

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.

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).

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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.

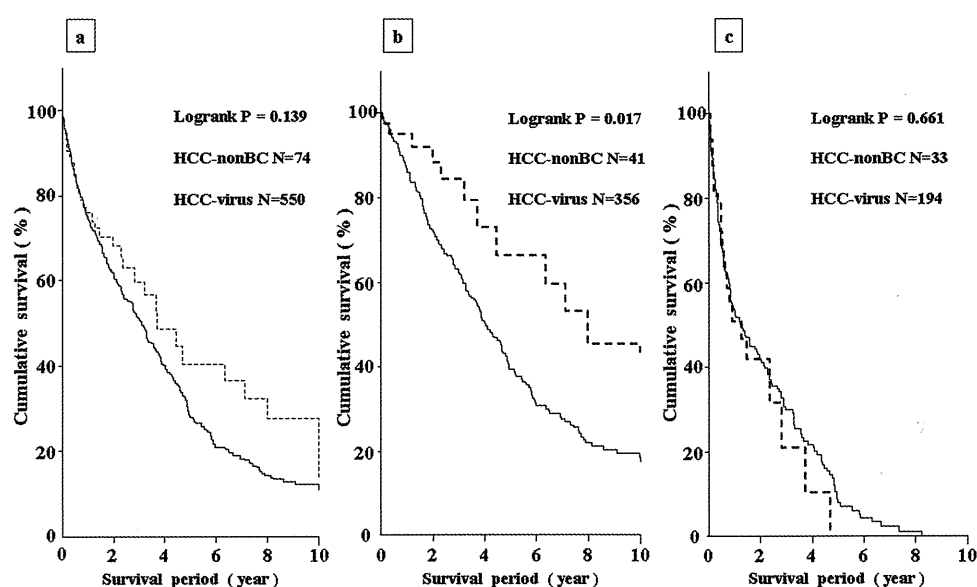


Figure 1. The cumulative survival rate in HCC patients without hepatitis virus infection (HCC-nonBC, dashed-line) and in HCC patients infected with hepatitis virus (HCC-virus, thin line) according to the TNM staging system.

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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