3．肝移植後の補助療法

肝移植は肝機能因子により肝切除不能な肝細胞癌に対して最も効果の高い治療法であり，5年無再発生存率が60～80%に達する．肝移植は肝細胞癌の根治切除と背景に存在する障害肝を同時に治療できる理想的な治療であるが，移植後の免疫抑制が必須であり，かつウィルス性肝炎の再発など複雑な要素が関連して，現在のところ肝細胞癌に対する肝移植後の補助療法は確立されていない．こういった現状で，Tosoらは治療戦略として以下の5点をあげている．

①循環腫瘍細胞が少ないレシピエントを選択する，

②術中操作による播種を最小限にする，

③循環腫瘍細胞の肝内着床を防止する(虚血再灌流障害は癌細胞の着床を促進する)，

④抗癌剤の使用(循環腫瘍細胞を死滅させる)，

⑤癌細胞を排除できるよう免疫系を調節すること，

である[47]．したがって再発予防のための補助療法として，新規分子標的薬を含めた抗癌剤の導入や，免疫療法などが試みられている．

4．肝細胞癌に対する肝移植後の再発予防としてのドナー肝由来活性化NK細胞療法：われわれの試み

肝移植後の肝細胞癌再発機構は，術前にすでに存在する癌細胞の肝外播種，あるいは手術操作に起因する癌細胞の物理的播種などが関連すると考えられる．肝移植後には免疫抑制剤の使用が不可欠であるが，これに伴う非特異的な生体防御機構の減弱のために，遺残する微量な癌細胞は排除されにくくなる．生体防御機構は自然免疫応答と獲得免疫応答からなるが，拒絶反応や免疫抑制療法に大きく影響を受けるのは獲得免疫応答である．そこでわれわれは，肝移植後に自然免疫応答を選択的に増強する制癌免疫療法の可能性について研究を重ねてきた．自然免疫応答をつかさどるNK細胞は，癌転移形成の初期段階に癌細胞を自己正常細胞から識別し，選択的に殺傷する能力を有する．自己の正常細胞に表出するMHC class Iを認識すると抑制性シグナル伝達により細胞傷害は生じないが，癌細胞上に表出する変異MHC classはNK細胞に抑制性シグナルを伝達できず傷害を受けると考えられている(missing-self theory)．われわれは，ヒト肝臓内には大量のNK細胞が含有され，末梢血由来のNK細胞と異なり，IL-2による刺激で強力な抗腫瘍分子(tumor necrosis factor-related apoptosis-inducing ligand，TRAIL：健常な細胞には影響せず腫瘍細胞のみを選択的に標的にする分子)を誘導し得ることを確認した[48]．さらに，術後再発率が高い中～低分化肝細胞癌はTRAIL受容体(death receptors)を高発現し，TRAILを介した細胞死が誘導されやすかった．マウス肝癌モデルにおいて，TRAIL表出肝NK細胞を外来的に移入することで肝癌細胞の肝内生着を抑止することが可能であった[49]．肝移植の際にはドナーから摘出した肝臓をレシピエントに移植する前に臓器保存液で肝臓内血液を置換するために灌流を行うが，この際に回収される灌流液から無菌操作でNK細胞を効率よく回収するシステムを開発した．以上の基礎研究に基づき，広島大学病院倫理委員会の承認(第414号)の下，肝癌症例に対する肝移植後の癌再発予防を目的としたドナー肝由来活性化NK細胞療法を2006年1月より臨床導入した．現在までにStage II以上の肝細胞癌合併肝硬変18例に対し本治療を行い，安全性を確認し，経過観察中である．今後長期の経過観察が必要であるが，現在のところ治療群で有意に再発が少ない傾向にある．また本治療法を脳死肝移植へ適応拡大する目的で，現在われわれはマイアミ大学と共同研究を行っている．本治療法は米国においてFDA承認を得て，Phase I studyが進行中である(Clinical Trial.gov#: NCT01147380)．

おわりに

肝細胞癌の再発には癌転移再発と多中心性発癌という再発形式が複雑に関与している．術後のインターフェロンを中心とするウィルス駆除療法は

一定のサブグループの補助療法として予後に寄与すると考えられるが，進行肝細胞癌に対して転移再発を抑制する有効な治療法はいまだ確立されていない．明らかになりつつある分子生物学的あるいは免疫学的機序を応用した新たな治療戦略を期待する．

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Impact of Adjuvant Immunotherapy Using Liver Allograft-Derived Lymphocytes on Bacteremia in Living-Donor Liver Transplantation

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Background. Bacteremia is one of the leading causes of mortality in living-donor liver transplant (LDLT) recipients. Lymphocytes, including natural killer cells, are believed to play a role in the first line of defense against invading infectious microbes.

Methods. From January 2004 to December 2009, 114 consecutive LDLT recipients were studied for postoperative bacteremia. The impact of adjuvant immunotherapy using activated liver allograft-derived lymphocytes on bacteremia was retrospectively evaluated by a one-to-one match using propensity score to overcome bias due to the different distribution of covariates for the two groups.

Results. After one-to-one matching, 21 patients who did not receive adjuvant immunotherapy and 21 who did not receive adjuvant immunotherapy had the same preoperative and operative characteristics. Six (28.6%) of the 21 patients who did not receive adjuvant immunotherapy had bacteremia, whereas only one (4.8%) of the 21 patients who received adjuvant immunotherapy had bacteremia; thus, the incidence of bacteremia in patients who had received adjuvant immunotherapy was significantly lower than that in patients who had not received adjuvant immunotherapy ($P=0.038$).

Conclusions. Adjuvant immunotherapy using liver allograft-derived lymphocytes may be a promising modality for reducing the postoperative bacteremia after LDLT.

Keywords: Living-donor liver transplantation, Natural killer cells, Bacteremia, Adjuvant immunotherapy.

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Infection is one of the leading causes of morbidity and mortality in liver transplant patients. The incidence of infection in patients after liver transplantation is higher than that after renal and heart transplantation (*1*). The high incidence of infection in liver transplant patients is likely attributed to the technical complexity of the surgery, latent contamination in the abdominal cavity, and the poor medical condition of the patients. After transplantation, the incidence of bacterial and fungal infection is approximately 50% and 10%, respectively (*2*, *3*). Most infections occur within the first month after liver transplantation and are primarily the result of surgical complications or are nosocomial in origin (*2–5*). Bacteremia has been reported to be the main cause of mortality in liver transplant recipients (*2*, *5*). Associated mortality rates of approximately 30% have been reported for bacteremia (*6–8*).

We have recently developed a novel strategy of using adjuvant immunotherapy to prevent the recurrence of hepatocellular carcinoma (HCC) or hepatitis C virus (HCV) infection after living-donor liver transplantation (LDLT) (*9*, *10*). This immunotherapeutic strategy involves intravenous injection of activated liver allograft-derived lymphocytes into LDLT patients. These lymphocytes can mount an antitumor immune response (*9*) and are known to play a role in the first line of defense against invading microbes.

In this study, we retrospectively investigated whether this adjuvant immunotherapy reduced the incidence of post-transplant bacteremia in LDLT recipients. However, there may be selection bias of liver function and operative characteristics between the liver transplant recipients who received adjuvant immunotherapy and who did not receive it. To overcome selection bias due to the different distribution of clinical characteristics such as severity of liver function impairment between the two groups, a one-to-one match was created using propensity score analysis. Thus, we obtained two groups that were comparable for baseline variables.

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RESULTS

The Adjuvant Immunotherapy Using Activated Liver Allograft-Derived Lymphocytes

We performed ex vivo perfusion of graft livers through the portal vein. The proportion of interleukin-2 (IL-2)-stimulated CD56+CD3-natural killer (NK) cells and CD56+CD3+ natural killer T (NKT) cells extracted from the liver perfusates was 40%±15% and 20%±9%, respectively (n=24). The recipients were administered a single intravenous injection of IL-2-stimulated liver allograft-derived lymphocytes 3 days after liver transplantation (793±350 × 10^6 cells injected per patient, n=24). During the follow-up period, no significant adverse effects or rejection episodes were observed.

Clinicopathologic Characteristics and Postoperative Course of the Entire Study Group

Differences in characteristics between patients who received adjuvant immunotherapy and those who did not are listed in Table 1. Specifically, patients who received immunotherapy were older (57 vs. 54; $P=0.03$), more likely to have HCC (87.5% vs. 38.9%; $P<0.001$), and had a lower score of model for end-stage liver disease (MELD) (11 vs. 16; $P<0.0038$). Of 90 patients who did not receive immunotherapy, 26 (28.9%) developed bacteremia, whereas only 1 (4.2%) of the 24 patients who received immunotherapy had bacteremia. The incidence of bacteremia was significantly lower in the adjuvant immunotherapy group than in the nonadjuvant immunotherapy group ($P=0.034$) (Table 2). The 1-year survival rate tended to be higher in the patients who had received adjuvant immunotherapy (100%) than in those who had not (80%) ($P=0.068$; Table 2). The sources and pathogens present in bacteremia are listed in Tables 3 and 4, respectively. Among the 27 recipients with episodes of bacteremia, five (18.5%) had primary bacteremia. The three most common sources of bacteremia were catheter-related infections (5/27 patients, 18.5%), peritonitis (8/27 patients, 29.6%), and cholangitis (5/27 patients, 18.5%). Gram-positive and gram-negative bacteria were detected in 15 (55.6%) and 12 (44.4%) of the 27 patients with bacteremia, respectively. The most common isolated pathogens were methicillin-resistant *Staphylococcus aureus* (MRSA) (n=9), coagulase-negative *staphylococcus* (n=5), *Escherichia coli* (n=5), *Enterobacter spp.* (n=3), and *Pseudomonas aeroginosa* (n=3). There was no difference in postoperative clinical data, including duration of central venous catheterization, hospital stay, and intensive care unit stay between the two groups (Table 5).

Results After Propensity Score Matching

Characteristics after propensity score matching analysis are shown in Table 1. Twenty-one of the 24 patients who received adjuvant immunotherapy were matched with 21 patients who did not receive therapy after covariate adjustment. Therefore, three patients who received immunotherapy and 69 patients who did not receive the therapy were excluded because their propensity scores could not be matched. The study group of 42 patients was well matched; in particular, all covariates that significantly affected overall survival in the entire study group were equally distributed over the two matched groups. Matched patients who received adjuvant immunotherapy had a similar age (56 vs. 57; $P=0.62$), donor age (36 vs. 36; $P=0.51$), graft-to-recipient weight ratio (0.89 vs. 0.85; $P=0.72$), score of MELD (13 vs. 11; $P=0.73$), blood loss (3270 vs. 3420 mL; $P=0.53$), and rate of postoperative bile leakage (14.3% vs. 19.0%; $P=0.67$) to those of matched patients who did not receive adjuvant immunotherapy. Similarly, other clinical variables were similar for patients who did and did not receive the therapy. Of the 21 patients who did not receive immunotherapy, six (28.6%) developed bacteremia. Of the 21 patients who received immunotherapy, one (4.8%) developed bacteremia. The incidence of bacteremia was significantly lower in the adjuvant immunotherapy group than in the nonadjuvant immunotherapy group, respectively ($P=0.038$) (Table 2). The 1-year survival rate tended to be higher in the patients who had received adjuvant immunotherapy (100%) than in those who had not received the therapy (80%) ($P=0.10$; Table 2). The sources and caus-

TABLE 1. Clinical characteristics of whole study and one-to-one matching study using propensity scores

	Whole study			Matched study population		
	IT (−) (n=90)	IT (+) (n=24)	*P*	IT (−) (n=21)	IT (+) (n=21)	*P*
Ageing (median, range)	54 (20–69)	57 (44–68)	0.03	57 (46–69)	56 (44–68)	0.62
Male	54 (60%)	16 (66.7%)	0.55	13 (61.9%)	15 (71.4%)	0.51
Age of donor	36 (17–67)	34 (20–54)	0.33	36 (18–57)	36 (20–54)	0.51
Illness						
HCC (%)	35 (38.9%)	21 (87.5%)	0.00021	16 (76.2%)	19 (90.1%)	0.21
Previous operation (%)	15 (16.7%)	5 (20.8%)	0.63	6 (28.6%)	4 (19.0%)	0.47
GRWR	0.91 (0.62–1.95)	0.87 (0.70–1.22)	0.11	0.85 (0.62–1.35)	0.89 (0.70–1.22)	0.72
MELD	16 (6–55)	11 (7–25)	0.0038	11 (7–25)	13 (7–23)	0.73
Presence of ascites	57 (63%)	15 (62.5%)	0.65	11 (52.4%)	9 (42.9%)	0.75
Blood loss (ml)	3675 (345–14,000)	3120 (1080–8990)	0.64	3420 (1100–8200)	3270 (1200–8990)	0.53
Operating time (min)	738 (530–1167)	706 (535–1009)	0.66	749 (530–930)	702 (535–1009)	0.27
Postoperative biliary leak (%)	14 (15.6%)	3 (12.5%)	0.7	4 (19.0%)	3 (14.3%)	0.67
Reoperation (%)	23 (25.6%)	4 (16.7%)	0.36	3 (14.3%)	5 (23.8%)	0.43

IT, adjuvant immunotherapy; HCC, hepatocellular carcinoma; GRWR, graft recipient weight ratio; MELD, model for end-stage liver disease.

TABLE 2. Outcomes of postoperative bacteremia after living-donor liver transplantation

Infection site	Whole study			Matched study population		
	IT (−) (n=90)	IT (+) (n=24)	*P*	IT (−) (n=21)	IT (+) (n=21)	*P*
Number of patients with bacteremia (%)	26 (28.9%)	1 (4.2%)	0.004	6 (28.6%)	1 (4.8%)	0.038
One-year survival	84%	100%	0.061	80%	100%	0.1

IT, immunotherapy.

TABLE 3. Causes of postoperative bacteremia after living-donor liver transplantation

Infection site	No. of patients with episodes of postoperative bacteremia			
	Whole study		Matched study population	
	IT (−) (n=90)	IT (+) (n=24)	IT (−) (n=21)	IT (+) (n=21)
Abdominal infection				
Intraabdominal abscess	3	0	0	0
Peritonitis	7	1	1	1
Cholangitis	5	0	2	0
Enteritis	1	0	0	0
Catheter infection	5	0	2	0
Primary	5	0	1	0

IT, immunotherapy.

TABLE 4. Etiological organisms of bacteremia after living-donor liver transplantation

Pathogen	No. patients with episodes of bacteremia			
	Whole study		Matched study population	
	IT (−) (n=90)	IT (+) (n=24)	IT (−) (n=21)	IT (+) (n=21)
Bacteria				
Gram (+)				
MRSA	9	0	2	0
Coagulase-negative *staphylococcus*	5	0	1	0
Others	1	0	0	0
Gram (−)				
Escherichia coli	4	1	2	1
Enterobacter sp	3	0	0	0
Pseudomonas aeruginosa	3	0	0	0
Serratia sp	1	0	1	0

IT, immunotherapy; MRSA, methicillin-resistant staphylococcus aureus.

ative pathogens of bacteremia are shown in Tables 3 and 4, respectively, and were as follows: one patient with primary bacteremia (MRSA), two patients with catheter-related infections (*Escherichia coli*, and *Serratia sp*), two patients with peritonitis (MRSA and *Escherichia coli*), and two patients with cholangitis (coagulase-negative *Staphylococcus* and *Escherichia coli*). There was no difference in postoperative clinical data, including duration of central venous catheterization, hospital stay, and intensive care unit stay between the two groups (Table 5).

DISCUSSION

Bacteremia is one of the main complications of liver transplantation, and it is also an important factor influencing the mortality associated with liver transplantation (*6*, *7*). Many studies have revealed that the period of hospitalization and stay in the intensive care unit is longer for patients with postoperative bacteremia (*4*, *11*, *12*).

Adjuvant immunotherapy using liver allograft-derived lymphocytes was principally performed to prevent HCC recurrence. As these lymphocytes may have antibacterial properties, this study was performed as a secondary aim to investigate whether this adjuvant immunotherapy reduced the incidence of posttransplant bacteremia in LDLT recipients. In this one-to-one matching study using propensity score, we have shown that adjuvant immunotherapy using liver allograft-derived lymphocytes significantly reduced the incidence of postoperative bacteremia after LDLT. We have previously shown that stimulation with IL-2 significantly increased the expression of tumor necrosis factor-related apoptosis-inducing ligand on liver NK cells exerting anti-HCV and tumoricidal effects (*9*, *10*). We have also confirmed that the number of interferon (IFN)-γ-secreting cells, including NK and NKT cells, in the peripheral blood of the liver transplant patients who received adjuvant immunotherapy was significantly higher than that in the peripheral blood of patients who did not receive the therapy at 14 days after LDLT (*10*). It is expected that the circulating activated NK and NKT cells in the peripheral blood may prevent postoperative bacteremia by exerting an antibacterial and antifungal effect, and the effect for preventing the postoperative infections may last during at least 2 weeks after LDLT. The adjuvant immunotherapy might reduce the number of postoperative infections as it involved the use of IL-2 stimulated NK and NKT cells. NK cell has protective functions for bacterial infection such as *Staphylococcus aureus* and *Pseudomonas aeroginosa* by NK cytotoxicity and augmentation of phagocytosis by macrophages by IFN-γ and tumor necrosis factor production secreted by NK cells (*13*, *14*). NKT cells also have protective effects for bacterial infection by IFN-γ production (*15*). In this study, no adverse effects, such as graft-versus-host disease, were observed. To prevent graft-versus-host disease, we added the anti-CD3 monoclonal antibody to the culture medium a day before the inoculation. As the current immunosuppressive regimen following after LDLT reduces the adaptive immune components, adjuvant

TABLE 5. Postoperative clinical data after living-donor liver transplantation

	Whole study			Matched study population		
	IT (−) (n=90)	IT (+) (n=24)	*P*	IT (−) (n=21)	IT (+) (n=21)	*P*
Duration of CV catheter	6 (5–23)	7 (5–11)	0.71	6 (5–16)	7 (5–10)	0.78
ICU stay (day)	4 (3–22)	6 (2–12)	0.56	4 (3–22)	5 (2–12)	0.67
Hospital stay (day)	54 (22–1414)	53 (16–190)	0.76	50 (22–1414)	53 (16–190)	0.95
WBC (mm^3)	7865 (4290–33,740)	7010 (2690–10,930)	0.09	7565 (4290–15,620)	7450 (2690–10,930)	0.21
Lymphocyte count (mm^3)	558 (89–1222)	491 (148–2142)	0.77	521 (89–1020)	545 (148–2142)	0.73
CRP	1.24 (0.15–11.3)	3.5 (0.3–14.1)	0.07	1.01 (0.15–9.6)	2.4 (0.3–14.1)	0.25
BT (°C)	37.1 (36.6–38.8)	37.1 (36.5–38.1)	0.86	37.0 (36.6–38.4)	36.9 (36.5–38.1)	0.89

CRP and BT were measured on day 7 after living-donor liver transplantation. Data were expressed as median (range).

IT, immunotherapy; CV, central venous; ICU, intensive care unit; WBC, white cell count; CRP, C-reactive protein; BT, body temperature; WBC, lymphocyte count.

immunotherapy may be a promising approach for reducing the posttransplant bacteremia.

In this study, we adopted a one-to-one matching study using propensity score analysis to overcome bias due to the different distribution of covariates among patients from the two groups (patients who received adjuvant immunotherapy and those who did not). Bert et al. (*7*) have shown that gender, kidney transplant, intraoperative transfusions, MELD score, return to surgery, retransplantation, and biliary complications are associated with bacteremia. Kim et al. (*8*) have revealed an association between bacteremia and age, intravenous catheterization, United Network for Organ Sharing status IIA, and posttransplant hemodialysis. The clinical characteristics that may influence outcomes tended to differ between the two groups in the whole study (Table 1). The proportion of the older patients with HCC was significantly higher in the adjuvant immunotherapy group than those in no adjuvant immunotherapy group because the liver transplant patients who received adjuvant immunotherapy with IL-2-stimulated lymphocytes were limited to patients with HCC or HCV. The scores of MELD were significantly lower in the adjuvant immunotherapy group than in the nonadjuvant immunotherapy group. A high MELD score is known to indicate a major risk of postoperative infections after liver transplantation (*16*, *17*). However, after matching using propensity scores analysis, there were no differences in clinical variables influencing outcomes such as age, MELD score, underlying liver disease, operative factors, and postoperative operative factors between the two groups. Iida et al. (*6*) have shown that the presence of preoperative massive pleural effusion or ascites requiring drainage were independent risk factors for postoperative bacteremia. In this study, there was no significant difference in the preoperative presence of ascites between the two groups. Although there was a significant difference in the incidence of bacteremia between the two groups, this study was unable to reveal a significant difference in outcomes, likely due to small study size. It is possible that the learning curve of individual surgeons or of the entire institution may be associated with an incidence of septic complications. Second, there was a selection bias confounding our results between the two groups, because adjuvant immunotherapy was selected into only patients with HCC or HCV, thus larger study is required to confirm the positive effect of adjuvant immunotherapy on outcomes of liver transplant recipients.

Bacteremia episodes were predominantly caused by gram-positive bacilli, which accounted for 55.6% of all isolated infections. Methicillin-resistant *staphylococci* were commonly observed among the gram-positive organism, whereas *Escherichia coli* strains were found to be the most prevalent species among the isolated gram-negative rods. During the 1990s, MRSA had become endemic and emerged as a major pathogen in many transplant centers. However, in the late 1990s, the incidence of gram-negative bacterial infection increased, probably because of the use of prophylactic antibiotics (*18*). In contrast, our study has shown that gram-positive bacteria have remained as the major pathogens (*8*, *19*). Our results support recent studies that report coagulase-negative *staphylococcus* as the major cause of bacteremia (*6*). Catheter-related infections were found to occur frequently after surgery. Appropriate management of the catheter, which disrupts the organization of the skin epithelium and the mucosa, and early catheter removal are important for lowering the incidence of catheter-related infections.

In conclusion, we found that adjuvant immunotherapy using liver allograft-derived lymphocytes significantly reduced the incidence of postoperative bloodstream infections after LDLT in the setting of one-to-one matching study using propensity score. As the immunosuppressive regimen currently used after LDLT reduces the adaptive immune components, adjuvant immunotherapy using liver allograft-derived lymphocytes may be a promising approach for reducing posttransplant bacteremia.

MATERIALS AND METHODS

Patients

We retrospectively studied 114 consecutive patients who had undergone LDLT at Hiroshima University Hospital, from April 2004 to December 2009. The LDLTs had been performed after the approval of the Liver Transplantation Ethics Committee of Hiroshima University, and informed consents were obtained from all the patients.

Perioperative Management Strategy

The procedures for donor evaluation, donor surgery, recipient surgery, and perioperative management followed in our hospital have been described in previously published studies (*20–22*). A central venous catheter (triple

lumen catheter, SA series, Arrow Corporation, Japan) was place in the internal jugular vein at induction of general anesthesia.

Antimicrobial prophylaxis consisted of intravenous cefmetazole (1.0 g) administration immediately before surgery and every 6 hr during surgery; thereafter, a dosage of 2 g/day was maintained for 5 days. Vancomycin (0.5 g/day, orally) was administered for 3 days before surgery. Itraconazole (200 mg/day) was orally administered for 7 days before surgery as prophylaxis against fungal infections. Trimethoprim/sulfamethoxazole (80/400 mg/day) was orally administered after surgery as prophylaxis against *Pneumocystis*.

The basic immunosuppression regimen comprised tacrolimus and methylprednisolone. If liver function stabilized, then the patients were weaned off the steroids 2 to 3 months after the operation. Rejection episodes were mainly treated with methylpredonisolone.

The use of adjuvant immunotherapy involving activated liver allograft-derived lymphocytes was approved by the Clinical Institutional Ethical Review Board of Hiroshima University, and the immunotherapy was started from January 2006. Gradient centrifugation with Ficoll-Paque was performed to isolate liver mononuclear cells from the perfusate effluents of liver grafts obtained from healthy donors. Liver mononuclear cells were cultured with human recombinant IL-2 for 3 days. A day before the infusion, the CD3-positive cell fraction was incubated with an anti-CD3 monoclonal antibody for opsonization. The purity of the isolated fractions was assessed by flow cytometric analysis, and the viability of the cells was assessed by dye-exclusion test before injection. The cells were suspended along with 5% human serum albumin in 0.9% sodium chloride; 3 days after liver transplantation, the cell suspension was then injected into patients with HCV or HCC (*10*).

Definitions of Bacteremia

Infections were defined according to the criteria proposed by the Centers for Disease Control (*23*). For this study, we included cases in which the infections had developed within 3 months after surgery and documented the first episode of bacteremia. Isolation of bacteria (other than common skin contaminants) from a single blood culture in the presence of clinical symptoms or signs of infection was considered proof of bacteremia. Bacteremia caused by common skin contaminants was considered significant only if the organism was also isolated from two blood cultures and was associated with clinical signs of infection. The bacteremia source was determined on the basis of clinical criteria and isolation from a clinically significant site of infection of the same organism found in the blood isolate on the basis of species identification and antibiotics susceptibility results. Bacteremia was classified as primary if it was of unknown origin (no physical, radiological, or pathological evidence of a definite infection source). Catheter-related infections were documented when the blood isolate was cultured from the catheter tip and blood cultures obtained by the catheter were found to be the same as the peripheral blood culture. Briefly, blood cultures were taken out by puncture. The blood culture was as follows: a 10-mL blood sample was aseptically inoculated at bedside into each bottle of a set of aerobic and anaerobic blood culture bottles. Bottles were incubated at 37°C for a period of 5 days or until a positive reading was detected by the instrument. Bacteremia was classified as primary if it was of unknown origin.

Statistical Analysis

Continuous variables were compared using the Mann-Whitney test. Categorical data were compared using the χ^2 test or Fisher's exact test, as appropriate. Overall survival analyses were carried out using the Kaplan-Meier method; comparisons between different groups were carried out using the log-rank test. To overcome bias due to the different distribution of covariates among patients from the two groups (patients who received adjuvant immunotherapy and those who did not), a one-to-one match was created using propensity score analysis (*24*, *25*). The propensity score represents the probability of each individual patient being assigned to a particular condition in a study given a set of known covariates. Propensity scores are used to reduce selection bias by equating groups on the basis of these covariates and are used to adjust for selection bias in observational studies through matching. Variables entered in the propensity model were age, sex, donor age, the presence of HCC, previous surgery, graft-to-recipient weight ratio, MELD score, operative factors (blood loss during operation and operation time), and postoperative complications (biliary leakage and reoperation). The model was then used to obtain a one-to-one match by using the nearest-neighbor matching method. We used a matching algorithm based on linear predictive values without replacement, until all possible matches had been formed. Initially, matching was performed to five decimal points, followed by 4, 3, 2, and 1 decimal point matching; cases whose propensity score deviated more than 0.10 were considered unmatched (*26*, *27*) Patients with unmatchable propensity scores were excluded from further analysis. Once the matched groups were obtained, differences in postoperative infection and prognosis were further analyzed to assess the unbiased influence of adjuvant immunotherapy on postoperative infection. Overall, survival analysis was performed within each matched subgroup to assess the influence of adjuvant immunotherapy on postoperative infection amended from the confounding factors. All analyses were performed using the SPSS 16.0, and P values less than 0.05 were considered statistically significant.

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ORIGINAL ARTICLE

Tumor-related factors do not influence the prognosis of solitary hepatocellular carcinoma after partial hepatectomy

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Abstract
Background/purpose Although many factors related to the tumor or the hepatic functional reserve may affect the outcome of partial hepatectomy for hepatocellular carcinoma (HCC), these factors have not yet been intensively investigated in patients with solitary HCC. The purpose of this study is to determine the clinicopathological factors influencing the long-term outcomes of partial hepatectomy for solitary HCC.
Methods Data on 266 consecutive patients with a solitary HCC who underwent curative hepatectomy between 1997 and 2006 were analyzed with regard to prognosis.
Results Overall survival rates at 3, 5, and 10 years were 89.5, 79.6, and 56.1%, respectively. The significant independent predictors for overall survival included hepatitis C virus infection, liver cirrhosis, and prolonged prothrombin activity. Disease-free survival rates at 3, 5, and 10 years were 51.7, 41.1, and 20.4%, respectively. The significant independent predictors for disease-free survival included elevated levels of aspartate amino transferase, decreased platelet counts, presence of liver cirrhosis, and prolonged prothrombin activity. Tumor-related factors such as tumor size and microscopic vascular invasion were not significant predictors of overall or disease-free survival.
Conclusions The long-term outcomes of patients with a solitary HCC who underwent partial hepatectomy mainly depended on the background liver status but not on tumor-related factors; this suggests that partial hepatectomy is a remarkably effective antitumor therapy. If the hepatic functional reserve is within the permissible range, partial hepatectomy should be considered as the treatment of choice for patients with a solitary HCC.

Keywords Solitary hepatocellular carcinoma · Partial hepatectomy · Prognostic factor · Hepatic functional reserve · Tumor-related factor

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide [1]. Several modalities have been used for treating HCC, including partial hepatectomy, orthotopic liver transplantation, radiofrequency ablation therapy, and transarterial chemoembolization therapy. Partial hepatectomy is the preferred treatment for patients with resectable HCC and offers low mortality and favorable long-

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term survival. However, the long-term survival rate remains unsatisfactory, mainly because of the high incidence of recurrence and metastasis after partial hepatectomy [2].

Several prognostic factors that negatively influence the outcomes of patients with HCC who undergo partial hepatectomy have been reported [3, 4]. They include the presence of cirrhosis, hepatitis C virus (HCV) infection, large tumors, bilobar distribution, multiple lesions, vascular invasion, pathological tumor–node–metastasis (pTNM) stage, surgical margin, high alpha-fetoprotein (AFP) level, and tumor differentiation [5, 6]. Although several tumor-related factors such as tumor size and microvascular invasion are recognized as significant adverse prognostic factors, it is still controversial whether these factors independently influence long-term outcomes of curative resection of a solitary HCC [7–9]. The purpose of this study was to determine the clinicopathological factors influencing the long-term outcomes of solitary HCC in patients undergoing curative hepatectomy.

Materials and methods

Patients

Between January 1997 and December 2006, 496 consecutive patients with primary HCC underwent hepatectomies in our hospital. We defined solitary HCC as a single tumor, without macroscopic vascular invasion and without distant metastasis, based on radiological imaging prior to surgery. Macroscopic vascular invasion was defined as a tumor invasion or a tumor thrombus in the vessels visible on radiological imaging prior to surgery. Microscopic vascular invasion was defined as a tumor invasion or a tumor thrombus in the vessels visible only on microscopy. We excluded 224 patients with multiple tumors, 1 with macroscopic vascular invasion, 3 with distant metastases, and 2 undergoing hepatectomies with intraoperative ablation therapy. Finally, 266 patients with a solitary HCC were enrolled in this retrospective study.

The patients' characteristics are shown in Table 1. A total of 182 patients (68.4%) were positive for HCV infection, 232 (87.2%) had viral hepatitis, and 259 (97.3%) had chronic liver disease.

Clinicopathological variables for age, gender, presence of viral hepatitis B and C, presence of diabetes mellitus, preoperative transarterial chemoembolization, preoperative liver function tests, Child–Pugh class, preoperative AFP and des-gamma-carboxy prothrombin (DCP) levels, types of hepatectomy, tumor characteristics according to the Liver Cancer Study Groups of Japan, and pathological findings of the resected specimen were obtained and analyzed [10]. Liver cirrhosis, microscopic vascular invasion, and intrahepatic metastasis were obtained by histological examination of resected specimens. Experimental protocols were approved by the institutional review committee and met the guidelines of responsible governmental agency.

Table 1 Patients' characteristics ($n = 266$)

Variables	Number of patients (%)
Age (years)[a]	65.3 ± 9.7 (23–86)
Gender	
Male	186 (69.9)
Female	80 (30.1)
Serology of viral hepatitis	
HBV only	50 (18.8)
HCV only	177 (66.5)
HBV + HCV	5 (1.9)
No infection	34 (12.8)
Child–Pugh classification	
A	236 (88.7)
B	30 (11.3)
Background liver status	
Normal liver	2 (0.8)
Chronic hepatitis or liver fibrosis	148 (55.6)
Liver cirrhosis	111 (41.7)
Unknown	5 (1.9)
Tumor size (mm)[a]	31.3 ± 18.6 (6–125)
Type of hepatectomy	
Limited resection	156 (58.6)
Segmentectomy	68 (25.6)
Sectionectomy	24 (9.0)
Hemihepatectomy	18 (6.8)

[a] Mean ± SD (range)

Hepatectomy

The indications for partial hepatectomy have been previously described [11]. The type of hepatectomy was decided on the basis of liver functional reserve and location of the tumor. Liver functional reserve was assessed on the basis of the Child–Pugh classification and the indocyanine green retention rate at 15 min (ICG-R15) [12]. Child–Pugh class C was regarded as a contraindication for partial hepatectomy. Types of hepatectomy were defined as follows: limited resection, a resection of less than 1 segment according to Couinaud's segmentation; segmentectomy, a resection of 1 segment according to Couinaud's segmentation; sectionectomy, a resection of 1 of the 4 sections (lateral, medial, anterior, or posterior) of the liver; and hemihepatectomy, a resection of the right or left hemiliver, according to the Brisbane 2000 terminology [13, 14]. The types of hepatectomy were selected as follows. If liver function permitted, anatomical resection (segmentectomy, sectionectomy, or

hemihepatectomy) was performed. In patients with insufficient hepatic functional reserve, limited resection was performed. For example, right hemihepatectomy could be tolerated if the ICG-R15 value was in the normal range. One-third of the liver parenchyma could be resected for patients with an ICG-R15 value between 10 and 19%. Segmentectomy could be tolerated by patients with an ICG-R15 value between 20 and 29%, and limited resection was indicated for patients with an ICG-R15 value of 30% or more. The types of hepatectomy performed are shown in Table 1.

Follow-up

The follow-up evaluation after surgery consisted of clinical physical examinations, blood chemistry tests, and measurements of the levels of tumor markers, including AFP and DCP, every month for 2 years. After 2 years, the patients were assessed every 3 months. Patients were examined by ultrasonography every 3 months and by computed tomography every 6 months. When recurrence was indicated by any of these examinations, the patients underwent hepatic angiography. If all recurrent tumors could be resected within the hepatic functional reserve, repeat hepatectomy was indicated. If they could not be resected within the hepatic functional reserve, the patients underwent percutaneous radiofrequency ablation or transarterial chemoembolization therapy. The median follow-up period for survivors was 45.2 months (range 1–120 months).

Statistical analysis

Overall survival rates and disease-free survival rates were calculated using the Kaplan–Meier method and were compared using the log-rank test. Disease-free survival rates were calculated by considering any deaths or recurrences as an event. The continuous variables were dichotomized based on the receiver operating characteristic curve analysis [15]. Independent prognostic factors were assessed using the multivariate Cox proportional hazards model among the variables found to be significant by univariate analysis. Statistical significance was defined as a p value less than 0.05. All statistical analyses were performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

No patient died within 1 month after partial hepatectomy, although 2 patients died of liver failure during the initial hospital stay (in-hospital mortality rate 0.8%). At the time of the last follow-up examination, 79 of 266 patients had died, and the causes of deaths were cancer progression in 38 patients (14.3%), liver failure in 12 patients (4.5%),

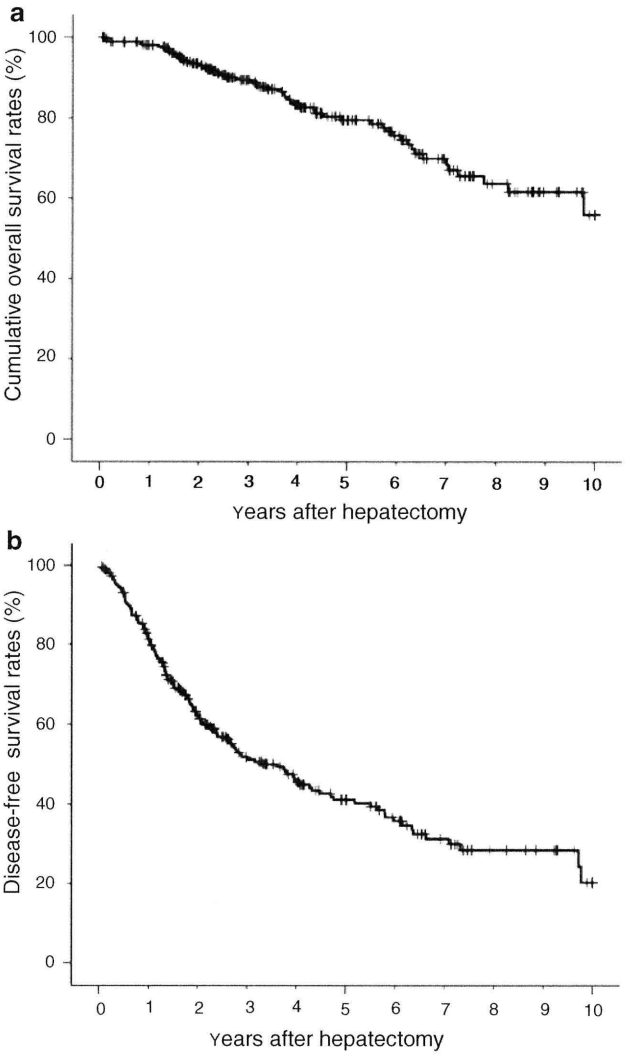

Fig. 1 The overall (**a**) and disease-free (**b**) survival curves of 266 patients with a solitary HCC after partial hepatectomy

bleeding from the gastrointestinal tract in 4 patients (1.5%), and other diseases in 25 patients (9.4%). The cumulative overall survival rates at 3, 5, and 10 years after partial hepatectomy were 89.5, 79.6, and 56.1%, respectively. The corresponding disease-free survival rates were 51.7, 41.1, and 20.4%, respectively (Fig. 1).

Table 2 shows a summary of the results of the univariate analyses for overall survival rates and disease-free survival rates according to the clinicopathological factors. HCV infection ($p = 0.003$), a platelet count of $<100 \times 10^3/mm^3$ ($p = 0.001$), prothrombin activity of <80% ($p = 0.001$), an aspartate aminotransferase (AST) level of ≥50 IU/l ($p = 0.002$), an albumin level of <3.5 g/dl ($p < 0.001$), an ICG-R15 of ≥20% ($p < 0.001$), an AFP level of ≥100 ng/ml ($p = 0.004$), a limited resection ($p = 0.011$), and the presence of cirrhosis in the background liver ($p < 0.001$) were significant adverse prognostic factors for overall survival. In

Table 2 Overall and disease-free survival rates according to clinicopathological factors

	Overall survival (%)			Disease-free survival (%)		
	3-year	5-year	*p* value	3-year	5-year	*p* value
All cases ($n = 266$)	89.5	79.6	–	51.7	41.1	–
Age (years)			0.564			0.336
<65 ($n = 102$)	89.1	82.4		53.4	43.7	
≥65 ($n = 164$)	89.7	77.7		50.5	39.1	
Gender			0.101			0.647
Male ($n = 186$)	89.8	83.3		52.9	41.2	
Female ($n = 80$)	88.8	69.4		49.4	41.6	
HCV infection			0.003			0.034
Negative ($n = 84$)	92.2	88.2		61.2	47.7	
Positive ($n = 182$)	88.0	75.3		46.9	37.8	
Diabetes mellitus			0.552			0.627
No ($n = 188$)	88.3	78.7		53.4	42.9	
Yes ($n = 72$)	92.0	83.8		48.8	40.2	
Preoperative TACE			0.526			0.417
No ($n = 121$)	90.4	80.9		50.7	44.1	
Yes ($n = 145$)	88.5	78.5		52.2	39.2	
Platelet count ($\times 10^3/m^3$)			0.001			<0.001
<100 ($n = 95$)	85.9	65.8		39.4	27.1	
≥100 ($n = 171$)	91.4	86.5		58.3	48.4	
Prothrombin activity (%)			0.001			0.004
<80 ($n = 99$)	81.8	71.2		42.0	31.4	
≥80 ($n = 167$)	94.7	85.9		57.7	48.1	
Total bilirubin level (mg/dl)			0.555			0.196
<1.0 ($n = 182$)	88.5	81.6		53.4	43.1	
≥1.0 ($n = 84$)	91.2	75.6		48.1	36.9	
AST (IU/l)			0.002			<0.001
<50 ($n = 181$)	89.9	84.3		59.5	49.2	
≥50 ($n = 85$)	88.6	68.6		33.3	22.6	
ALT (IU/l)			0.077			0.023
<50 ($n = 174$)	88.5	82.0		57.6	44.9	
≥50 ($n = 92$)	91.2	75.3		41.0	34.1	
Albumin level (g/dl)			<0.001			0.024
<3.5 ($n = 57$)	77.2	64.3		39.2	29.1	
≥3.5 ($n = 209$)	92.8	83.8		55.1	44.4	
ICG-R (%)			<0.001			0.007
<20 ($n = 170$)	93.1	85.9		57.7	45.2	
≥20 ($n = 96$)	82.9	68.1		41.0	33.9	
Child–Pugh			0.108			0.233
A ($n = 236$)	89.9	80.3		53.3	42.9	
B ($n = 30$)	85.7	74.9		38.2	27.3	
AFP (ng/ml)			0.004			0.426
<100 ($n = 185$)	92.6	86.8		54.5	41.1	
≥100 ($n = 81$)	82.9	64.9		45.6	41.5	
DCP (mAU/ml)			0.115			0.054
<100 ($n = 166$)	88.6	77.2		46.0	36.5	
≥100 ($n = 98$)	91.0	83.6		60.2	47.6	

Table 2 continued

	Overall survival (%)			Disease-free survival (%)		
	3-year	5-year	*p* value	3-year	5-year	*p* value
Type of hepatectomy			0.011			0.044
Limited resection ($n = 156$)	87.8	76.7		48.3	35.9	
Segmentectomy or more ($n = 110$)	91.7	83.5		56.4	48.4	
Tumor size (mm)			0.345			0.965
<30 ($n = 149$)	91.0	82.5		53.4	41.4	
≥30 ($n = 117$)	87.7	76.2		49.6	40.8	
Tumor size (mm)			0.411			0.068
<50 ($n = 230$)	89.7	79.7		48.9	39.6	
≥50 ($n = 36$)	88.5	78.7		62.5	52.8	
Tumor differentiation			0.522			0.202
Well or moderately ($n = 215$)	89.5	79.0		51.4	41.0	
Poorly ($n = 29$)	81.5	69.1		41.0	29.3	
Microscopic vascular invasion			0.221			0.714
Negative ($n = 200$)	91.6	81.2		51.1	40.7	
Positive ($n = 66$)	83.3	74.4		52.2	41.6	
Intrahepatic metastasis			0.220			0.921
Negative ($n = 245$)	89.1	80.6		51.9	40.6	
Positive ($n = 17$)	94.1	70.6		48.9	48.9	
Background liver			<0.001			<0.001
No cirrhosis ($n = 150$)	97.0	90.3		57.8	50.5	
Cirrhosis ($n = 111$)	79.7	64.8		43.0	27.6	
Surgical margin (mm)			0.940			0.410
<5 ($n = 164$)	88.2	80.6		50.0	39.3	
≥5 ($n = 102$)	91.7	78.2		54.5	44.0	

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contrast, DCP, tumor size, tumor differentiation, microscopic vascular invasion, and intrahepatic metastasis did not significantly influence overall survival. Furthermore, HCV infection ($p = 0.034$), a platelet count of $<100 \times 10^3/mm^3$ ($p < 0.001$), prothrombin activity of <80% ($p = 0.004$), an AST level of ≥50 IU/l ($p < 0.001$), an alanine aminotransferase (ALT) level of ≥50 IU/l ($p = 0.023$), an albumin level of <3.5 g/dl ($p = 0.024$), an ICG-R15 of ≥20% ($p = 0.007$), limited resection ($p = 0.044$), and the presence of cirrhosis in the background liver ($p < 0.001$) were significant adverse prognostic factors for disease-free survival. In contrast, AFP, DCP, tumor size, tumor differentiation, microscopic vascular invasion, and intrahepatic metastasis did not significantly influence disease-free survival. The cut-off value of 3 cm in tumor size was selected based on the receiver operating characteristic curve analysis. Even if the cut-off value of 5 cm had been selected, tumor size did not affect the overall and disease-free survival of the patients with solitary HCC.

Multivariate analysis for overall survival rates after partial hepatectomy revealed that HCV infection ($p = 0.002$), the presence of cirrhosis in the background liver ($p < 0.001$), and a prothrombin activity of <80% ($p = 0.006$) were independent factors related to poor overall survival rates (Table 3). Figure 2 shows the overall survival curves stratified with a number of adverse prognostic factors for overall survival. The overall survival rates of patients with 3 adverse prognostic factors were significantly lower than those of patients with 2 or fewer of these factors. Furthermore, the overall survival rates of patients with 2 prognostic factors were significantly lower than those of patients with 1 factor.

Multivariate analysis for disease-free survival rates after partial hepatectomy revealed that an AST level of ≥50 IU/l ($p = 0.001$), a platelet count of $<100 \times 10^3/mm^3$ ($p = 0.041$), the presence of cirrhosis in the background liver ($p = 0.040$), and a prothrombin activity of <80% ($p = 0.049$) were independent factors related to poor disease-free survival rates (Table 3). Figure 3 shows the disease-free survival curves stratified with the number of adverse prognostic factors for disease-free survival. The disease-free survival rates of patients with 4 adverse

Table 3 Results of multivariate analysis for survival rates after partial hepatectomy

Variables	Beta value	SE	*p* value	Relative risk	95% confidence interval
Overall survival					
HCV infection	1.242	0.408	0.002	3.462	1.557–7.698
Liver cirrhosis	1.224	0.305	<0.001	3.402	1.871–6.188
Prothrombin activity <80%	0.830	0.300	0.006	2.294	1.275–4.126
Disease-free survival					
AST ≥50 IU/l	0.597	0.177	0.001	1.817	1.285–2.569
Platelet count $<100 \times 10^3/m^3$	0.389	0.190	0.041	1.476	1.016–2.142
Liver cirrhosis	0.381	0.186	0.040	1.464	1.017–2.018
Prothrombin activity <80%	0.342	0.174	0.049	1.408	1.002–1.978

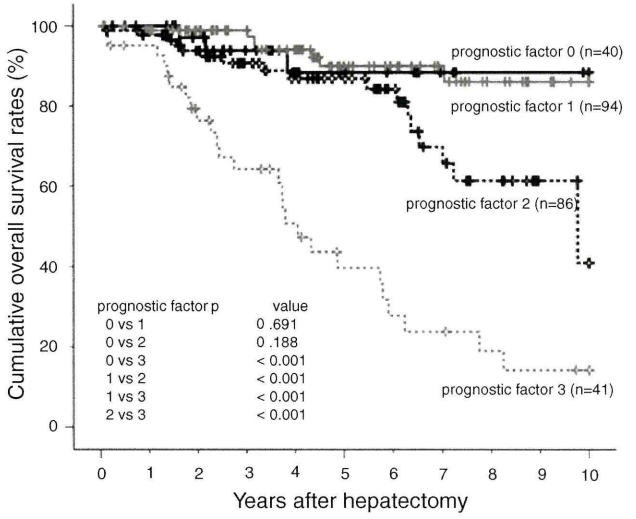

Fig. 2 Comparison of the cumulative overall survival curves stratified with a number of adverse prognostic factors (HCV infection, liver cirrhosis, and prothrombin activity of <80%)

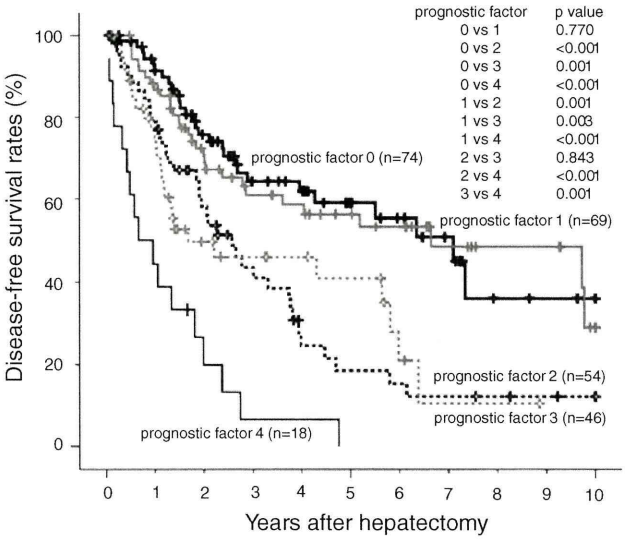

Fig. 3 Comparison of the disease-free survival curves stratified with a number of adverse prognostic factors (AST ≥50 IU/l, platelet count $<100 \times 10^3/m^3$, liver cirrhosis, and prothrombin activity <80%)

prognostic factors were significantly lower than those of patients with 3 or fewer of these factors. Furthermore, the disease-free survival rates of patients with 2 or 3 prognostic factors were significantly lower than those of patients with none or 1 of these factors.

To evaluate whether the types of hepatectomy were influenced by tumor-related factors, we compared patients with limited resection to those with segmentectomy or more, according to clinicopathological factors (Table 4). By univariate analysis, the patients who underwent segmentectomy or more had lower rates of HCV infection, higher platelet counts, higher prothrombin activity, lower ICG-R15 values, higher rates of Child–Pugh class A, higher AFP levels, higher DCP levels, larger tumors, higher rates of microscopic vascular invasion, and lower rates of liver cirrhosis compared to those who underwent limited resection. By multivariate analysis, the independent factors affecting the selection of the hepatic resectional procedure were prothrombin activity, ICG-R15 value, AFP level, tumor size, and presence of liver cirrhosis.

We further determined the type of recurrence and the main therapeutic modality for recurrence according to number of adverse prognostic factors, including HCV infection, liver cirrhosis, and prothrombin activity of <80% (Table 5). At the time of the last follow-up, 134 of 266 patients showed tumor recurrence. In patients having more adverse prognostic factors, tumor recurrence occurred more frequently. Among the patients with tumor recurrence, there was no significant difference in the type of recurrence according to the number of adverse prognostic factors ($p = 0.415$). Meanwhile, patients with multiple adverse prognostic factors infrequently underwent repeat hepatectomy for intrahepatic recurrence ($p = 0.022$).

Table 4 Comparison of the types of hepatectomy according to clinicopathological factors

	Univariate analysis			Multivariate analysis		
	Limited resection	Segmentectomy or more	*p* value	Relative risk	95% confidence interval	*p* value
All cases ($n = 266$)	156	110	–			
Age (years)			0.523			
<65 ($n = 102$)	57	45				
≥65 ($n = 164$)	99	65				
Gender			0.509			
Male ($n = 186$)	107	79				
Female ($n = 80$)	49	31				
HCV infection			0.007			
Negative ($n = 84$)	39	45				
Positive ($n = 182$)	117	65				
Diabetes mellitus			0.325			
No ($n = 188$)	106	82				
Yes ($n = 72$)	46	26				
Preoperative TACE			0.321			
No ($n = 121$)	75	46				
Yes ($n = 145$)	81	64				
Platelet count ($\times 10^3/m^3$)			<0.001			
<100 ($n = 95$)	71	24				
≥100 ($n = 171$)	85	86				
Prothrombin activity (%)			0.014	1.941	1.019–3.697	0.044
<80 ($n = 99$)	68	31				
≥80 ($n = 167$)	88	79				
Total bilirubin level (mg/dl)			0.082			
<1.0 ($n = 182$)	100	82				
≥1.0 ($n = 84$)	56	28				
AST (IU/l)			0.062			
<50 ($n = 181$)	99	82				
≥50 ($n = 85$)	57	28				
ALT (IU/l)			0.795			
<50 ($n = 174$)	101	73				
≥50 ($n = 92$)	55	37				
Albumin level (g/dl)			0.293			
<3.5 ($n = 57$)	37	20				
≥3.5 ($n = 209$)	119	90				
ICG-R (%)			<0.001	3.632	1.837–7.183	<0.001
<20 ($n = 170$)	80	90				
≥20 ($n = 96$)	76	20				
Child–Pugh			0.017			
A ($n = 236$)	132	104				
B ($n = 30$)	24	6				
AFP (ng/ml)			0.015	3.034	1.578–5.832	0.001
<100 ($n = 185$)	118	67				
≥100 ($n = 81$)	38	43				
DCP (mAU/ml)			<0.001			
<100 ($n = 166$)	112	54				
≥100 ($n = 98$)	43	55				

Table 4 continued

	Univariate analysis			Multivariate analysis		
	Limited resection	Segmentectomy or more	*p* value	Relative risk	95% confidence interval	*p* value
Tumor size (mm)			<0.001	3.768	2.087–6.802	<0.001
<30 ($n = 149$)	106	43				
≥30 ($n = 117$)	50	67				
Tumor differentiation			0.070			
Well or moderately ($n = 215$)	130	85				
Poorly ($n = 29$)	12	17				
Microscopic vascular invasion			0.001			
Negative ($n = 200$)	129	71				
Positive ($n = 66$)	27	29				
Intrahepatic metastasis			1.000			
Negative ($n = 245$)	143	102				
Positive ($n = 17$)	10	7				
Background liver			<0.001	2.438	1.293–4.598	0.006
No cirrhosis ($n = 150$)	71	79				
Cirrhosis ($n = 111$)	84	27				
Surgical margin (mm)			0.898			
<5 ($n = 164$)	97	67				
≥5 ($n = 102$)	59	43				

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Table 5 The type of recurrence and the main therapeutic modality for recurrence according to the number of adverse prognostic factors

Variables	Number of adverse prognostic factors				*p* value
	0	1	2	3	
Case	40	94	86	41	
Recurrence	21 (52.5)	34 (36.2)	49 (57.0)	30 (73.2)	<0.001
Type of recurrence					0.415
Intrahepatic solitary	7 (33.3)	20 (58.8)	28 (57.1)	16 (53.3)	
Intrahepatic multiple	9 (42.9)	8 (23.5)	16 (32.6)	8 (26.7)	
Extrahepatic recurrence	5 (23.8)	6 (17.7)	5 (10.2)	6 (20.0)	
Main therapeutic modality					0.022
Repeat hepatectomy	6 (28.6)	18 (52.9)	14 (28.6)	5 (16.7)	
Liver transplantation	0	0	0	2 (6.7)	
Radiofrequency ablation	4 (19.1)	4 (11.8)	17 (34.7)	4 (13.3)	
TACE	8 (38.1)	6 (17.7)	12 (24.5)	11 (36.7)	
Systemic chemotherapy	2 (9.5)	1 (2.9)	1 (2.0)	1 (3.3)	
Others	1 (4.8)	5 (14.7)	5 (10.2)	7 (23.3)	

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[a] Adverse prognostic factors: HCV infection, liver cirrhosis, prothrombin activity of <80%

Discussion

Although several previous studies have reported the predictive factors affecting surgical outcomes in patients with resectable HCC, few studies have reported the outcomes in patients with a solitary HCC [7–9]. The present study showed that in patients with a solitary HCC the prognosis after curative hepatectomy depends on the background liver status and is independent of tumor factors. The adverse prognostic factors affecting these patients' overall

survival rates were HCV infection, liver cirrhosis, and prolonged prothrombin activity. Furthermore, the adverse prognostic factors affecting their disease-free survival rates were a high AST level, decreased platelet count, liver cirrhosis, and prolonged prothrombin activity. Patients with more of these adverse prognostic factors had worse overall and disease-free survival rates. In addition, the prognosis of patients with well-preserved liver function after partial hepatectomy of a solitary HCC was extremely good. These findings confirm the importance of hepatic functional reserve and sustained hepatitis activity in the outcomes of patients after partial hepatectomy.

In general, the prognosis of patients with HCC is influenced by factors related to the background liver and the tumor [3]. It is well known that recurrence after the initial treatment of HCC is caused by multicentric occurrence and intrahepatic metastasis [16]. It was reported that multicentric occurrence was more frequently observed in patients with a poor hepatic functional reserve [17]. Bilimoria et al. [18] reported that the presence of moderate to severe fibrosis/cirrhosis was the most important predictor of death, overshadowing all other tumor factors. Wayne et al. [19] reported that the fibrosis score, Edmondson–Steiner grade, and Child–Pugh score were significant predictors of survival after resection in a cohort of patients undergoing resection for small HCCs. In addition, the incidence of multicentric recurrences was greater in HCV-positive patients than in hepatitis B virus-positive patients [20, 21]. Thus, the prognosis of patients with HCC after partial hepatectomy is influenced by poor hepatic functional reserve probably due to fibrosis or cirrhosis, especially in the case of patients with HCV infection. Additionally, the preoperative AST level was identified as an adverse prognostic factor for the disease-free survival rate in this study. There are several reports suggesting that active inflammation in the nontumoral liver is an independent risk factor for intrahepatic recurrence [22]. It has been reported that the elevation of preoperative liver enzymes may be a factor that affects intrahepatic recurrence, which is more likely to originate from metachronous carcinogenesis [23]. Tarao et al. [24] reported a close association between inflammatory necrosis and rapid recurrence in hepatectomized patients with HCC who had HCV-associated cirrhosis. Thus, inflammation of the nontumoral liver may also influence multicentric carcinogenesis. In this study, all of the factors that were identified as adverse prognostic factors for overall and disease-free survival rates reflected the background liver status.

Several tumor-related factors that negatively influence the outcomes of patients with HCC who have undergone partial hepatectomy have been reported. They include tumor size, tumor number, vascular invasion, and pTNM stage [5, 25]. Although tumor size is recognized as one of the significant adverse prognostic factors, it remains controversial whether larger tumors solely influence the outcome after curative hepatectomy in patients with solitary HCCs. Several reports indicated that a larger tumor resulted in poorer survival rates [26–28]. Yeh et al. [6] reported that significant indicators of adverse prognosis in patients with HCC and cirrhosis included an elevated alkaline phosphatase value, a tumor size of >2 cm, the presence of satellite lesions, and vascular invasion. Pawlik et al. [29] reported that tumor size predicted microvascular invasion and advanced histological grade. Tsai et al. [30] reported that macroscopic and microscopic venous invasion, surgical margin, ICG-R15, and tumor size and number were significant predictors of postresectional survival. They concluded that the larger the size of the tumor, the higher the incidence of venous invasion. However, some authors have reported that tumor size did not influence the postoperative outcome [31–33]. Liau et al. [31] reported that overall and disease-free survival of patients with HCCs that were >10 cm in diameter were similar to those of patients with HCCs of ≤5 cm in diameter. Vauthey et al. [32] proposed a simplified staging for HCCs in which they classified a single HCC without vascular invasion as sT1 because tumor size had no effect on survival in patients with a single HCC and no vascular invasion. More recently, Yang et al. [33] reported a specific subtype of HCC, which is large in size but exhibits a low invasive and metastatic potential and a good outcome after resection. They categorized these tumors as solitary, large HCCs, which grow expansively within an intact capsule or pseudocapsule. The reason for this conflict might be the fact that most reports included larger tumors with macroscopic vascular invasion. In the present study, we demonstrated that tumor-related factors do not influence the prognosis of solitary HCC after curative partial hepatectomy in patients who did not show macroscopic vascular invasion. Thus, tumor-related factors other than macroscopic vascular invasion were not adverse prognostic factors in patients with solitary HCC, although our results might be influenced by the biased study population, i.e. the high proportion of patients infected with HCV in our series.

In the present study, limited resection was one of the significant adverse prognostic factors for overall and disease-free survivals by using univariate analysis. It might suggest that the selection of types of hepatectomy could be influenced by tumor-related factors. To evaluate whether the types of hepatectomy was influenced by tumor-related factors, we compared patients with limited resection to those with segmentectomy or more, according to clinicopathological factors. The independent factors affecting the selection of hepatic resectional procedure were prothrombin activity, ICG-R15 value, AFP level, tumor size, and

presence of liver cirrhosis. In our institution, the types of hepatectomy were determined on the basis of liver functional reserve and location of the tumor. Although tumor-related factors were in theory not considered to determine the hepatic resectional procedure, we could not exclude the possibility that the tumor size and AFP level influenced the types of hepatectomy selected. The patients who underwent segmentectomy or more had more unfavorable tumor-related factors such as a larger tumor or a higher AFP level compared with those who underwent limited resection. Meanwhile, compared with those who underwent limited resection, patients who underwent segmentectomy or more had more favorable liver-related factors, such as higher prothrombin activity, a lower ICG-R15 value, and the absence of liver cirrhosis. Despite unfavorable tumor-related factors, the patients who underwent segmentectomy or more showed higher overall and disease-free survival than those who underwent limited resection. These findings may be attributable to the fact that the patients who underwent segmentectomy or more had more favorable liver-related factors than those who underwent limited resection. Therefore, we concluded that although tumor-related factors might affect the selection of the types of hepatectomy, they do not influence the prognosis of solitary HCC after curative partial hepatectomy. Additionally, as determined by multivariate analysis, the types of hepatectomy were not found to be independent prognostic factors for either overall or disease-free survival.

We also demonstrated that the background liver status is the major factor limiting the long-term outcome after partial hepatectomy of solitary HCC. If recurrence occurred, the patients with adverse liver-related factors tended to undergo nonsurgical therapy for recurrent tumor, such as radiofrequency ablation or transcatheter arterial chemoembolization. This observation may be due to the therapeutic difficulty of the recurrent tumor. If the patients with a solitary HCC have several adverse prognostic factors such as HCV infection, liver cirrhosis, and prolonged prothrombin activity, they may benefit from liver transplantation rather than partial hepatectomy as the treatment for HCC. However, donor organ availability is unfortunately limited in Japan.

In conclusion, the long-term prognosis of patients with a solitary HCC who undergo curative partial hepatectomy mainly depends on the background liver status and not on tumor-related factors; this suggests that hepatectomy provides a significant therapeutic benefit. The postoperative prognosis of patients with a solitary HCC and well-preserved liver function is extremely good, irrespective of the tumor size. Thus, if the hepatic functional reserve is within the permissible range, partial hepatectomy should be considered as the treatment of choice for patients with a solitary HCC.

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