Preoperative Neutrophil-to-Lymphocyte Ratio Is a Predictor of Survival After Hepatectomy for Hepatocellular Carcinoma

A Retrospective Analysis

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Objective: To clarify the prognostic value of the preoperative blood neutrophil-to-lymphocyte ratio (NLR) in patients undergoing hepatectomy for hepatocellular carcinoma (HCC).

Background: Although a high NLR has been reported to be a predictor of poor survival in patients with various cancers, it has not been extensively examined in patients with HCC.

Methods: This retrospective study enrolled 958 patients who underwent hepatectomy without preoperative therapy for HCC from 1996 to 2009. Clinicopathological parameters, including NLR, were evaluated to identify predictors of overall and recurrence-free survival after hepatectomy. Univariate and multivariate analyses were performed, using the Cox proportional hazards model. The best cutoff was determined with time-dependent receiver operating characteristic curve. To determine the mechanism of NLR elevation, immunohistological examination using CD163 staining was performed in 150 patients.

Results: Univariate and multivariate analyses showed that NLR was an independent prognostic factor in overall and recurrence-free survival. The best cutoff of NLR was 2.81, and 238 of 958 patients (24.8%) had NLR of more than 2.81. The 5-year survival rate after hepatectomy was 72.9% in patients with NLR less than 2.81 and 51.5% in those with NLR 2.81 or more ($P < 0.0001$). CD163-positive cell counts were significantly higher in tumors in the group with NLR 2.81 or more than in the group with NLR less than 2.81 ($P = 0.0004$).

Conclusions: Our results show that NLR is an independent predictor of survival after hepatectomy in patients with HCC. Accumulation of tumor-associated macrophages in the tumor is associated with a high NLR.

Keywords: blood neutrophil-to-lymphocyte ratio, hepatocellular carcinoma, liver resection, prognosis, tumor-associated macrophage

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Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide.[1] Hepatic resection is considered to be the treatment of choice for solitary lesions in patients with noncirrhotic livers or with Child-Pugh–grade cirrhosis, indicating well-preserved liver function.[2] However, the 5-year overall survival rate after hepatic resection is only 50% to 70%.[3–5]

The most significant factor affecting survival is the high postoperative recurrence rate. The reasons for this high recurrence rate remain unclear and seem to be complex and multifactorial.[6,7] One of the important causes of recurrence is metachronous carcinogenesis, caused by hepatic inflammation.[8] Another cause is the malignant potential of cancer cells. Pathological examination shows that microscopic portal vein invasion and intrahepatic metastasis are prognostic factors for survival.[9] Tumor-associated macrophages (TAMs) have been shown to have tumor-promoting effects, with a high density of TAMs in the tumor reported to be associated with a poor prognosis.[10,11] High serum *des-γ*-carboxy prothrombin level and expression of focal adhesion kinase have also been reported to reflect a high malignant potential in HCC.[12,13]

There is increasing evidence that increased systemic inflammation correlates with poorer cancer-specific survival in various cancers.[14–18] Recent studies have shown that the host's inflammatory response to cancer and/or the systemic effects exerted by the cancer cells lead to upregulation of the inflammatory process, predisposing the cancer to proliferation and metastasis through the inhibition of apoptosis, promotion of angiogenesis, and repair of DNA damage.[19,20] The presence of a systemic inflammatory response can be detected by the elevation of the C-reactive protein (CRP) level[21] and neutrophil-to-lymphocyte ratio (NLR)[22]. A high serum CRP level has been shown to be associated with portal vein invasion of cancer cells, and some reports have indicated that a high preoperative serum CRP level is associated with early recurrence of HCC and poorer survival after hepatic resection.[23] A high NLR has been reported to be a predictor of poor survival after hepatic resection, radio-frequency ablation, transarterial chemoembolization, and liver transplantation for HCC.[24–27] To our knowledge, only one relatively small study of fewer than 100 patients by Gomez et al[24] has reported that the preoperative NLR was a prognostic indicator of survival after hepatic resection for HCC.

This study aimed to evaluate the relationship between systemic inflammation and focal infiltration of inflammatory cells, represented by the preoperative NLR and TAMs, and outcome after hepatic resection in 958 patients in 3 high-volume centers in Japan.

METHODS

Patients

From January 1996 to December 2009, a total of 422 patients at the Second Department of Surgery, Kyushu University, 253 patients at the Department of Surgery, Hiroshima Red Cross Hospital, and 316 patients at the Department of Surgery, Iizuka Hospital, underwent hepatic resection for HCC. Thirty-three patients who underwent

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preoperative therapy, such as transarterial chemoembolization, radiofrequency ablation, or percutaneous ethanol injection, were excluded, and the remaining 958 patients (689 males, 269 females) were enrolled in this study. The mean age of patients was 67 years.

Curative resection was defined as complete macroscopic removal of the tumor and was performed in 874 patients (91.2%). Of these, 591 patients (61.7%) were seropositive for hepatitis C antibody (HCV-Ab), 161 (16.8%) were seropositive for hepatitis B surface antigen (HBs-Ag), 204 (21.3%) were seronegative for both HCV-Ab and HBs-Ag, and 5 (0.5%) were seropositive for both HBs-Ag and HCV-Ab. Of the 422 patients who underwent hepatic resection in Kyushu University, 150 consecutive patients who underwent resection from January 1997 to March 2005 were selected for immunohistological examination using CD163 staining.

Prognostic Factors in Overall and Recurrence-Free Survival After Hepatectomy

Neutrophil-to-lymphocyte ratios of all the patients in this study were calculated on the basis of preoperative blood value. Univariate analysis in overall survival and recurrence-free survival was performed, using the Cox proportional hazards model. The overall survival was evaluated in all the 958 patients, and the recurrence-free survival was evaluated only in 874 patients who underwent curative resection. The following variables were examined with respect to overall survival and recurrence-free survival rate: age, sex, serum albumin level, indocyanine green retention rate at 15 minutes (ICGR15), tumor size, serum α-fetoprotein (AFP) level, portal vein thrombus, number of tumors, TNM stage according to the Liver Cancer Study Group in Japan[28] (I or II vs III or IV), and curative resection (resection without remnant tumors). In the analysis of recurrence-free survival, variable: curative resection was excluded, because postoperative recurrence was defined only in the patients without remnant HCC who underwent curative resection. The contiguous variables were entered into the model.

The best cutoff of NLR was determined by receiver operating characteristic curve. The recurrence pattern of HCC was compared between patients with the best cutoff value of NLR. The recurrence pattern was defined as nodular ($\leq$3 nodules), multiple (>3 nodules), and extrahepatic metastasis (metastasis to organs other than the liver), as previously described.[29]

Follow-up Strategy and Recurrence Pattern

After discharge, all patients underwent monthly screening for recurrence, using ultrasonography and tumor markers such as AFP, and 6-monthly computerized tomography scanning. If recurrence was suspected, additional investigations such as hepatic angiography were performed.

Immunohistochemical Examination

Sections of resected specimens were fixed in 10% buffered formalin, embedded in paraffin, and stained using the Envision+ system and DAB kit (DAKO, Grostrup, Denmark). Immunohistochemical staining was performed using CD163 antibodies (10D6, 1:200; Novocastra). Sections were pretreated before being incubated with primary antibodies in a microwave oven for 20 minutes. Serial sections were stained and examined by 2 pathologists (Y.M. and S.A.). The total number of cells with cytoplasmic or membrane staining in 3 high-power fields was counted.

Statistical Analysis

All data are expressed as the mean $\pm$ standard deviation. Independent χ^2 tests were used to compare categorical variables. Continuous variables were compared using unpaired t tests. Survival curves were analyzed using the Kaplan-Meier method and compared using the log-rank test. The Cox proportional hazards model was used for univariate and multivariate analyses. The best cutoff of NLR was determined by time-dependent receiver operating characteristic curve.[30] Adjustment for covariates and the Cox proportional hazards model was conducted using JMP software (SAS Institute, Cary, NC) on a Windows computer. P values of less than 0.05 were considered statistically significant.

RESULTS

NLR as an Independent Prognostic Factor

The statistically significant prognostic factors identified by univariate analyses are shown in Table 1. Indicators of poor liver function, such as low serum albumin level and high ICGR15, were identified as significant predictors of poor prognosis. Among tumor-related factors, large tumor size, high AFP level, presence of portal vein thrombus, multiple tumors, advanced clinical stage, and noncurative resection were identified as predictors of poor prognosis. Furthermore, NLR was also identified as a predictor of prognosis. Multivariate analyses identified low serum albumin level, large tumor size, high NLR level, presence of portal vein thrombus, multiple tumors, and advanced clinical stage as independent predictors of poor prognosis (Table 2).

The statistically significant factors in recurrence-free survival identified by univariate analyses are shown in Table 3. Indicators of poor liver function, such as low serum albumin level and high ICGR15, were identified as significant predictors of poor prognosis. Among tumor-related factors, large tumor size, high AFP level,

TABLE 1. Univariate Analyses of Factors in Relation to Overall Survival, Using the Cox Proportional Hazards Model

Prognostic Variables	Hazard Ratio	P	95% CI
Age	1.226	0.4145	0.993–1.018
Sex	1.964	0.6105	0.821–1.400
Albumin	7.813	<0.0001	0.271–0.457
ICGR15, %	3.274	0.0011	1.007–1.030
Tumor size	8.527	<0.0001	1.117–1.193
AFP	5.608	<0.0001	1.000–1.000
Portal vein thrombus	7.666	<0.0001	0.194–0.378
Multiple	5.520	<0.0001	0.375–0.627
Stage (I + II)	8.150	<0.0001	0.292–0.471
NLR	3.716	0.0002	1.022–1.074
Curative resection	2.392	0.0168	0.445–0.923

TABLE 2. Multivariate Analyses of Factors in Relation to Overall Survival, Using the Cox Proportional Hazards Model

Prognostic Variables	Hazard Ratio	P	95% CI
Albumin	6.779	<0.0001	0.279–0.495
NLR	3.745	0.0002	1.027–1.088
Tumor size	3.736	0.0002	1.036–1.122
Portal vein thrombus	3.445	0.0006	0.315–0.728
Stage (I + II)	2.603	0.0092	0.467–0.898
Multiple	2.211	0.0270	0.512–0.960
ICGR15	1.532	0.1254	0.997–1.022
Curative resection	1.044	0.2967	0.534–1.211
AFP	1.000	0.5100	1.000–1.000
Age	1.003	0.6721	0.990–1.016
Sex	1.058	0.6947	0.797–1.405

presence of portal vein thrombus, multiple tumors, and advanced clinical stage were identified as predictors of poor prognosis for recurrence-free survivals. Furthermore, NLR was also identified as a predictor of tumor recurrence. Multivariate analyses identified high AFP levels, low serum albumin level, high IGGR15, high NLR level, and presence of portal vein thrombus as independent predictors of tumor recurrence (Table 4).

Selection of the Best Cutoff Point for NLR

The best cutoff of NLR was determined for postoperative prognosis, using time-dependent receiver operating characteristic curve. An NLR of 2.81 was the best cutoff point for operative prognosis. All the patients were divided into 2 groups: a low (<2.81) NLR group (n = 720) and a high (≥2.81) NLR group (n = 238).

Prognostic Comparisons of the Low and High NLR Groups

The overall survival rates of patients in the low and high NLR groups are shown in Figure 1. The overall 1-, 3-, and 5-year survival rates were 95.5%, 83.9%, and 72.9% in the low (<2.81) NLR group and 87.1%, 68.9%, and 51.5% in the high (≥2.81) NLR group, which was a significant difference ($P < 0.0001$). The mean survival time was 8.0 ± 0.23 years in the low NLR group and 6.1 ± 0.38 years in the high NLR group.

The recurrence-free survival rates of patients in the low and high NLR groups are shown in Figure 2. The recurrence-free survival rate was significantly higher in the low NLR group than in the high NLR group ($P = 0.0272$).

Comparison of tumor recurrence patterns between the groups is shown in Table 5. Considering those patients with recurrence,

TABLE 3. Univariate Analyses of Factors in Relation to Recurrence Free Survival, Using the Cox Proportional Hazards Model

Prognostic Variables	Hazard Ratio	*P*	95% CI
Age	1.002	0.6467	0.993–1.011
Sex	1.121	0.2622	0.919–1.366
Albumin	3.928	<0.0001	0.546–0.817
ICGR15, %	3.603	0.0003	1.007–1.024
Tumor size	1.452	0.1465	0.991–1.063
AFP	6.271	<0.0001	1.000–1.000
Portal vein thrombus	2.659	0.0078	0.452–0.887
Multiple	2.657	0.0079	0.580–0.921
Stage (I + II)	3.438	0.0006	0.561–0.854
NLR	2.359	0.0183	1.005–1.059

TABLE 4. Multivariate Analyses of Factors in Relation to Recurrence free Survival, Using the Cox Proportional Hazards Model

Prognostic Variables	Hazard Ratio	*P*	95% CI
AFP	5.376	<0.0001	1.000–1.000
Albumin	3.517	0.0004	0.551–0.844
ICGR15	2.509	0.0121	1.003–1.021
NLR	2.096	0.0361	1.002–1.060
Portal vein thrombus	2.337	0.0194	0.487–1.032
Multiple	2.211	0.0728	0.512–0.960
Stage (I + II)	2.603	0.2673	0.659–1.123
Sex	1.096	0.368	0.892–1.345
Tumor size	1.008	0.8641	0.965–1.044
Age	1.003	0.9082	0.991–1.010

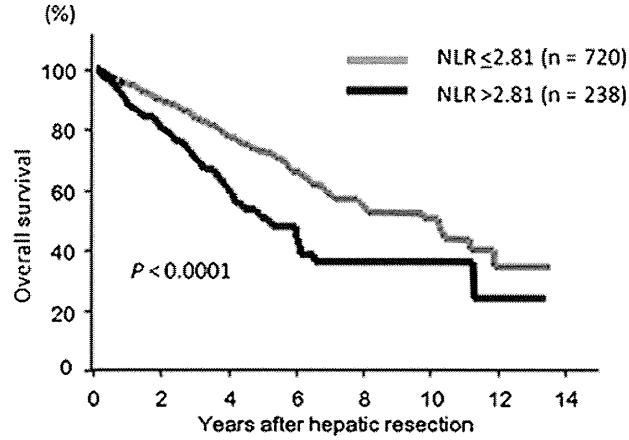

FIGURE 1. Comparison of overall survival rates in the low (<2.81) and high (≥2.81) blood NLR groups. The overall 1-, 3-, and 5-year survival rates were 95.5%, 83.9%, and 72.9% in the low (< 2.81) NLR group and 87.1%, 68.9%, and 51.5% in the high (≥2.81) NLR group, which was a significant difference ($P < 0.0001$).

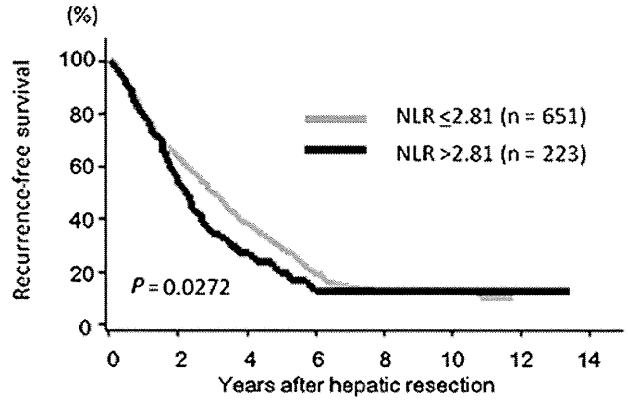

FIGURE 2. Comparison of recurrence-free survival rates in the low (<2.81) and high (≥2.81) NLR groups. The recurrence-free survival rate was significantly higher in the low NLR group than in the high NLR group ($P = 0.0272$).

TABLE 5. Comparison of Recurrence Patterns Between the Low and High NLR Groups

NLR	Nodular	Multiple	Extrahepatic	*P*
<2.81 (n = 351)	243 (69.2%)	86 (24.5%)	22 (6.3%)	0.0002
≥2.81 (n = 115)	55 (47.8%)	48 (41.7%)	12 (10.4%)	—

Nodular indicates fewer than 3 recurrent intrahepatic tumors; multiple, 3 or more recurrent intrahepatic tumors.

multiple tumors in the liver were significantly more frequent in the high NLR group than in the low NLR group ($P = 0.0002$).

Immunohistochemical Examination

We performed immunohistochemical staining for CD163 in 150 consecutive cases at Kyushu University Hospital. Figure 3A

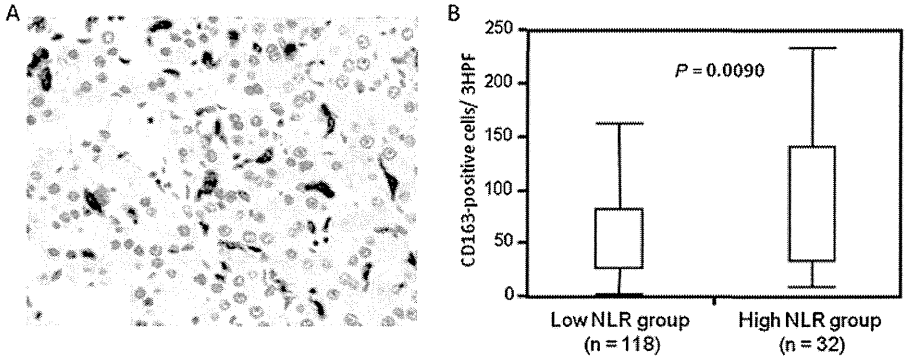

FIGURE 3. A, Immunohistochemical CD163 staining of a hepatocellular carcinoma specimen (×200). B, CD163-positive cell counts in the low and high NLR groups ($P = 0.0090$). HPF indicates high-power field.

shows CD163 staining of TAMs. We compared tumor infiltration by CD163-positive cells between the high and low NLR groups. CD163-positive cell counts were significantly higher in tumors in the high NLR group than in the low NLR group (91.0 ± 82.5 vs 61.2 ± 47.4, $P = 0.0090$; Fig. 3B).

DISCUSSION

Indicators of poor liver function, such as low serum albumin level and high ICGR15, and tumor invasion factors, such as large tumor size, presence of portal vein thrombosis, multiple HCC, and high serum AFP level, have previously been reported to be predictors of poor prognosis in patients with HCC.[31,32] The results of this study clearly show that the high preoperative NLR was an independent predictor of poor survival after hepatectomy in patients with HCC.

Although a high NLR is thought be associated with systemic inflammation, the cause of this inflammation remains unclear. Hashimoto et al[22] reported that a high CRP level was an independent prognostic factor in patients who underwent hepatectomy for HCC. Fever and high CRP level are suspected to be caused by humoral factors, especially inflammatory cytokines such as interleukin (IL)-6, IL-8, and tumor necrosis factor-α. However, fever is extremely rare in patients with HCC, and this mechanism cannot be applied to all patients with a high NLR.

Some reports have indicated that macrophage infiltration into HCC is related to the aggressiveness of the tumor.[10,11] Macrophages can assume a range of different phenotypes based on environmental stimuli. The extremes of this range in vitro are the M1 phenotype, associated with active microbial killing, and the M2 phenotype, associated with tissue remodeling and angiogenesis.[10,11,32] When monocytes in the tumor are exposed to tumor-derived anti-inflammatory molecules such as IL-4, IL-10, transforming growth factor-β1, and prostaglandin E2, they polarize into M2 macrophages.[11] The M2 phenotype macrophage seems to be the dominant type in tumors, with TAMs characterized by high expression of M2 macrophage antigens such as CD163 and high constitutive expression of IL-6 and IL-10.[33,34] Our immunohistochemical analysis showed that high infiltration of TAMs was associated with a high NLR. TAMs express some cytokines, such as IL-6 and IL-8, within the lesion, and these cytokines may promote systemic neutrophilia.[35–37] Ubukata et al[38] demonstrated that a high NLR is significantly correlated with high numbers of Th2 cells in patients with gastric cancer. Th2 cells express IL-4 and IL-10, which polarize macrophages to TAMs. A high NLR is associated with a high infiltration of TAMs and high inflammatory cytokine production in the tumor. On the contrary, our histological examination revealed that local accumulation of neutrophils into HCC might not play an important role in NLR elevation (date not shown). This phenomenon may be explained by complex expression of several cytokines. Kuang et al[39] demonstrated that intratumoral neutrophils did not have a critical role in tumor progression but peritumoral neutrophils did, and proinfilammatory IL-17 secreted by lymphocytes recruits neutrophils to peritumoral stroma. IL-17 is one of the proinflammatory cytokines. Peritumoral IL-17 may enhance systematic neutrophils in our study. Close relationship between TAMs and IL-17–producing cells was reported previously.[34,40] Thus, similar mechanism may be one of the cause of NLR elevation in HCC patients. From this point of view, a high infiltration of TAM is a first and important step of NLR elevation. Further examination is necessary to determine this clear mechanism.

There are a many reports regarding the promotion of distant metastasis of cancer cells by TAMs. Rolny et al[41] demonstrated that inhibition of TAM infiltration into tumors, by neutralizing antibodies to monocyte chemoattractants, reduces metastasis. Recent studies have provided evidence that TAMs and cytokines, such as IL-1, tumor necrosis factor, IL-6, and IL-8, increase metastasis. IL-6 levels are much higher in HCC patients than in healthy adults.[42] Harimoto et al[43] reported an HCC patient with a high IL-8 level, high CRP level, and pyrexia who had an extremely poor outcome after hepatectomy. Liu et al[44] demonstrated that IL-6 induced antiapoptotic activity via the STAT3 signaling pathway in human HCC cell lines. These phenomena may be related to TAMs, which can produce IL-6 and IL-8. Anti-inflammatory treatment may be beneficial in the treatment of HCC, and further study is necessary to investigate this.

CONCLUSIONS

Neutrophil-to-lymphocyte ratio is an easily measurable inflammatory biomarker. Our results show that NLR is an independent predictor of survival after hepatectomy in patients with HCC and that accumulation of TAMs in the tumor may be one of the causes of NLR elevation.

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LETTER FROM THE FRONTLINE

Fairly Rare Spontaneous Disappearance of a Hepatic Artery Aneurysm Following Living Donor Liver Transplantation

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TO THE EDITORS

The patient was a 54-year-old female with end-stage liver disease secondary to primary biliary cirrhosis without a hepatic artery aneurysm. She underwent ABO-incompatible living donor liver transplantation (LT) with a left lobe graft donated by her 58-year-old husband. Preoperatively, the patient underwent plasma exchange several times and rituximab administration for the removal of anti–blood type antibodies. The hepatic artery of the graft (A2/3) was anastomosed end to end to the recipient's left hepatic artery, and A4 was anastomosed to the middle hepatic artery. The cold and warm ischemia times were 67 and 39 minutes, respectively. Postoperative immunosuppression was induced with cyclosporine with mycophenolate mofetil and steroids. Routine follow-up dynamic computed tomography 1 week after LT revealed no hepatic artery aneurysm (Fig. 1A). However, a tiny globular pseudoaneurysm at the distal side of the anastomosis with the thrombus at the main trunk of the portal vein was revealed 2 weeks after LT (Fig. 1B). Coumadin administration at 2 mg/day was initiated, and good control was achieved with an international normalized ratio of 1.5 to 2.0; this prevented the development of the portal thrombus. The pseudoaneurysm developed with a spindle-shaped form 1 month after LT (Fig. 1C), and 2 months after LT, it had a diameter of 7 mm (Fig. 1D). Open surgery for resecting and reconstructing the pseudoaneurysm was planned. However, a computed tomography examination revealed the spontaneous disappearance of the hepatic artery pseudoaneurysm 10 days after a pause in the anticoagulant administration (Fig. 1E). There was no new development of the pseudoaneurysm 1 month after its disappearance.

DISCUSSION

A hepatic artery pseudoaneurysm is an unusual and potentially serious complication that can occur after LT, and it is characterized by a high mortality rate.[1] Early diagnosis and treatment (eg, surgical reconstruction and catheter-based endovascular treatment of stent or coil embolization) are essential for preventing life-threatening hemorrhaging.[2] However, these therapies involve considerable associated risks.[3] The mechanism of hepatic artery pseudoaneurysm development after LT is usually a technical problem involving a bacterial infection and inflammation around the hepatic artery, which cause weakening of the vessel wall.[1-3] In the case reported here, there was excessive local anticoagulant around the hepatic artery anastomosis site, which may have been unable to adapt to any qualitative or quantitative changes because of decreased elasticity and strength. The minute intimal hemorrhage consequently may have induced the development of the hepatic artery pseudoaneurysm.[3,4] In this case, the sequence of anticoagulant treatment, treatment of the portal thrombus, and no surgical resection of the pseudoaneurysm allowed the development of the hepatic pseudoaneurysm and its later disappearance to be observed for the first time. The pseudoaneurysm developed first as a tiny, spindle-shaped form before it became a larger globular body and vanished without a trace. If the anticoagulation had been discontinued earlier as the pseudoaneurysm was developing from the spindle-shaped form, the risk of rupture would have been very low.

Fistouris et al.[4] extensively reviewed their cases and showed that an infectious etiology (particularly bile leakage) may be closely related to the occurrence of pseudoaneurysms.[4] They also showed the major

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The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration and was approved by our institutional review board.

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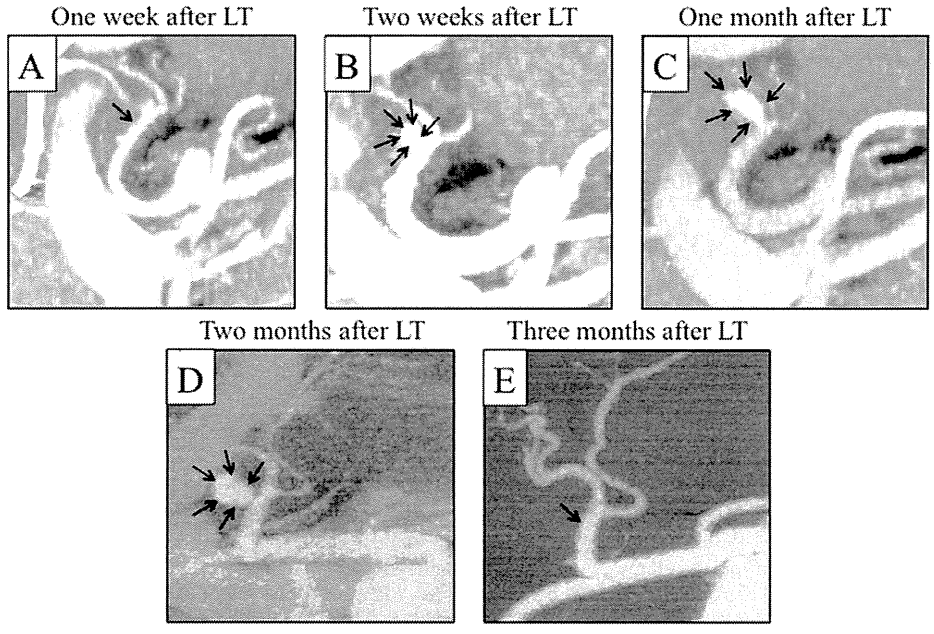

Figure. 1. Development of a hepatic artery pseudoaneurysm as observed with dynamic computed tomography after LT. (A) One week after LT, there was no hepatic artery aneurysm. (B) Two weeks after LT, a tiny globular pseudoaneurysm was evident at the distal side of the anastomosis. (C) One month after LT, the pseudoaneurysm was developing as a spindle-shaped form. (D) Two months after LT, the pseudoaneurysm had grown to 7 mm in diameter. (E) Three months after LT, the hepatic artery pseudoaneurysm spontaneously disappeared (10 days after a pause in the anticoagulant administration).

responsible bacterium to be *Candida albicans* and identified hepaticojejunostomy as one of the risk factors. Molecular biological analysis has shown that tumor necrosis factor α production from endothelial cells, which are often highly expressed in infectious insults, may prevent the fibrotic organization of the internal elastic lamina and aggravate hepatic artery pseudoaneurysms.[5] In light of such evidence, only the manipulation of the anticoagulant series could have clinically caused the pseudoaneurysm in this case because there were no intraoperative and postoperative infectious insults.

Here we report a rare case of a hepatic artery pseudoaneurysm that disappeared after living donor LT. This case suggests that a wait-and-see strategy may be appropriate with careful case-by-case consideration when an anticoagulant treatment is being used.

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Obstructing Spontaneous Major Shunt Vessels is Mandatory to Keep Adequate Portal Inflow in Living-Donor Liver Transplantation

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Background. It has not been addressed whether the major spontaneous portosystemic shunt vessels should be ligated in living-donor liver transplantation (LDLT).
Methods. We performed a retrospective analysis of 324 cases of adult-to-adult LDLT.
Results. Factors associated with the presence of major (>10 mm) shunt vessels (n=130) included portal vein (PV) thrombosis (27.7%), lower PV pressure at laparotomy, Child-Pugh class C, and transplantation of right-side grafts. The types of major portosystemic shunt vessels included splenorenal shunts (46.2%), gastroesophageal shunts (26.9%), mesocaval shunts (13.8%), and others (13.1%). Ligation of the major shunt vessels increased PV pressure (mean [SD], from 16.8 [3.9] mm Hg to 18.6 [4.3] mm Hg; $P<0.001$) and PV flow (mean [SD], from 1.35 [0.67] L/min to 1.67 [0.67] L/min; $P<0.001$) into the grafts. Post-LDLT computed tomography showed patent major shunts in 14 patients. Nine of such patients (64.3%) with unligated major shunt vessels (undetected shunt vessels, n=5; incomplete ligation, n=2; and the shunt was newly created or left open to maintain high PV pressure after reperfusion, n=3) required secondary interventions. Two of these patients died because of graft dysfunction. PV flow was significantly lower in the nine patients who underwent secondary ligation of the major shunt vessels compared with patients with successful primary ligation (mean [SD], 0.96 [0.34] L/min vs. 1.65 [0.63] L/min; $P=0.001$).
Conclusions. It is an appropriate option to obstruct the major portosystemic shunt vessels to ensure adequate graft inflow in LDLT.

Keywords: Shunt vessels, Portal vein, Living-donor liver transplantation, Splenectomy.

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Portal hypertension is a common outcome of end-stage liver disease, and it causes gastrointestinal bleeding, hypersplenism, intractable ascites, and hepatic encephalopathy (*1*). For treating such portal hypertension, transjugular intrahepatic portosystemic shunt has been commonly applied mainly in Western countries, resulting in uncommon development of tremendous or major shunt vessels (*2, 3*). In Eastern countries including Japan, however, transjugular shunting approach is less commonly used and replaced by other types of endoscopic or interventional treatment (*4, 5*), and therefore, patients who are referred for liver transplantation frequently have major portosystemic shunt vessels.

Liver transplantation is the ultimate treatment of portal hypertension, and it is known that splenomegaly and major spontaneous portosystemic shunts recover after whole-liver transplantation (*6–9*). On the other hand, after living-donor liver transplantation (LDLT), transient or persistent portal hypertension with increased portal vein (PV) resistance could occur even after surgery because of significant graft regeneration and small-for-size graft dysfunction (*10–12*). Thus, in LDLT recipients with major portosystemic shunt vessels, increased PV pressure might cause portal steal phenomenon, resulting in insufficient portal inflow and graft dysfunction (*13–15*).

In this study, we retrospectively analyzed 324 adult LDLT cases to determine the clinical characteristics of patients with major spontaneous portosystemic shunt vessels and, ultimately, to assess whether such major shunts need to be obstructed in LDLT.

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RESULTS

Characteristics of the Recipients, Donors, and Grafts

The mean (SD) age of the recipients was 52.4 (11.3) years. Indications for LDLT included cholestatic cirrhosis (n=85, 26.2%), postnecrotic viral or nonviral cirrhosis (n=229, 70.7%), and others (n=10, 3.1%). Approximately half of the patients were hepatitis C virus positive (n=154, 47.5%). Almost two-third of the patients were classified as Child-Pugh class C (n=206, 63.6%). The mean (SD) model for end-stage liver disease score was 15.8 (6.1). Major shunt vessels were present in 130 recipients (40.1%). The mean (SD) age of the donors was 36.5 (11.3) years. Graft types included left-lobe (n=197, 60.8%), right-lobe (n=120, 37.0%), and posterior segment (n=7, 2.2%) grafts. Twelve donors (4.3%) provided blood type–incompatible grafts. The mean (SD) graft volume (GV) and GV/standard liver volume (SLV) were 478 (105) g and 41.5 (8.5), respectively. Splenectomy was performed in 165 recipients (50.9%). The mean (SD) operation time was 807 (185) min, and the mean (SD) blood loss was 7.7 (16.1) L.

TABLE 1. Patient demographics

Variables	Presence of Major Shunt Vessels		P
	No (n=194)	Yes (n=130)	
Recipient age, mean (SD), y	51.9 (11.6)	53.0 (10.8)	0.409
Recipient gender, male, n (%)	104 (53.6)	56 (43.1)	0.063
Child-Pugh class C, n (%)	113 (58.2)	93 (71.5)	0.015
MELD score, mean (SD)	15.3 (6.6)	16.4 (5.3)	0.109
Hospitalized status, n (%)	68 (35.1)	44 (42.3)	0.187
PV thrombosis before LDLT, n (%)	8 (4.1)	36 (27.7)	<0.001
Hepatocellular carcinoma, n (%)	99 (51.9)	63 (48.5)	0.650
Donor age, mean (SD), y	36.9 (11.2)	35.9 (11.5)	0.407
Donor gender, male, n (%)	130 (67.0)	76 (58.5)	0.117
Blood type–incompatible donor, n (%)	7 (3.6)	7 (5.4)	0.447
Left-lobe graft, n (%)	131 (67.5)	66 (50.7)	0.002
GV, mean (SD), g	472 (106)	486 (104)	0.282
GV/SLV ratio, mean (SD), %	41.1 (8.5)	41.9 (8.4)	0.391
PV pressure at laparotomy, mean (SD), mm Hg	25.0 (5.8)	23.8 (5.4)	0.048
PV pressure at the closure, mean (SD), mm Hg	17.1 (4.2)	17.1 (4.3)	0.856
PV flow, mean (SD), L/min/graft	1.68 (0.40)	1.68 (0.65)	0.995
HA flow, mean (SD), mL/min	110 (70)	103 (68)	0.449
Operation time, mean (SD), min	802 (179)	813 (192)	0.585
Operative blood loss, mean (SD), L	8.4 (19.1)	6.6 (10.1)	0.305
Acute cellular rejection, n (%)	30 (15.4)	16 (12.3)	0.425
PV thrombosis, n (%)	3 (1.5)	5 (3.8)	0.190
Bacteremia, n (%)	21 (10.8)	18 (13.9)	0.398
Primary graft dysfunction, n (%)	21 (10.8)	22 (16.9)	0.112

GV, graft volume; HA, hepatic artery; LDLT, living-donor liver transplantation; MELD, model for end-stage liver disease; PV, portal vein; SLV, standard liver volume.

Factors associated with the presence of major spontaneous portosystemic shunt vessels were evaluated (Table 1). Patients with major shunt vessels more frequently had PV thrombosis before transplantation compared with patients without major shunt vessels (27.7% vs. 4.1%, $P<0.001$) before transplantation. Patients with major portosystemic shunt vessels also had lower PV pressure at laparotomy (mean [SD], 23.8 [5.4] mm Hg vs. 25.0 [5.8] mm Hg; $P=0.048$), were more often classified as Child-Pugh class C (71.5% vs. 58.2%, $P=0.015$) and were more likely to receive right-side grafts, including right-lobe and posterior segment grafts (49.3% vs. 32.5%, $P=0.002$). There were no other differences in preoperative and operative factors between patients with and without major portosystemic shunt vessels. Although the incidence of hepatic artery thrombosis was higher in patients with major portosystemic shunts before LDLT (2.3% vs. 0.0%, $P=0.033$), there were no differences in other postoperative factors.

Types of Shunt Vessels

The types of major portosystemic shunt vessels (n=130; Table 2) included splenorenal shunts (n=60, 46.2%), gastroesophageal shunts (n=35, 26.9%), mesocaval shunts (n=18, 13.8%), paraumbilical shunts (n=16, 12.3%), and cavernous transformation in the hepatoduodenal ligament (n=1, 0.8%). A total of 36 patients (27.7%) had PV thrombosis, including an atrophic PV (n=3), or complete (n=14), partial (n=15), or luminal PV thrombosis (n=4). In four patients, a splenorenal shunt was used for renoportal anastomosis to establish PV inflow (Table 2).

Ligation of the Shunt Vessels and PV Pressure

Ligation of major portosystemic shunt vessels significantly increased mean (SD) PV pressure from 16.8 (3.9) mm Hg to 18.6 (4.3) mm Hg ($P<0.001$) and PV flow from 1.35 (0.67) mm Hg to 1.67 (0.67) mm Hg ($P<0.001$). Concomitant splenectomy decreased mean (SD) PV pressure by 3.9 (0.8) mm Hg.

PV Reconstruction in Patients with PV Thrombosis

For patients with PV thrombosis, graft PV inflow was established by renoportal anastomosis (n=4), interposing an internal jugular (IJ) vein graft (n=3) between the superior mesenteric vein and graft PV, thrombectomy (n=25), or direct anastomosis (n=4). The other 94 patients (72.3%) did not have PV thrombosis before LDLT.

For patients with a splenorenal shunt and severe PV thrombosis, including an atrophic PV (n=4), renoportal anastomosis was performed to establish PV inflow. Right-lobe grafts were transplanted for appropriate vascular alignment and to accommodate the vast inflow from the mesenteric, splenic, and left renal veins (Table 2).

For patients with a mesocaval shunt and severe PV thrombosis, including patients with an atrophic PV (n=2), we used IJ jump grafts. In one patient with an atrophic PV, the atrophied PV was dissected down to the junction of the mesenteric and splenic veins, and the IJ vein graft was directly anastomosed onto the exposed junction. In the other

TABLE 2. The types of the shunts and portal vein thrombosis

Shunts	PV Thrombosis	Procedures for PV Thrombosis and Major Shunts
Splenorenal shunt (n=60)	Atrophic PV (n=2)	Renoportal anastomosis using IJ vein (n=2)
	Complete (n=7)	Renoportal anastomosis using IJ vein (n=2)
		Thrombectomy+shunt ligation (n=5)
	Partial (n=6)	Thrombectomy+shunt ligation (n=6)
	Luminal (n=1)	Direct anastomosis+shunt ligation (n=1)
	No PV thrombosis (n=44)	Shunt ligation (n=36)
		No (n=7) or incomplete (n=1) shunt ligation, followed by secondary ligation (n=4)
Gastroesophageal shunt (n=35)	Complete (n=5)	Thrombectomy+shunt ligation (n=5)
	Partial (n=3)	Thrombectomy+shunt ligation (n=3)
	Luminal (n=2)	Direct anastomosis+shunt ligation (n=2)
	No PV thrombosis (n=25)	Shunt ligation (n=21)
		No shunt ligation (n=4), followed by secondary ligation (n=3)
Mesocaval shunt (n=18)	Atrophic PV (n=1)	Interposition using IJ vein+shunt ligation (n=1)
	Complete (n=1)	Interposition using IJ vein (behind pancreas)+shunt ligation (n=1)
	Partial (n=3)	Thrombectomy+shunt ligation (n=2)
		Thrombectomy+incomplete shunt ligation, followed by secondary ligation (n=1)
	No PV thrombosis (n=13)	Shunt ligation (n=13)
Paraumbilical shunt (n=16)	Partial (n=3)	Thrombectomy+shunt ligation (n=3)
	Luminal (n=1)	Direct anastomosis+shunt ligation (n=1)
	No PV thrombosis (n=12)	Shunt ligation (n=12)
Cavernous transformation (n=1)	Complete (n=1)	Interposition using IJ vein (behind pancreas)

IJ, internal jugular, PV, portal vein.

patient with complete PV thrombosis and a very fragile PV wall caused by cholangitis, the IJ vein graft was anastomosed to the mesenteric vein in an end-to-side fashion, tunneled behind the pancreas neck and connected to the graft's PV.

For the patient with cavernous transformation, the shunt vessels in the hepatoduodenal ligament were divided under mechanical portocaval shunting from the inferior mesenteric vein into the axillar vein. The IJ vein graft was connected to the mesenteric vein in an end-to-end fashion, tunneled behind the pancreas neck, and was connected to the graft's PV.

Untied or Newly Created Shunt Vessels

Ligation of the major shunt vessels was not performed in 13/130 patients (10.0%), and a new hemiportocaval shunt was created in another patient. Therefore, 14 patients had major shunt vessels after LDLT. The reasons for having patent major shunt vessels after LDLT included undetected vessels (n=9), incomplete ligation (n=2), or the shunt was newly created or left open to maintain high PV pressure after reperfusion (n=3). Nine patients (62.3%) required secondary interventions (Table 3, Fig. 1). Among them, six cases had small-for-size graft syndrome like primary graft dysfunction, and one had cholangitis caused by biliary anastomotic stricture after LDLT.

PV flow was significantly lower in the patients who underwent secondary ligation of the major shunt vessels (n=9) than in the patients without the secondary procedure (n=121) (mean [SD], 0.96 [0.25] L/min vs. 1.62 [0.62] L/min; P=0.007). By contrast, PV pressure at the end of surgery was not significantly different between these two groups of patients (18.6 [3.9] mm Hg vs. 17.1 [4.4] mm Hg, P=0.604).

Graft Survival

The presence of major shunt vessels or PV thrombosis did not significantly affect graft survival. The 1- and 5-year cumulative graft survival rates were 90.7% and 83.0%, respectively, in patients without major shunts vessels versus 86.1% and 77.2%, respectively, in patients with major shunt vessels (P=0.195). The 1- and 5-year cumulative graft survival rate was 97.8% and 82.3%, respectively, in patients without PV thrombosis versus 84.0% and 70.6%, respectively, in patients with PV thrombosis (P=0.119).

DISCUSSION

In deceased-donor whole-liver transplantation, it is generally considered that special interventions are not necessary for spontaneous portosystemic shunts or hypersplenism. In Western countries in particular, nonsurgical transjugular intrahepatic portosystemic shunts have supplanted surgical shunts for pretransplantation management of portal hypertension and achieved sufficient portal decompression (*4–6*). In LDLT, however, there is no consensus on whether spontaneous portosystemic shunts should be obstructed, although some reports have described secondary interventions for patent portosystemic shunts with portal steal phenomenon (*13–15*). Moreover, the beneficial effects of surgically created portocaval shunts for small-for-size grafts have been widely advocated in recent years (*16, 17*). Nevertheless, there is no consensus on whether the shunt should be closed, kept, or

TABLE 3. The patients who underwent secondary intervention for obstructing shunt vessels after LDLT

Case No.	Age, Sex	Graft	GV/SLV ratio (%)	Shunt Type	Reason for Left Open	PV Pressure (mm Hg)	PV Flow (mL/min)
1	40, M	Left	28.9	Splenorenal	Unrecognized shunts	N/A	1150
2	47, F	Left	40.5	Splenorenal	Unrecognized shunts	N/A	770
3	53, F	Left	38.2	Splenorenal	Unrecognized shunts	22	1500
4	40, M	Left	44.7	Mesocaval	Incomplete shunt ligation	15	980
5	53, M	Right	42.9	Gastroesophageal	Unrecognized shunts	12	900
6	51, F	Post	38.6	Splenorenal	Unrecognized shunts	15	790
7	47, F	Left	23.7	Hemiportocaval	Created for high PV pressure	21	270
8	47, F	Left	35.6	Gastroesophageal	Left open for high PV pressure	21	1340
9	62, F	Left	35.4	Gastroesophageal	Left open for high PV pressure	24	990

Case No.	Intervention After LDLT	Indication	Method	Outcome
1	1st day	Decreased PV flow Graft dysfunction	Relaparotomy	Treated
2	133th day	Risky gastric varices	BRTO	Treated
3	82th day	Risky gastric varices	BRTO	Treated
4	1st day	Decreased PV flow Graft dysfunction	Relaparotomy	Treated
5	8th day	Decreased PV flow Graft dysfunction	Relaparotomy	Treated
6	22th day	Decreased PV flow Graft dysfunction	BRTO	Dead
7	4th day	Decreased PV flow Graft dysfunction	Relaparotomy	Treated
8	57th day	Encephalopathy	Relaparotomy	Treated
9	8th day	Decreased PV flow Graft dysfunction	Relaparotomy	Dead

BRTO, balloon-occluded retrograde transvenous obliteration; GV, graft volume; LDLT, living-donor liver transplantation; N/A, not applicable; PV, portal vein; SLV, standard liver volume.

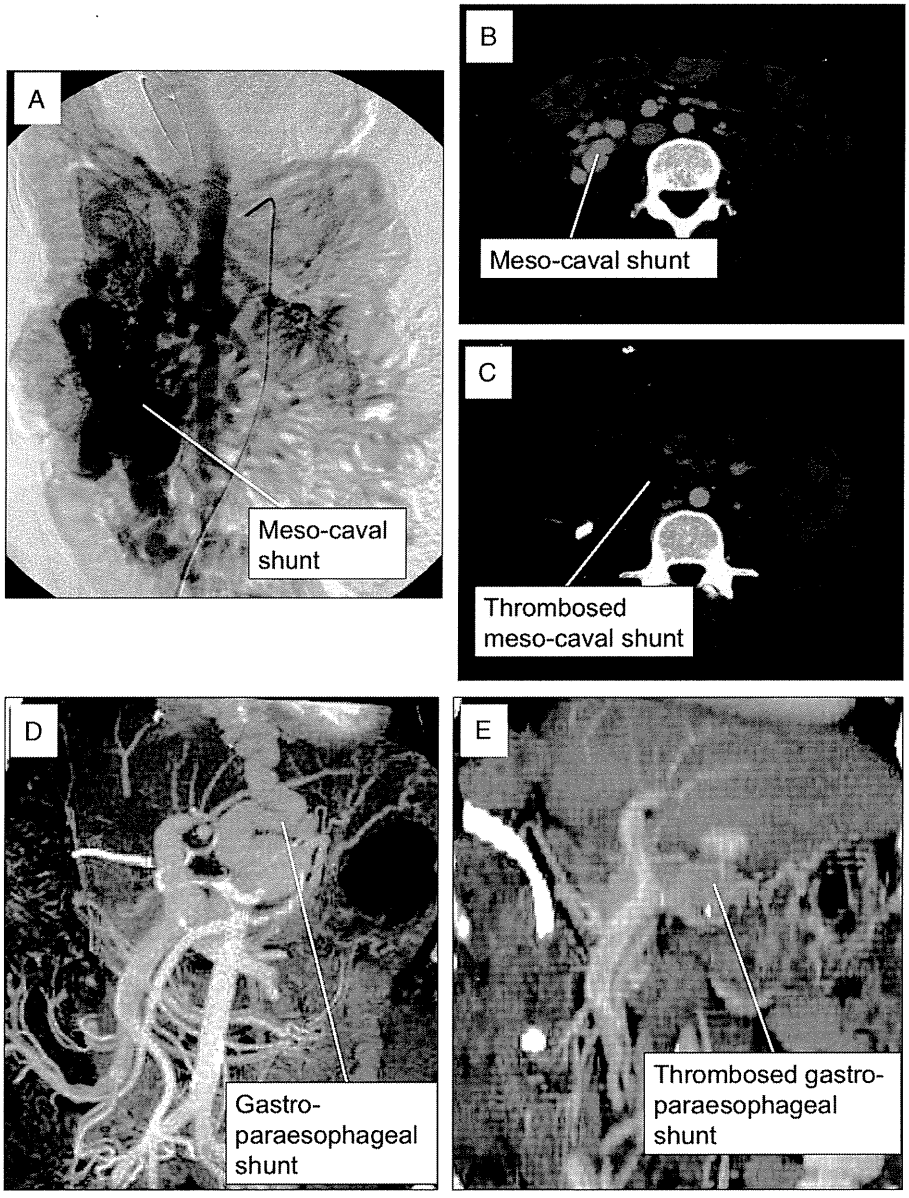

FIGURE 1. The cases of patient major shunt vessels requiring secondary intervention: Case 4 (A–C) and Case 8 (C, D). Mesenteric arterioportogram (A) and computed tomographic scan (B) showed portal steal caused by patent major mesocaval shunt, followed by surgical ligation (C). Multidetector computed tomographic image of patent gastroesophageal shunt after LDLT (D), followed by surgical ligation (E).

created, nor have the indications, such as PV pressure of PV flow, for shunt management been assessed.

In our institute, the management of portal hemodynamic status has changed considerably with our accumulating experience. Before 2000, we did not perform shunt ligation or splenectomy. However, since 2000, when we experienced a case of graft dysfunction caused by portal steal through a splenorenal shunt, we have ligated the major shunts, whenever possible. In 2001, we started to apply splenic artery ligation for patients with small-for-size graft dysfunction and increased PV pressure and then adopted splenectomy in 2005 (*18, 19*). Following several reports published in the mid-2000s showing the beneficial effects of creating portocaval shunt for reducing PV pressure and PV flow, we created portocaval shunts or kept the major shunts in three patients to reduce high PV pressure after reperfusion, albeit with poor outcomes (*20*). These three cases showed more graft portal resistance caused by dynamic graft regeneration and reduced PV flow, which was diverted into the portosystemic shunts (*10, 12, 15*). Hemiportocaval shunting is considered to be neither stable nor safe because the flow rate into the graft and shunt cannot be controlled in a dynamically regenerating liver graft (*20*). In fact, a

similar discussion was previously reported in the context of portal steal phenomenon in auxiliary partial liver transplantation, and a consensus was obtained for ligating the PV of the native liver to provide constant PV flow into a regenerating auxiliary graft (*21, 22*). Thus, our current strategy for the management of PV hemodynamic status in LDLT (i.e., shunt ligation to prevent portal stealing and splenectomy for PV decompression) simplifies and normalizes PV hemodynamic management.

We have implemented several technical refinements to approach and treat portosystemic shunts, including en bloc stapling division of gastroesophageal varices and a direct approach for splenorenal shunts. Gastroesophageal shunts are often coiled or tortuous, engorged with a thin wall, sometimes multiple in number, and are usually buried in the retroperitoneum on the diaphragmatic crus (*15, 23*). Because manual isolation and ligation of such vessels is technically very difficult, our technique to divide these vessels, including the left gastric artery en bloc, is safe and simultaneously decreases portal inflow through the left gastric artery (*23*).

The approach for splenorenal shunts is also technically demanding. Lee et al. (*24*) recently reported a novel technique involving ligation of the left renal vein to prevent portal stealing through the splenorenal shunt. However, they also reported that ligation of the left renal vein decreased kidney size in 75% of the recipients. Therefore, they concluded that the procedure should be limited to a life-saving procedure (*24*). We expose the left renal vein and follow it to identify splenorenal shunts originating from the left adrenal vein. We think that normal anatomic structures, including the left renal vein and left adrenal vein, have appropriate venous shapes and thicknesses, unlike abnormal venous shunts, and following such veins can be performed safely.

The surgical technique to establish PV inflow is another issue that needs to be addressed for patients with portosystemic shunts. We use corkscrew technique for PVs extending into the splenomesenteric junction. For atrophic PV, we consider patch plasty, renoportal anastomosis, or placing an interposition graft under the pancreas neck between the superior mesenteric vein and the graft's PV. Renoportal anastomosis may be applied for patients with a splenorenal shunt (*25*). To place an interposition graft, it is not always possible to procure a long venous segment to join the superior mesenteric vein with the graft PV over the pancreas, especially in Japan, where deceased-donor venous grafts are rare (*26*). Instead, we use the IJ vein, which is tunneled under the pancreas neck. Nevertheless, it is necessary that sufficient PV inflow is maintained after obstructing the major shunts. Indeed, we recently reported that a postreperfusion PV flow of less than 1 L/min is a significant risk factor for relaparotomy for inadequate PV flow (*27*).

Pretransplantation evaluation of PV hemodynamic status is useful to identify the major shunt to be ligated. To achieve this, we have used multidetector-row computed tomography (MDCT) to provide three-dimensional images of PV circulation since 2007. Numerous reports have confirmed the usefulness of MDCT for visualizing the arterial, portal, and venous vasculature systems (*15*). Although six patients before 2006 had undetected or incompletely ligated shunts requiring secondary intervention, there have been no further cases after the introduction of three-dimensional MDCT. If PV flow after reperfusion was less than 1 L/min, cineportograms could be performed, as suggested by Moon et al. (*15*).

PV hemodynamics is influenced by various factors including graft inflows and outflows, graft compliance, and central venous pressure. Partial LDLT grafts are more susceptible to these changes than the whole-liver graft (*28*). In the nine cases undergoing secondary intervention for PV stealing phenomenon, six had primary graft dysfunction (*29*), and one had cholangitis caused by biliary anastomotic stricture after LDLT. Moreover, graft regeneration itself causes decreased graft compliance, and all the partial regenerating grafts have such risks as portal stealing during regeneration (*28*). Such events are difficult to be forecasted, and because PV stealing with insufficient graft perfusion causes secondary graft injuries, we believe that major portosystemic shunts should be obstructed during transplantation.

Our principle in managing PV hemodynamics in LDLT is represented by simplification and normalization. By obstructing the abnormal portosystemic shunts, all the mesenteric PV flow runs into the LDLT graft without stealing, although PV pressure increases. We obstruct major (defined as ≥10 mm in size on preoperative MDCT) shunts after reperfusion. Thereafter, splenectomy is performed if PV pressure after shunt ligation is 20 mm Hg or greater for PV decompression. Although both procedures seem opposite, they are common in treating abnormal PV hemodynamics caused by end-stage liver diseases. The combination of both procedures simplify and normalize PV hemodynamics and is in contrast to preserved major portosystemic shunts without splenectomy resulting in keeping hyperdynamic PV flow with stealing.

In conclusion, it is an appropriate option to obstruct the major portosystemic shunt vessels to ensure adequate graft inflow in LDLT.

MATERIALS AND METHODS

Patients

Between May 1997 and June 2012, 381 adult-to-adult LDLTs were performed at Kyushu University Hospital, under the approval from the Ethics and Indications Committee of Kyushu University. Cases of acute liver failure (n=56) and LDLT using dual grafts (n=1) were excluded from the present analyses. Thus, 324 adult-to-adult LDLTs for chronic hepatic disorders were included in the current analyses. A total of 130 patients had major spontaneous portosystemic shunts (diameter, ≥10 mm on computed tomography) before LDLT, and 194 patients did not. The mean (SD) follow-up time was 4.8 (3.6) years.

Graft Selection

Grafts were selected as previously described (*30*). Left-lobe grafts were used as the primary graft type if the desired GV/SLV ratio was 35% or greater. Right-lobe grafts were used if the simulated GV/SLV ratio of the left-lobe graft was less than 35% and the donor's remnant liver volume was 35% or greater. Posterior segment grafts were used if GV/SLV ratio of the posterior segment graft was 35% or greater with isolated branching of the posterior PV from the main PV and if both left and right-lobe grafts were not available.

Transplant Procedures

Donor parenchymal transection was performed using the Cavitron Ultrasonic Surgical Aspirator (Valleylab Inc., Boulder, CO) with the hanging maneuver (*31*). After donor hepatectomy, the graft was perfused, weighed, and stored in University of Wisconsin solution (Viaspan; DuPont Inc., Wilmington, DE).

After recipient hepatectomy, the grafts were transplanted in a piggyback fashion. The orifice of the recipient's hepatic vein was enlarged with an incision

on the vena cava for the venous anastomosis to provide sufficient outflow. After venous anastomoses, the PV was reconstructed and reperfused. Subsequent arterial reconstruction was performed under a microscope. If indicated, splenectomy was performed as previously described (*31*). Biliary reconstruction was performed by duct-to-duct biliary anastomosis whenever possible.

Ligation or Division of the Major Shunt Vessels

Since 2000, we have ligated all of the identified major (≥10 mm) portosystemic shunt vessels during LDLT, whenever indicated. The presence of such major portosystemic shunts was diagnosed using MDCT after operation. The shunts are controlled and left open during the anhepatic phase to minimize portal venous congestion and are ligated after reperfusion.

To control splenorenal or gastrorenal shunts, intraoperative sonography was used to identify the left renal vein in the retroperitoneum near the left side of the inferior mesenteric vein. The transverse mesocolon was retracted in a cephalad direction to provide an adequate surgical field. The retroperitoneum was opened, and the left renal vein was identified. By following the left renal vein, a dilated splenorenal shunt was identified on the cranial side and was then controlled as appropriate.

For gastroesophageal shunts, the dilated coronary vein was carefully dissected from the retroperitoneum and controlled, followed by its ligation, before May 2011. However, because this is a technically difficult procedure, caused by the tortuously dilated coronary veins with minor collateral vessels, we have since applied endostapling devices to the base of the left gastric ligament, including the left gastric artery, engorged coronary vein, and collateral vessels, followed by en bloc division using endostapling devices (Echelon Flex Endopath TM Staplers 60-2.5; Ethicon Endo-Surgery Inc., Cincinnati, OH) and mass suturing with continuous 3-0 Prolene sutures with an SH needle (Ethicon Inc., Somerville, NJ) (*23*).

Mesocaval shunts were identified in the retroperitoneum on a case-by-case basis. We routinely ligate the major inflow into the mesocaval shunts and the major outflow into the vena cava.

Establishment of PV Inflow

The presence of major shunt vessels might indicate PV thrombosis or atrophy. Eversion or corkscrew procedures were performed for PV thrombectomy (*32*). Renoportal anastomosis was indicated for complete PV thrombosis with a fragile or atrophic PV vein wall with numerous splenorenal shunts. The left renal vein was controlled after mobilization of the duodenum. End-to-end PV reconstruction was performed using an IJ vein graft with continuous 6-0 polydiaxanone sutures.

For an atrophied PV or complete PV thrombosis with a fragile PV wall, without splenorenal shunts, PV reconstruction was performed using IJ jump grafts, which were connected to the mesenteric-splenic junction or the mesenteric vein. The IJ graft was then tunneled behind the pancreas neck to connect to the PV.

Splenectomy

The indications for splenectomy during LDLT include hypersplenism, PV pressure of 20 mm Hg or greater, and patients with hepatitis C treated with interferon after LDLT. We have introduced tieless splenectomy (*19*) using a vessel-sealing system (LigaSure Atlas; Valleylab Inc., Boulder, CO) and endostapling devices (Echelon Staplers 60-2.5; Ethicon Endo-Surgery Inc.).

Measurement of Portal Hemodynamic Properties

PV pressure was continuously monitored during surgery using a cannula (Medicut LCV-UK catheter 14 GTM; Nippon Sherwood Inc., Tokyo, Japan) placed in the superior mesenteric vein through a terminal jejunal vein. Intraoperative PV flow (L/min) was measured in the recipients using an ultrasonic transit time flow meter (Transonic System, Ithaca, NY) after establishing hepatic artery flow and reperfusion.

Posttransplantation Medical Care

The basic immunosuppression protocol consisted of tacrolimus or cyclosporine with mycophenolate mofetil and steroids. The target tacrolimus level was 10 to 14 ng/mL at 1 month after LDLT and was decreased to 7 to 10 ng/mL during the next few months. The target cyclosporine level was 150 to 250 ng/mL at 1 month after LDLT and was decreased to 100 to 150 ng/mL during the next few months.

Primary graft dysfunction was defined as graft insufficiency with possible early graft loss, without technical, anatomic, immunologic, or hepatitis-related issues. Primary graft dysfunction was detected as delayed hyperbilirubinemia, with a total bilirubin of 20 mg/dL or greater, usually occurring 7 days after surgery and persisting for 7 or more consecutive days. It was treated by plasma exchange (*29*).

Statistical Analysis

All analyses were conducted in a retrospective manner. Values are expressed as the mean (SD). Variables were analyzed using chi-square tests for categorical values or the Mann-Whitney test for continuous variables. Cumulative survival was determined using the Kaplan-Meier method with log-rank tests. $P<0.05$ was considered statistically significant.

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Original Article

Risk factors for recurrence after curative resection of hepatitis C-related hepatocellular carcinoma in patients without postoperative interferon therapy

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Aim: Hepatitis C (HC)-related hepatocellular carcinoma (HCC; HC-HCC) is highly recurrent.

Methods: From 1995–2007, 183 curative hepatic resections for primary solitary HC-HCC without postoperative interferon therapy were included in this study. The patients were divided into three groups: (i) 2 cm or less ($n = 56$); (ii) more than 2 cm to less than 5 cm ($n = 79$); and (iii) 5 cm or more ($n = 48$). Independent risk factors for HC-HCC recurrence for each group were determined.

Results: Independent risk factors for recurrence were aspartate aminotransferase or alanine aminotransferase (AST/ALT) of 80 IU/L or more (hazard ratio [HR], 2.1; $P = 0.02$) in patients with HCC of 2 cm or less, des-γ-carboxy prothrombin of 100 mAU/mL or more (HR, 2.5; $P = 0.02$) and AST/ALT of 80 IU/L or more (HR, 2.1; $P = 0.04$) in patients with HCC of more than 2 cm to less than 5 cm, and the presence of macroscopic portal vein tumor thrombus (HR, 2.8; $P = 0.02$) and AST/ALT of 80 IU/L or more (HR, 2.1; $P = 0.04$) in patients with HCC of 5 cm or more. All 13 late recurrences of 1 year or more after hepatic resection (27.1%) in patients with HCC of 5 cm or more were accompanied by AST/ALT of 80 IU/L or more.

Conclusion: AST/ALT of 80 IU/L or more is an independent risk factor for the recurrence of primary solitary HC-HCC after curative resection irrespective of the primary HC-HCC size.

Key words: hepatitis C virus, hepatocellular carcinoma, risk factors, tumor recurrence

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common malignancies worldwide, with at least 1 million new cases annually.[1] The cause of HCC is infection with a hepatitis virus such as hepatitis B virus (HBV) or hepatitis C virus (HCV).[2] The mechanism of carcinogenesis by HCV is still unknown. HCV is an RNA virus that does not integrate into the DNA of hepatocytes. Theoretically, HCV, unlike HBV, does not have a direct oncogenic mechanism.[3] Chronic inflammation, liver cell necrosis and regeneration, and extensive fibrosis are important in the development of hepatitis C-related HCC (HC-HCC).[3]

Despite improvements in imaging and surgical procedures, outcomes after hepatic resection for HCC are still unsatisfactory because of the high rate of recurrence.[4] Recurrence is considered to be caused by two factors, metastasis of cancer cells and multicentric occurrence, in patients after curative resection of HCC. We extensively examined the recurrence pattern of HC-HCC and found that recurrence-free survival after hepatic resection for HC-HCC is deeply related to hepatitis activity.[5–8] This activity, represented by high serum aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) levels, was thought to be related to multicentric occurrence,[9,10] but metastasis of cancer cells was related to characteristics of advanced

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tumor stage such as large tumor size or portal vein tumor thrombus.[11,12] Several randomized controlled trial studies confirmed the effectiveness of postoperative interferon therapy for HC-HCC,[13,14] but the target of this therapy is thought to be a multicentric occurrence, not metastasis. To establish a postoperative treatment strategy for HC-HCC, the risk factors for the recurrence from the aspects of metastasis or multicentric occurrence need to be evaluated.

In the present study, we analyzed 183 consecutive patients who had undergone curative hepatic resections for primary solitary HC-HCC from 1995–2007, and who had received more than 5 years' follow up without postoperative interferon therapy at a single institution. We determined the independent risk factors for recurrence by dividing the primary HC-HCC size into three size groups: (i) HCC of 2 cm or less; (ii) HCC of more than 2 cm to less than 5 cm; and (iii) HCC of 5 cm or more.

METHODS

Patients

A TOTAL OF 219 anti-HCV antibody positive patients who had undergone curative hepatic resections for primary solitary HC-HCC at the Department of Surgery, Hiroshima Red Cross and Atomic Bomb Survivors Hospital, between January 1995 and December 2007 were included in this study. Thirty-six patients were excluded for the following reasons: five patients had tested negative for serum HCV RNA, one patient had died during hospital stay, and 30 patients had received postoperative interferon therapy. Hence, there were 183 evaluative patients in our series. Curative resection was defined as a complete tumor resection without tumor exposure, as confirmed by pathological examination. Patients were divided into three groups according to primary HC-HCC size: (i) HCC of 2 cm or less ($n = 56$); (ii) HCC of more than 2 cm to less than 5 cm ($n = 79$); and (iii) HCC of 5 cm or more ($n = 48$). The medical records of all patients were followed up through March 2012. The median follow-up periods in our series were 7.6, 6.8 and 5.4 years, respectively.

Surgical techniques and follow-up methods

Details of the surgical techniques and patient selection criteria have been reported previously.[15,16] The resection volume was decided based on the indocyanine green retention rate at 15 min (ICG-R15). Patients with an ICG-R15 of 35% or more were selected for limited resection.[16] In almost all hepatic resections, Pringle's maneuver, consisting of clamping the portal triad for 15 min and then releasing the clamp for 5-min intervals was applied; alternatively, hemivascular occlusion[17,18] was performed. The CUSA system (Valleylab, Boulder, CO., USA) was used to transect the liver parenchyma.

After discharge, all patients were examined for recurrence by ultrasonography and tumor markers such as α-fetoprotein and des-γ-carboxy prothrombin (DCP) each month, and by dynamic computed tomography every 3 or 4 months.[19] No patients in this series received adjuvant chemotherapy. We treated recurrent HCC by repeat hepatectomy,[20] ablation therapy or lipiodolization[21] according to a previously described strategy.[22]

Statistical analysis

The disease-free survival (DFS) curves were generated by the Kaplan–Meier method and compared by the log-rank test. To evaluate the independent risk factors for recurrence of HC-HCC after curative hepatic resection in each group, we performed multivariate analysis with the Cox proportional hazard model, using a variable-selection method involving the backward-elimination procedure. The cut-off for elimination was set at $P < 0.15$. The following 16 clinical, surgical and tumor-related variables were analyzed:[16] age (≥65 years vs younger); the mean serum total bilirubin level after hepatic resection (≥1.0 mg/dL vs less); preoperative ICG-R15 (≥20% vs less); the mean serum albumin level after hepatic resection (≥3.5 g/dL vs less); the mean serum AST/ALT (either AST or ALT) levels assessed by the data obtained each month for 1 year after hepatic resection (≥80 IU/L vs less); histological cirrhosis (present vs absent); macroscopic portal vein tumor thrombus (VP; present vs absent); preoperative α-fetoprotein (≥100 ng/mL vs less); preoperative DCP (≥100 IU/L vs less); pathological cancer spread including portal vein invasion (vp) and intrahepatic metastasis (im) (present vs absent); tumor cell differentiation (well or moderate vs poor); surgical time (≥300 min vs less); surgical blood loss (≥1000 mL vs less); operative procedure (anatomical resection vs limited resection); history of intraoperative blood cell transfusion (yes vs no); and surgical margin (≥5 mm vs smaller). Categorical variables were compared using either the χ^2-test or Fisher's exact test, as appropriate. All analyses were performed with Statview 5.0 software (Abacus Concepts, Berkeley, CA, USA). *P*-values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Rates of vp and im

TABLE 1 SHOWS the rates of vp and im in each group. In patients with HCC of 2 cm or less, the vp and im rates were 5.4% and 1.8%, respectively. In patients with HCC of more than 2 cm to less than 5 cm, the vp rate increased significantly to 29.1% ($P = 0.03$). In patients with HCC of 5 cm or more, the vp and im rates increased markedly to 81.3% ($P < 0.01$) and 58.3% ($P < 0.01$), respectively.

Independent risk factors for recurrence of HC-HCC in each group

Table 2 summarizes the results of univariate analysis according to the risk factors for recurrence of HC-HCC in patients with HCC of 2 cm or less. Table 3 shows that the independent risk factor for recurrence was found to be AST/ALT of 80 IU/L or more (hazard ratio [HR], 2.1; $P = 0.02$). The DFS curves of the two groups divided by AST/ALT or ICG-R15 values are illustrated in Figure 1. The DFS was significantly worse in patients with AST/ALT of 80 IU/L or more ($P = 0.03$). In this group, the 1- and 3-year DFS in patients with AST/ALT of 80 IU/L or more versus those with AST/ALT of less than 80 IU/L were 80% versus 95%, and 49% versus 72%, respectively.

Table 4 summarizes the results of univariate analysis according to the risk factors for recurrence of HC-HCC in patients with HCC of more than 2 cm to less than 5 cm. As Table 5 shows, the independent risk factors for recurrence were DCP of 100 mAU/mL or more (HR, 2.5; $P = 0.02$) and AST/ALT of 80 IU/L or more (HR, 2.1; $P = 0.04$). The DFS curves of the two groups divided by DCP or AST/ALT values are illustrated in Figure 2. The DFS was significantly worse in patients with DCP of 100 mAU/mL or more ($P = 0.02$). In this group, the 1- and 3-year DFS in patients with AST/ALT of 80 IU/L or more versus with AST/ALT of less than 80 IU/L were 82% versus 84% and 53% versus 80%, respectively.

Table 1 Rates of vp and im according to the primary HC-HCC size

Tumor diameter	vp	im
HCC ≤2 cm ($n = 56$)	3 (5.4%)	(1.8%)
HCC >2 cm to <5 cm ($n = 79$)	23 (29.1%)*	3 (3.8%)
HCC ≥5 cm ($n = 48$)	39 (81.3%)**	28 (58.3%)**

*$P = 0.03$, **$P < 0.01$.
HC, hepatitis C; HCC, hepatocellular carcinoma; im, intrahepatic metastasis; vp, portal vein invasion.

Table 2 Univariate analysis for risk factors of recurrence: HCC ≤2 cm

Variables	5-year disease free survival	*P*-value
Background characteristics		
Age ≥65 years	Yes, 24%; no, 30%	0.87
T-bil ≥1.0 mg/dL	Yes, 23%; no, 32%	0.44
ICG-R15 ≥20%	Yes, 17%; no, 39%	0.09
Alb <3.5 g/dL	Yes, 31%; no, 33%	0.82
AST/ALT ≥80 IU/L	Yes, 20%; no, 50%	0.03
Surgical outcomes		
Surgical time ≥300 min	Yes, 21%; no, 40%	0.22
Surgical blood loss ≥1000 mL	Yes, 29%; no, 32%	0.97
Transfusion (+)	Yes, 28%; no, 49%	0.19
Limited resection	Yes, 13%; no, 46%	0.05
Surgical margin <5 mm	Yes, 17%; no, 38%	0.21
Tumor-related factors		
VP (+)	Yes, 0%; no, 39%	0.89
Cancer spread (+)	Yes, 18%; no, 23%	0.88
Poorly differentiated	Yes, 40%; no, 50%	0.25
AFP ≥100 ng/mL	Yes, 0%; no, 36%	0.09
DCP ≥100 mAU/L	Yes, 35%; no, 38%	0.37
Histological cirrhosis (+)	Yes, 12%; no, 44%	0.17

Alb, albumin; AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; cancer spread, pathological cancer spread including portal vein invasion and intrahepatic metastasis; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; ICG-R15, indocyanine green retention rate at 15 min; T-bil, total bilirubin; VP, macroscopic portal vein tumor thrombus.

Table 6 summarizes the results of univariate analysis according to the risk factors for recurrence of HC-HCC in patients with HCC of 5 cm or more. As Table 7 shows, the independent risk factors for recurrence were VP+ (HR, 2.8; $P = 0.02$) and AST/ALT of 80 IU/L or more (HR, 2.1; $P = 0.04$). The DFS curves of the two groups divided by the VP or AST/ALT value are illustrated in

Table 3 Independent risk factors for recurrence: HCC ≤2 cm

Variables	Hazard ratio	95% CI	*P*-value
AST/ALT ≥80 IU/L	2,1	1.28–3.84	0.02
ICG-R15 ≥20%	1.6	0.82–3.68	0.15
AFP ≥100 ng/mL	1.5	0.18–1.33	0.16
Limited resection	1.4	0.67–3.17	0.19

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; HCC, hepatocellular carcinoma; ICG-R15, indocyanine green retention rate at 15 min.

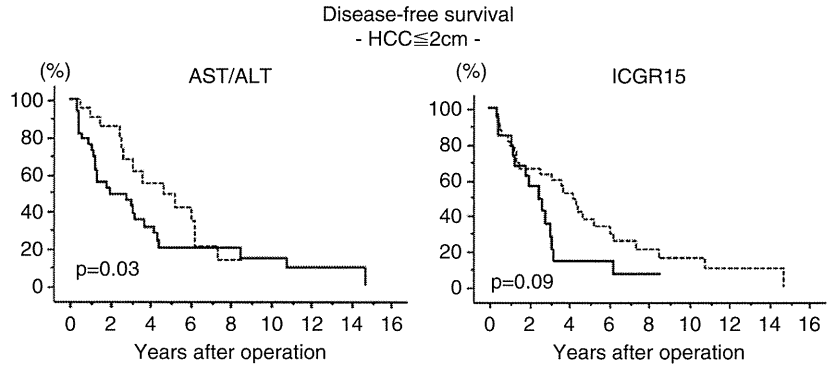

Figure 1 In patients with hepatocellular carcinoma (HCC) ≤2 cm, the disease-free survival (DFS) curves of the two groups divided by aspartate aminotransferase (AST)/alanine aminotransferase (ALT) (≥80 IU/L or <80 IU/L) or indocyanine green retention rate at 15 min (ICG-R15) (≥20% or <20%) values are illustrated. The DFS was significantly worse in patients with AST/ALT ≥80 IU/L ($P = 0.03$). ----, AST/ALT <80 IU/L ($n = 22$); ——, AST/ALT ≥80 IU/L ($n = 34$); ----, ICG-R15 <20% ($n = 35$); ——, ICG-R15 ≥20% ($n = 21$).

Table 4 Univariate analysis for risk factors of recurrence: HCC of more than 2 cm to less than 5 cm

Variables	5-year disease free survival	*P*-value
Background characteristics		
Age ≥65 years	Yes, 28%; no, 39%	0.11
T-bil ≥1.0 mg/dL	Yes, 39%; no, 39%	0.95
ICG-R15 ≥20%	Yes, 17%; no, 43%	0.03
Alb <3.5 g/dL	Yes, 23%; no, 36%	0.16
AST/ALT ≥80 IU/L	Yes, 27%; no, 51%	0.06
Surgical outcomes		
Surgical time ≥300 min	Yes, 31%; no, 52%	0.32
Surgical blood loss ≥1000 mL	Yes, 37%; no, 39%	0.59
Transfusion (+)	Yes, 23%; no, 48%	0.47
Limited resection	Yes, 20%; no, 41%	0.01
Surgical margin <5 mm	Yes, 29%; no, 32%	0.86
Tumor-related factors		
VP (+)	Yes, 27%; no, 31%	0.62
Cancer spread (+)	Yes, 29%; no, 32%	0.55
Poorly differentiated	Yes, 24%; no, 35%	0.35
AFP ≥100 ng/mL	Yes, 25%; no, 36%	0.18
DCP ≥100 mAU/L	Yes, 27%; no, 42%	0.04
Histological cirrhosis (+)	Yes, 21%; no, 44%	0.02

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; cancer spread, pathological cancer spread including portal vein invasion and intrahepatic metastasis; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; ICG-R15, the indocyanine green dye retention rate at 15 minutes; T-bil, total bilirubin; VP, macroscopic portal vein tumor thrombus.

Figure 3. The DFS was significantly worse in patients with VP+ ($P = 0.04$). In this group, the 1- and 3-year DFS in patients with AST/ALT of 80 IU/L or more versus with AST/ALT of less than 80 IU/L were 47% versus 72% and 32% versus 56%, respectively.

Recurrence patterns according to the primary HC-HCC size

Table 8 summarizes the pattern of recurrence, such as solitary liver recurrence, multiple liver recurrence or distant recurrence after curative resection of HC-HCC in each group. In patients with HCC of 2 cm or less, 39 patients (69.6%) had tumor recurrence. The rate of solitary liver recurrence (62.5%) was significantly higher in this group ($P < 0.01$). Among the patients with HCC of

Table 5 Independent risk factors for recurrence: HCC more than 2 cm to less than 5 cm

Variables	Hazard ratio	95% CI	*P*-value
DCP ≥100 mAU/L	2.5	1.10–4.00	0.02
AST/ALT ≥80 IU/L	2.1	1.02–6.33	0.04
Age ≥65 years	1.9	1.00–3.70	0.06
Limited resection	1.4	0.67–3.17	0.12
ICG-R15 ≥20%	1.0	0.43–2.12	0.91
Histological cirrhosis (+)	1.1	0.40–2.12	0.92

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; HCC, hepatocellular carcinoma; ICG-R15, the indocyanine green dye retention rate at 15 minutes.

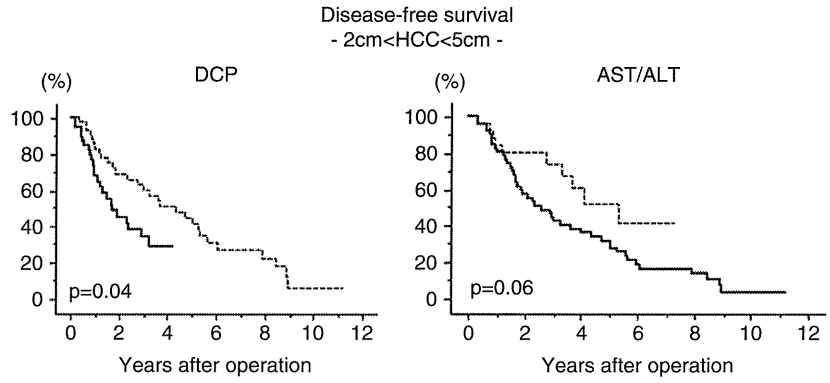

Figure 2 In patients with hepatocellular carcinoma (HCC) of more than 2 cm to less than 5 cm, the disease-free survival (DFS) curves of the two groups divided by des-γ-carboxy prothrombin (DCP) (≥100 or <100 mAU/mL) or aspartate aminotransferase (AST)/alanine aminotransferase (ALT) (≥80 or <80 IU/L) values are illustrated. The DFS was significantly worse in patients with DCP ≥100 mAU/mL ($P = 0.02$). ----, DCP <100 mAU/mL ($n = 43$); —, DCP ≥100 mAU/mL ($n = 36$); ----, AST/ALT <80 IU/L ($n = 26$); —, AST/ALT ≥80 IU/L ($n = 53$).

Table 6 Univariate analysis for risk factors of recurrence: HCC ≥5 cm

Variables	5-year disease free survival	*P*-value
Background characteristics		
Age ≥65 years	Yes, 7%; no, 22%	0.23
T-bil ≥1.0 mg/dL	Yes, 0%; no, 13%	0.39
ICG-R15 ≥20%	Yes, 0%; no, 21%	0.02
Alb <3.5 g/dL	Yes, 0%; no, 12%	0.63
AST/ALT ≥80 IU/L	Yes, 12%; no, 25%	0.09
Surgical outcomes		
Surgical time ≥300 min	Yes, 0%; no, 24%	0.25
Surgical blood loss ≥1000 mL	Yes, 0%; no, 17%	0.56
Transfusion (+)	Yes, 0%; no, 18%	0.89
Limited resection (+)	Yes, 5%; no, 22%	0.14
Surgical margin <5 mm	Yes, 8%; no, 23%	0.38
Tumor-related factors		
VP (+)	Yes, 0%; no, 20%	0.04
Cancer spread (+)	Yes, 0%; no, 18%	0.93
Poorly differentiated	Yes, 11%; no, 17%	0.28
AFP ≥100 ng/mL	Yes, 7%; no, 22%	0.81
DCP ≥100 mAU/L	Yes, 0%; no, 14%	0.85
Histological cirrhosis (+)	Yes, 0%; no, 19%	0.18

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; cancer spread, pathological cancer spread including portal vein invasion and intrahepatic metastasis; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; ICG-R15, the indocyanine green dye retention rate at 15 minutes; T-bil, total bilirubin; VP, macroscopic portal vein tumor thrombus.

more than 2 cm to less than 5 cm, 54 (68.4%) had tumor recurrence. The rates of solitary liver recurrence, multiple liver recurrence and distant recurrence were 53.2%, 11.4% and 3.8%, respectively. In patients with HCC of 5 cm or more, 36 patients (75.0%) had tumor recurrence. The rates of multiple liver recurrence (37.5%) and distant recurrence (20.8%) were significantly higher in this group ($P = 0.02$ and $P = 0.04$, respectively).

Duration of recurrence according to the primary HC-HCC size

Table 9 summarizes the durations of recurrence, such as within 1 year (<1 year; early recurrence) or after 1 year (≥1 year; late recurrence) in each group. In patients with HCC of 2 cm or less, 32 patients (57.0%) had late recurrence, a significantly higher rate ($P = 0.04$). In patients with HCC of more than 2 cm to less than 5 cm, 13

Table 7 Independent risk factors for recurrence: HCC ≥5 cm

Variables	Hazard ratio	95% CI	*P*-value
VP (+)	2.8	1.52–7.41	0.02
AST/ALT ≥80 IU/L	2.1	1.04–5.34	0.04
ICG-R15 ≥20%	1.6	0.21–1.74	0.28
Limited resection	1.3	0.47–1.57	0.53

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; HCC, hepatocellular carcinoma; ICG-R15, the indocyanine green dye retention rate at 15 minutes; VP, macroscopic portal vein tumor thrombus.