Serosal Invasion Strongly Associated With Recurrence After Curative Hepatic Resection of Hepatocellular Carcinoma

A Retrospective Study of 214 Consecutive Cases

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Abstract: The purpose of this study was to clarify the individual prognostic factors after curative and primary resection of hepatocellular carcinoma (HCC).

Reliable prognostic factors and tumor staging for HCC have been required to predict an appropriate prognosis. However, in HCC, no staging system has received universal acceptance, and several tumor factors seem to relate to HCC prognosis, but they are not definitive. At present, few studies have mentioned the importance of serosal invasion as a prognostic factor.

A retrospective search of our database identified 214 consecutive patients who underwent primary and curative hepatectomy for HCC at our department between January 1998 and December 2011. Risk factors for recurrence-free survival (RFS) and overall survival (OS) were analyzed with Cox proportional hazard model, Kaplan-Meier method, and log-rank tests.

Multivariate analyses showed that serosal invasion (hazard ratio [HR], 2.75; $P = 0.0005$) and vascular invasion (HR, 1.71; $P = 0.0331$) were independently correlated with RFS. Serosal invasion was significantly correlated with HCC recurrence ($P = 0.0230$). The Kaplan–Meier method and log-rank tests revealed that the patients with serosal invasion showed significantly worse prognosis both in RFS ($P < 0.0001$) and OS ($P = 0.0016$).

Serosal invasion should be regarded as a strong independent predictor for recurrence in curatively resected HCC cases.

(*Medicine* 94(9):e602)

Abbreviations: AFP = alpha fetoprotein, CT = computed tomography, DCP = des-gamma-carboxy prothrombin, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HR = hazard ratio, ICG = indocyanine green, ICG-R15 = ICG-15-min retention rates, OS = overall survival, RFS = recurrence-free survival, TNM = tumor-node-metastasis, UICC = Union for International Cancer Control.

INTRODUCTION

Hepatocellular carcinoma (HCC) represents the fifth most common malignancy and the third most common cause of cancer-related death worldwide.[1,2] Hepatic resection is still a major treatment for patients with HCC.[3–5] However, even after curative resection, 80% of patients develop intrahepatic recurrence and 50% die within 5 years.[6]

Tumor staging at the time of pathological diagnosis is needed to determine the patients' overall survival (OS) probability after treatment, decide the most appropriate type of therapy, and enable an objective comparison among the outcomes of research studies. Furthermore, it allows us to predict the prognosis of resected cancer cases. For these reasons, staging systems should separate patients into groups with homogeneous prognosis, and serve to select appropriate treatment.[7] The Union for International Cancer Control (UICC) tumor-node-metastasis (TNM) staging system incorporates tumor size, number of tumor nodules, and vascular invasion in the HCC classification. In general, the TNM staging system is the main outcome predictor for various neoplasms, but prognostic modeling in HCC is more complex. There are some classifications of HCC such as the Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire Prognostic classification,[8] Cancer of the Liver Italian Program,[9] Barcelona-clinic Liver Cancer staging,[10] the Chinese University Prognostic Index,[11] and staging according to the guidelines of the Liver Cancer Study Group of Japan.[12] However, none of these classification systems have received universal acceptance.[13] One of the reasons why HCC staging is difficult is its characteristic recurrence pattern: intrahepatic metastasis and multicentric occurrence. Therefore, prognostic factors for HCC should be related to both the above-mentioned recurrence patterns.

The risk factors for postoperative recurrence and OS after resection of HCC can be categorized into tumor risk factors and background risk factors. Tumor-related factors mainly include pathological information such as tumor differentiation, growth form, capsule formation, capsule infiltration, septal formation, serosal invasion, and vascular invasion. Among those tumor-related factors, vascular invasion is the most consistently reported risk factor for recurrence after resection of HCC.[14–16] In some studies, it was the single most important factor for HCC recurrence.[17–19] The effects of other tumor pathologic features such as tumor encapsulation, serosal invasion, and tumor differentiation on the risk of recurrence are less conclusive. Some studies reported that tumor encapsulation was associated with a reduced incidence of local venous invasion and direct invasion of adjacent liver tissue.[18,20,21] However, another study found that the presence of a tumor capsule was a strong predictor of vascular invasion.[22] Regarding tumor

differentiation, one study demonstrated a significantly higher recurrence rate with poorly differentiated HCC,[23] whereas other studies showed that tumor differentiation did not have a significant impact on the risk of recurrence.[24,25] Few studies have analyzed serosal invasion as a prognostic factor in HCC cases after hepatic resection.[26] To date, there is no consensus on the tumor prognostic risk factors associated with HCC except for vascular invasion.

The purpose of this study was to clarify the individual prognostic risk factors after primary and curative resection of HCC by conducting a search of consecutive resected cases from our institution.

METHODS

In a search of our database, 254 consecutive patients who underwent primary and curative hepatectomy for HCC at the Department of Gastroenterological Surgery, Nagoya University Graduate School of Medicine between January 1998 and December 2011 were identified. Written informed consent, as required by the Institutional Review Board, was obtained from all patients to use the anonymized information. The cases with inappropriate information were excluded, and 214 cases were analyzed in this retrospective study.

Preoperative blood samples were drawn 1 or 2 days prior to surgery. Serum albumin concentrations were measured, as well as white blood cell, neutrophil, lymphocyte, and platelet counts. Preoperative total bilirubin, alpha fetoprotein (AFP), des-gamma-carboxy prothrombin (DCP), hepatitis C virus (HCV) antibody, and hepatitis B surface antigen concentrations were also measured, as well as prothrombin time. Indocyanine green (ICG) testing was usually performed ahead of surgery, and 15-min retention rates (ICG R15) and the disappearance rate of ICG were calculated. Other host-related variables included age, sex, Child–Pugh classification, and liver damage score.

Indications for hepatectomy and extent of hepatic resection were based on the size, number and location of tumors, presence of ascites, serum albumin and total bilirubin concentrations, prothrombin time, computed tomography (CT) volumetry findings, and results of the ICG test.

In our department, livers were usually dissected using a CUSA system (Valley Lab, Boulder, CO, USA) and VIO soft-coagulation system (ERBE Elektromedizin, Tübingen, Germany) used since 2007. Sometimes, particularly in the patients with liver cirrhosis, precoagulation was performed on the resection line with a microwave coagulating apparatus to avoid excess bleeding until the early 2000s. Many patients underwent surgery using the intermittent Pringle maneuver, clamping the portal triad for 15 min each at 5-min intervals. In appropriate cases, the liver hanging maneuver[27] and the Glissonean pedicle transection method[28] were performed both respectively and jointly. Resection was defined as curative when all gross tumors were completely removed; cases of incidentally found small lesions—suspected to be HCC—that were treated by radiofrequency therapy or microwave coagulation therapy during the hepatic resection were regarded as curative cases (n = 5).

After discharge, patients were followed up once per month for 3 months and every 3 months thereafter. Blood tests, including those for serum AFP and DCP, were performed at every outpatient care visit, and dynamic contrast-enhanced CT was performed every 6 months. Patients with abnormal data or suspected lesions underwent further examinations, including contrast ultrasonography, magnetic resonance imaging with proper contrast media such as gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid, CT with hepatic arterioportography, and/or positron emission tomography for the diagnosis of HCC recurrence. The recurrence-free survival (RFS) was defined as the time between the curative resection of HCC and confirmation of recurrence. OS was defined as the time between the operation and all-cause death.

Surgery-related variables included operation time, estimated intraoperative blood loss, and requirement for intraoperative blood transfusion. Tumor-related variables included tumor number and size, and postoperative pathological variables (tumor differentiation, serosal invasion, capsule formation, capsule infiltration, septal formation, vascular invasion, bile duct invasion, and surgical margin). Serosal invasion was histologically defined as infiltration into visceral peritoneum including perforation of visceral peritoneum and vascular invasion included either portal vein invasion or hepatic vein invasion. Tumors were categorized as well/moderately or poorly differentiated, whereas the other pathological variables were categorized as positive or negative, as described by the guidelines of the Liver Cancer Study Group of Japan.[12]

All statistical analyses were performed using JMP Pro software version 11.0.0 (SAS International Inc, Cary, NC). Continuous variables were expressed as medians (ranges) and compared using the Wilcoxon rank sum test, and categorical variables were compared using the chi-square test or Fisher exact test, as appropriate. Univariate and multivariate Cox proportional hazards models were used to determine the independent risk factors associated with the RFS and OS. RFS and OS associated with each individual risk factor elucidated by multivariate analysis were also analyzed by the Kaplan–Meier method and log-rank tests. The level of statistical significance was set at $P < 0.05$.

RESULTS

Patient demographic and clinical characteristics are shown in Table 1. The 214 patients with HCC included 171 men (79.9%) and 43 women (20.1%). The median age of the patients was 65 years (range, 33–80 years). The distribution of pathologic Japanese stage was I: 19 (8.9%) cases, II: 115 (53.7%) cases, III: 55 (25.7%) cases, and IV (IVa): 24 (11.2%) cases. The median patient follow-up time was 49.5 months (range, 0.3–193.8 months). At the end of the follow-up period, 104 (48.6%) patients had died, and the median duration from the time of surgery to death in these cases was 34.6 months (range, 0.3–154.9 months). The number of patients who died within 30 days of their surgery was 4 (1.87%), and there was no case of intraoperative patient death. The cause of death included HCC recurrence (n = 37, 35.6%), liver failure (n = 15, 14.4%), other neoplasms (n = 6, 5.8%), rupture of esophageal varices (n = 3, 2.9%), heart disease (n = 3, 2.9%), pneumonia (n = 2, 1.9%), cerebrovascular disease (n = 2, 1.9%), and unknown disease (n = 36, 34.6%). Tumor recurrence occurred in 140 (65.4%) patients, and the median time to recurrence was 17.4 months (range, 0.6–137.9 months).

Significant correlations were identified between HCC recurrence and virus infection (HCV or others, $P = 0.0115$), tumor size (≥ 2 or < 2 cm, $P = 0.0155$), formation of capsule ($P = 0.0123$), and serosal invasion ($P = 0.0230$). In addition, the presence of liver cirrhosis seemed to be associated with tumor recurrence; however, the correlation was not statistically significant ($P = 0.0533$) (Table 2).

TABLE 1. Characteristics of 214 Patients With Hepatocellular Carcinoma

Characteristics	Value
Age (years)	65 (33–80)
Sex, men:women	171:43
Viral infection, HBV:HCV:non HBV/HCV	67:115:37
Child–Pugh classification, A:B	201:13
Liver damage classification, A:B:C	150:37:1
Albumin (mg/dL)	3.9 (2–4.9)
Total bilirubin (mg/dL)	0.7 (0.2–7.3)
Prothrombin time (%)	89.1 (46.9–138)
Alpha fetoprotein (ng/mL)	19 (0.8–222228)
Operation time (min)	306 (100–792)
Estimated blood loss (mL)	779 (13–17090)
Intraoperative transfusion, +:−	52:149
Tumor size (cm)	3.5 (0.08–15)
Tumor number, solitary:multiple	159:55
ICG R15 (%)	11.9 (1.6–35.2)
Japanese stage, I:II:III:IV	19:115:55:24

HBV = hepatitis B virus, HCV = hepatitis C virus, ICG R15 = indocyanine green 15-min retention rate.

TABLE 2. Clinicopathological Findings in Patients With Hepatocellular Carcinoma According to Recurrence

Clinicopathological Factor	Recurrence		
	(+)	(−)	*P*
Age, y			0.6907
≥65	74	37	
<65	66	37	
Sex			0.1384
Male	116	55	
Female	24	19	
Virus infection			**0.0115**
HCV	84	31	
Others	56	43	
Albumin			0.5820
<3.5	31	14	
≥3.5	109	60	
PT, %			0.7729
<70	17	8	
≥70	123	66	
ICG R15, %			0.0765
≥15	33	10	
<15	57	36	
Liver cirrhosis			0.0533
(+)	60	22	
(−)	77	51	
Child–Pugh			0.7182
B	9	4	
A	131	70	
Liver damage			0.4142
B or C	27	11	
A	96	54	
Tumor number			0.7376
Multiple	37	18	
Solitary	103	56	
Tumor size, cm			**0.0155**
≥2	124	54	
<2	13	15	
AFP, ng/mL			0.9714
≥20	63	34	
<20	66	36	
Differentiation			0.1409
Poor	11	2	
Well/moderate	126	69	
Growth form			0.5602
Infiltrative	23	10	
Expansive	116	64	
Formation of capsule			**0.0123**
(−)	37	32	
(+)	103	42	
Infiltration to capsule			0.3323
(+)	75	34	
(−)	65	39	
Septal formation			0.5570
(−)	54	25	
(+)	85	47	
Serosal invasion			**0.0230**
(+)	38	10	
(−)	102	64	
Portal vein or hepatic vein invasion			0.0823
(+)	44	15	
(−)	96	59	
Surgical margin			0.8952
(+)	16	9	
(−)	115	61	
Japanese stage			0.2597
III/IV	56	24	
I/II	83	50	

Chi-square or Fisher exact test was applied as appropriate. Significant *P* values are bolded. AFP = alpha fetoprotein, HCV = hepatitis C virus, ICG R15 = indocyanine green 15-min retention rate, PT = prothrombin time.

We investigated the relationship between 28 clinical factors and RFS by univariate analyses (Table 3). Ten factors were associated with RFS, such as HCV infection, serum albumin level (<3.5 vs ≥3.5 mg/dL), ICG R15 (≥15 vs <15 %), liver cirrhosis, liver damage score (B or C vs A), tumor number (multiple vs solitary), tumor size (≥2 vs <2 cm), serosal invasion, vascular invasion, and Japanese stage (III/IV vs I/II). Next, we investigated the relationship between 28 clinical factors and OS (Table 4). Univariate analyses showed that 11 factors such as age (≥65 vs <65 years), HCV infection, serum albumin level, ICG R15, liver damage score, tumor number, serum AFP level, tumor differentiation, serosal invasion, vascular invasion, and Japanese stage were associated with OS.

Multivariate analysis of 8 factors that were found to be significantly correlated with RFS on univariate analysis, except for liver damage score and Japanese stage, was performed; serosal invasion (hazard ratio [HR], 2.75; $P = 0.0005$) and vascular invasion (HR, 1.71; $P = 0.0331$) were independently correlated with RFS (Table 3). Multivariate analysis of 9 factors that were found to be significantly correlated with OS on univariate analysis, except for liver damage score and Japanese stage, was also performed; HCV infection, tumor differentiation, and vascular invasion were independently correlated with OS (Table 4). According to these results, serosal invasion can be considered an individual risk factor related to recurrence after curative resection of HCC.

Kaplan–Meier analysis revealed that the patients with serosal invasion ($n = 48$, 22.4%) showed significantly worse RFS than those without serosal invasion ($n = 166$, 77.6%) ($P < 0.0001$) (Figure 1A). Furthermore, serosal invasion was significantly associated with worse OS ($P = 0.0016$) (Figure 1B).

The distributions of clinicopathological findings, excluding serosal invasion, were investigated according to the presence or absence of serosal invasion (see Supplemental Table 1, http://links.lww.com/MD/A220). Tumor size ($P = 0.0193$), vascular invasion ($P = 0.0131$), and Japanese stage ($P = 0.0182$) showed significant correlations with serosal invasion. In addition, the tumors with serosal invasion were significantly larger than tumors without serosal invasion ($P = 0.0008$) (see Supplemental Figure, http://links.lww.com/MD/A220, which

TABLE 3. Univariate and Multivariate Analysis of Recurrence-free Survival

Clinicopathological Factor		Univariate Analysis			Multivariate Analysis		
		HR	95% CI	*P*	HR	95% CI	*P*
Age, y	≥65 vs <65	1.24	0.91−1.70	0.1585			
Sex	Men vs women	1.33	0.90−2.03	0.1439			
Virus infection	HCV vs others	1.51	1.10−2.08	**0.0096**	1.08	0.70−1.68	0.7088
Albumin, mg/dL	<3.5 vs ≥3.5	1.86	1.26−2.68	**0.0019**	1.39	0.79−2.37	0.2413
PT, %	<70 vs ≥70	1.10	0.67−1.71	0.6712			
ICG R15, %	≥15 vs <15	2.13	1.40−3.21	**0.0005**	1.62	0.97−2.65	0.0629
Liver cirrhosis	(+) vs (−)	1.38	1.00−1.89	**0.0434**	1.46	0.95−2.23	0.0772
Child−Pugh	B vs A	1.28	0.67−2.23	0.4226			
Liver damage	B or C vs A	2.04	1.37−2.97	**0.0006**			
Tumor number	Multiple vs solitary	1.74	1.22−2.45	**0.0023**	1.22	0.71−2.02	0.4428
Tumor size, cm	≥2 vs <2	1.75	1.07−3.06	**0.0221**	0.93	0.50−1.83	0.8270
AFP, ng/mL	≥20 vs <20	1.24	0.90−1.71	0.1849			
Differentiation	Poor vs well/moderate	1.74	0.88−3.07	0.1001			
Growth form	Infiltrative vs expansive	1.08	0.69−1.64	0.7023			
Formation of capsule	(−) vs (+)	0.76	0.53−1.07	0.1261			
Infiltration to capsule	(+) vs (−)	1.17	0.86−1.60	0.3094			
Septal formation	(−) vs (+)	1.00	0.72−1.38	0.9645			
Serosal invasion	(+) vs (−)	2.28	1.58−3.24	**<0.0001**	2.75	1.57−4.72	**0.0005**
Portal vein or hepatic vein invasion	(+) vs (−)	1.93	1.37−2.69	**0.0002**	1.71	1.04−2.76	**0.0331**
Surgical margin	(+) vs (−)	1.13	0.71−1.89	0.6001			
Japanese stage	III/IV vs I/II	1.47	1.07−2.02	**0.0172**			

A multivariate Cox proportional hazard model was used to investigate independent risk factors of recurrence-free survival. Significant *P* values are bolded. AFP = alpha fetoprotein, CI = confidence interval, HCV = hepatitis C virus, HR = hazard ratio, ICG R15 = indocyanine green 15-min retention rate, PT = prothrombin time.

shows the comparison of tumor sizes between tumors with [+] and [−] serosal invasion).

Among the 48 serosal invasion cases, 38 patients (79.2%) presented recurrence. The most dominant site of recurrence was the remnant liver, followed by the bone, lung, peritoneum, adrenal gland, lymph nodes, and pleura (Table 5).

DISCUSSION

A major obstacle for the treatment for HCC is the high frequency of tumor recurrence even after curative resection and liver transplantation.[29] The postoperative prognosis for HCC is still unsatisfactory because of recurrence in the remnant liver.

TABLE 4. Univariate and Multivariate Analysis of Overall Survival

Clinicopathological Factor		Univariate Analysis			Multivariate Analysis		
		HR	95% CI	*P*	HR	95% CI	*P*
Age, y	≥65 vs <65	1.72	1.16−2.58	**0.0060**	1.43	0.79−2.60	0.2304
Sex	Men vs women	1.17	0.73−1.96	0.5074			
Virus infection	HCV vs others	1.77	1.19−2.68	**0.0044**	1.76	1.00−3.20	**0.0495**
Albumin, mg/dL	<3.5 vs ≥3.5	0.80	1.12−2.79	**0.0145**	1.04	0.50−2.05	0.9068
PT, %	<70 vs ≥70	1.41	0.81−2.33	0.2098			
ICG R15, %	≥15 vs <15	2.24	1.32−3.78	**0.0030**	1.85	0.99−3.40	0.0536
Liver cirrhosis	(+) vs (−)	1.43	0.96−2.11	0.0732			
Child−Pugh	B vs A	1.48	0.72−2.72	0.2612			
Liver damage	B or C vs A	2.36	1.47−3.68	**0.0005**			
Tumor number	Multiple vs solitary	1.79	1.16−2.68	**0.0082**	1.83	0.96−3.32	0.0615
Tumor size, cm	≥2 vs <2	1.59	0.85−3.41	0.1526			
AFP, ng/mL	≥20 vs <20	1.66	1.11−2.50	**0.0131**	1.61	0.89−2.91	0.1127
Differentiation	Poor vs well/moderate	2.37	1.10−4.47	**0.0280**	2.79	1.05−6.75	**0.0398**
Growth form	Infiltrative vs expansive	1.06	0.61−1.74	0.8175			
Formation of capsule	(−) vs (+)	0.95	0.61−1.44	0.8236			
Infiltration to capsule	(+) vs (−)	1.00	0.67−1.48	0.9915			
Septal formation	(−) vs (+)	1.14	0.76−1.69	0.5081			
Serosal invasion	(+) vs (−)	1.96	1.26−2.98	**0.0032**	1.56	0.77−3.01	0.2047
Portal vein or hepatic vein invasion	(+) vs (−)	2.28	1.47−3.45	**0.0004**	2.76	1.50−4.98	**0.0014**
Surgical margin	(+) vs (−)	1.37	0.76−2.31	0.2710			
Japanese stage	III/IV vs I/II	1.54	1.03−2.27	**0.0335**			

A multivariate Cox proportional hazard model was used to investigate independent risk factors of overall free survival. Significant *P* values are bolded. AFP = alpha fetoprotein, CI = confidence interval, HCV = hepatitis C virus, HR = hazard ratio, ICG R15 = indocyanine green 15-min retention rate, PT = prothrombin time.
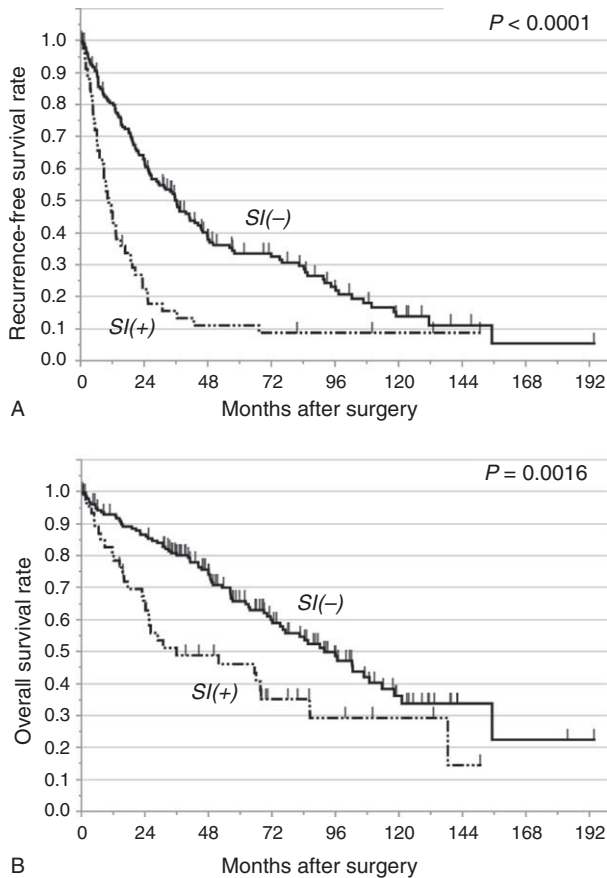
FIGURE 1. RFS (A) and OS (B) of 214 patients after hepatic resection of hepatocellular carcinoma, according to the presence of serosal invasion. The Kaplan–Meier method and log-rank tests show that patients with evidence of serosal invasion had significantly worse RFS and OS. OS = overall survival, RFS = recurrence-free survival, SI = serosal invasion.

The poor prognosis of this disease is associated with both tumor factors and background nontumorous liver factors. Tumor factors include tumor invasiveness such as differentiation, size, growth form, and serosal invasion. Background non-tumorous liver factors include viral infection, liver cirrhosis, and age, among others. According to the general rules for the clinical and pathological study of primary liver cancer—published by the

TABLE 5. Site of Recurrence in 38 Hepatocellular Carcinoma Patients With Serosal Invasion

Site of Recurrence	N	%
Remnant liver	31	68.9
Bone	5	11.1
Lung	5	11.1
Peritoneum	1	2.2
Adrenal grand	1	2.2
Lymph nodes	1	2.2
Pleura	1	2.2

Percentages were calculated as (number of recurrence tumors in a specific site)/(total number of recurrence tumors). The total number of recurrent tumors was 45.

Liver Cancer Study Group of Japan—one of the tumor factors that should be considered after hepatic resection is serosal invasion.[12] However, few reports mention the importance and effect of serosal invasion in HCC.

Uenishi et al[30] showed no survival impact after hepatic resection for mass-forming intrahepatic cholangiocarcinoma, which is one of the components of primary liver neoplasms. In contrast to cholangiocarcinoma, our study showed the effect of serosal invasion in RFS and OS among HCC patients. However, several studies describe vascular invasion as a risk factor associated with recurrence and survival, and the UICC TNM and Japanese staging system consider it a determinant factor. In this study, multivariate analyses revealed that serosal invasion—aside from vascular invasion—was one of the independent risk factors associated with RFS. The current UICC TNM classification (7th edition) defines T4 as tumors with direct invasion to the adjacent organs other than the gall bladder or with perforation of the visceral peritoneum. However, it does not describe the method for treating microscopic serosal invasion. The definition of T4 according to the Japanese staging system has been presented in the supplemental table (see Supplemental Table 2, http://links.lww.com/MD/A220, which shows the definition of the T factor as per the Japanese staging system).[12] This system defines tumor rupture as the perforation of the visceral peritoneum with intraperitoneal bleeding. Thus, serosal invasion is not a sufficient condition for T4 staging according to both the UICC TNM staging and Japanese staging systems. If further evidence is obtained, serosal invasion may be worth considering in tumor staging after HCC resection.

The distribution of cases with serosal invasion was significantly correlated with tumor size, vascular invasion, and Japanese stage, as shown in the Supplemental Table 1, http://links.lww.com/MD/A220. The Japanese staging system seems to be affected by tumor size, although the tumor size was not an independent factor for RFS in the present study. A tumor located deep in the liver would need to have a large size to reach the serosal area, which may be one of the reasons for the correlation of serosal invasion with tumor size. However, this large size does not appear to be sufficient to have a greater influence on HCC recurrence, as indicated in our analysis. The correlation of serosal invasion with vascular invasion may depend on the invasive characteristics of the tumor with serosal invasion.

Although serosal invasion seems to be associated with peritoneal recurrence, it is not, and rather, it is associated with remnant liver recurrence. The subserosal lymphatic cycle may be one of the hypothetical mechanisms of this phenomenon. The hepatic serosa is composed of single-layered flat mesothelium and lower connective tissue, which is considerably thick ($40–70\,\mu m$) and protects the liver[31]; this tissue is also called Glisson capsule.[32] The subserosal tissue continues into the liver parenchyma and separates the hepatic lobules. There are numerous descriptions of anastomoses between the superficial and the deep lymph vessels, between portal and venous lymph vessels, between lymph vessels of the liver and those of the gall bladder, and also between lymph vessels and intrahepatic branches of the portal vein.[33,34] Lymphatic vessels are abundant in the immediate vicinity of the HCC and liver metastasis.[35] HCCs expressing vascular endothelial growth factor-C are more likely to metastasize, indicating that lymphangiogenesis is associated with their enhanced metastasis.[35] Therefore, when the tumor tissue reaches the serosa, metastases via lymphatic vessels seems to occur more easily. This might be one of the reasons why HCC patients with serosal invasion showed a worse prognosis in the

present study. We postulated that serosal invasion would be associated with dissemination into the peritoneum cavity. In fact, in the present study, the recurrence in HCC patients in whom the primary lesions exhibited serosal invasion, predominantly occurred in their remnant liver. This finding supports the hypothesis of the indirect association between the subserosal lymphatic cycle and serosal invasion. However, particularly in the case of liver cirrhosis, high pressure in the lymphatic vessels seems to disturb this putative way of metastasis; thus, further studies are needed to explain this.

This study has several limitations, including its retrospective, single-institution design. Therefore, the dynamics of metastasis via superficial hepatic lymphatics should be shown by further histological study.

In conclusion, this study showed that serosal invasion was one of the strongest prognostic factors associated with recurrence after curative resection of HCC. Such cases should be observed carefully and might require adjuvant therapy if it is applicable in the future.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. *CA Cancer J Clin.* 2005;55:74–108.

2. Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer.* 2010;127:2893–2917.

3. Rahbari NN, Mehrabi A, Mollberg NM, et al. Hepatocellular carcinoma: current management and perspectives for the future. *Ann Surg.* 2011;253:453–469.

4. Kobayashi A, Kawasaki S, Miyagawa S, et al. Results of 404 hepatic resections including 80 repeat hepatectomies for hepatocellular carcinoma. *Hepatogastroenterology.* 2006;53:736–741.

5. Chen MS, Li JQ, Zheng Y, et al. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg.* 2006;243:321–328.

6. Taura K, Ikai I, Hatano E, et al. Implication of frequent local ablation therapy for intrahepatic recurrence in prolonged survival of patients with hepatocellular carcinoma undergoing hepatic resection: an analysis of 610 patients over 16 years old. *Ann Surg.* 2006;244:265–273.

7. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet.* 2003;362:1907–1917.

8. Chevret S, Trinchet JC, Mathieu D, et al. A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. Groupe d'Etude et de Traitement du Carcinome Hepatocellulaire. *Journal of hepatology.* 1999;31:133–141.

9. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. *Hepatology.* 1998;28:751–755.

10. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis.* 1999;19:329–338.

11. Leung TW, Tang AM, Zee B, et al. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program staging system: a study based on 926 patients. *Cancer.* 2002;94:1760–1769.

12. Japan lcsgo. General Rules for the Clinical and Pathological Study of Primary Liver Cancer. 3rd English ed. Tokyo: Kanehara and Co; 2010.

13. Faria SC, Szklaruk J, Kaseb AO, et al. TNM/Okuda/Barcelona/UNOS/CLIP International Multidisciplinary Classification of Hepatocellular Carcinoma: concepts, perspectives, and radiologic implications. *Abdom Imag.* 2014;39:1070–1087.

14. Kosuge T, Makuuchi M, Takayama T, et al. Long-term results after resection of hepatocellular carcinoma: experience of 480 cases. *Hepato-gastroenterology.* 1993;40:328–332.

15. Izumi R, Shimizu K, Ii T, et al. Prognostic factors of hepatocellular-carcinoma in patients undergoing hepatic resection. *Gastroenterology.* 1994;106:720–727.

16. Hanazaki K, Kajikawa S, Koide N, et al. Prognostic factors after hepatic resection for hepatocellular carcinoma with hepatitis C viral infection: univariate and multivariate analysis. *Am J Gastroenterol.* 2001;96:1243–1250.

17. Vauthey JN, Klimstra D, Franceschi D, et al. Factors affecting long-term outcome after hepatic resection for hepatocellular carcinoma. *Am J Surg.* 1995;169:28–34discussion 34–25.

18. Nagasue N, Uchida M, Makino Y, et al. Incidence and factors associated with intrahepatic recurrence following resection of hepatocellular carcinoma. *Gastroenterology.* 1993;105:488–494.

19. Yamamoto J, Kosuge T, Takayama T, et al. Recurrence of hepatocellular carcinoma after surgery. *Brit J Surg.* 1996;83:1219–1222.

20. Ng IO, Lai EC, Ng MM, et al. Tumor encapsulation in hepatocellular carcinoma. A pathologic study of 189 cases. *Cancer.* 1992;70:45–49.

21. Nzeako UC, Goodman ZD, Ishak KG. Hepatocellular carcinoma in cirrhotic and noncirrhotic livers. A clinico-histopathologic study of 804 North American patients. *Am J Clin Pathol.* 1996;105:65–75.

22. Adachi E, Maeda T, Kajiyama K, et al. Factors correlated with portal venous invasion by hepatocellular carcinoma: univariate and multivariate analyses of 232 resected cases without preoperative treatments. *Cancer.* 1996;77:2022–2031.

23. Ikeda K, Saitoh S, Tsubota A, et al. Risk factors for tumor recurrence and prognosis after curative resection of hepatocellular carcinoma. *Cancer.* 1993;71:19–25.

24. Adachi E, Maeda T, Matsumata T, et al. Risk factors for intrahepatic recurrence in human small hepatocellular carcinoma. *Gastroenterology.* 1995;108:768–775.

25. Shirabe K, Matsumata T, Adachi E, et al. Prognosis of well differentiated small hepatocellular carcinoma–is well differentiated hepatocellular carcinoma clinically early cancer? *Hepatogastroenterology.* 1995;42:923–930.

26. el-Assal ON, Yamanoi A, Soda Y, et al. Proposal of invasiveness score to predict recurrence and survival after curative hepatic resection for hepatocellular carcinoma. *Surgery.* 1997;122:571–577.

27. Belghiti J, Guevara OA, Noun R, et al. Liver hanging maneuver: a safe approach to right hepatectomy without liver mobilization. *J Am Coll Surg.* 2001;193:109–111.

28. Takasaki K. Glissonean pedicle transection method for hepatic resection: a new concept of liver segmentation. *J Hepatobiliary Pancreat Surg.* 1998;5:286–291.

29. Ha TY, Hwang S, Moon DB, et al. Long-term survival analysis of liver transplantation for hepatocellular carcinoma with bile duct tumor thrombus. *Transplant Proc.* 2014;46:774–777.

30. Uenishi T, Yamazaki O, Yamamoto T, et al. Serosal invasion in TNM staging of mass-forming intrahepatic cholangiocarcinoma. *J Hepatobiliary Pancreat Surg.* 2005;12:479–483.

31. Burt AD, Portmann B, Ferrell L. MacSween's Pathology of the Liver. 6th ed. Elsevier Churchill Livingstone; 2011.

32. Young B, Woodford P, O'Dowd G, et al. Wheater's Functional Histology: A Text and Colour Atlas. 6th ed. Elsevier; 2013.

33. Trutmann M, Sasse D. The lymphatics of the liver. *Anat Embryol.* 1994;190:201–209.

34. Szabo G, Magyar Z, Jakab F. The lymphatic drainage of the liver capsule and hepatic parenchyma. *Res Exp Med.* 1975;166:193–200.

35. Ohtani O, Ohtani Y. Lymph circulation in the liver. *Anat Rec.* 2008;291:643–652.