44
Platelet count ($\times 10^4$/µl)	Mean ± SD	14 ± 5	13 ± 7	0.41
log AFP (ng/ml)	Mean ± SD	1.8 ± 1.2	2.2 ± 1.4	0.14
Child–Pugh	A	67 (96%)	13 (87%)	0.21
Cirrhosis	Present	37 (53%)	10 (67%)	0.33
Surgical procedure	Sectionectomy	42 (60%)	10 (67%)	0.63
Macroscopic type	Simple nodular	49 (70%)	13 (87%)	0.19
Portal invasion	Present	11 (16%)	4 (27%)	0.31
Intrahepatic metastasis	Present	4 (6%)	2 (13%)	0.29
Multicentric occurrence	Present	12 (17%)	4 (27%)	0.47

HBe-Ab, hepatitis B envelope antibody; AST, aspartate aminotransferase; ICGR15, indocyanine green retention rate at 15 min; log AFP, logarithmic alpha-fetoprotein; sectionectomy, sectionectomy or larger resection.

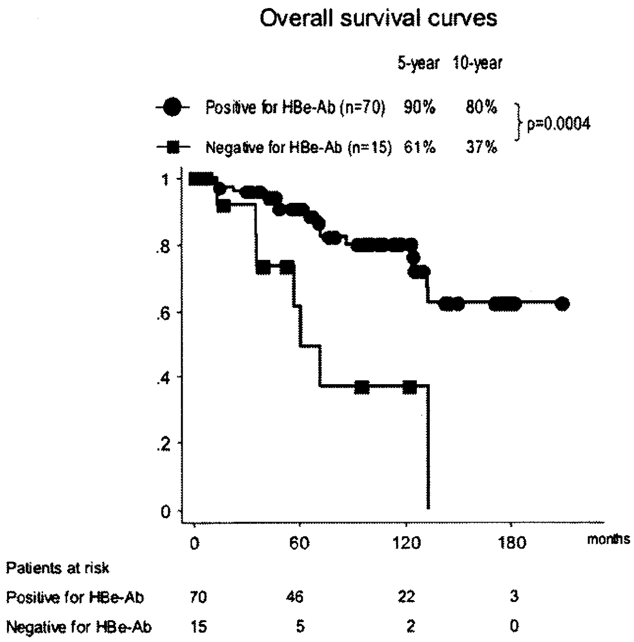

Fig. 1. The overall survival curves of patients with HCC within the Milan criteria in relation to the HB envelope are shown.

HBe-Ab was an independent prognostic factor for survival and recurrence-free survival in patients with HCC who met the Milan criteria.

DISCUSSION

It is well known that positive HBe-Ab means seroconversion in patients with HBV, and activity or progression of chronic hepatitis differs between patients who are positive for HBe-Ab (negative for HBe-Ag) and negative for HBe-Ab (positive for HBe-Ag) even in patients with HBs-Ag. Therefore, the higher carcinogenesis of HCC or poorer prognosis in patients who are positive for HBe-Ag than in patients who are negative for HBe-Ag has been reported [1]. Furthermore, poorer surgical outcomes after hepatectomy for small HCC in patients who are positive for HBe-Ag than in patients who are negative for HBe-Ag have been reported [6,7] However, there have been no previous reports of surgical outcomes of HCC within Milan criteria in relation to the HB envelope. In this study, lower risk of recurrence and longer survival were seen in patients who were positive for HBe-Ab than in patients who were negative for HBe-Ab. Moreover, multivariate analysis showed positive HBe-Ab to be a significant independent prognostic factor for survival and recurrence-free survival in patients with HBV-related HCC within the Milan criteria.

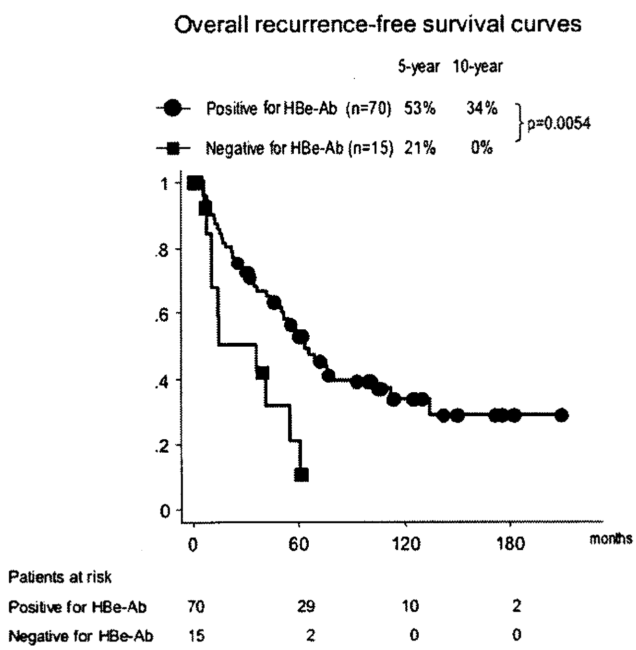

Fig. 2. The overall recurrence-free survival curves of patients with HCC within the Milan criteria in relation to the HB envelope are shown.

Liver transplantation in patients with a solitary HCC of 5 cm or less or 2 or 3 HCCs of 3 cm or less has obtained better results with regard to both survival and tumor-free survival rates [12–16]. Greater survival rate and recurrence-free survival rate after liver transplantation for patients with HCC within the Milan criteria than after hepatectomy have been reported [17–19]. However, Poon et al. also reported that, although recurrence after hepatectomy was higher than after liver transplantation, there was no significant difference in the long-term survival after resection and transplantation for patients with HBV-related HCC within the Milan criteria [15]. In the present study, favorable surgical outcomes, not only 5-year survival (90%) but also 10-year survival (80%) were achieved in patients with HBV-related HCC within the Milan criteria and positive HBe-Ab. Hepatectomy is therefore recommended in patients with HCC within the Milan criteria and positive HBe-Ab.

Recently, practice guidelines for the treatment of HCC have been published by American Association for the Study of Liver Disease (AASLD) [20,21] and in Japan [22]. According to the AASLD, surgical resection is recommended for patients who have a single HCC, Child–Pugh class A, and no cirrhotic or cirrhosis but still well-preserved liver function (normal bilirubin and hepatic vein pressure gradient <10 mmHg). According to the Japanese guidelines, liver resection is recommended for patients who have a single HCC or 2 or 3 HCCs with the liver damage categorized into class A or B. There is no consideration of either HBV or HCV infection in both algorithms. Routine tests for HB envelope are required to consider the treatment strategy for HBV-related HCC within the Milan criteria, because greater long-term survival after hepatectomy can be expected in patients with HBV-related HCC within the Milan criteria and positive HBe-Ab.

There have been reports regarding the effects of antiviral drugs on the prevention of initial HCC and recurrence of HCC after treatment. Kubo et al. reported that 24 patients with high-serum HBV DNA concentrations who underwent liver resection for HBV-related HCC and postoperative lamivudine therapy had improved tumor-free survival rates after liver resection [4]. In the present study, we did not compare outcomes in relation to the antiviral drug because the study period was long, from 1991 to 2005, and postoperative antiviral therapy was recently performed in only six patients from 2000. However, antiviral therapy after hepatectomy for patients with HCC and negative HBe-Ab is required because shorter survival and higher risk of recurrence after hepatectomy were seen in patients who were negative for HBe-Ab than in patients who were positive for HBe-Ab.

There have been only a few reports on outcomes after hepatectomy for small HCC in relation to the HB envelope [6,7]. In the present study, favorable long-term surgical outcomes were achieved in patients with HBV-related HCC within the Milan criteria and positive HBe-Ab. Multivariate analysis showed that positive HBe-Ab was an independent prognostic factor for survival and recurrence-free survival. Routine tests for HB envelope are required for patients with

TABLE II. Factors Associated With Survival Rate and Recurrence-Free Survival Rate of Patients by Univariate Analysis

	Number	5-year survival (%)	P-value	5-year recurrence-free survival (%)	P-value
Sex					
Male	67	87	0.34	53	0.0437
Female	18	85		26	
Age (years)					
<55	45	84	0.44	42	0.20
≥55	40	89		55	
Hepatitis B envelope antibody					
Positive	70	90	0.0004	53	0.0054
Negative	15	61		21	
Serum hepatitis B viral load[a]					
Present	15	100	0.09	43	0.58
Absent	16	79		46	
AST (U/l)					
<40	44	90	0.95	51	0.17
≥40	41	83		45	
$ICGR_{15}$ (%)					
<12	52	87	0.99	51	0.19
≥12	33	86		44	
Platelet count ($\times 10^4/\mu l$)					
≤14	49	81	0.06	43	0.06
>14	36	93		56	
Child–Pugh class					
A	80	88	0.0306	51	<0.0001
B	5	50		0	
Cirrhosis					
Present	47	81	0.21	45	0.18
Absent	38	93		45	
log AFP (ng/ml)					
≤1.9	50	82	0.08	45	0.24
>1.9	35	93		53	
Surgical procedure					
Sectionectomy	52	90	0.33	57	0.0235
Partial sectionectomy	33	80		33	
Macroscopic type					
Simple nodular	62	87	0.33	47	0.99
Others	23	84		53	
Portal invasion					
Present	15	77	0.41	49	0.90
Absent	70	88		48	
Intrahepatic metastasis					
Present	6	80	0.10	20	0.0089
Absent	79	87		50	
Multicentric occurrence					
Present	16	85	0.86	22	0.0019
Absent	69	86		54	

C.I., Confidence interval; HBe Ab, hepatitis B envelope antibody.
AST, aspartate aminotransferase; ICGR15, indocyanine green retention rate at 15 min; log AFP, logarithmic alpha-fetoprotein; sectionectomy, sectionectomy or larger resection.
[a]Thirty-one patients were assessed.

TABLE III. Multivariate Analysis Using Cox's Proportion Hazards Model

Risk factor			Relative risk	95% CI	P-value
Survival	Hepatitis B envelope antibody	Positive	0.250	0.096–0.651	0.0045
Recurrence	Hepatitis B envelope antibody	Positive	0.323	0.149–0.697	0.0040
	Intrahepatic metastasis	Present	5.546	1.821–16.862	0.0026

CI, confidence interval.

HBV-related HCC, and hepatectomy is recommended in patients with HCC within the Milan criteria and positive HBe-Ab.

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