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000 **Perioperative Synbiotic Treatment to Prevent Infectious Complications in Patients After Elective Living Donor Liver Transplantation. A Prospective Randomized Study**

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According to this randomized controlled study, infectious complications after elective living-donor liver transplantation significantly decreased with the perioperative administration of synbiotic therapy.

Differences in prognostic factors according to viral status in patients with hepatocellular carcinoma

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Abstract. The number and ratio of both HBsAg- and HCV Ab-negative hepatocellular carcinoma (HCC-nonBC) cases have been steadily increasing in Japan. The aim of this study was to examine the frequency of detection of HCC-nonBC by screening methods and to elucidate the clinical characteristics of HCC-nonBC compared with those of hepatitis C and/or B virus-associated HCC (HCC-virus). We recruited 624 patients with HCC who were diagnosed between 1982 and 2007 at the Department of Gastroenterology and Hepatology, Nagasaki University Hospital. They were categorized into 2 groups as follows: i) 550 were included in the HCC-virus group: positive for HBsAg and/or positive for HCV Ab, and ii) 74 were included in the HCC-nonBC group: negative for both HBsAg and HCV Ab. The follow-up patterns until the initial detection of HCC and the survival rates were analyzed and compared between the 2 groups. Multivariate analysis identified follow-up, alcohol consumption, albumin level, total bilirubin level, α-fetoprotein (AFP) level, and tumor-node-metastasis (TNM) stage as independent and significant risk factors for prognosis. Among the 397 patients with HCC in TNM stage I or II, multivariate analysis identified the cause of liver disease, gender, Child-Pugh score, serum albumin level and TNM stage as independent and significant risk factors for prognosis. We reported that the poor prognoses of patients with HCC-nonBC were attributable to its late detection in an advanced condition due to the absence of a surveillance system for the early detection of HCC. However, in early-stage patients, patients with HCC-nonBC showed significantly better prognosis than those in the HCC-virus group.

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Introduction

Primary liver cancer is the most common cancer of the liver, accounting for approximately 6% of all human cancers. It is estimated that half a million cases of this disease occur worldwide each year, making primary liver cancer the fifth most common malignancy in men and the ninth in women (1-6). Hepatocellular carcinoma (HCC) accounts for 85 to 90% of primary liver cancers, (7) and the age-adjusted HCC mortality rate has increased over the past few decades in Japan (8). Similarly, a trend in increasing incidence rates of HCC has been reported for several developed countries in North America, Europe and Asia (9,10). HCC often develops in patients with liver cirrhosis caused by hepatitis B virus (HBV), hepatitis C virus (HCV), excessive alcohol consumption or nonalcoholic fatty liver disease. HCV is the predominant causative agent of HCC in Japan (11-14). However, it has been reported that the number and ratio of both HBsAg- and HCV Ab-negative HCC (HCC-nonBC) have been steadily increasing in Japan (15,16).

The prognosis for patients with HCC is still poor. Surgical resection and liver transplantation are the standard treatment methods available. Radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI) have recently been recognized as effective methods of achieving complete tumor necrosis in small HCCs (17); however, the chances of curative treatment are often limited by several features of HCC. HCCs usually grow to a large size before symptom manifestation. Bilobar or multifocal tumors are common, and the incidence of associated cirrhosis is high, being over 80% in most cases (18-20). Transcatheter arterial chemoembolization (TACE), which is considered to be an ineffective method of achieving complete necrosis of HCCs, also depends on the above factors (21). Early detection of HCC by α-fetoprotein (AFP) and/or imaging screening has been implemented in many countries to increase the chances of successful intervention and to improve survival (22-26).

The aim of this study was to examine the frequency of detection of HCC-nonBC by screening methods and elucidate the differences in the clinical characteristics between non-B, non-C HCC and hepatitis C and/or B virus-associated HCC (HCC-virus).

Patients and methods

Patients and study groups. We recruited 624 patients with HCC who were diagnosed between January, 1982 and December, 2007 at the Department of Gastroenterology and Hepatology, Nagasaki University Hospital. Informed consent was obtained from all patients. The diagnosis of HCC was based on AFP levels; results of imaging techniques such as ultrasonography (USG), computerized tomography (CT), magnetic resonance imaging (MRI) and hepatic angiography (HAG); and/or liver biopsy. The diagnostic criteria included characteristic liver biopsy findings, elevated AFP (≥20 ng/ml) and neovascularization on HAG and/or CT.

Sera were stored at -80˚C until they were used for the following assays. The diagnosis of chronic hepatitis C virus (HCV) infection was based on the presence of HCV Ab (microparticle enzyme immunoassay; Abbott Laboratories) and HCV RNA as detected by polymerase chain reaction. The diagnosis of chronic HBV infection was based on the presence of HBsAg (enzyme-linked immunosorbent assay; Abbott Laboratories); the serum AFP level was measured by radioimmunoassay (Abbott Laboratories). The history of alcohol intake was noted from medical records; habitual drinking was defined as an average daily consumption of an amount equivalent to 80 g of pure ethanol for a period of more than 10 years.

The patients were categorized into 2 groups as follows: i) HCC-virus group (550) comprising patients positive for HBsAg and/or positive for HCV Ab and ii) HCC-nonBC group (74) comprising patients negative for both HBsAg and HCV Ab. We analyzed and compared the 2 groups for age distribution, gender ratio, body-mass index, alcohol intake, serum AFP level, tumor-node metastasis (TNM) stage of hepatocellular carcinoma tumors at the time of initial detection, Child-Pugh score, follow-up pattern until the initial detection of HCC and the survival rates.

Follow-up. All patients were categorized into 2 groups: the follow-up group included 365 (58%) patients with subclinical HCC diagnosed by screening; the non-follow-up group consisted of 259 (42%) patients who were diagnosed at our hospital owing to the appearance of symptoms indicative of HCC. AFP levels and liver function were assessed every 3 to 6 months, and USG or CT imaging was performed every 3 to 12 months over a period of at least 12 months prior to the diagnosis of HCC in patients of the follow-up group. The non-follow-up group patients presented with clinical symptoms such as abdominal pain, discomfort, nausea or weight loss which led to the evaluation and diagnosis of HCC.

Treatment modalities. Patients diagnosed with HCC were assessed for surgery on the basis of the extent of lobar involvement and liver function status. The extent of lobar involvement was evaluated by a combination of USG, CT, MRI and HAG. Patients were considered unfit for resection when they met the following criteria: i) bilobar involvement, ii) evidence of tumor infiltration into the main portal vein or thrombosis of the vein, iii) evidence of extrahepatic metastases, iv) Child's grade C cirrhosis or v) poor cardiac and respiratory statuses. If the patients were deemed unfit for operation or refused to undergo operation, PEI therapy was the second choice of treatment offered to such patients with HCCs <3 cm in diameter. The remaining patients without main portal vein thrombosis or extrahepatic metastasis were advised to undergo TACE irrespective of the size and number of tumors.

After initial treatment, AFP levels and liver function of the patients were assessed every 1 to 3 months, and USG imaging was performed every 3 to 6 months during the follow-up period. Patients suspected to have HCC recurrence were further evaluated by CT and/or MRI. The assessment of treatment for recurrent HCC was based on lobar involvement and liver function status as described for the initial treatment. RFA or liver transplantation to treat HCC was started at our institution in 2002; none of the patients were treated by these methods between 1982 and 2001. Furthermore, none of the subjects in our study received either of these treatments for recurrent HCC during the follow-up period.

Statistical analysis. The time of survival was measured from the time of the diagnosis of HCC to the time of death or until the time of preparation of the manuscript. The data were analyzed by the Mann-Whitney test for continuous ordinal data, and the Chi-square test with Yates' correction and Fisher's exact test were performed for intergroup comparisons to determine the association between 2 qualitative variables. The survival rate was analyzed using the Kaplan-Meier method, and the differences between the survival probability curves were tested using the log-rank test. The independent risk factors associated with the rate of survival were estimated by the non-time-dependent stepwise Cox regression analysis. The standard error was calculated based on the binomial model to estimate the response rate. A value $P<0.05$ was considered statistically significant. Data analysis was performed with SPSS version 16.0 software for Windows.

Results

Patient characteristics at enrollment. We diagnosed 624 patients with HCC during the study period. Patient characteristics at the time of diagnosis of HCC are presented in Table I. The underlying causes of HCC were determined to be as follows: 120 (19%) patients were positive for HBsAg, 411 (66%) were positive for HCV Ab, 19 (3%) were positive for both HBsAg and HCV Ab and 74 (12%) were negative for HBsAg and anti-HCV.

Comparison of clinical characteristics and survival between patients with and without hepatitis virus infection. The patients were divided into 2 groups: the HCC-nonBC group (74 patients) and the HCC-virus group (550 patients); the characteristics of each group were compared (Table I). There were no significant differences in gender, BMI, Child-Pugh score, prothrombin time, or albumin and total bilirubin levels. However, there were significant differences between the 2 groups in terms of median age ($P=0.001$), habitual drinkers ($P=0.015$), TNM stage ($P=0.030$), AFP ($P=0.002$) and follow-up group ($P=0.010$). The HCC-nonBC group had a lower proportion of patients who were followed up when compared to those of the HCC-virus group.

Table II indicates the results of univariate and multivariate analyses of the prognosis factors for HCC using the

Table I. Comparison between HCC patients with and without virus infection.

	All patients		HCC-nonBC		HCC-virus		P-value
Total	624		74		550		
Median age, years	65	(13)	70	(6)	64	(12)	0.001
Gender (%)							
Male	478	(77)	54	(73)	424	(77)	
Female	146	(23)	20	(27)	126	(23)	NS
BMI	22.4	(4.2)	23.1	(6.0)	22.3	(4.8)	NS
Alcohol consumption (%)							
Not excessive	497	(80)	51	(69)	446	(81)	
Excessive	127	(20)	23	(31)	104	(19)	0.015
Follow-up (%)							
Follow-up group	365	(58)	33	(45)	332	(60)	
Non-follow-up group	259	(42)	41	(55)	218	(40)	0.010
Child-Pugh score	6	(1)	5	(2)	6	(2)	NS
Hepatitis virus							
HBsAg (+)/HCV Ab (-)	120	(19)	0	(0)	120	(22)	
HBsAg (-)/HCV Ab (+)	411	(66)	0	(0)	411	(75)	
HBsAg (+)/HCV Ab (+)	19	(3)	0	(0)	19	(3)	
HBsAg (-)/HCV Ab (-)	74	(12)	74	(100)	0	(0)	-
TNM stage (%)							
I	158	(25)	11	(15)	147	(27)	
II	239	(38)	30	(40)	209	(39)	
III	142	(23)	20	(27)	122	(22)	
IV	85	(14)	13	(18)	72	(12)	0.030
Laboratory data							
Albumin (g/dl)	3.7	(0.8)	3.8	(0.9)	3.7	(0.8)	NS
Prothrombin time (%)	80	(22)	85	(22)	80	(22)	NS
Total bilirubin (mg/dl)	1.0	(0.8)	0.9	(0.7)	1.0	(0.8)	NS
AFP (ng/ml)	51	(446)	16	(290)	59	(452)	0.002

Data are median (IQR) or frequency (%). NS, not significant.

Cox proportional hazards model. Univariate analysis revealed that 9 of 12 factors (male, excessive alcohol intake, Child-Pugh score ≥7, albumin <3.7 g/dl, prothrombin time <80%, total bilirubin ≥1.1 mg/dl, AFP ≥52 ng/ml, TNM stage III or IV, and the follow-up group) significantly affected the survival rate in patients with HCC. Multivariate analysis identified follow-up (follow-up group, relative risk 0.71), alcohol consumption (excessive drinker, relative risk 1.32), albumin (<3.7 g/dl, relative risk 1.37), total bilirubin (≥1.1 mg/dl, relative risk 1.53), AFP (≥52 ng/ml, relative risk 1.44), and TNM stage (III or IV, relative risk 2.50), as independent and significant risk factors (P=0.002, 0.043, 0.046, <0.001, 0.001 and <0.001, respectively) for prognosis.

Comparison of clinical characteristics and survival between patients with and without hepatitis virus infection in those patients with TNM stage I or II. Characteristics of patients with TNM stage I or II at the time of HCC diagnosis are presented in Table III. No significant differences were observed in gender, habitual drinkers, BMI, TNM stage, prothrombin time, or total bilirubin level. However, there were significant differences in the median age (P<0.001), Child-Pugh score (P=0.012), albumin level (P=0.009), AFP (P<0.001) and follow-up group (P=0.010).

Table IV indicates the results of univariate and multivariate analyses of the prognosis factors for HCC using the Cox proportional hazards model. Univariate analysis revealed that 6 of 12 factors (male, Child-Pugh score ≥7, albumin <3.7 g/dl, AFP ≥52 ng/ml, TNM stage II and HCC-nonBC) significantly affected the survival rate in HCC patients. Multivariate analysis identified HCC-nonBC (HCC-nonBC, relative risk 0.55), gender (male, relative risk 1.58), Child-Pugh score (≥7, relative risk 1.47), albumin (<3.8 g/dl, relative risk 1.62) and TNM stage (stage II, relative risk

Table II. Univariate and multivariate analyses of prognostic factors for HCC in the 624 patients.

Variable		Univariate analysis		Multivariate analysis	
		P-value	Relative risk (95% CI)	P-value	Relative risk (95% CI)
Age (years)	≥65	0.058	0.82 (0.67-1.01)		
Gender	Male	0.003[a]	1.46 (1.14-1.88)	0.800	1.28 (0.97-1.68)
BMI	≥25	0.177	0.84 (0.65-1.08)		
Alcohol consumption	Excessive	0.011[a]	1.37 (1.08-1.75)	0.043[a]	1.32 (1.01-1.72)
Follow-up	Followed up	<0.001[a]	0.63 (0.52-0.77)	0.002[a]	0.71 (0.56-0.89)
Child-Pugh score	≥7	<0.001[a]	2.10 (1.70-2.59)	0.134	1.30 (0.92-1.82)
Albumin (g/dl)	<3.7	<0.001[a]	1.98 (1.62-2.43)	0.046[a]	1.37 (1.01-1.85)
Prothrombin time (%)	<80	0.002[a]	1.37 (1.12-1.68)	0.959	0.99 (0.78-1.27)
Total bilirubin (mg/dl)	≥1.1	<0.001[a]	1.67 (1.36-2.05)	<0.001[a]	1.53 (1.22-1.92)
AFP (ng/ml)	≥52	<0.001[a]	1.83 (1.49-2.24)	0.001[a]	1.44 (1.16-1.79)
TNM stage	III or IV	<0.001[a]	3.02 (2.45-3.72)	<0.001[a]	2.50 (2.00-3.13)
Etiology of liver disease	HCC-nonBC	0.139	0.77 (0.54-1.09)		

CI, confidence interval.

Table III. Comparison between HCC in TNM stage I or II patients with and without virus infection.

	All patients		HCC-nonBC		HCC-virus		P-value
Total	397		41		356		
Median age, years	65	(13)	72	(13)	65	(13)	<0.001
Gender (%)							
Male	288	(73)	27	(66)	261	(73)	
Female	109	(27)	14	(34)	95	(27)	NS
BMI	22.3	(4.0)	23.7	(5.2)	22.3	(3.9)	NS
Alcohol consumption (%)							
Not excessive	328	(83)	31	(76)	297	(83)	
Excessive	69	(17)	10	(24)	59	(17)	NS
Follow-up (%)							
Follow-up group	268	(68)	21	(51)	247	(60)	
Non-follow-up group	129	(32)	20	(49)	109	(40)	0.019
Child-Pugh score	6	(2)	5	(1)	6	(2)	0.012
Hepatitis virus							
HBsAg (+)/HCV Ab (-)	70	(18)	0	(0)	70	(20)	
HBsAg (-)/HCV Ab (+)	274	(69)	0	(0)	274	(77)	
HBsAg (+)/HCV Ab (+)	12	(3)	0	(0)	12	(3)	
HBsAg (-)/HCV Ab (-)	40	(10)	40	(100)	0	(0)	-
TNM stage (%)							
I	158	(40)	11	(15)	147	(27)	
II	239	(60)	30	(40)	209	(39)	NS
Laboratory data							
Albumin (g/dl)	3.8	(0.7)	4.0	(0.6)	3.8	(0.8)	0.009
Prothrombin time (%)	82	(22)	87	(20)	80	(21)	NS
Total bilirubin (mg/dl)	0.9	(0.6)	0.8	(0.4)	1.0	(0.7)	NS
AFP (ng/ml)	32	(222)	9	(32)	36	(254)	<0.001

Data are median (IQR) or frequency (%). NS, not significant.

Table IV. Univariate and multivariate analyses of prognostic factors for HCC in patients with TNM stage I or II.

Variable		Univariate analysis		Multivariate analysis	
		P-value	Relative risk (95% CI)	P-value	Relative risk (95% CI)
Age (years)	≥65	0.514	0.91 (0.69-1.20)		
Gender	Male	0.039[a]	1.40 (1.02-1.94)	0.008[a]	1.58 (1.13-2.21)
BMI	≥25	0.062	0.71 (0.50-1.02)		
Alcohol consumption	Excessive	0.083	1.36 (1.96-1.93)		
Follow-up	Followed up	0.270	0.85 (0.64-1.13)		
Child-Pugh score	≥7	<0.001[a]	2.04 (1.52-2.73)	0.041[a]	1.47 (1.02-2.11)
Albumin (g/dl)	<3.8	<0.001[a]	2.04 (1.56-2.68)	0.007[a]	1.62 (1.15-2.30)
Prothrombin time (%)	<82	0.083	1.27 (0.97-1.67)		
Total bilirubin (mg/dl)	≥0.9	0.067	1.30 (0.98-1.72)		
AFP (ng/ml)	≥32	<0.001[a]	1.64 (1.26-2.16)	0.065	1.31 (0.98-1.74)
TNM stage	II	0.004[a]	1.52 (1.14-2.01)	0.004[a]	1.53 (1.14-2.04)
Etiology of liver disease	HCC-nonBC	0.020[a]	0.51 (0.29-0.90)	0.048[a]	0.55 (0.30-0.99)

CI, confidence interval.

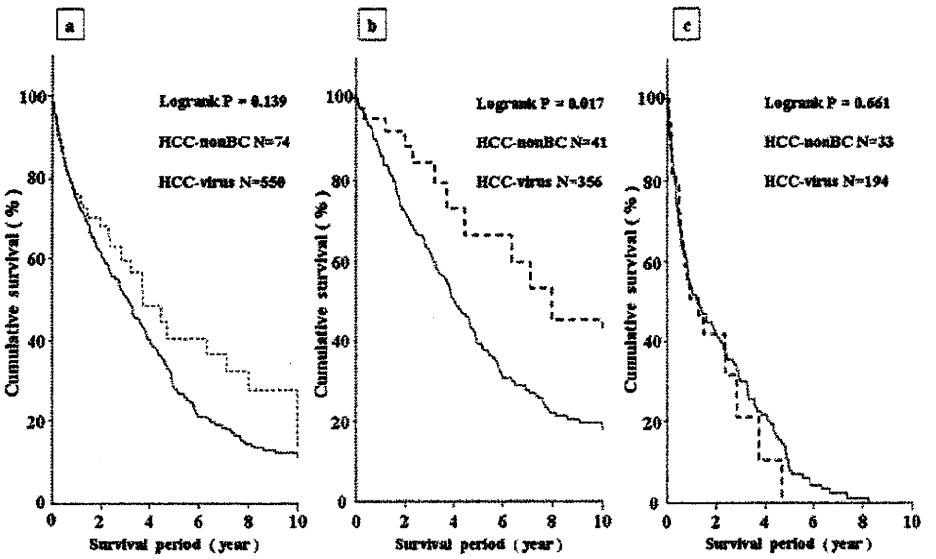

Figure 1. The cumulative survival rate in HCC patients without hepatitis virus infection (HCC-nonBC, dashed-line) and in HCC patients infected with hepatitis virus (HCC-virus, thin line) according to the TNM staging system.

1.53), as independent and significant risk factors (P=0.048, 0.008, 0.041, 0.007 and 0.004, respectively) for prognosis.

Patient survival. Overall, the median survival of all 624 patients was 1.84 years. No significant difference was detected in the survival rate between patients with and without hepatitis virus infection (Fig. 1a). When patients were classified according to the TNM stage, patients in the HCC-nonBC group with TNM stage I or II had a higher cumulative survival rate than those in the HCC-virus group (Fig. 1b; P=0.017). Patients who had TNM stage III or IV and HCC-nonBC and HCC-virus patients did not show significant differences in survival rates (Fig. 1c).

Discussion

The age-adjusted mortality rate for HCC has increased over the past few decades in Japan (27). However, the majority of patients are still diagnosed at an advanced stage and so have a short survival time after diagnosis. Patients with chronic HBV and/or HCV infection complicated by cirrhosis should be monitored with ultrasonography, CT or MRI of the liver to detect tumors at an early stage. In 58% of our patients, the tumors were detected on follow-up. Patients in the follow-up group had smaller tumors at the time of diagnosis and were more likely to be eligible for treatment. In addition, there was a significant improvement in survival rates among the

follow-up group (24-26,28-32). We recognized that the 2 groups of patients could not be evaluated in a prospective study, and improved survival in the follow-up group patients may be owing to the effect of lead-time bias. Nevertheless, our data corroborate those of previous studies indicating that follow-up may have increased rates of early detection and eligibility for curative treatment, which may in turn translate to improved survival.

In the TNM stages I and II, patients with HCC-nonBC had a better prognosis than those with HCC-virus. This difference may be explained as follows. HCC secondary to liver cirrhosis is less frequent in patients with HCC-nonBC than in those with HCC-virus (12). Patients with HCC-nonBC are less likely to progress to liver cirrhosis (33). However, in the TNM stage III and IV, the patients with HCC-nonBC had a similar prognosis to those with HCC-virus. The percentages of advanced stage HCC and non-follow-up patients were significantly higher in the HCC-nonBC group than in the HCC-virus group. Taken together, these results indicate that the prognosis of patients with HCC-nonBC is linked to the follow-up studies for detecting HCC.

A large proportion of people infected with HCV, HBV or both have latent cancer. Therefore, it is essential that HCC is detected at an early stage in individuals who harbor chronic HCV or HBV infections. In this study, more than 80% of patients had HCC associated with HBV and/or HCV; therefore, the target population for the surveillance of HCC must be easily identifiable. However, the incidence of hepatitis virus associated with HCC will decrease in Japan (15,34,35) because of the following reasons. In Japan, the population of individuals infected with chronic HCV is rapidly aging (36,37), and chronic HBV infection has been preventable since the licensing of the hepatitis B vaccine in 1982. In fact, primary tumors in 12% of our patients with HCC were negative for both HBsAg and HCV Ab. Of these, non-alcoholic fatty liver disease (NAFLD) may be a cause of HCC. Bugianesi *et al* suggested that liver disease was caused by NAFLD in 23/641 (4%) patients with HCC (38). However, it will be difficult to select patients for the screening of HCC, who are negative for both HBsAg and HCV Ab.

HCC surveillance for patients eligible for imaging tests is usually performed at 6-month intervals. Additionally, a combined imaging test and a serological test such as AFP or des-γ carboxy prothrombin is a sensitive method to detect HCC (29,39). The target population for the surveillance of HCC may not be easily identified in Japan. It has been reported previously that more than 60% of patients in the follow-up group had HCCs measuring less than 3 cm in diameter (26). It is possible that 12-month intervals for the imaging test were reasonable to ensure the detection of treatable tumors in patients with HCC.

In summary, the poorer prognosis of patients with HCC-nonBC was attributable to its late detection in an advanced condition, owing to the lack of a surveillance system for early detection of HCC. However, among early-stage patients, those with HCC-nonBC showed a significantly better prognosis than those with HCC-virus. To conclude, we suggest that the entire population of Japan should be tested using imaging techniques at least every 12 months along with an abdominal examination.

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INTERNAL MEDICINE

□ CASE REPORT □

Diffuse Liver Metastasis of Small Cell Lung Cancer Causing Marked Hepatomegaly and Fulminant Hepatic Failure

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Abstract

A 62-year-old female was admitted for examination of an abnormal liver function. Plain CT and MRI of the abdomen showed marked hepatomegaly but no visible nodular lesion in the liver. On the 3rd hospital day she had hepatic encephalopathy and was treated with a course of high-dose steroids, but ultimately died of disease progression on the 7th hospital day. An autopsy revealed a small pulmonary nodule with the histological findings showing small cell carcinoma. There was almost complete parenchymal replacement with metastatic tumor in the liver. Neoplastic involvement of the liver should be considered in the differential diagnosis of fulminant hepatic failure of unknown etiology.

Key words: small cell carcinoma, fulminant hepatic failure, hepatomegaly

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Introduction

Fulminant hepatic failure (FHF) is defined as liver disease that causes encephalopathy within 8 weeks of the onset of symptoms in a patient with no prior evidence of liver disease. The most common causes of FHF are viral hepatitis and drug toxicity (1). FHF due to primary and metastatic carcinoma is rare. This generally occurs as a result of diffuse neoplastic infiltration or vascular involvement. The most common cause of FHF secondary to the infiltration of the liver is hematologic malignancies (2-4). Small cell lung cancer manifesting as acute hepatic failure resulting from diffuse parenchymal infiltration by a metastatic tumor is rare. This report presents a patient with FHF due to massive metastatic small cell lung cancer that was unrecognized until autopsy.

Case Report

A 62-year-old female was admitted to the nephrology department because of hyponatremia (Na 117 mEq/L). Though it was improved by mineral corticoid, she showed marked hepatomegaly with spontaneous pain and abnormal liver function. She was transferred to the department of gastroenterology and hepatology for an examination of her liver function. She had a history of aortitis syndrome. She had no history of blood transfusion, hepatitis, or alcohol abuse, but she smoked an average of one pack of cigarettes a day for 40 years. A physical examination revealed jaundice, massive ascites and hepatomegaly. Her consciousness level was clear. Relevant laboratory test results were: hemoglobin 11.1 g/dL; leukocyte count 14,200/mm^3; platelet count 70,000/mm^3; prothrombin time 54%; serum albumin 2.8 g/dL; total bilirubin 8.1 mg/dL; serum aspartate transaminase (AST) 266 IU/L; serum alanine transferase (ALT) 126 IU/L; alkaline phosphatases 1,672 IU/L, lactate dehydrogenase (LDH) 934 IU/

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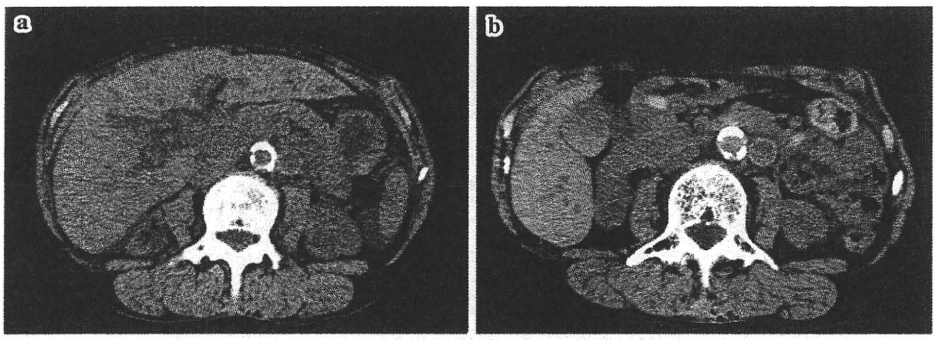

Figure 1. **a: CT scan of the abdomen at the liver shows marked hepatomegaly. No obvious nodular lesions are depicted in the liver. b: CT at almost the same level taken one year previously shows a normal-sized liver.**

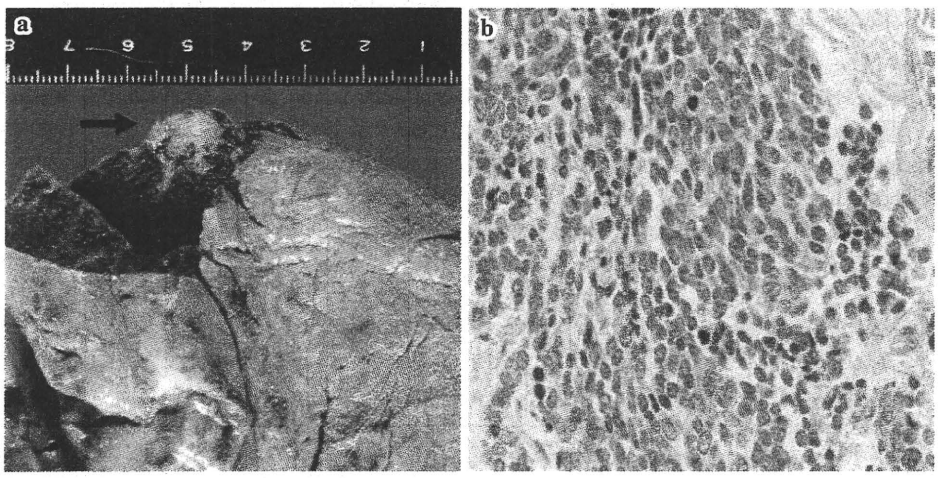

Figure 2. **a: Macroscopically, the resected right upper lung lobe shows a small nodule lesion. b: Microscopically, the specimen shows the cytoplasm to be scant, while the nuclear chromatin is fine.**

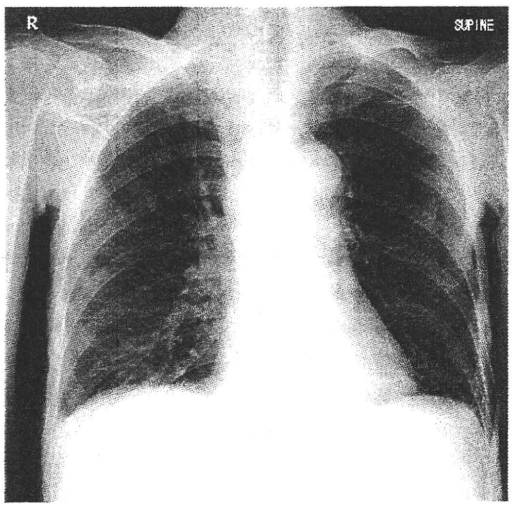

Figure 3. **Chest radiograph shows no abnormal shadow in either lung field.**

L; and ammonia (NH3) 119 μ/dL. Hepatitis B surface (HBs) antigen and HCV antibody were negative.

Abdominal ultrasound revealed hepatomegaly without dilated bile ducts or a focal mass. A computed tomography (CT) and magnetic resonance imaging (MRI) without contrast showed hepatomegaly and edema of a periportal lesion (Fig. 1). Contrasted CT and MRI were not performed because her renal function was not good. The patient's clinical course continued to deteriorate after admission. On the 3rd hospital day she had hepatic encephalopathy and was treated with a course of high-dose steroids but ultimately died of the disease progress on the 7th hospital day. Metastatic liver disease was suspected to be the cause of fulminant hepatic failure, but this could not be confirmed, because imaging modalities did not show any tumor lesion. An autopsy revealed a small pulmonary nodule with histology showing a small cell carcinoma (Fig. 2a, 2b) that was not seen on chest radiographs (Fig. 3) and CT. The liver was enlarged, weighting 3,550 g. The cut surface of the liver showed widely distributed tumor nodules of varying sizes. Only a few hepatocytes could be identified microscopically, and the parenchyma was almost completely replaced with metastatic small cell carcinoma, identified as oat cells (Fig. 4a, 4b). These carcinomas showed marked lymph-vascular invasion. Immunohistochemical staining of these tumor cells was strongly and diffusely positive for CD56 and diffusely positive for TTF-1 and faintly positive for synaptophysin. These

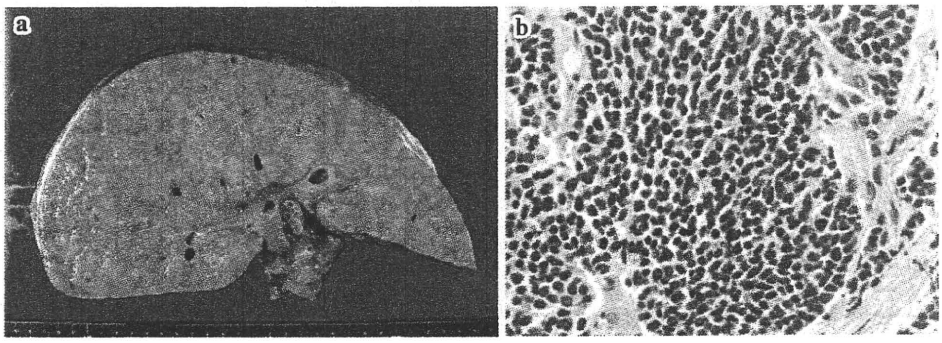

Figure 4. a: Macroscopically, resected liver shows marked enlargement. b: Microscopically, almost complete parenchymal replacement is observed with a metastatic tumor in the liver.

immunohistochemical findings indicated small cell carcinoma.

Discussion

The liver is the most common site for metastatic tumor deposits with evidence of hepatic metastases reported in 36% of all patients who died from cancer (4). Despite this, liver dysfunction may not be evident. FHF secondary to a metastatic tumor is rare. In some cases, tumors may replace up to 90% of the liver without any manifestation of jaundice. Only 7.2% of 292 patients with metastatic liver disease developed a coma, and it occurred mostly in patients with breast, gastric cancer, colon cancer, and lymphoma (5). Small cell lung cancer is so highly invasive that hepatic metastasis is common, but a rapid progression to FHF is extremely rare. The cause of the present admission was hyponatremia. Patients with small cell carcinoma, which produces AVP, sometimes also demonstrate hyponatermia. In the present patient, the cause of hyponatremia could have been the presence of the AVP producing tumor, because the plasma AVP concentration increased to 46.3 pg/mL. The incidence as defined by clinical and biochemical findings ranges from 7-16% (6, 7). Four of 40 patients who showed clinically significant hyponatremia were identified from 1 to 4 months before the malignancy was clinically detected (7). Although the presence of hyponatemia as a first sign of small cell carcinoma is not rare, it remains difficult to detect an AVP-producing tumor at an early stage.

Previous reports have explained that FHF in cases of diffuse intrasinusoidal liver metastases is due to destruction of the liver cell by spreading diffuse carcinoma cells, ischemia by vascular occlusion of the portal vein by of the tumor or nonoccculusive infarction of liver due to shock from other causes such as sepsis or cardiac dysfunction (8, 9). The tumor cells spread diffusely in the liver of the current patient and only a few hepatocytes could be identified. Moreover, there was significant vascular involvement of the portal vein. A previous report discussed that CT showed hepatomegely but it was not visible in a case of diffuse metastases of small cell lung cancer (10). US, CT and MRI also showed marked hepatomegaly in that case but did not visualize a nodular liver lesion. These findings are similar to the present case.

The sensitivity of plain CT scan in the detection of liver tumors larger than 2 cm is 92%, but the sensitivity in detection of a liver tumor smaller than 2 cm is only 8%. The sensitivity of MRI in detection of the liver tumor smaller than 2 cm is 33% (11). These patients cannot undergo contrast CT because of poor renal function. The sensitivity of contrast CT for a liver tumor smaller than 2 cm is 20% (11). The hepatic tumor in the current patient had invaded diffusely and each tumor was too small to be visualized.

It can be difficult to differentiate other disease associated with hepatomegely when marked hepatomegely is seen on CT and MRI and no hepatic nodular lesion is visualized. Likewise, in most previous cases, appropriate chemotherapy was not performed, and no diagnosis was made before death, because FHF develops secondary to diffuse liver metastasis, grows rapidly and no effective treatment has yet been established, except for chemotherapy (10, 12, 13). In conclusion, diffuse liver metastasis must be considered when imaging modalities show hepatomegely in patients with fulminant hepatic failure, especially when viral hepatitis and drug reactions are excluded.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Alpha-fetoprotein above normal levels as a risk factor for the development of hepatocellular carcinoma in patients infected with hepatitis C virus

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Abstract
Background Noninvasive risk factors are required for predicting the development of hepatocellular carcinoma (HCC) not only in patients with cirrhosis but also in those with chronic hepatitis who are infected with hepatitis C virus (HCV).
Methods A total of 707 patients with chronic HCV infection without other risks were evaluated for the predictive value of noninvasive risk factors for HCC, including age, sex, viral load, genotype, fibrosis stage, aspartate and alanine aminotransferase levels, bilirubin, albumin, platelet count, and alpha-fetoprotein (AFP) at entry to the study, as well as interferon (IFN) therapy they received.
Results The ten-year cumulative incidence rates of HCC for patients with fibrosis stages F0/F1, F2, F3, and F4 were 2.5, 12.8, 19.3, and 55.9%, respectively. Multivariate analysis identified age ≥57 years [hazard ratio (HR) 2.026, $P = 0.004$], fibrosis stage F4 (HR 3.957, $P < 0.001$), and AFP 6–20 ng/mL (HR 1.942, $P = 0.030$) and ≥20 ng/mL (HR 3.884, $P < 0.001$), as well as the response to IFN [relative risk (RR) 0.099, $P < 0.001$], as independent risk factors for the development of HCC. The ten-year cumulative incidence rates of HCC in the patients with AFP levels of <6, 6–20, and ≥20 ng/mL at entry were 6.0, 24.6, and 47.3%, respectively.
Conclusions Not only high (>20 ng/mL), but also even slightly elevated (6–20 ng/mL) AFP levels, could serve as a risk factor for HCC to complement the fibrosis stage. In contrast, AFP levels <6 ng/mL indicate a low risk of HCC development in patients infected with HCV, irrespective of the fibrosis stage.

Keywords Alpha-fetoprotein · Hepatitis C virus · Hepatocellular carcinoma

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Introduction

Worldwide, an estimated 170 million people are persistently infected with hepatitis C virus (HCV) [1, 2], and they are at high risk of developing hepatocellular carcinoma (HCC) [1, 3–5]. Several factors have been identified that increase the risk of HCC, including, age, male gender, and alcohol intake, as well as cirrhosis and the duration of infection [3, 5]. Of these factors, the stage of liver fibrosis parallels the risk for HCV-associated HCC. The annual incidence of HCC in patients with HCV-related cirrhosis ranges from 1 to 7% [6, 7]. Although liver biopsy is the gold standard for the assessment of hepatic fibrosis [8, 9], it is too invasive a procedure to be acceptable as a routine test [10, 11]. In place of liver biopsy, the platelet count is used to estimate the degree of fibrosis [12–14], and low platelet counts have been shown to be a risk factor for the development of HCC in cirrhotic patients [13, 15, 16]. In this study, we tried to identify noninvasive markers for predicting the development of HCC in a large cohort of

patients with chronic HCV infection during a long observation period.

Patients and methods

Study design

Between January 1992 and December 2003, 832 patients were identified who were positive for both anti-HCV, by a second or third-generation enzyme-linked immunosorbent assay (ELISA), and for HCV RNA by polymerase chain reaction (PCR). These patients underwent liver biopsy guided by ultrasonography (US) at the National Nagasaki Medical Center. Of the 832 patients, 125 (15.0%) were excluded according to the following criteria: (1) positive for hepatitis B surface antigen (HBsAg) ($n = 12$); (2) heavy habitual drinking defined as an average daily consumption of >100 g ethanol ($n = 26$); (3) presence of autoimmune hepatitis (AIH), primary biliary cirrhosis, or idiopathic portal hypertension ($n = 8$); (4) positive antinuclear antibody (defined as a titer of >320×) without a diagnosis of AIH ($n = 8$); or (5) a short follow-up period (<180 days) ($n = 71$). The remaining 707 patients were analyzed retrospectively for the incidence of HCC. Their medical histories had been recorded, with the results of routine tests for blood cell counts, liver biochemical parameters, and markers for HCV infection at the time of US-guided liver biopsy at regular intervals. Complete blood cell counts and biochemical tests were performed, using automated procedures, at the clinical pathology laboratories of the National Nagasaki Medical Center. Iinformed consent was obtained from each patient included in the study, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a-priori approval by the institution's human research committee.

Staging of hepatic fibrosis

Liver biopsy was taken by fine-needle aspiration (18G or 16G sonopsy) guided by US. Liver tissue specimens were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. They were evaluated for the stage of hepatic fibrosis by a pathologist according to the criteria of Desmet et al. [17].

HCV RNA, HCV core antigen, and HCV genotypes

HCV RNA was determined by reverse transcriptase (RT)-PCR using a commercial kit (Amplicor HCV; Roche Diagnostic Systems, Basel, Switzerland). HCV core antigen was determined using the lumispot EIKEN HCV antigen assay (Eiken Chemicals, Tokyo, Japan). HCV core antigen levels were classified as low or high with the cutoff at 1,000 fmol/L [18, 19]. Genotypes of HCV were determined by RT-PCR with genotype-specific primers (HCV RNA core genotype; Roche Diagnostics, Tokyo, Japan) [20, 21].

Interferon therapy

During the observation period, 373 of the 707 (52.8%) patients received interferon (IFN) monotherapy, pegylated (PEG)-IFN monotherapy, combination therapy with IFN and ribavirin, or PEG-IFN and ribavirin. Sustained virological response (SVR) was defined as the absence of detectable HCV RNA by the end of treatment that persisted for longer than 6 months thereafter, while failure in meeting these criteria was judged as non-SVR. There was no relapse of viremia after 6 months among SVR patients.

Diagnosis of hepatocellular carcinoma

Patients were followed up with hematological and biochemical tests at intervals of 1–12 months. Liver imaging was performed by US at 6- to 12-month intervals in most patients at fibrosis stages F0–F2, while computed tomography (CT), magnetic resonance imaging (MRI), or US was performed at 3- to 6-month intervals in patients at fibrosis stages F3 and F4. HCC was diagnosed by typical vascular patterns on CT, MRI, or angiography, or by fine-needle biopsy of space-occupying lesions detected in the liver.

Statistical analysis

Continuous variables [platelet counts, albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alpha-fetoprotein (AFP), HCV core antigen] were dichotomized with respect to the median value or clinically meaningful values in a multivariate analysis. To estimate the cumulative risk of developing HCC, the Kaplan–Meier method and the log-rank test were used. Cox proportional hazards regression analysis was performed to evaluate risk factors for HCC. Analysis was performed by Bonferroni's correction and data analysis was performed with SPSS ver. 11.0 (SPSS, Chicago, IL, USA).

Results

Characteristics at enrollment

Table 1 lists the characteristics of the 707 patients at enrollment. The median age was 57.0 years; 120 (17.0%)

Table 1 Demographic, clinical, and virological characteristics of 707 patients persistently infected with hepatitis C virus (HCV)

Age (years)	57.0 (19–79)
Male	351 (49.6%)
Observation period (years)	8.2 ± 4.4[a]
Interferon therapy	373 (52.8%)
Habitual alcohol intake	135 (19.1%)
Fibrosis stage	
F0/F1	273 (38.6%)
F2	193 (27.3%)
F3	121 (17.1%)
F4	120 (17.0%)
Platelet count ($\times 10^3/mm^3$)	156 (30–391)
Albumin (g/dL)	4.2 (2.7–5.3)
Total bilirubin (mg/dL)	0.7 (0.1–2.5)
Aspartate aminotransferase (AST; IU/L)	53 (11–422)
Alanine aminotransferase (ALT; IU/L)	82 (1–1,057)
Alpha-fetoprotein (AFP; ng/mL)	6 (1–510)
HCV core antigen	
≥1,000 fmol/L	539 (76.2%)
HCV genotype	
1b	510 (72.1%)
2a/2b	195 (27.6%)
Unknown	2 (0.3%)

Values are medians with ranges in parentheses, or means with SD in parentheses

[a] Mean ± SD

patients were diagnosed histologically with liver cirrhosis (fibrosis stage: F4) and the remaining 587 had chronic hepatitis (fibrosis stage F0, F1, F2, or F3). The median value of AFP was 6 ng/mL. The average follow-up period was 8.2 years. The patients were classified into three categories by the level of AFP; 350 patients (49.5%) had AFP levels of <6 ng/mL, 254 (35.9%) had levels between 6 and 20 ng/mL, and the remaining 103 (14.6%) had levels of ≥20 ng/mL.

IFN therapy and IFN response

Of the 120 patients with cirrhosis (fibrosis stage F4), 46 (38.3%) received IFN while the remaining 74 (61.7%) did not. The proportions of IFN-treated patients showing an SVR were 40.8% (56/137) in patients with F1; 37.6% (44/117) in those with F2; 32.8% (24/73) in those with F3; and 32.6% (15/46) in those with F4.

Risk factors for HCC

Cox regression analysis was performed on several variables, including age, sex, alcohol consumption, IFN therapy during the observation period, and biochemical as well as virological parameters. The following factors were identified as showing an increased risk for HCC by the univariate analysis: age; IFN therapy; fibrosis stage; platelet count; albumin; AST, ALT, and AFP levels; and HCV genotype (Table 2). Multivariate analysis was performed on these factors (Table 3), and the following were identified as independent risk factors: fibrosis stage (F4), AFP (6–20 and ≥20 ng/mL), age (≥57 years), and IFN therapy (SVR).

Development of HCC

During the follow-up period, HCC developed in 110 (15.6%) patients. Of the 110 patients with HCC, 58 (52.7%) were diagnosed with the disease by histological examination of biopsy-obtained or resected liver specimens. Of these 58 patients, 24 (41.3%) had hypovascular HCC.

Among the patients with HCC, only eight (7.2%) had AFP <6 ng/mL at the time of diagnosis of HCC. Figure 1 shows Kaplan–Meier estimates of the cumulative risk of HCC with respect to fibrosis stage at entry. The 10-year cumulative incidence rates of HCC for stages F0/F1, F2, F3, and F4 were 2.5, 12.8, 19.3, and 55.9%, respectively.

There were significant differences in cumulative incidence rates among the three groups of patients with different AFP levels. The 10-year cumulative risk of HCC was 6.0% in the 350 patients with AFP <6 ng/mL at the study entry, 24.6% in the 254 patients with AFP 6–20 ng/mL, and 47.3% in the 103 patients with AFP ≥20 ng/mL ($P < 0.001$) (Fig. 2). Of the 350 patients with AFP <6 ng/mL, 21 eventually developed HCC during the observation period. Fourteen of these 21 patients were ≥57 years old and 10 had fibrosis stage F3 or F4. In remarkable contrast, HCC ultimately developed in 84.5% of the patients with AFP ≥20 ng/mL.

The 10-year cumulative incidence rates of HCC were 3.1% in patients with SVR to IFN, 14.6% in patients with non-SVR, and 29.5% in the patients without IFN therapy (Fig. 3). Of the 139 patients with SVR, three (2.2%) eventually developed HCC during the observation period. These three patients had advanced fibrosis stages at the study entry (1 with F3 and 2 with F4). Figure 4 shows the cumulative incidence of HCC in the patients with different AFP levels, stratified by the fibrosis stage. In the patients with fibrosis stage F4, there were significant differences in HCC incidence between those with AFP levels of <6 and those with levels of ≥20 ng/mL.

Figure 5 shows the proportions of patients with different AFP levels stratified by the fibrosis stage. The proportion of patients with AFP <6 ng/mL decreased with the advance of fibrosis stage, and conversely, the proportion of patients with AFP ≥20 ng/mL increased with the advance of fibrosis stage. There was a strong correlation between AFP levels and the fibrosis stage.

Table 2 Factors increasing the risk for hepatocellular carcinoma (HCC), determined by univariate analysis

Features	Hazard ratio	*P* value
Age		
<57 years	1	
≥57 years	3.889	<0.001
Sex		
Female	1	
Male	1.146	0.475
Alcohol intake		
None	1	
Habitual	1.012	0.962
Interferon therapy		
None	1	
Non-SVR	0.523	0.002
SVR	0.063	<0.001
Fibrosis stage		
F0/F1	1	
F2	1.863	0.096
F3	3.985	<0.001
F4	13.045	<0.001
Platelet count		
$\geq 150 \times 10^3/mm^3$	1	
$<150 \times 10^3/mm^3$	4.644	<0.001
Albumin		
≥4.2 g/dL	1	
<4.2 g/dL	2.952	<0.001
Total bilirubin		
<0.7 mg/dL	1	
≥0.7 mg/dL	1.438	0.065
AST		
<53 IU/L	1	
≥53 IU/L	2.501	<0.001
ALT		
<82 IU/L	1	
≥82 IU/L	1.514	0.035
AFP		
<6 ng/mL	1	
6–20 ng/mL	4.628	<0.001
≥20 ng/mL	10.335	<0.001
HCV core antigen		
<1,000 fmol/L	1	
≥1,000 fmol/L	1.112	0.645
HCV genotype		
2a/2b	1	
1b	1.730	0.027

SVR sustained virological response

Table 3 Factors increasing the risk for HCC, determined by multivariate analysis

Features	Hazard ratio (95% CI)	*P* value
Fibrosis stage		
F0/F1	1	
F2	1.030 (0.471–2.253)	0.942
F3	1.682 (0.632–3.713)	0.198
F4	3.957 (1.861–8.411)	<0.001
AFP		
<6 ng/mL	1	
6–20 ng/mL	1.942 (1.066–3.538)	0.030
≥20 ng/mL	3.884 (2.014–7.433)	<0.001
Age		
<57 years	1	
≥57 years	2.026 (1.261–3.255)	0.004
Interferon therapy		
None	1	
Non-SVR	0.704 (0.453–1.094)	0.119
SVR	0.099 (0.029–0.334)	<0.001

CI confidence interval

Discussion

In the present study, four variables were identified as risk factors for HCC in patients with chronic HCV infection: fibrosis stage, AFP level, age, and IFN therapy. Previous reviews have analyzed risk factors for the development of HCC [3, 22–25]. Yoshida et al. [6] have reported that the annual incidence increases with the stage of liver fibrosis, from 0.5% in patients with stage F0 or F1 to 7.9% in patients with stage F4 (cirrhosis). In our study, the cumulative incidence of HCC increased along with the advance of fibrosis stage. AFP is used as a serological marker of HCC, and is employed in combination with US for screening HCC [3]. Several reports have shown an elevated AFP level as a risk factor for the development of HCC among patients infected with HCV [16, 25–32]. Most of the studied patients had cirrhosis that was not definitely diagnosed by clinical symptoms and ultrasonographic findings. There have been few studies on patients with chronic hepatitis C, in addition to those with cirrhosis [27]. Thus, it has been unclear whether elevated AFP levels are a risk factor for the development of HCC in patients infected with HCV. Against this background, we were prompted to analyze the utility of AFP as a risk factor for the development of HCC in patients who had been histologically diagnosed by US-guided liver biopsy. In the present study,

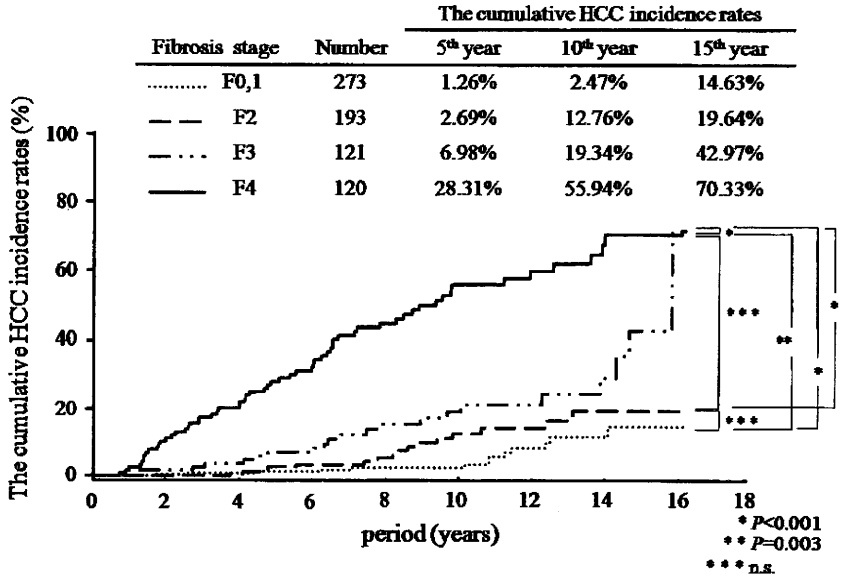

Fig. 1 Cumulative incidence of hepatocellular carcinoma (*HCC*) according to the fibrosis stage

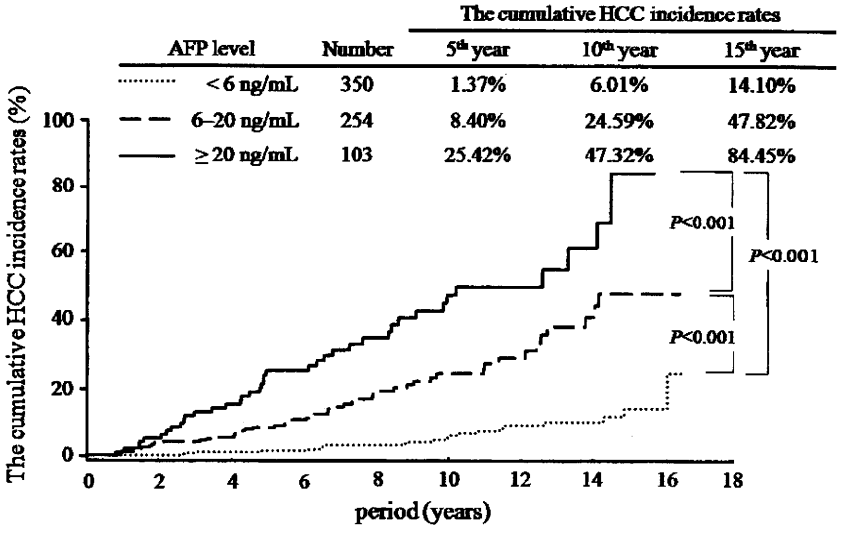

Fig. 2 Cumulative incidence of HCC according to alpha-fetoprotein (*AFP*) levels

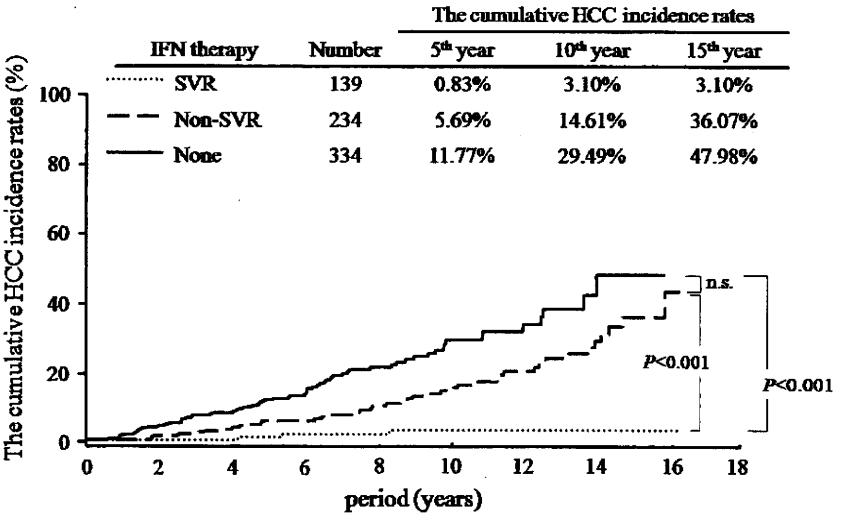

Fig. 3 Cumulative incidence of HCC according to interferon (*IFN*) therapy. *SVR* Sustained virological response

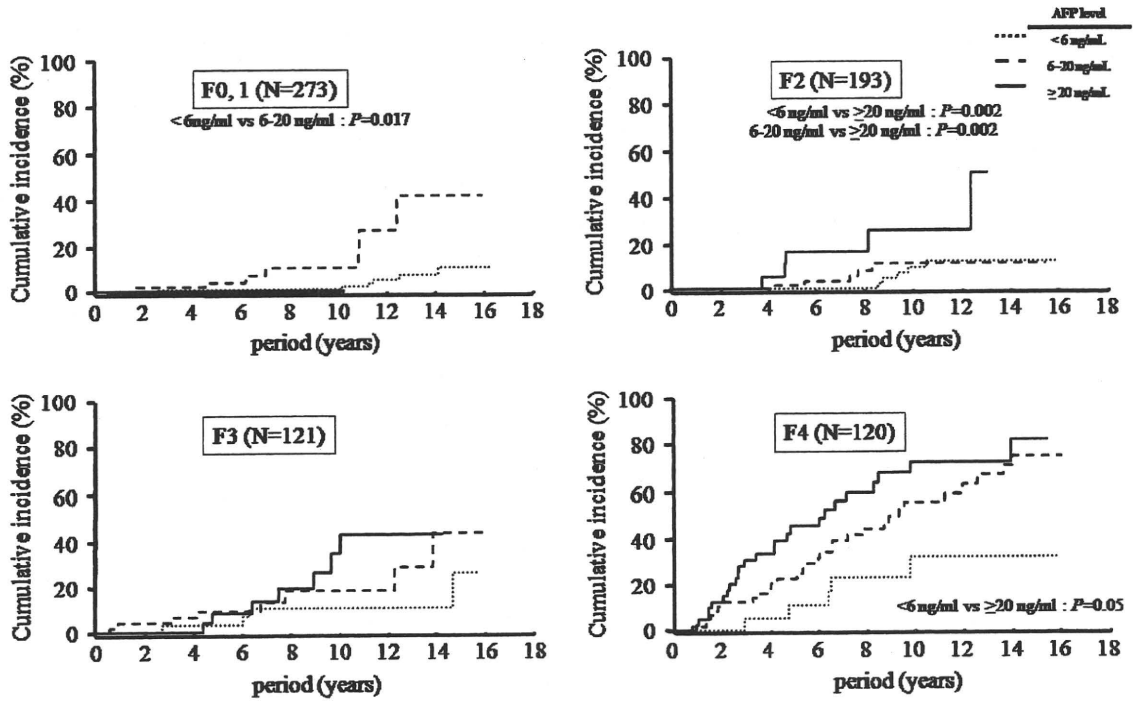

Fig. 4 Cumulative incidence of HCC according to AFP levels, stratified by the fibrosis stage

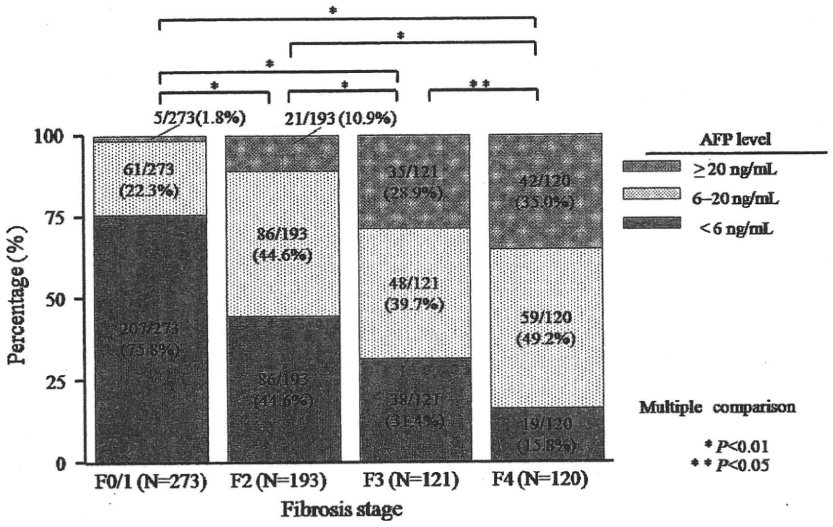

Fig. 5 Proportions of patients with three different AFP levels (<6 ng/mL, 6–20 ng/mL, and ≥20 ng/mL) at different fibrosis stages

among patients infected with HCV, including not only those with cirrhosis but also those with chronic hepatitis, we found AFP levels to be a dependable risk factor for HCC, in addition to the fibrosis stage. Of particular note, not only the patients with high AFP levels (≥20 ng/mL) but also those with even slightly elevated AFP levels (between 6 and 20 ng/mL) had increased risks for the development of HCC. In the patients in this study, the median AFP level was 6 ng/mL. It deviated slightly from serum levels of AFP in healthy adults that have been reported to range from 0.1 to 5.8 ng/mL [33]. Hence, we performed analyses by setting various AFP cutoff levels for evaluating their performance as risk factors. However, there were no significant differences in the analysis with the use of AFP cutoff levels exceeding 7 ng/mL. On the basis of these observations, an AFP cutoff level of 6 ng/mL was adopted in this study. In previous reports, AFP levels were associated with advanced fibrosis stage in patients infected with HCV in the absence of HCC [34–38]. In the present study, AFP levels were elevated in parallel with advanced fibrosis stages and correlated well with the fibrosis stage. As the patients with even slightly elevated AFP levels, between 6 and 20 ng/mL, had moderately advanced liver fibrosis stages, these AFP levels could

indicate an elevated risk for HCC in patients with chronic HCV infection.

Hu et al. [36] found that an AFP level of 15.0 mg/mL could detect severe fibrosis with a sensitivity of 22.8% and specificity of 94.5%. Moreover, they reported, during observation for 6 months of patients with chronic hepatitis C, that AFP levels stayed within the normal range (<10 ng/mL) in 60%, were persistently elevated in 24%, and fluctuated in the remaining 15%. By multivariate analysis, they identified AST, INR, and fibrosis as risk factors for AFP levels of >10 ng/mL. In view of the correlation between AFP levels and fibrosis stages, the AFP level at the time of liver biopsy was taken into account in the analysis in the present study; ALT levels are reported to be persistently elevated in the majority (60%) of patients with chronic hepatitis C.

Liver biopsy is the gold standard for assessing hepatic fibrosis [8, 9]. However, the needle liver biopsy has a sampling error and is too invasive as a routine procedure [10, 11]. Therefore, AFP levels may be used as a noninvasive and predictive marker in place of the fibrosis stage. The platelet count is known to reflect the severity of chronic hepatitis C [12, 13], and is used to estimate the degree of fibrosis without resort to liver biopsy [12–14]. Previous reports have shown low platelet counts to represent a risk factor for HCC in cirrhotic patients [13, 15, 16]. Matsumura et al. [13] reported that age and serum platelet count were significant risk factors for the development of HCC, and as such, they were a major clinico-laboratory means of evaluating the fibrosis stage. In the present study, however, the platelet count was not an independent risk factor for HCC development. When Cox regression analysis was performed on variables other than the fibrosis stage, platelet count and serum albumin levels were identified as independent risk factors for the development of HCC (data not shown).

IFN has been used to treat patients with HCV infection. Failure to achieve an SVR to IFN-based therapies, and preexisting advanced hepatic fibrosis and/or cirrhosis, are major predictors of HCC [6, 23, 25, 39, 40]. In the present study, SVR emerged as an independent risk factor for the development of HCC, while non-SVR was not. However, the cumulative incidence rate of HCC in patients with non-SVR was lower than that in those without IFN therapy. These results suggest that the use of IFN in patients with HCV-related liver disease may be beneficial in preventing the development of HCC. Several Japanese cohort studies have demonstrated that IFN therapy reduces the incidence of HCC, not only in sustained virological responders but also in transient responders who have failed to eliminate HCV [6, 41–45]. In cirrhotic patients, Nishiguchi et al. [39] reported that the relative risk of patients with IFN-alpha treatment developing HCC was 0.067 in comparison with the control group. In contrast, Valla et al. [46] could not prove any significant benefit for the prevention of HCC between patients with and without IFN treatment. Camma et al. [47] suggested a slight preventive effect of IFN on HCC development in patients with HCV-related cirrhosis. Shiffman et al. [48] have reported that continuous IFN therapy led to a decline in hepatic fibrosis despite the persistence of viremia. In addition, there are case reports that IFN therapy reduced AFP levels in virological nonresponders [49]. Murashima et al. [50] showed that IFN therapy, but not Strong Neo-Minophagen C (SNMC) (Glycyrrhizin, Tokyo, Japan), universally reduced basic AFP levels. In an in vitro study of the effects of IFN on an HCC cell line, IFN exhibited antitumor effects [51]. Taken together, these findings suggest that AFP levels may be useful for predicting the development of HCC during IFN-based treatments, including long-term low-dose IFN therapy.

There have been several reports on the relationship between chronological trends in platelet counts, AST or AFP levels, and the development of HCC [11, 26, 27, 52–54]. Tarao et al. [52, 53] showed that in patients with HCV-related cirrhosis, those with persistently high serum ALT levels had a high risk of developing HCC and multicentric carcinogenesis, whereas those with persistently low ALT levels faced a very low risk. Likewise, the dynamics of AFP levels in patients with chronic HCV infection may be useful to estimate the risk of developing HCC. Recently, Bruce et al. [32] found serial measurements of AFP helpful in identifying persons with advanced fibrosis. They used an AFP level of 8 ng/mL, the test manufacturer's upper limit of normal, as the evaluation of the risk of development of HCC. It is not certain whether or not AFP would be a risk factor of HCC development in patients with chronic liver disease of etiologies other than persistent HCV infection. Velazquez et al. [55] reported that an AFP level of >5 ng/mL at study entry was associated with the development of HCC in their univariate analysis but not in their multivariate analysis. They speculated that this could have been because the main causative factor of liver cirrhosis in their series was alcohol. Taken together, the findings of various studies suggest that the baseline AFP level may be more reliable as a predictive factor for the development of HCC in patients with HCV-related liver disease than in those with liver disease of other etiologies.

In conclusion, AFP is a noninvasive predictive marker for the development of HCC in patients infected with HCV. The present study indicates that not only high AFP levels (≥20 ng/mL) but also slightly elevated AFP levels, between 6 and 20 ng/mL, could indicate substantial risks for the development of HCC, complementing the fibrosis stage. In contrast, AFP levels of <6 ng/mL indicate a low risk of HCC development, irrespective of the liver fibrosis stage. IFN therapy significantly reduces the risk of the