Effects of Continuous Hepatitis with Persistent Hepatitis C Viremia on Outcome after Resection of Hepatocellular Carcinoma

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The effect of persistent hepatitis C viremia on the outcome after resection of hepatocellular carcinoma (HCC) was investigated in 59 consecutive patients with a single small HCC (≤3.0 cm in diameter). The presence of serum hepatitis C virus (HCV) RNA was evaluated using a reverse transcription polymerase chain reaction method as well as a branched DNA probe method. Clinicopathologic findings were compared between patients with and without viremia and the risk factors for poor outcome were evaluated. Hepatitis C virus (HCV) RNA was not detected in the sera from 7 patients (group 1), but was detected in the sera from the other 52 patients (group 2). Alanine aminotransferase (ALT) activity was significantly higher in group 2 than in group 1. The proportion of patients with active hepatitis was significantly higher in group 2. In group 2, new HCC often developed after the operation and four patients died of liver dysfunction. HCV viremia, high ALT activity, high concentration of total bilirubin, and liver cirrhosis were related to recurrence after the operation. Multivariate analysis indicated that HCV viremia and high ALT activity were independent risk factors for recurrence of HCC. Continuous hepatitis with persistent HCV viremia worsened the outcome after the resection of HCC by causing new development of HCC and deterioration of liver function. In patients with HCV-related HCC, but without HCV viremia, satisfactory results can be expected after liver resection.

Key words: Hepatocellular carcinoma — Hepatitis C virus — Chronic hepatitis C — Liver resection — Histologic activity index

Despite improvements in medical imaging, surgical techniques, and perioperative management, outcomes after liver resection for hepatocellular carcinoma (HCC) still are unsatisfactory because of a high recurrence rate.[1] There have been many studies concerning factors related to recurrence and outcome after resection of HCC.[2–13] In some of these reports, hepatitis viral status was not evaluated. In others, hepatitis C virus (HCV) infection was determined by whether the patients had anti-HCV antibodies in their sera before the operation. The subjects in these studies thus might have included patients without HCV viremia at the time of or after the operation.

In patients infected with HCV, cirrhosis with chronic inflammation, liver cell necrosis and regeneration, and extensive fibrosis are important in the development of HCC.[14–18] HCC tends to develop or to become detectable when DNA synthesis increases in patients with HCV-associated cirrhosis. Increased DNA synthesis in hepatocytes also appears to accelerate recurrence after resection for HCC in patients with HCV-associated cirrhosis.[19] We have found that a high spermidine/spermine ratio, which is closely related to cell proliferation, in the noncancerous hepatic tissue is a risk factor for recurrence after liver

resection for HCC.[20] It has also been reported that the sustained elevation of the serum alanine aminotransferase (ALT) activity in HCV infection is closely associated with progression from cirrhosis to HCC, as well as with recurrence after the resection of HCC.[21] These findings indicate that continuous hepatitis caused by persistent HCV viremia strongly affects the outcome after the operation. Recently we found that HCV RNA was not detectable in the sera of some patients with HCC and anti-HCV.[22] Benvegnù et al.[23] have reported that HCV RNA was not detected in some patients with chronic liver disease and anti-HCV, and that persistently high or fluctuating levels of ALT during follow-up were rarely seen in such patients. The natural disappearance rate of serum HCV RNA in patients infected with HCV has been reported to be 2.8% per year in Japan.[24] In addition, interferon therapy results in the disappearance of HCV RNA from not only the serum, but also the hepatic tissue.[25–27] However, persistent HCV viremia has not been studied as a prognostic factor.

The aim of the present study was to investigate the effect of continuous hepatitis with persistent HCV viremia on the clinicopathologic findings in patients with HCC and on the outcome after resection.

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PATIENTS AND METHODS

Patients From April 1990 to June 1997, 204 patients with anti-HCV antibodies underwent liver resection for HCC. Fifty-nine of the 204 patients had a single small HCC (≤3.0 cm in diameter) without portal or hepatic vein invasion. Patients whose resected liver specimens showed portal or hepatic vein invasion on pathologic examination were excluded from the study to avoid instances of intrahepatic metastasis that might be present at the time of the operation. Four of the 59 patients had received interferon therapy before the detection of HCC. Patients were examined preoperatively by ultrasonography, computed tomography (plain and enhanced CT), Lipiodol CT (Ultra-Fluide, Laboratorie Guerbet, Villepointe, France), magnetic resonance imaging (in most patients) and angiography. Direct ultrasonography was performed during the operation. Since May 1993, CT during arteriography and CT during arterioportography have been done if possible. Patients with hepatitis B surface antigen (HBsAg) in their sera were excluded from the study. The patients comprised 44 men and 15 women with ages ranging from 46 to 79 years. The subjects were divided into two groups. Group 1 consisted of patients in whom HCV RNA was not detected in their sera taken before and after operation, and group 2 consisted of patients in whom HCV RNA was detected in their sera. None of the patients received adjuvant therapy or interferon therapy after the operation.

The study was conducted in accordance with the Helsinki Declaration and the guidelines of the ethics committee of our institution. Written informed consent was obtained from each patient.

Viral markers Serum samples obtained before and after operation from all patients were assayed for hepatitis B virus (HBV) and HCV. Serum was examined for HBsAg with an enzyme immunoassay (International Reagents Corp., Kobe). Samples were examined for anti-HCV by second- or third-generation ELISA (Ortho Diagnostic Systems, Tokyo). Serum HCV RNA was detected using a polymerase chain reaction with reverse transcription and primers derived from a conserved 5′-untranslated region of the viral genome[28] as well as a branched DNA probe method (Quantiplex HCV-RNA, Chiron Corp., Emeryville, CA). When HCV RNA was not detected in the sera by these two methods, the results were further confirmed by a single-tube assay kit (Amplicor HCV test, Roche Diagnostic Systems/Nippon Roche Co., Branchburg, NJ). HCV RNA in HCC tissue and noncancerous hepatic tissue, obtained at operation, was also examined using a reported method.[29]

Results of laboratory tests Laboratory tests included measurements of serum concentrations of α-fetoprotein (AFP), total bilirubin, and albumin, activities of aspartate aminotransferase (AST) and ALT, platelet count, and 15-min indocyanine green retention test (ICGR$_{15}$). Changes in ALT activity after the operation also were evaluated, using only results measured in our hospital. Values obtained in other hospitals were not used because the methods (kits) used in other hospitals sometimes were different from ours.

Detection of recurrence When tumor recurrence was suspected on the basis of tumor marker assays, ultrasonography, CT, or some combination of these, angiography and/or a biopsy under ultrasonographic guidance were employed to make a definitive diagnosis.

Pathologic examination The histologic grade of tumor differentiation was assigned using a modification[30] of the classification by Edmondson and Steiner[31] and Kondo et al.[32] Well-differentiated HCC had a high cell density, a high nucleus/cytoplasm ratio, strong cytoplasmic eosinophilia, and an irregular pattern with thin trabeculae; in some tumors, the pattern was pseudoglandular. Portal tracts were found within the cancerous tissue. There was replacement growth at the boundary between the tumor and healthy tissue. Noncancerous tissues were also examined pathologically. A histology activity index (HAI) score[33] was used to evaluate the severity of hepatitis and the degree of fibrosis. At least two pathologists inspected each specimen.

Risk factors for poor outcome after operation We evaluated various risk factors and for each, we calculated the relative risk of poor outcome. The variables were selected based on their potential relationship to outcome according to previous studies or our clinical experience. The variables chosen were HCV RNA in the serum (presence or absence), age (<65 or ≥65 years), sex, history of heavy drinking (intake of at least 86 g of ethanol daily for at least 10 years),[1, 34] history of blood transfusion, Child-Pugh classification (A or B),[35] serum ALT activity (≤45 IU/liter or >45 IU/liter), serum concentration of total bilirubin (<1.0 mg/dl or ≥1.0 mg/dl), differentiation of the main tumor (well-differentiated or other), the severity of hepatitis [grade ≤1 (no or minimal hepatitis) or grade >1 (mild to severe hepatitis)], the degree of fibrosis [stage ≤3 (no fibrosis to severe fibrosis) or stage 4 (cirrhosis)], and type of resection (anatomic or nonanatomic).

Statistics We used Student's *t* test to analyze differences in age and the Mann-Whitney test to analyze differences in serum concentrations of albumin and total bilirubin, activities of AST and ALT, platelet count, ICGR$_{15}$, and tumor size. The χ^2 or Fisher's exact test was used to compare categorical data between groups. When the changes in ALT activity after the operation were evaluated, the mean ALT activity obtained for each patient after the operation was calculated and the mean of the values obtained for each test in one group was compared with that in the other group. The Wilcoxon rank sum test was then used to assess differences in these means. Tumor-free

and cumulative survival rates were calculated using the Kaplan-Meier method and the significance of differences in survival rates between groups was assessed using the log-rank test. Cox's proportional hazards model was used for multivariate analysis. A difference with a *P* value <0.05 was considered significant.

RESULTS

Clinicopathologic findings in patients HCV RNA was not detected in the sera obtained from 7 patients before operation or at any follow-up appointment (group 1); therefore these 7 patients did not have persistent HCV viremia. HCV RNA was detected in the sera from the remaining 52 patients before and during the whole obser-

vation period (group 2); these 52 patients had persistent HCV viremia. In group 1, 3 of 7 patients had received interferon therapy before the detection of HCC and HCV RNA was not detected in their sera just before the operation. Tests for HCV RNA in the HCC tissue and the non-cancerous tissue, which were performed in 4 patients in group 1, were negative. Clinical features and the results of laboratory tests are shown in Table I. Ages ranged from 46 to 79 years in group 1 and from 51 to 64 years in group 2. There was no significant difference between the two groups in the mean age, sex distribution, history of blood transfusion, or history of alcohol abuse. No significant differences were noted between the two groups in Child-Pugh score, proportion of patients with elevated AFP concentration (greater than 20 ng/ml), or tumor size.

Table I. Clinicopathologic Findings and the Results of Laboratory Tests in Patients with and without Hepatitis C Viremia

	Group 1 (*n*=7)	Group 2 (*n*=52)	*P*
Age (years, mean±SD)	58±6	62±6	0.152
Sex (M:F)	6:1	38:14	0.666
History of blood transfusion	3 (43%)	14 (27%)	0.400
Alcohol abuse	4 (57%)	13 (25%)	0.176
Child-Pugh score			
A	5 (71%)	37 (71%)	>0.999
B	2 (29%)	15 (29%)	
Albumin (g/dl)	4.0 (3.8, 4.3)	3.5 (3.1, 4.1)	0.0034
AST (IU/liter)	38 (29, 50)	58 (32, 105)	0.0244
ALT (IU/liter)	39 (34, 52)	70 (24, 127)	0.0197
Total bilirubin (mg/dl)	0.5 (0.4, 0.5)	0.8 (0.5, 1.4)	0.0012
Platelet count ($\times10^4$/mm^3)	14.1 (6.9, 19.7)	10.1 (6.7, 18.3)	0.367
ICGR$_{15}$ (%)	16.4 (11.1, 26.3)	16.7 (10.4, 34.0)	0.669
Abnormal level of α-fetoprotein (>20 ng/ml)	2 (29%)	29 (56%)	0.240
Tumor size (cm)	2.2 (1.9, 2.5)	2.0 (1.2, 3.0)	0.580
Differentiation of tumor			
Well-differentiated	3 (43%)	18 (35%)	0.691
Other	4 (57%)	34 (65%)	
Severity of hepatitis (grading)			
None or minimal (0 or 1)	6 (86%)	13 (25%)	0.0033
Mild to severe (2 to 4)	1 (14%)	39 (75%)	
Degree of fibrosis (staging)			
None to severe (0 to 3)	3 (43%)	18 (35%)	0.691
Cirrhosis (4)	4 (57%)	34 (65%)	
Type of resection			
Anatomic resection	4 (57%)	17 (33%)	0.233
Nonanatomic resection	3 (43%)	35 (67%)	

Results of laboratory tests are given as medians and 10th and 90th percentiles (in parentheses).
AST, aspartate aminotransferase; ALT, alanine aminotransferase; ICGR$_{15}$, 15-min indocyanine green retention test.
Activity of hepatitis and degree of fibrosis were evaluated according to histologic activity index.[33]
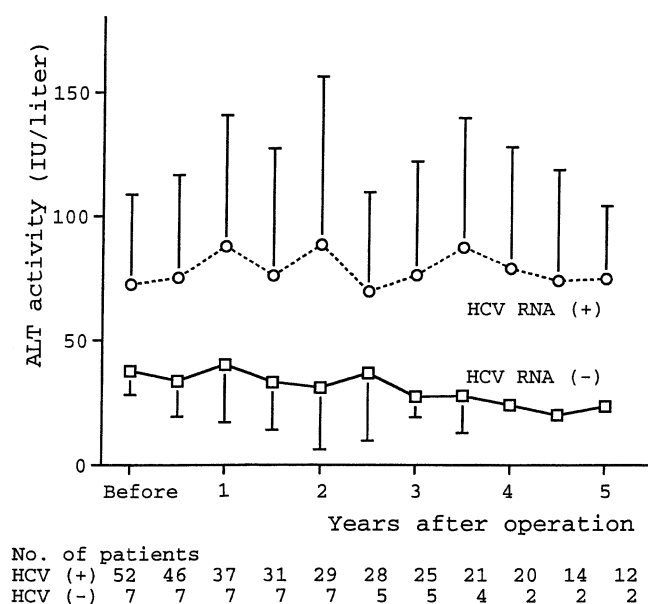
Fig. 1. Changes in serum alanine aminotransferase activity after operation. Vertical bars indicate SD. Numbers of patients at each time point are shown at the bottom. ○ alanine aminotransferase activity in patients with serum HCV RNA, □ alanine aminotransferase activity in patients without serum HCV RNA.

The activities of AST and ALT and serum concentration of total bilirubin were significantly higher in group 2 than in group 1 (P=0.0244, P=0.0197, and P=0.0012, respectively). The serum concentration of albumin was significantly lower in group 2 than in group 1 (P=0.0034). Platelet count and ICGR$_{15}$ were not significantly different between the two groups. Although differentiation of the main tumor and the degree of fibrosis (staging) were not significantly different between the two groups, the proportion of patients with no or minimal hepatitis (grade 0 or 1) was significantly higher in group 1 than in group 2 (P=0.0033).

Although the mean serum ALT activity after the operation in group 1 was within the reference range (≤45 IU/liter), the mean in group 2 was higher than the reference range (>45 IU/liter, Fig. 1). The mean serum ALT activity after the operation was significantly higher in group 2 than in group 1 (P=0.0051).

Outcome after operation and factors related to recurrence and outcome In group 1, recurrence was seen in the remaining liver of one patient, who died 3 years and 3 months after the operation. Recurrence in the remaining liver was noted in 36 patients in group 2. In 8 of these 36 patients, pathologic examination of the recurrent tumors obtained by a biopsy or second resection was possible. The recurrent tumors in 7 of the 8 patients included a component of well-differentiated HCC, suggesting that the

Table II. Tumor-free Survival Rates after the Operation

Variable (n)	Survival rates			P
	3-year	5-year	7-year	
Age				
<65 (38)	52	27	15	0.246
≥65 (21)	17	17	0	
Sex				
Male (44)	42	28	12	0.740
Female (15)	57	22	—	
History of blood transfusion				
− (17)	52	30	12	0.0082
+ (42)	22	—	—	
Alcohol abuse				
− (17)	50	31	13	0.594
+ (42)	31	24	0	
HCV RNA				
− (7)	87	87	87	0.0027
+ (52)	38	16	0	
Child-Pugh score				
A (42)	53	32	14	0.0621
B (17)	29	—	—	
Alanine aminotransferase (IU/liter)				
≤45 (20)	70	38	10	0.0303
>45 (39)	28	16	—	
Total bilirubin (mg/dl)				
<1.0 (38)	49	32	18	0.0192
≥1.0 (21)	29	11	0	
Differentiation of main tumor				
Well-differentiated (19)	62	35	—	0.128
Other (40)	34	22	8	
Adenomatous hyperplasia				
− (55)	46	25	—	0.725
+ (4)	38	38	38	
Severity of hepatitis (grading)				
0 or 1 (19)	48	48	48	0.0641
2 to 4 (40)	43	19	0	
Degree of fibrosis (staging)				
0 to 3 (21)	68	50	—	0.0472
4 (38)	31	12	12	
Type of resection				
Anatomic (21)	54	17	—	0.446
Nonanatomic (38)	37	14	12	

recurrent tumors were newly developed HCC.[7, 36–39] Tumor-free survival rates are shown in Table II. A history of blood transfusion (P=0.0082), presence of HCV viremia (group 2, P=0.0027, Fig. 2), high activity of ALT (P=0.0303), high concentration of total bilirubin (P=0.0192), and liver cirrhosis (stage 4 fibrosis, P=0.0472) were significantly related to recurrence after operation. Child-Pugh score B (P=0.0621) and mild to severe hepatitis (grade 2 to 4, P=0.0641) tended to be related to
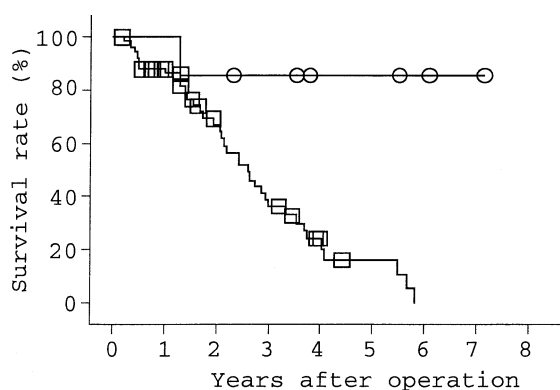
Fig. 2. Tumor-free survival rates after liver resection in patients with and without serum HCV RNA. ○ patients without HCV viremia (*n*=7), □ patients with HCV viremia (*n*=52).

recurrence, but without statistical significance. In the patient with recurrence in group 1, transcatheter arterial embolization was performed for the recurrent tumors. In group 2, second resection was performed in 7 patients, transarterial treatment such as transcatheter arterial embolization and hepatic arterial infusion chemotherapy in 19 patients, percutaneous ethanol injection therapy in 5 patients, and microwave coagulonecrotic therapy in 5 patients.

Twenty patients in group 2 died from recurrence, and 4 patients died of liver dysfunction without recurrence. Cumulative survival rates for the two groups are shown in Table III. Child-Pugh score B (*P*=0.0155), high concentration of total bilirubin (*P*=0.0341), liver cirrhosis (stage 4 fibrosis, *P*=0.0409), and nonanatomic resection (*P*=0.0303) were significantly related to shorter survival time. A history of alcohol abuse (*P*=0.0881), presence of HCV viremia (group 2, *P*=0.0983, Fig. 3), and mild to severe hepatitis (grade 2 to 4, *P*=0.0591) tended to be related to shorter survival time, but without statistical significance.

Multivariate analysis indicated that history of blood transfusion, HCV viremia, and high activity of ALT were independent risk factors for recurrence, and Child-Pugh score B was an independent risk factor for shorter survival time (Table IV). Odds ratios of HCV viremia (group 2), compared with absence of HCV viremia (group 1), were 15.87 for recurrence and 4.05 for shorter survival time.

DISCUSSION

Chronic liver disease, such as active hepatitis or cirrhosis, is generally considered a premalignant condition. Since the advent of the test for anti-HCV antibodies, HCV has been thought to be a major cause of chronic

Table III. Cumulative Survival Rates after the Operation

Variable	Survival rates			P
	3-year	5-year	7-year	
Age				
<65	86	57	43	0.0687
≥65	87	53	23	
Sex				
Male	86	59	27	0.977
Female	87	43	—	
History of blood transfusion				
−	85	52	35	0.322
+	87	58	—	
Alcohol abuse				
−	92	62	28	0.0881
+	75	21	0	
HCV RNA				
−	100	84	84	0.0983
+	85	48	19	
Child-Pugh score				
A	88	70	41	0.0155
B	83	12	0	
Alanine aminotransferase (IU/liter)				
≤45	90	53	31	0.0887
>45	85	52	19	
Total bilirubin (mg/dl)				
<1.0	94	60	41	0.0341
≥1.0	72	42	0	
Differentiation of main tumor				
Well-differentiated	86	41	21	0.856
Others	86	62	28	
Adenomatous hyperplasia				
−	87	56	22	0.929
+	68	34	34	
Severity of hepatitis (grading)				
0 or 1	88	74	74	0.0591
2 to 4	85	47	11	
Degree of fibrosis (staging)				
0 to 3	88	88	33	0.0409
4	85	37	22	
Type of resection				
Anatomic	94	82	47	0.0303
Nonanatomic	82	41	25	

liver disease and HCC in areas where the prevalence of HCV is high, such as Japan and Europe.[40–43] In this study, laboratory test results and the severity of hepatitis were better in group 1 than in group 2, although the degree of fibrosis in the HAI score was not different between the two groups. These findings indicate that, in group 1, liver function and activity of hepatitis had improved after the remission of HCV viremia, although fibrosis had already developed before remission.
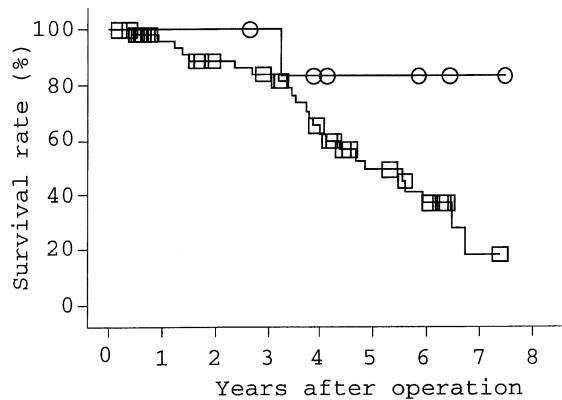
Fig. 3. Cumulative survival rates after liver resection in patients with and without serum HCV RNA. ○ patients without HCV viremia (*n*=7), □ patients with HCV viremia (*n*=52).

Recurrence of HCC after the operation may manifest itself as either intrahepatic metastasis from the original tumor or newly developed HCC.[2–7,36–39] Tarao *et al.*[21] have reported that the recurrence rate after liver resection for HCV-related HCC was higher in patients with high ALT activity ($\geq$80 IU/liter) than in patients with low ALT activity (<80 IU/liter). They suggested that a necroinflammatory process in the hepatocytes is important for new carcinogenesis after the operation. Chiu *et al.*[44] have reported that a marked increase in the proliferative capacity of the noncancerous hepatic tissue is a significant risk factor for tumor recurrence. In this study, we found that HCV viremia, high activity of ALT (>45 IU/liter), high concentration of total bilirubin ($\geq$1.0 mg/dl), and liver cirrhosis (stage 4 fibrosis) were significant risk factors for recurrence, using univariate analysis. HCV viremia and high activity of ALT were independent risk factors in multivariate analysis. Mild to severe hepatitis (grade 2 to 4) also tended to be related to recurrence. The mean ALT activity was significantly higher in group 2 than in group

1. In addition, some recurrent tumors in group 2 were strongly suspected to be newly developed HCC. These findings suggest that persistent HCV viremia is related to active hepatitis in the adjacent hepatic tissue, resulting in a high risk of hepatocarcinogenesis even after resection of the primary HCC.

Child-Pugh score B, high serum concentration of total bilirubin, and liver cirrhosis (stage 4 fibrosis) were significant risk factors for poor outcome. The three factors are related to advanced liver disease that had already developed before operation. Although nonanatomical resection was not a significant factor for recurrence, it was a significant factor for poor outcome. These findings indicate that new HCC often developed even after anatomical resection and that the patients who underwent anatomical resection might have better remaining liver function. Alcohol abuse, HCV viremia, and mild to severe hepatitis (grade 2 to 4) tended to be related to shorter survival time. We have reported that the outcome after liver resection was significantly poorer in patients with alcohol abuse than in patients without alcohol abuse.[34] Progression of liver dysfunction due to persistent HCV infection seems to be an additional risk factor; in 4 patients in group 2, worsening liver function was the cause of death.

Recent randomized controlled trials have shown that interferon therapy leads to a rapid decrease in ALT activity and to a disappearance of serum HCV RNA in about one-third of patients with chronic hepatitis C.[28, 45, 46] Patients who respond to interferon therapy with long-term remission of disease and sustained loss of HCV RNA generally are regarded as being unlikely to develop cirrhosis of the liver or HCC. Thus, interferon therapy may suppress postoperative carcinogenesis. Another possible mechanism is that interferon may have a suppressive effect on HCC progression, because Lai *et al.*[47] have shown that patients with advanced HCC treated with interferon have a significantly higher survival rate than patients not given interferon.

In conclusion, continuous hepatitis with persistent HCV viremia adversely affects outcome after liver resection for

Table IV. Prognostic Factors for Recurrence and Outcome by Multivariate Analysis

Variable	Regression coefficient	Standard error	Odds ratio (95% CI)	*P*
Recurrence				
HCV viremia	2.762	1.036	15.87 (2.08–125.00)	0.0076
Blood transfusion	1.288	0.396	3.62 (1.67–7.87)	0.0011
High ALT activity	0.838	0.392	2.31 (1.07–5.00)	0.0325
Survival				
Child-Pugh score B	0.898	0.417	2.46 (1.08–5.56)	0.0313
HCV viremia	1.399	1.024	4.05 (0.54–30.30)	0.172

HCC, probably by causing new development of HCC and deterioration of liver function. In HCV-related HCC patients without HCV viremia, satisfactory results can be expected after liver resection for HCC. Not only treatment for HCC, but also treatments for HCV viremia, including interferon therapy, may be important in the management of patients with HCC and HCV viremia.

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