Predictor for Histological Microvascular Invasion of Hepatocellualr Carcinoma: A Lesson from 229 Consecutive Cases of Curative Liver Resection

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Abstract

Background Microscopic vascular invasion is an important risk factor for recurrent hepatocellular carcinoma (HCC), even after curative liver resection or orthotopic liver transplantation. To predict microscopic portal venous invasion, the following two questions were examined retrospectively: 1. Is it possible to detect microvascular invasion preoperatively? and 2. What are characteristics of group of early recurrence of HCC even with no microvascular invasion?

Methods Study 1: Of 229 patients with HCC who underwent curative liver resection between 1991 and 2008, 127 had HCC without microscopic portal venous invasion, while 52 had HCC with microscopic portal venous invasion. These two distinct groups were analyzed with regard to various clinicopathological factors. Subsequently, we specifically investigated if HCCs less than 5cm with vascular invasion (n=32) have some characteristics in order to detect latent microvascular invasion.

Study 2: Of the 127 HCC patients without MVPI, 42 had recurrence within 2 years, while 85 patients were recurrence-free for at least 2 years. These two distinct groups were analyzed with regard to various clinicopathological factors.

Results Diameter of HCC more than 5 cm, the macroscopic appearance of HCC and high levels of preoperative des-gamma-carboxy prothrombin (DCP) are significant

prognostic factors in identifying microvascular invasion in HCC. The strongest predictor of early recurrence within 2 years.was serum levels of alpha-fetoprotein (AFP) in patients without clear microvascular invasion.

Conclusions Tumor size, macroscopic appearance and high tumor markers are important elements in identifying the group of patients with a low recurrence rate of HCC after curative liver resection.

Introduction

Microvascular invasion is a very strong prognostic factor for hepatocellular carcinoma (HCC), even after curative liver resection. Even after orthotopic liver transplantation (OLT), which is the ultimate removal of malignant tumors, microvascular invasion remains a significant prognostic factor since HCC becomes systemic through invasion to peripheral portal or hepatic veins and subsequent spread [1-3]. Therefore, vascular invasion has always been included as an indication of OLT, including in the Milan criteria [4]. Macrovascular invasion to the 1st and 2nd ramifications of the portal vein can be diagnosed by computed tomography or other imaging techniques, however, microvascular invasion to minute peripheral areas, such as the 3rd branch of the portal vein, is difficult to detect with current imaging modalities. Therefore, to predict the outcome of liver transplantation or curative liver resection more accurately, it is necessary to identify factors that indicate or predict the microvascular invasion of HCC.

We performed 229 curative liver resections for HCC during a period of 17 years. We carried out a retrospective analysis in order to identify factors that can predict the recurrence of HCC using histological findings of HCC in the resected liver.

Patients and Methods

Between 1991 and 2008, 229 curative liver resections were performed in the Department of Surgery of Nagasaki University Hospital, Nagasaki, Japan. Of 229 HCCs, 50 HCCs had vascular invasion up to 2nd branches of the portal vein. Therefore, remaining 179 patients were analyzed in the study. The average age of the patients was 65 years old (range 20-85). There were 143 males and 36 females, and the median follow-up period was 45.5 months.

The macroscopic appearance of HCC was classified into 4 types: type 1 (single nodular type), type 2 (single nodular type with extranodular growth), type 3 (contiguous multinodular type formed by a cluster of small and contiguous nodules) and type 4 (infiltrative type) [5]. As tumor markers, serum levels of alpha-fetoprotein (AFP), and des-gamma-carboxy prothrombin (DCP) were measured. We conducted two distinct retrospective studies.

Study 1

To identify factors for detecting microscopic portal venous invasion preoperatively, we examined 179 of our 229 patients excluding the patients with vascular invasion to the 1st and/or 2nd branches of the portal vein. Of these 179 patients, 127 had no

histologically proven microscopic portal venous invasion, hepatic venous invasion or intrahepatic metastasis, while the remaining 52 had microscopic vascular invasion regardless of the presence or absence of intrahepatic metastasis of HCC. Subsequently, after we learned that larger tumor size became than 5cm, more microvascular invasion occurred, we were interested in whether HCCs less than 5cm with microvascular have some characteristics in order to detect latent microvascular. When we limited our examination to patients with a single HCC lesion of less than 5 cm in diameter, we had 102 patients without and 31 patients with microvascular invasion.

Study 2

This study was performed to identify the group of patients who suffered early recurrence, which was defined as recurrence within 2 years, even though they showed no microscopic portal venous invasion. Of the 127 patients without proven microvascular invasion, 42 suffered early recurrence and 85 experienced recurrence after 2 years. These two distinct groups were analyzed with regard to various clinicopathological factors. For this study, necroinflammatory activity (Grading) and the degree of fibrosis (Staging) determined by Knodell were calculated by routine histological examination [6].

Statistical analysis

All analyses were conducted with Stat-View. Univariate analysis was performed using the Pearson chi-square test for categorical factors and the Mann-Whitney test for numerical values. Multivariate analysis was conducted with logistic regression model. Odds ratios (OR) and the corresponding 95% confidence interval (CI) were computed to assess the strength of association. *P* values of less than 0.05 were considered to be statistically significant.

Results

Study 1

Univariate analysis revealed that the size of the HCC, the number of HCC lesions, the macroscopic appearance of HCC, and tumor markers (AFP, DCP) had a significant predictive value (Table 1). Multivariate logistic regression analysis revealed size of HCC and macroscopic appearance of HCC as a significant independent risk factor for microvascular invasion of HCC (Table 2).

When we limited our examination to patients with a single HCC lesion of less than 5 cm in diameter, we had 102 patients without and 31 patients with microvascular invasion. Significant predictive factors for microvascular invasion were the

macroscopic appearance of HCC, and high DCP level (Table 3). With respect to the macroscopic appearance of HCC, Types 2 and 3 had a significant predictive value for the microvascular invasion of HCC.

Study 2

Only AFP had a significant predictive value for identifying patients likely to experience recurrence within 2 years even if there was no histological evidence of microvascular invasion (Table 4). Also positive rate of hepatitis C antibody in the early recurrence group was higher than its counterpart. Grading (necroinflammatory response) and Staging (fibrosis) were no statistical difference between the group with early recurrence or not.

Discussion

In the present study, a tumor diameter of more than 5 cm, macroscopic appearance and DCP level were significant predictive factors for microvascular invasion, which can not be detected by current imaging techniques; this is consistent with the findings of a previous report by Shirabe et al. [7] The macroscopic appearance of Type 2 or Type 3 HCC, which can be evaluated in imaging studies, also predicts microvascular invasion. Therefore, in cases of Type 2 or 3 HCC, early recurrence can be carefully monitored even after OLT.

With respect to tumor markers, DCP level was an important factor in estimating the malignant potential of HCC without microscopic vascular invasion even after curative liver resection. Even if the HCC is limited to a single lesion of less than 5 cm in diameter (as described in the Milan criteria), elevation of DCP implies a poor prognosis even after curative resection. For these small single HCCs, DCP level was found to be a better predictor of vascular invasion than the macrovascular appearance of HCC.

According to the results of the present Study 2, AFP predicted the early recurrence of HCC even without proven microvascular invasion in the resected specimen. Since the documentation of microvascular invasion may be difficult because

of the width of the slice in the tumor, the possibility of microvascular invasion in the group of patients with early recurrence can not be ruled out. Our findings indicate that even when HCC within the Milan criteria (a single lesion of less than 5 cm in diameter) is removed by curative liver resection and OLT, patients should be carefully monitored for early recurrence when AFP is elevated. Usually, after curative liver resection, recurrence within 2 years mostly occur as intrahepatic metastasis through vascular invasion while recurrences occurring 2 years after R0 are regarded as multicentric occurrence of HCCs, which are different clone from first resected HCC. In other words, it is not usual that recurrence occurs after 2 years through MVI.

When considering the expansion of the indication criteria of OLT for HCC, the prediction of vascular invasion should be a key point because previous report showed its importance even after total eradication of the diseased liver [8]. The present study found that macroscopic appearance and tumor markers are important as predictors of microvascular invasion, and that DCP in particular can be used to detect latent microvascular invasion of HCC even in patients with a single lesion of less than 5 cm in diameter. Furthermore, AFP can be used to predict early recurrence after curative removal of HCC, which implies latent microvascular invasion, since early recurrence is generally considered to indicate intrahepatic metastasis of primary HCC through the

portal vein [9]. In contrast, recurrence after 2 years is usually regarded as a second occurrence of HCC in the diseased liver (multicentric occurrence) [10]. Since the diseased liver is removed in OLT, intrahepatic metastasis through microvascular invasion is more important than the multicentric occurrence of HCC after the procedure.

Recently, we showed that preoperatively undetectable HCC does not have a prognostic impact on outcome and recurrence of HCC after liver transplantation using thin-sliced explant liver [11]. The characteristics of undetectable HCCs included a minute (median size 6 mm), well-differentiated appearance (80%), with indistinct margins (85.3%) and without vascular invasion (94%). There was no recurrence in any patients at the time of follow up (median follow-up period, 30.1 months). In fact, tumor markers in almost all patients were within normal limits. Together with this results, it was found that small HCC with low tumor marker imply absence of microvascular invasion of HCC. As a subgroup analysis, we investigated the group of patients with only HCC less than 5cm to elucidate the predictor for microscopic vascular invasion. Since it has been already widely reported that HCC larger than 5cm in diameter has more chance to spread through microvascular invasion. Therefore for those patients, prediction of MVI is not really important. Even for the criteria for liver transplantation, size of HCC larger than

5cm is contraindicated because of high risk for recurrence even after OLT. On the other hand, in this manuscript, we try to find potential microscopic vascular invasion aside from size of HCC.

In conclusion, tumor size, macroscopic appearance of HCC and DCP level are important factors that can be used to identify the group of patients with a low probability of recurrence of HCC after curative liver resection. AFP can also be used as a predictor of latent microscopic vascular invasion and early recurrence.

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Table 1　Association of microvascular invasion of HCC

-all HCCs-

		microvascular invasion		
		positive (n= 52)	negative (n= 127)	p value
Age, median (range)		64 (20-85)	65 (44-81)	N.S.
Gender (M:F)		41:11	102:25	N.S.
HBs Ag positive		20 (38.5%)	36 (28.3%)	N.S.
HCV Ab positive		20 (38.5%)	63 (50.8%)	N.S.
Liver damage (A:B)		48:4	114:13	N.S.
Size of HCC, median (range)		5.2 (0.5-17)	3 (0.8-11.5)	<0.001
Numbers of HCC, median (range)		1 (1-5)	1 (1-6)	<0.01
Macroscopic appearance of HCC		8/17/19/1	65/26/20/2	<0.001
(Type 1/2/3/4)		(7 unclassified)	(14 unclassified)	
Tumor markers	AFP	95 (1.6-454,300)	12.7 (1.2-13,840)	<0.001
median (range)	DCP	845 (6-76,600)	24 (0-69,150)	<0.05

HCC: Hepatocellular carcinoma, HBs Ag: hepatitis B virus surface antigen, HCV Ab: hepatitis C virus antibody, AFP: alpha fetoprotein, DCP: des-gamma-carboxy prothrombin

Table 2　Logistic regression of factors associated with microvascular invasion of HCC

-all HCCs-

	Coefficient	Odds ratio (95% CI)
Size of HCC	0.517	1.678* (1.275-2.208)
Numbers of HCC	-0.42	0.657 (0.181-2.384)
Macroscopic appearance of HCC		
Type I		Reference
Type II	2.569	13.047# (1.514-112.439)
Type III	3.229	25.253* (3.289-193.913)
Type IV	4.098	60.205# (2.574-1408.257)

* $p<0.01$, # $p<0.05$

Table 3　Association of microvascular invasion of HCC

-single HCC less than 5cm in diameter-

		microvascular invasion		
		positive (n=31)	negative (n=102)	p value
Age, median (range)		61 (37-85)	65 (44-81)	N.S.
Gender (M:F)		27 : 4	81 : 21	N.S.
HBs Ag positive		12 (38.7%)	28 (27.4%)	<0.05
HCV Ab positive		12 (38.7%)	52 (50.9%)	N.S.
Child-Pugh (A:B)		30 : 1	94 : 8	N.S.
Macroscopic appearance		6/11/10/1	55/23/13/1	<0.001
(Type 1/2/3/4)		(3 data missing)	(10 data missing)	
Tumor markers	AFP	97.9 (1.6-454,300)	13 (1.2-81)	N.S.
median (range)	DCP	1,307 (1.8-76,600)	71 (0-8,520)	<0.001

HCC: Hepatocellular carcinoma, HBs Ag: hepatitis B virus surface antigen, HCV Ab: hepatitis C virus antibody, AFP: alpha fetoprotein, DCP: des-gamma-carboxy prothrombin

Table 4 Factors on early recurrence within 2 years without proven microvascular invasion

		Early recurrence		
		Yes (n= 42)	No (n=85)	p value
Age, median (range)		65 (45-77)	66 (44-81)	N.S.
Male:Female		35 : 7	67 : 18	N.S.
HBsAs positive		12 (28.6%)	27 (31.7%)	N.S.
HCV Ab positive		23 (54.8%)	39 (45.9%)	p<0.05
Child Pugh A:B		36 : 6	80 : 5	N.S.
Size of HCC, median (range)		3 (1-11.5)	3 (0.8-11.4)	N.S.
Number of HCC, median (range)		1 (1-6)	1 (1-2)	N.S.
Macroscopic appearance		21/10/5/1	45/14/16/2	N.S.
(Type 1/2/3/4)		(5 unclassified)	(8 unclassified)	
Tumor markers	AFP	41.4 (2-1,714)	8.3 (1.2-13,840)	p<0.05
median (range)	DCP	61 (0-69,150)	24 (6-3,999)	N.S.
HAI	Grading	5 (0-13)	5 (0-13)	N.S.
	Staging	3 (0-4)	2 (0-4)	N.S.

HCC: Hepatocellular carcinoma, HBs Ag: hepatitis B virus surface antigen, HCV Ab: hepatitis C virus antibody, AFP: alpha fetoprotein, DCP: des-gamma-carboxy prothrombin HAI: hepatitis activity index