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A Long-term Follow-up Study on Risk Factors for Hepatocellular Carcinoma among Japanese Patients with Liver Cirrhosis

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To identify virological parameters (serostatus of hepatitis B surface antigen [HBsAg] and antibodies to hepatitis C virus [anti-HCV], HCV genotypes and HCV-RNA titer) and other clinico-biological and lifestyle variables that may influence or predict the development of hepatocellular carcinoma (HCC) in cirrhosis, we followed 100 cirrhotic patients without HCC, who visited Kyushu University Hospital between 1985 and 1987, until the end of 1995 (follow-up rate: 98%; average follow-up period: 5.3 years). After elimination of 4 patients who developed HCC or were censored within the initial 6 months, 37 (39%) out of 96 patients developed HCC during follow-up. As compared with HBsAg(+) patients, anti-HCV(+) HBsAg(–) patients demonstrated significantly elevated HCC risk (adjusted hazard ratio [HR]=5.85, 95% confidence interval [CI] 1.65–20.67). Genotype 1 HCV infection was not associated with increased risk compared with genotype 2 (HR=0.64, 95% CI 0.21–1.99). For genotype 1 HCV infection, patients with HCV-RNA levels <1 Meq/ml tended to present lower risk than patients with ≥1 Meq/ml ($P=0.03$). Male sex, advanced Child's class, lower serum albumin, and higher serum aminotransferase and α-fetoprotein were also found to be strong predictors. Overall, drinking and smoking habits were not associated with significantly elevated risk. Among virological parameters, anti-HCV positivity and, possibly high HCV-RNA titer, were predictive of HCC occurrence in cirrhosis in our clinical setting.

Key words: Hepatocellular carcinoma — Cirrhosis — Follow-up study — Hepatitis C virus — Risk factor

Hepatocellular carcinoma (HCC) develops mostly in patients with liver cirrhosis (LC), with the reported yearly incidence ranging from 3 to 7%.[1–3] Because of this high incidence and the poor prognosis, identification of risk factors or predictors of HCC occurrence in cirrhosis is a major clinical concern from both etiological and managerial points of view. Male sex, increasing age, and elevated α-fetoprotein levels have been well documented as established determinants,[1, 3, 4] yet the roles of other factors, particularly virological parameters, remain to be elucidated.

Since the discovery of hepatitis C virus (HCV), a number of clinical and epidemiological investigations have been focused on the following questions: 1) whether risk of developing HCC for HCV infection is as high as that for chronic hepatitis B virus (HBV) infection[3, 5]; 2) whether genotype 1b HCV is more likely than other genotypes to lead to severe forms of chronic liver disease or HCC[6]; and 3) whether HCV-RNA titer in serum or liver tissue is associated with the severity of chronic liver disease or the development of HCC.[7, 8] However, most studies employed cross-sectional and retrospective designs prone to potential biases and provided controversial results. Follow-up data remain insufficient.

In an attempt to address the above questions, we performed a follow-up study of cirrhotic patients, utilizing previously accumulated data and stored sera. The effects of other clinico-biological and lifestyle factors were also investigated and were controlled through the Cox proportional hazards model in order to examine the associations with virological parameters.

MATERIALS AND METHODS

Study subjects This study involved 100 consecutive Japanese patients with LC who were attending or were admitted to the Third Internal of Medicine and the Second Department of Surgery at Kyushu University Hospital between December 1985 and December 1987. These patients were recruited as one of the case groups in previous case-control studies[9, 10] and met the following selection criteria at enrollment (i.e., on the date of the interview survey described below): a) without evidence of HCC based on ultrasonography, computed tomography, and/or serum α-fetoprotein level; b) aged 40–69 years; c) residents in Fukuoka or Saga prefecture (adjacent to Fukuoka prefecture); and d) of Japanese nationality. In addition, patients with special forms of LC (primary or secondary biliary cirrhosis, and cirrhosis due to autoim-

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mune hepatitis, parasitosis, congestive heart failure, or metabolic disorders) were excluded. Both prevalent and newly diagnosed cases were eligible, since it was practically difficult to collect a sufficient number of incident cases. At outpatient clinics, eligible outpatients were interviewed regarding their lifestyles and other relevant factors, and, if scheduled by attending physicians, venous blood was drawn on the same day; serum was separated within 3 h, and portions of the sera were stored for future use. For inpatients, interviews and serum collection were conducted during their admission. Laboratory data on the date of interview or, if unavailable, within 1 month before or after interview, were also used as baseline data.

After the follow-up data became complete, we further eliminated 4 patients whose observation periods were 6 months or less, as described later. The remaining 96 patients (62 males and 34 females; 60 patients from the internal medicine department and 36 patients from the surgery department; 65 outpatients and 31 inpatients; 92 residents in Fukuoka prefecture and 4 residents in Saga prefecture) were analyzed in this study. The diagnosis of LC was established by histology for 31 patients (32%), by laparoscopy for 16 patients (17%), and by evident clinical signs (e.g., ascites and esophageal varices) and imaging/laboratory findings for 49 patients (51%). Based on the Child-Turcotte classification,[11] 43 (45%) patients were considered to be in Child's class A at enrollment, 37 patients (39%) in Child's class B, and 16 patients (17%) in Child's class C. None of the 96 patients had received interferon therapy during follow-up.

Follow-up The endpoint in this study was defined as the development of HCC as a final diagnosis more than 6 months after enrollment. The original 100 patients were followed from the date of enrollment until the diagnosis of the endpoint, death from other causes, loss to follow up, or December 31, 1995, whichever came first. Sixty-five patients had been attending the cooperating departments or relevant hospitals until the last date of follow-up. They had undergone monthly checkup of serum α-fetoprotein level and had been examined at 3–6 month intervals by ultrasonography and/or computed tomography, followed by angiography and/or liver biopsy if HCC occurrence had been suspected on the basis of the former diagnostic procedures.

The remaining 35 patients had moved to other hospitals or had ceased attending the cooperating departments. For these patients, the vital status as of December 31, 1995 was investigated through municipal public offices retaining their permanent address records ("koseki" in Japanese); 24 were dead, and 11 were alive. For the 24 deceased, copies of death certificates were obtained from local government offices (Bureau of Legal Affairs), and we contacted the finally attending physicians or their colleagues to inquire about the presence or absence of HCC at the time of death and, if present, the date of a final diagnosis of HCC and diagnostic methods; complete follow-up information was obtained for all but 2 patients.

We located the remaining 11 survivors by utilizing all available information in past medical records. Ten patients were still attending clinics, and with the patients' permission we referred to their attending physicians for information about their current condition; all were receiving periodical examinations by routine methods, and none had evidence of HCC as of December 31, 1995. One patient who had not been attending any hospital since his last attendance at the cooperating medicine department visited the department after telephone contact, and ultrasonography revealed no evidence of HCC.

According to the definition of the endpoint, patients had to be observed for more than 6 months. We thus excluded 1 patient who had been lost to follow up 1 month after enrollment, 1 patient who had died 1 month after enrollment, and 2 patients who had developed HCC within the initial 6 months, leaving 96 patients for the analysis. Of the 96 patients, 37 (39%) had developed HCC as the end point; 11 were diagnosed from histology, 21 from angiography, and 5 from computed tomography and/or ultrasonography; all of the last 5 patients had subsequently died of the disease. The average follow-up period was only 5.3 years despite the high follow-up rate, because of a low survival rate among the 96 patients (5 and 10 year cumulative survival rates: 61% and 25%).

Interviews Lifetime drinking and smoking habits as well as past history of blood transfusion were ascertained by a trained interviewer, using structured questionnaires. All interviews were tape-recorded and double-checked against the questionnaires. In this study, heavy drinking history was defined as having consumed 80 ml or more of ethanol per day for at least 10 years. Cumulative alcohol consumption in drink-years and cumulative smoking amount in pack-years were computed as reported previously.[12] Transfusion events definitely related to liver disease (e.g., transfusion due to variceal bleeding) were excluded in defining a transfusion history.

Laboratory tests Serum levels of albumin (normal range: 3.9–5.0 g/dl), asparate aminotransferase (AST, normal range: 0–40 U/liter), alanine aminotransferase (ALT, normal range: 0–40 U/liter), bilirubin (normal range: 0.2–1.2 mg/dl), and α-fetoprotein (AFP, normal range <20 ng/ml) were determined by standard clinical laboratory procedures, and serum hepatitis B surface antigen (HBsAg) by a reverse passive hemagglutination method (Auscell, Abbott, Chicago, IL). For 72 (75%) out of the 96 patients, sera were kept frozen at −70°C and were made available for testing HCV markers. Serum antibodies to HCV (anti-HCV) were measured by a second-generation immunoradiometric assay (IRMA II, Ortho, Raritan, NJ). Positive results in this assay were further confirmed by a second-

generation recombinant immunoblot assay (RIBA II, Chiron Corp., Emeryville, CA). Reactivity by RIBA II, or an indeterminate result by RIBA II with the presence of serum HCV-RNA were regarded as positive for anti-HCV.

For all 72 serum specimens, the presence or absence of HCV-RNA was examined by a commercially available assay (Amplicor HCV, Roche Diagnostic Systems, Basel, Switzerland). HCV genotypes (I, II, III, and IV) were determined by amplifying a putative core-region sequence with genotype-specific primers as described by Okamoto *et al.*[13] According to the nomenclature system for major genotypes (or *types*) proposed by Simmonds *et al.*,[14] we classified genotypes I and II as genotype 1 and genotypes III and IV as genotype 2. Furthermore, the major HCV genotypes were serologically determined by an enzyme-linked immunosorbent assay (Imucheck HCV, International Reagents Corp., Kobe).[15] The HCV-RNA titer in serum was measured by a first-generation branched DNA signal amplification assay (Quantiplex HCV-RNA, Chiron Corp.).

Statistical analysis Statistical analyses were performed with the SAS/PC statistical package (SAS Institute Inc., Cary, NC). χ^2 tests were used for unadjusted comparisons based on frequency. The Kruskal-Wallis test was conducted to compare distributions of continuous variables. The cumulative incidence of HCC was calculated by the method of Kaplan and Meier.[16] The difference among the incidence curves was evaluated by means of a log-rank test. Cox proportional hazards models were employed to estimate the hazard ratios (HRs) of developing HCC for main exposure variables with adjustment for potential confounders.[17] The adjusted HCC incidence according to a main exposure variable for specific covariate values was computed as the product limit estimate for the Cox model.[17] All reported *P* values are two-tailed, and those values less than 0.05 were considered statistically significant.

RESULTS

Hepatitis virus markers and other clinico-biological/lifestyle variables Of all 96 patients, 15 (16%) were positive for serum HBsAg, whereas 53 (74%) out of 72 patients with stored sera tested positive for anti-HCV; only 1 patient was positive for both markers. Based on these results, the 96 patients were classified into the following 4 categories: 15 HBsAg-positive (B+) patients, 52 HBsAg-negative and anti-HCV-positive (B−C+) patients, 12 patients negative for both markers (B−C−), and 17 HBsAg-negative patients with undetermined anti-HCV (B−CU). Of the 12 B−C− and 17 B−CU patients, 1 B−C− and 1 B−CU patients had a firm diagnosis of alcoholic

Table I. Baseline Characteristics of Study Subjects according to Hepatitis Virus Markers

Factor	Hepatitis virus marker[a]				*P* for difference[c]
	B+ (*n*=15[b])	B−C+ (*n*=52)	B−C− (*n*=12)	B−CU (*n*=17)	
Males (%)	67%	65%	58%	65%	0.97
Age (years, median)	50	56	58	57	0.04
<2 years since LC diagnosis (%)	67%	40%	33%	47%	0.56
Internal medicine patients (%)	87%	63%	42%	53%	0.08
Outpatients (%)	47%	81%	83%	35%	0.001
History of blood transfusion[d] (%)	33%	31%	17%	18%	0.56
Heavy drinking history[e] (%)	13%	17%	33%	24%	0.55
Current smokers (%)	33%	35%	42%	35%	0.97
Child's class A (%)	40%	50%	67%	18%	0.04
Serum albumin (g/dl, median)	3.5	3.6	4.1	3.4	0.007
Serum AST (U/liter, median)	56	82	53	87	0.002
Serum ALT (U/liter, median)	56	77	37.5	79	0.002
Serum bilirubin (mg/dl, median)	0.8	1.1	1.0	1.1	0.74
Serum AFP <20 ng/ml (%)	60%	58%	100%	65%	0.09

a) B+, HBsAg-positive; B−C+, HBsAg-negative and anti-HCV-positive; B−C−, HBsAg-negative and anti-HCV-negative; B−CU, HBsAg-negative with undetermined anti-HCV.
b) Serum anti-HCV was negative for 7 patients, positive for 1 patient, and undetermined for 7 patients.
c) Based on χ^2 tests (for proportions) or Kruskal-Wallis tests (for continuous variables).
d) Histories of blood transfusion due to liver diseases were excluded (see text).
e) Heavy drinking history was defined as having consumed ≥80 ml of ethanol per day for ≥10 years.

Table II. HCC Incidence among Study Subjects according to Child's Class and Laboratory Measurements at Enrollment

Factor	No. (%)	No. of HCC occurrence	5-year HCC incidence[a](%)	Log-rank P[b]
Child's class				
A	43 (44.8)	13	26	0.04
B	37 (38.5)	17	39	
C	16 (16.7)	7	59	
Serum albumin (g/dl)				
<3.0	14 (14.6)	7	61	0.008
3.0–3.5	33 (34.4)	16	45	
≥3.6	49 (51.0)	14	23	
Serum AST (U/liter)				
<50	19 (19.8)	0	0	0.0002
50–99	50 (52.1)	22	40	
≥100	27 (28.1)	15	55	
Serum ALT (U/liter)				
<50	27 (28.1)	1	5	0.0003
50–99	43 (44.8)	22	45	
≥100	26 (27.1)	14	49	
Serum bilirubin (mg/dl)				
<1.0	41 (42.7)	15	34	0.49
1.0–1.9	43 (44.8)	18	34	
≥2.0	12 (12.5)	4	55	
Serum AFP (ng/ml)				
<20	62 (64.6)	14	17	0.00001
20–99	26 (27.1)	17	71	
≥100	8 (8.3)	6	55	

a) Based on the Kaplan-Meier method.
b) *P* value for the difference in HCC incidence curves by log-rank test.

cirrhosis, although other 3 B–C– and 3 B–CU patients had a history of heavy drinking.

Table I summarizes selected characteristics of the 96 patients according to hepatitis virus markers. Significant differences were observed for age, hospitalization status at enrollment (inpatients vs. outpatients), Child's class, and serum levels of albumin, AST, and ALT. Among the factors listed in Table I, male sex (vs. female sex, P=0.02), <2 years since LC diagnosis (vs. ≥2 years since LC diagnosis, P=0.005), inpatients (vs. outpatients, P=0.0003), advanced Child's class, lower serum albumin, and higher serum AST, ALT and AFP (Table II) were significantly associated with increased incidence of HCC by log-rank tests. The corresponding associations with age (3 categories of 40–49, 50–59, and 69 years, P=0.18), departments (internal medicine vs. surgery, P=0.30), transfusion history (P=0.26), and serum bilirubin level (Table II) were statistically insignificant.

Positive history of heavy drinking (P=0.05) and increased cumulative alcohol consumption in drink-years (P=0.06 by the Cox model) were associated with somewhat lower HCC risk among males, but not among females (P=0.50 and 0.66, respectively). Drinking habit at enrollment (3 categories of never, past, and current drinkers) was not significantly associated with elevated risk (P=0.18 for males and 0.86 for females). Current smoking status (3 categories of never, past, and current smokers; P=0.54 for males and 0.40 for females) or pack-years (P=0.69 for males and 0.88 for females by the Cox model) was also not significantly related to risk increase.

Among all 96 patients, the overall cumulative incidence of HCC was estimated to be 36% at the fifth year and 48% at the tenth year. Table III and Fig. 1A show the cumulative incidence of HCC according to hepatitis virus markers. The B–C+ and B–CU patients demonstrated substantially higher HCC incidence (5-year incidence: 41% and 55%, respectively) than did the B+ patients (29%) and the B–C– patients (9%); only 1 patient developed HCC among the B–C– patients. There was a significant difference in the HCC incidence curves (log-rank test, P=0.03).

HCV genotypes and HCV-RNA titer Of the 53 anti-HCV-positive patients, 43 (81%) had detectable HCV-RNA in serum; 39 patients were classified as genotype 1

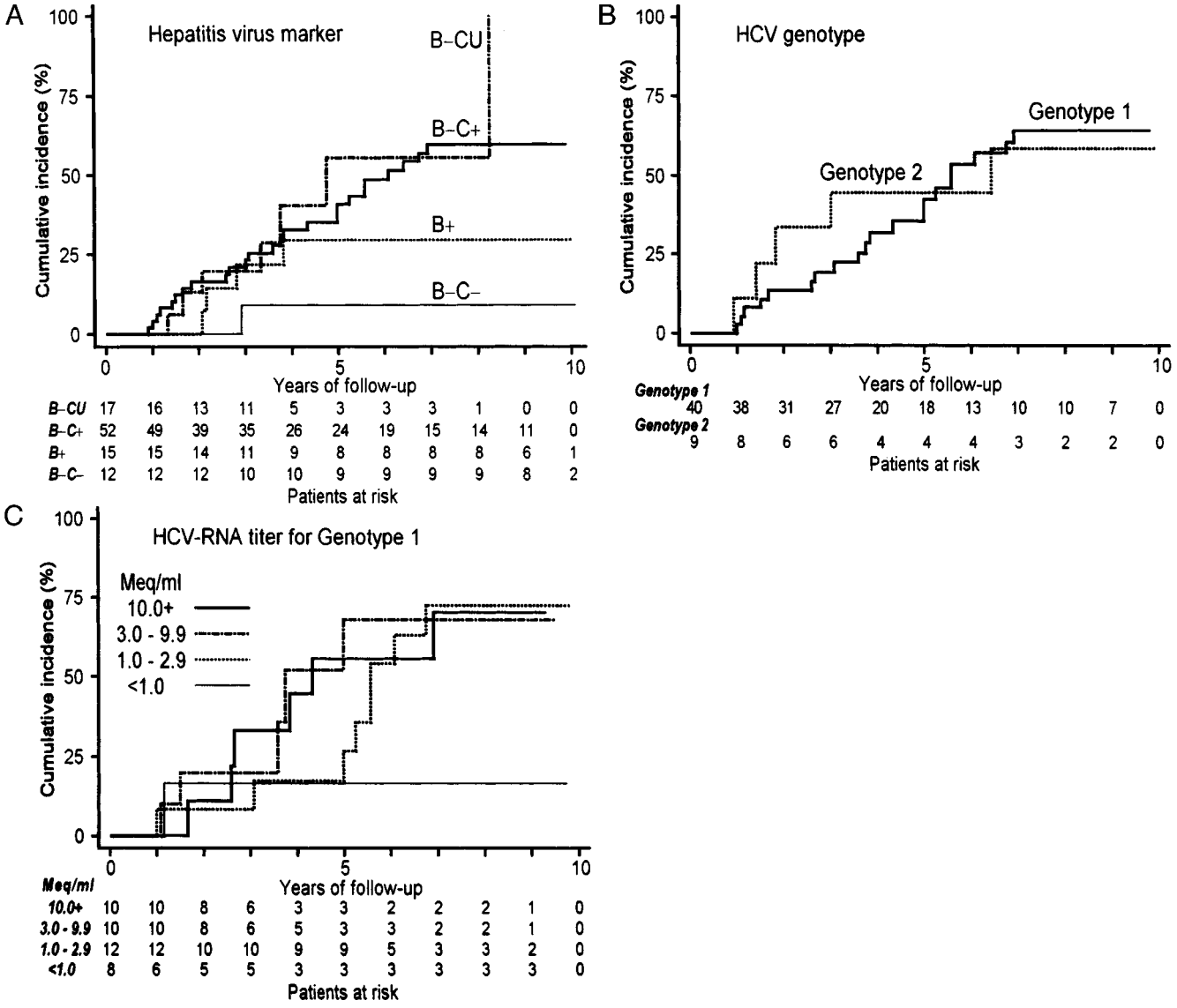

Fig. 1. Cumulative incidence of HCC according to hepatitis virus markers (A), HCV genotypes (B), and serum HCV-RNA titer for genotype 1 infection (C). Abbreviations used are as follows: B+, HBsAg-positive; B–C+, HBsAg-negative and anti-HCV-positive; B–C–, HBsAg-negative and anti-HCV-negative; B–CU, HBsAg-negative with undetermined anti-HCV.

(all subtype 1b) and 4 patients as genotype 2 (subtype 2a for 3 patients and subtype 2b for 1 patient) by reverse transcription-polymerase chain reaction (RT-PCR). Of the 10 HCV-RNA-negative patients, 2 had antibodies to the genotype 1 antigen, 5 had antibodies to the genotype 2 antigen, and 3 tested indeterminate or unreactive by the serological assay. After elimination of 1 patient with genotype 1 infection, who also tested seropositive for HBsAg, 40 B–C+ patients were regarded as having genotype 1 infection and 9 B–C+ patients as having genotype 2 infection. In Table III and Fig. 1B, the cumulative incidence of HCC according to HCV genotypes are presented. No measurable difference was seen between genotypes 1 and 2 (P=0.36).

The HCC incidence according to serum HCV-RNA titer was also estimated among patients with either genotype 1 or 2 infection (Table III and Fig. 1C); HCV-RNA-negative patients with genotype-specific antibodies were included in the lowest level (<1 Meq/ml). For genotype 1, patients with HCV-RNA levels <1 Meq/ml appeared to

Table III. HCC Incidence among Study Subjects according to Hepatitis Virus Markers, HCV Genotypes, and Serum HCV-RNA Titer

Factor	No. (%)	No. of HCC occurrence	5-year HCC incidence[a](%)	Log-rank *P*[b]
Hepatitis virus marker[c]				
B+	15 (15.6)	4	29	0.03
B–C+	52 (54.2)	25	41	
B–C–	12 (12.5)	1	9	
B–CU	17 (17.7)	7	55	
HCV genotype[d]				
Genotype 1	40 (76.9)	20	43	0.36
Genotype 2	9 (17.3)	5	44	
Indeterminate	3 (5.8)	0	0	
HCV-RNA titer (Meq/ml)[d, e]				
Genotype 1				
<1.0	8 (20.0)	1	17	0.45
1.0–2.9	12 (30.0)	8	27	
3.0–9.9	10 (25.0)	6	56	
≥10.0	10 (25.0)	5	68	
Genotype 2				
<1.0	7 (77.8)	3	43	0.20
≥1.0	2 (22.2)	2	50	

a) Based on the Kaplan-Meier method.
b) *P* value for the difference in HCC incidence curves by log-rank test.
c) B+, HBsAg-positive; B–C+, HBsAg-negative and anti-HCV-positive; B–C–, HBsAg-negative and anti-HCV-negative; B–CU, HBsAg-negative with undetermined anti-HCV.
d) One patient who tested positive for both HBsAg and anti-HCV (genotype 1b, HCV-RNA titer <0.5 Meq/ml) was excluded. HCV genotyping was based on both RT-PCR and a serological assay (see text).
e) HCV-RNA-negative patients with genotype-specific antibodies were included in the lowest level (<1.0 Meq/ml).

experience lower HCC incidence, although this was not statistically significant (P=0.45). For genotype 2, no difference in HCC risk was evident according to HCV-RNA titer (<1.0 or ≥1.0 Meq/ml).

Adjusted analyses of hepatitis virus markers, HCV genotypes, and HCV-RNA titer As described above, several clinico-biological variables differed according to hepatitis virus markers, and also were strongly predictive of HCC incidence. We selected the following variables to be adjusted for in multivariate analyses: sex, age (in years), years since LC diagnosis (<2 vs. ≥2), departments (internal medicine vs. surgery), hospitalization status (inpatients vs. outpatients), serum albumin (g/dl), serum AST (U/liter), and serum AFP (<20 vs. ≥20 ng/ml). In our study subjects, the Child's class largely depended on serum albumin level, and so only albumin level was included in the model. Serum AST and ALT were strongly correlated with each other (Pearson correlation coefficient=0.89), but the AST level was slightly more predictive of HCC risk. Additional adjustment for other factors (transfusion history, drinking and smoking habits, or serum bilirubin level) did not essentially change the results. For comparability, identical adjustments were carried out for analyses of HCV genotypes and serum HCV-RNA titer.

Table IV shows the adjusted HR of HCC occurrence for hepatitis virus markers, HCV genotypes and HCV-RNA titer. As compared with the B+ patients, the B–C+ and B–CU patients showed elevated HRs of 5.85 (P=0.006) and 4.15 (P=0.06), respectively, while no such risk increase was evident for the B–C– patients (HR=1.49). Genotype 1 infection was not related to increased risk (HR=0.64), as compared with genotype 2 infection. Overall, there was no significant monotonic association with serum HCV-RNA titer (P for trend=0.17), yet patients with HCV-RNA levels <1.0 Meq/ml appeared to experience decreased risk; the adjusted HR for <1.0 vs. ≥1.0 Meq/ml was calculated as 0.06 (95% confidence interval 0.005–0.75, P=0.03).

The adjusted 5-year incidence (and 95% confidence interval) of HCC occurrence was estimated at 8% (0–18%) for the B+ patients, 39% (4–62%) for the B–C+ patients, 12% (0–32%) for the B–C– patients, and 30% (0–58%) for the B–CU patients, based on predictions from the Cox model for the following covariate levels:

Table IV. Adjusted Hazard Ratios (HRs) of HCC according to Hepatitis Virus Markers, HCV Genotypes, and HCV-RNA Titer

Factor	Adjusted HR[a)]	95% confidence interval
Hepatitis virus marker[b)]		
B+	1.00	(reference)
B−C+	5.85	1.65−20.67
B−C−	1.49	0.13−16.73
B−CU	4.15	0.93−18.56
HCV genotype		
Genotype 1	0.64	0.21−1.99
Genotype 2	1.00	(reference)
HCV-RNA level (Meq/ml) for genotype 1 infection		
<1.0	0.07	0.005−0.98
1.0−2.9	1.00	(reference)
3.0−9.9	1.34	0.36−5.04
≥10.0	1.50	0.39−5.74
P for trend[c)]	0.17	

a) Adjusted for sex, age (in years), years since LC diagnosis (<2 or 2+), department (internal medicine or surgery), hospitalization status (outpatient or inpatient), serum albumin (g/dl), serum AST (U/liter), and AFP level (<20 or 20+ ng/ml).
b) B+, HBsAg-positive; B−C+, HBsAg-negative and anti-HCV-positive; B−C−, HBsAg-negative and anti-HCV-negative; B−CU, HBsAg-negative with undetermined anti-HCV.
c) To each HCV-RNA level, a median value was assigned, and this variable was included in the multivariate Cox model as an independent variable.

male sex, age of 56 years (median among all 96 patients), <2 years since LC diagnosis, internal medicine patients, outpatients, serum albumin of 3.6 g/dl (median), serum AST of 74.5 U/liter (median), and serum AFP <20 ng/ml; these covariate levels were determined from a consideration of the outpatient clinic setting, where patients in relatively early stages of cirrhosis were usually seen.

DISCUSSION

Although the sample size of the present study is rather small, this limitation is partly compensated by the long observation period and the almost complete follow-up information, which have allowed us to identify a substantial number of HCC occurrences. Another limitation is that etiology could not clearly be defined for most B−C− and B−CU patients. The majority of the B−CU patients, however, were likely to be HCV-associated, since the anti-HCV positivity in this group was probably close to that among HBsAg-negative patients with stored sera (52/64 or 81%). This may account for the high HCC incidence among the B−CU patients, which was comparable to that among the B−C+ patients.

Although the main objective of this study was to elucidate the roles of virological parameters, we have also identified other clinico-biological predictors of HCC occurrence. Firstly, more advanced stage of cirrhosis, as indicated by advanced Child's class and lower serum albumin levels, was closely associated with increased risk. Secondly, patients with low serum AST or ALT levels near or within the normal range at enrollment exhibited a very low HCC incidence. Similar findings were reported by Benvegnu *et al*.[18)] and Sato *et al*.[19)] In this connection, it is noteworthy that the B−C+ patients presented higher aminotransferase levels than the B+ or B−C− patients. Ongoing inflammation and liver cell injury may play a key role in hepatocarcinogenesis in HCV-associated cirrhosis, in comparison with a potential direct oncogenic role of chronic HBV infection.[20)] Thirdly, the AFP level at enrollment was strongly predictive of HCC incidence, which is consistent with previous observations.[1, 3)] Lastly, an unexpected finding was that longer time (years) since LC diagnosis was associated with decreased risk. Presumably, this phenomenon may reflect the slowly progressive nature of the disease among patients with longer duration.

Overall, no measurements of drinking and smoking habits were associated with significantly elevated risk in this study. Although the role of heavy alcohol consumption in the development of HCC is well recognized,[21)] it remains uncertain whether this exposure is directly

involved in hepatocarcinogenesis or is indirectly linked to HCC through leading to LC. The results of this study, together with our previous findings in a case-control study of HCC,[12] support the latter possibility. Although Tsukuma *et al*.[3] and Chiba *et al*.[22] observed a significant increase in HCC risk among current smokers, our study failed to detect such a risk increase.

In the current study, the overall annual incidence of HCC was estimated to be about 7%, which appears to be one of the highest figures ever reported.[1–3, 5, 18, 19, 23–25] In terms of hepatitis virus markers, the B−C+ patients showed a higher HCC risk (annual 8%) than did the B+ patients (annual 6%) or the B−C− patients (annual 2%). Among the B+ patients, contamination with anti-HCV-positive patient(s) may be an issue, which could have caused a slight overestimation of the HCC incidence due to potential positive interaction between HBV and HCV.[18] Although the seropositivity of antibodies to hepatitis B core antigen has been correlated with elevated HCC risk irrespective of HBsAg status,[26] we could not address this issue because of the lack of the corresponding data.

Four prospective studies reported a greater HCC incidence in HCV-associated cirrhosis than in HBV-associated cirrhosis,[1, 5, 19, 23] whereas 2 afforded opposite results.[3, 18] Part of this difference may have arisen from different clinical backgrounds of patients from study to study. In an attempt to eliminate the effects of other clinico-biological variables, we performed multivariate analyses based on the Cox model, yet the higher incidence for the B−C+ group remained unchanged. The years since LC diagnosis may be an especially important confounder, and the dichotomous classification (<2 vs. ≥2 years) may appear insufficient for adjustment. However, it did not materially change the current results to employ more levels of classification (<2, 2−3 and ≥4 years) or a continuous variable (in years) (data not shown). Departments (medicine vs. surgery) and hospitalization status were also controlled for as potential confounders, although it was difficult to define clearly the clinical significance of these variables.

This study did not show elevated HCC risk in cirrhosis for genotype 1 HCV infection. A limited number of follow-up studies of patients with chronic hepatitis or cirrhosis on HCV genotypes gave conflicting results.[27–29] Although possible confounding by longer duration of infection for genotype 1b has been suggested,[30] such an effect was not evident among our study subjects.[10] In our case-control study contrasting HCC patients with control subjects undergoing health examinations, we observed about 3−4 fold increase in HCC risk for genotype 1b vs. genotype 2a.[31] This finding may not contradict the current results, if one considers the possibility that genotype 1b HCV may play a role in relatively early stages of liver disease progression prior to the establishment of LC.[10] However, Poynard *et al*.[32] did not find any evidence of the role of HCV genotypes in liver fibrosis progression. Larger cohort studies involving patients with a whole spectrum of liver diseases are needed to address the above possibility.

Although the overall relation to serum HCV-RNA titer in this study was weak, HCC risk for <1 Meq/ml levels appeared to be lower than that for higher levels among patients with genotype 1 infection. Follow-up studies on this association remain very few, although a number of cross-sectional or case-control studies, which investigated the correlation of the HCV-RNA level in serum or liver tissue with the severity of liver disease and the histology activity index, afforded controversial findings.[7] In a follow-up study of patients with chronic hepatitis C, Kobayashi *et al*.[29] found that the HCV-RNA titer at enrollment was positively associated with the deterioration of the stage of liver disease, but, to our knowledge, no other prospective data are available.

In conclusion, among virological parameters, the presence of anti-HCV appeared to be predictive of the development of HCC among cirrhotic patients, at least in our clinical setting. However, other conventional clinico-biological parameters, such as those reflecting severity of cirrhosis or liver cell injury, as well as the AFP level, also turned out to be equally or more important. Genotype 1 HCV infection was not related to risk increase compared with genotype 2 HCV among our study subjects. The positive association with serum HCV-RNA titer may deserve further investigation by a prospective approach because of the lack of sufficient data.

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