Follow-up Studies

After liver biopsy, follow-up examinations were conducted on a monthly to tri-monthly basis by monitoring biochemical and virological data. Although 27 (28.4%) patients started to receive interferon (IFN) therapy because of ALT elevation, none underwent lamivudine therapy during the course of the study. "Activation of hepatitis" or "flare-up of ALT" was defined as an increase of ALT value of twice or more of the upper limit of normal.

Imaging studies were performed annually in each patient and included computed tomography (CT) or ultrasound (US) after 1985. At least 2 imaging procedures were performed in those patients with cirrhosis. HCC was diagnosed by typical hypervascular characteristics on angiography in addition to certain features of CT and US. Pathological confirmation of surgically resected specimens or autopsy was made in 3 (60.0%) of 5 patients who developed HCC during the study.

A total of 17 patients were lost to follow-up after 5 years. The observation period ranged from 5.1 to 24.7 years, with a median of 10.2 years.

Assays of HBV Markers

Serum HBsAg was measured by radioimmunoassay (Dainabot, Tokyo, Japan), and HBeAg and anti-HBe were determined by enzyme-linked immunosorbent assay (ELISA, Institute of Immunology) using commercially available kits. Anti-HCV antibody was analyzed using second-generation anti-HCV kits (enzyme-linked immunosorbent assay, Dainabot). HBV DNA was assayed using frozen sera stored at −80°C and measured with Amplicor HBV Monitor kits (Roche Diagnostics Japan Co., Tokyo). The effective measurement of the concentration ranged from $10^{2.6}$ (400) copies/mL to $10^{7.6}$ (40 000 000) copies/mL. All measurements of HBV DNA were performed at the same time using the same assay kits. Quantitative HBV DNA was conducted at the time of liver biopsy, at the end of 6 months, and at the end of the 1st, 2nd, 3rd, and 5th years.

Statistical Analysis

The chi-square test, Fisher's exact test, and Kruskal-Wallis test were used to analyze the relationship between HBV markers and the clinical course. Cumulative flare-up rate of hepatitis and carcinogenesis rate were calculated with the Kaplan-Meier method,[26] and differences in the rates were analyzed by the log-rank test. Multivariate Cox proportional hazard model[27] was adopted to analyze the prediction of future hepatitis activation and carcinogenesis. A *P*-value of less than .05 with 2-tailed test was considered significant. Data analysis was performed using the SPSS software version 11.[28]

RESULTS

Histological Findings

According to the classification of Desmet et al,[25] liver biopsy showed minimal hepatitis (F0) in 9 (9.5%), F1 in 53 (55.8%), F2 in 21(22.1%), F3 in 6 (6.3%), and F4 or cirrhosis in 6 (6.3%). Occult cirrhosis with normal ALT and negative HBe antigen was found in 6.3%. Inflammatory activity was minimal (A0) in 18 (18.9%), A1 in 72 (75.8%), and A2 in 5 (5.3%).

Reasons for Liver Biopsy

The reasons for liver biopsy in spite of "stable hepatitis" included a history of ALT elevation in the past (group A, n = 41), patient's apprehension based on family history of advanced liver diseases (group B, n = 25), possible advanced liver disease on ultrasonography (US) or liver function tests (group C, n = 8), or simple desire for thorough examination including biopsy (group D, n = 21). We also assessed the relationship between background features and reasons for undergoing liver biopsy (Table 2).

Advanced liver disease of F3 or F4 was present in 4 of 41 (9.8%) patients in group A, 4 of 25 (16.0%) in group B, 3 of 8 (37.5%) in group C, and 1 of 18 (5.6%) in group D. The median platelet count was the lowest in group C, which suggested that the group included more numbers of patients with advanced stages of the disease. The median HBV DNA concentration at the beginning was the highest in group A. HBV genotype A was exclusively found in group D and the incidence of genotype B was higher in groups A and D.

Initial and Serial Assays of HBV DNA Concentration

The median DNA concentration at the time of liver biopsy was $10^{4.4}$ copies/mL, with 25th percentile of $10^{3.2}$, and 75th percentile of $10^{5.2}$ (range, $<10^{2.6}$ to $>10^{7.6}$).

The relationship between the initial HBV DNA values and serial measurements of DNA during the observation were assessed, classifying the initial DNA concentration into 4 groups: low DNA group ($<10^4$ copies/mL), DNA concentration of 10^4-$10^{4.9}$ copies/mL, DNA concentration of 10^5-$10^{5.9}$ copies/mL, and high DNA of $\geq 10^6$ copies (Figure 1). Among 33 patients with initial HBV DNA concentration of $<10^4$ copies/mL, 29 (87.9%) showed a continuously low DNA of less than 10^6 copies/mL, and 4 (12.1%) showed an intermittent rise of the DNA to $>10^6$ copies/mL. Among 34 patients with initial HBV DNA of 10^4 to $10^{4.9}$ copies/mL, 25 (73.5%) showed persistently low DNA, and 9 (26.5%) an intermittent rise of the DNA. Of 18 patients with initial DNA of 10^5 to $10^{5.9}$ copies/mL, 10 (55.5%) showed persistently low DNA course, and 8 (44.4%) an intermittent elevation of the DNA. Among 10 patients with initial DNA of $\geq 10^6$ copies/mL, an intermittent rise of the DNA was found in 9 patients (90.0%) and persistently high DNA in one (10.0%). The initial HBV DNA concentration was significantly associated with the subsequent serial HBV DNA course ($P < .0001$).

Table 2 Demographic, Histological, Biochemical and Virological Findings According to the Reasons for Examination Including Liver Biopsy

	Reasons for Liver Biopsy			
	Group A	Group B	Group C	Group D
Number of patients	41	25	8	21
Male/Female	36/5	15/10	5/3	19/2
Age (years – median, range)	39 (18-61)	38 (30-67)	41 (30-57)	41 (21-58)
Family history of liver disease or HBV infection	18 (37.5%)	38 (100%)	5 (62.5%)	6 (28.6%)
Liver histology				
F0 (minimal)	1	5	0	3
F1	21	15	2	15
F2	15	1	3	2
F3	2	2	1	1
F4 (cirrhosis)	2	2	2	0
AST (IU/L) (median, range)	27 (17-37)	22 (10-37)	24 (16-28)	21 (14-34)
ALT (IU/L)	34 (18-50)	20 (6-48)	21 (12-48)	20 (8-50)
Platelet ($\times 1000/mm^3$)	169 (94-274)	185 (96-311)	156 (86-216)	196 (128-296)
ICG_{R15} (%)	12 (6-24)	9.5 (3-33)	9 (7-13)	12 (5-72)
Albumin (g/dL)	4.3 (3.6-5.1)	4.4 (3.9-5.4)	4.15 (3.9-5.4)	4.4 (3.7-4.9)
Bilirubin (mg/dL)	1.0 (0.2-1.5)	1.1 (0.4-1.8)	0.75 (0.3-1.1)	0.8 (0.3-1.9)
Prothrombin (%)	89 (67-111)	100 (70-108)	100 (76-101)	100 (73-101)
HBV DNA (Log_{10} copy/ml) (median, 25-75 percentiles)	4.6 (4.2-5.3)	4.2 (3.1-5.4)	3.45 (<2.6-4.8)	3.9 (<2.6-4.8)
HBV genotype				
A	0	0	0	3
B	11	0	0	7
C	29	25	7	10
Others/unidentified	1	0	1	1

Group A: History of ALT elevation in the past.
Group B: Advanced liver disease in family members.
Group C: Possible advanced disease on ultrasonography or blood examination.
Group D: Simple desire for thorough examination

Activation of Hepatitis and Its Prediction

Twenty-five patients showed twice as high as normal ALT concentration, and the other 27 patients showed activation of hepatitis with ALT elevation followed by IFN therapy. Consequently, a total of 52 patients (54.7%) showed an abnormal ALT concentration during a median observation period of 10.2 years. The cumulative rate of hepatitis activation was 34.7% at the end of the 3rd year, 45.4% at the 5th year, and 55.7% at the 10th year.

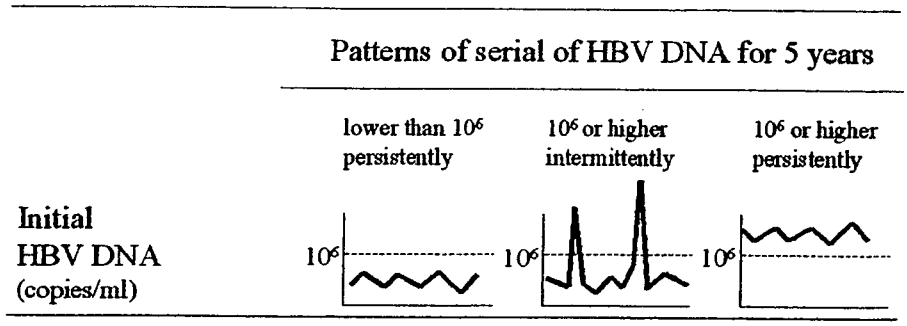
The incidence of hepatitis activation was assessed in the following 4 groups according to the initial HBV DNA concentration: $<10^4$ copies/mL (n = 33), 10^4 to $10^{4.9}$ (n = 34), 10^5 to $10^{5.9}$ (n = 18), and $\geq 10^6$ (n = 10). Cumulative hepatitis flare-up rates in each group were 12.0%, 41.2%, 44.4%, and 70.0%, respectively, at the end of the 3rd year; 12.0%, 61.8%, 55.5%, and 80.0%, respectively, at the 5th year; and 18.6%, 70.3%, 80.0%, and 80.0%, respectively, at the 10th year (Figure 2). The initial DNA load correlated significantly with future hepatitis activation rate ($P < .0001$). Average DNA concentration during the initial 5 years was closely associated with hepatitis activation for the period: 52 patients with ALT elevation had a high average DNA value of $10^{3.3}$ (median, range: $<10^{2.6}$-$10^{5.3}$), and 43 patients without ALT elevation showed $10^{4.8}$ (median, range: $10^{2.9}$->$10^{7.6}$).

The hepatitis activation rates in groups A, B, C, and D were 70.7%, 36.0%, 25.0%, and 14.3%, respectively, at the end of the 5th year; and 81.7%, 45.1%, 25.0%, and 26.7%, respectively, at the end of the 10th year (Figure 3). The ALT flare-up rate in patients of group A was the highest ($P < .0001$).

Multivariate proportional hazard analysis disclosed that initial HBV DNA concentration, a past history of ALT elevation, and histological staging were significantly associated with reactivation of hepatitis in the follow-up (Table 3). Patients with HBV DNA of 10^4 to $10^{5.9}$ copy/mL had a high hazard ratio of 5.73, and those with 10^6 copy/mL or higher showed a hazard ratio of 10.43, compared with those with lower DNA concentration $<10^4$. Past history of ALT elevation also was associated with hepatitis activation with a hazard ratio of 3.62. Histological staging of F2 or higher also showed a high risk for carcinogenesis with a hazard ratio of 3.06.

Relationship between HBV DNA in First 2 Years and Hepatitis Activation

The prediction of hepatitis activation was analyzed using HBV DNA level in the first 2 years (DNA measurements at the time of observation, 6th, 12, and 24th month). Using various cutoff values of DNA, patients were classified into



Initial HBV DNA (copies/ml)		lower than 10^6 persistently	10^6 or higher intermittently	10^6 or higher persistently
$<10^4$	(N=33)	29 (87.9%)	4 (12.1%)	0
10^4-$10^{4.9}$	(N=34)	25 (73.5%)	9 (26.5%)	0
10^5-$10^{5.9}$	(N=18)	10 (55.6%)	8 (44.4%)	0
$>=10^6$	(N=10)	0	9 (90%)	1 (10%)

Figure 1 Relationship between initial HBV DNA values and serial DNA during the initial 5 years.

low DNA group (persistently low) and high DNA group (a high value at least once during the observation period).

When patients were divided using a cutoff value of 10^6 copies/mL (Figure 4a), hepatitis activation rates in low DNA group (n = 69) and high DNA group (n = 26) were 27.5% and 53.8%, respectively, at the end of the 3rd year; 37.7% and 65.4%, respectively, at the 5th year; and 46.0% and 79.2%, respectively, at the 10th year. The hepatitis activation rate in the high DNA group was significantly higher than that of the low DNA group ($P = .0014$). If DNA concentration remained $<10^6$ copies/mL for 2 years, 53.6% (37/69) of the patients did not show flare-up of ALT during the observation period.

Next, when patients were divided using a cutoff value of 10^5 copies/mL (Figure 4b), hepatitis activation rates in the low DNA group (n = 47) and high DNA group (n = 48) were 14.9% and 54.2%, respectively, at the end of the 3rd year; 23.4% and 66.7%, respectively, at the 5th year; and 30.8% and 79.4%, respectively, at the 10th year. Hepatitis activation rate in the high DNA group was significantly

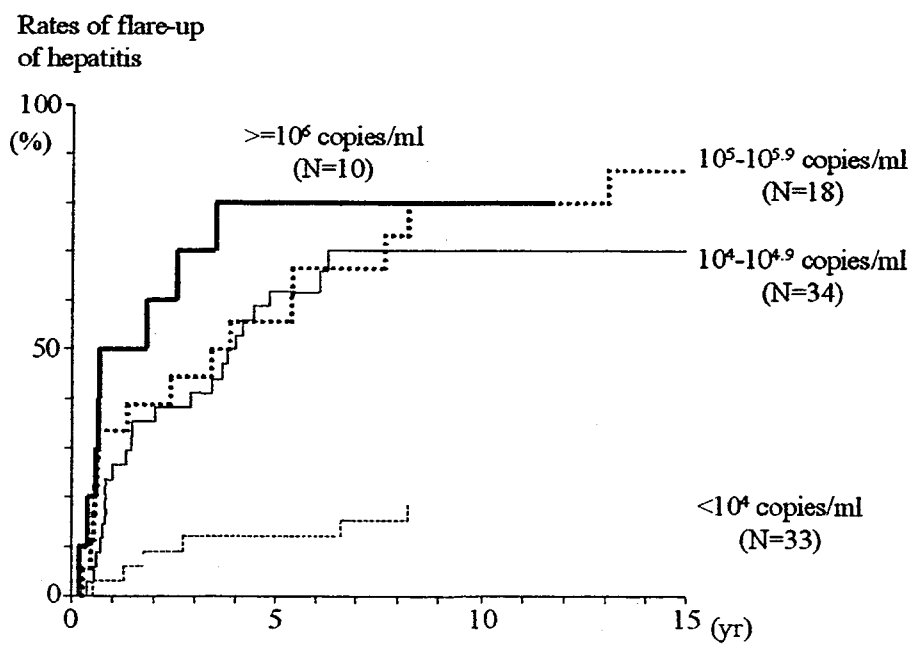


Figure 2 Cumulative hepatitis activation rates in the 4 patient groups according to initial HBV DNA values: Log_{10} HBV DNA of <4.0, 4.0–4.9, 5.0–5.9, and ≥6.

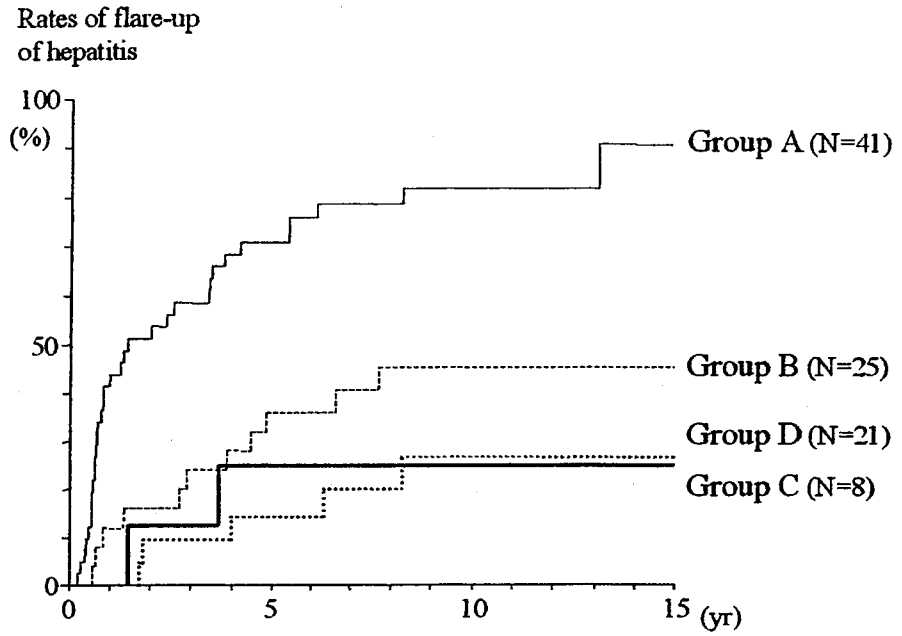


Figure 3 Cumulative hepatitis activation rates in subgroup A, B, C, and D: Patients in group A had a history of aminotransferase elevation in the past, those in group B had apprehension of their own diseases based on a significant family history, those in group C were suspected of having an advanced liver disease on ultrasonography or liver function tests, and those in group D had a simple desire for thorough examination with biopsy.

higher than that of the low DNA group ($P < .0001$). If DNA concentration remained $<10^5$ copies/mL for 2 years, 70.2% (33/47) of the patients did not show flare-up of ALT during the observation period.

Similarly, when patients were divided using a cutoff value of 10^4 copies/mL (Figure 4c), hepatitis activation rates in the low DNA group (n = 24) and high DNA group (n = 71) were 4.2% and 45.1%, respectively, at the end of the 3rd year; 4.2% and 59.1%, respectively, at the 5th year; and 8.3% and 72.1%, respectively, at the 10th year. Hepatitis activation rate in the high DNA group was significantly higher than that of the low DNA group ($P < .0001$). If DNA concentration remained $<10^4$ copies/mL for 2 years, 91.7% (22/24) of the patients did not show flare-up of ALT during the observation period.

A receiver-operating characteristic (ROC) curve was generated to assess the best cutoff value of HBV DNA for the prediction of future hepatitis activation (Figure 5). Cutoff value of $10^{6.0}$ or higher predicted 86.0% of future hepatitis activation, but persistently lower value than $10^{6.0}$ predicted only 36.5% of stable disease. On the contrary, cutoff value of $10^{4.0}$ or higher predicted only 51.2% of future ALT elevation, but constantly lower value than $10^{4.0}$ predicted 96.2% of stable hepatitis.

Table 3 Factors Associated with Future Hepatitis Activation in Patients with Positive HBs Antigen who Showed Normal Aminotransferase and Negative HBe Antigen at the Time of Biopsy

Factor	Category	Hazard Ratio	(95% CI)	*P* Value
Initial HBV DNA	0: $-10^{3.9}$	1		
	1: 10^4-$10^{5.9}$	5.73	(2.36-13.9)	<.0001
	2: $10^{6.0}$-	10.43	(3.44-31.60)	<.0001
History of ALT elevation	0: no	1		
	1: yes	3.62	(1.97-6.67)	<.0001
Histological staging	0: F0-F1	1		
	1: F2-F4	3.06	(1.73-5.42)	<.0001

Hepatocellular Carcinogenesis and Its Prediction

During a median observation period of 10.2 years, 5 patients (5.3%) developed HCC. Among the 5 patients who developed HCC, one had low HBV DNA concentration of $\leq 10^4$ copies/mL, 3 had intermediate value of 10^4 to 10^6, and one had high DNA concentration of $\geq 10^6$ copies/mL. Among 90 patients who did not develop HCC, 32 had low DNA, 50 had intermediate viral load, and 8 had high concentration ($P = .62$). The cumulative carcinogenesis rates in patients with and without high DNA concentration of $\geq 10^6$ copies/mL during the initial 3 years were 6.9% and 0%, respectively, at the end of the 5th year, and 11.5% and 1.8%, respectively, at the 10th year (Figure 6).

Those patients with high DNA concentration of $\geq 10^6$ copies/mL at least once during the initial 3 years had a significantly higher carcinogenesis rate than those without DNA elevation ($P = .021$). Average DNA concentration during the initial 5 years also was associated with hepatitis

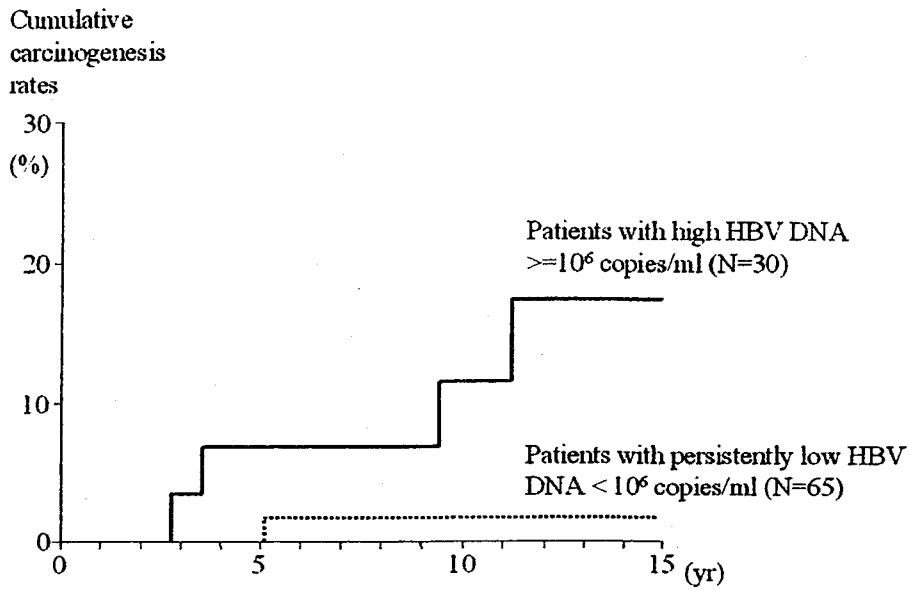


Figure 6 Cumulative hepatocellular carcinogenesis rates in patients with and without high HBV DNA concentration during the initial 3 years.

hepatitis activity often fluctuates with significant changes of DNA concentration in the patients, even sensitive DNA assays demonstrate an obvious limitation from a single time measurement.[22] Our retrospective study, therefore, analyzed the chronological changes in DNA and biochemistry in a patient cohort over a period of more than 5 years (median, 10.2 years).

We found all patients with negative HBeAg and normal ALT had a detectable value of serum HBV DNA by PCR assay, and that the DNA value correlated well with high ALT concentration and the incidence of future ALT elevation, in agreement with other reports.[19-22,32-35] A few studies[19,20,36] reported that a cutoff value of 10^5 copies/mL was practical in discriminating chronic hepatitis from asymptomatic carrier state, whereas Manesis et al[21] reported that a cutoff DNA level of 3×10^4 copies/mL was more appropriate for the same purpose. In forecasting future ALT elevation in a narrower patient cohort with negative HBe antigen and normal ALT concentrations, however, a cutoff value of 10^4 copies/mL effectively predicted reactivation of hepatitis. The efficacy of the cutoff value of 10^4 copies/mL was true in using both the initial DNA value at a single-time assay and multiple assays during 2-year follow-up. From the longitudinal data, only 8.3% of patients with persistently low DNA of $<10^4$ copies/mL in the initial 2 years showed ALT elevation during a median of 10.2 years of observation.

The fate of patients regarding future DNA elevation and ALT fluctuation also depends on the reason for undergoing invasive diagnostic procedures including liver biopsy. Patients of group A (patients with history of ALT elevation) showed the highest initial DNA concentration and future ALT elevation rate. Those of group B (patients with family history of advanced disease) showed the second highest value of initial DNA and high incidence of ALT elevation. Although we analyzed more than 80% of consecutive patients in our hospital, the background features of patients should be taken into account in the study of HBV-related disease, when a tertiary medical center performs a retrospective cohort study using a selected patient group.

Among the stable patients with negative HBe antigen and normal ALT, HCC occurred in 5 patients (5.3%) during a median of 10.2 years. As was reported previously,[33,37,38] hepatocarcinogenesis was significantly associated with persistently high HBV DNA. In this cohort study, patients with DNA of $\geq 10^6$ copies/mL had a significantly higher carcinogenesis rate than those with low DNA. The cutoff concentration of DNA of 10^6 copies/mL was consistent with other cohorts with cirrhosis[33,37,38] in the prediction of carcinogenesis. The reason for the higher cutoff value for carcinogenesis than that for hepatitis reactivation might be explained by differences in the pathogenetic processes. Although hepatocarcinogenesis in these patients may well be associated with DNA concentration severity of the liver disease, or merely persistence of high ALT concentration, general practitioners or physicians should be aware of the risk of HCC development in these stable patients.

The precise role of HBV in oncogenesis is still unknown, but increasing evidence indicates that the virus plays an important role in the development of HCC. Because the disease activity and carcinogenic potency can change significantly during the course of HBV-related liver disease, a longitudinal analysis is desirable for the study of carcinogenesis from HBV-related cirrhosis. Future studies should be aimed, therefore, at defining the basic oncogenic mechanisms and roles of serum DNA concentration in carcinogenesis in patients with HBV infection.

In conclusion, advanced stages of hepatitis should not be overlooked in patients with HBe antigen-negative, aminotransferase-normal HBV carriers. Serial DNA assessment

in the early few years predicted future hepatitis activation and the risk of carcinogenesis.

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Key Words
Cirrhosis · Fibrosis · Hepatitis C virus · Hepatocellular carcinoma · Interferon

Abstract
Background: The anticarcinogenic capacity of interferon (IFN) was assessed in a cohort of Japanese patients with chronic hepatitis C en masse. ***Patients and Methods:*** The rate of hepatocarcinogenesis was analyzed in 2,166 patients with chronic hepatitis C, of whom 1,654 had received IFN therapy while 512 had not. ***Results:*** Crude rates of hepatocarcinogenesis in treated and untreated patients were 2.6 and 4.6% at the end of the 5th year, 5.8 and 12.7% at the 10th year and 13.9 and 23.9% at the 15th year (after completion of IFN therapy for those treated) ($p < 0.001$). IFN decreased the hazard ratio of carcinogenesis to 0.42 ($p < 0.001$) in multivariate analysis with adjustments for significant covariates including fibrotic stage, γ-glutamyl transpeptidase level, gender, platelet count and age. Among the 1,654 patients treated with IFN, 606 (36.6%) achieved persistent loss of hepatitis C virus (HCV) RNA and an additional 266 (16.1%) gained normal levels of alanine aminotransferase without loss of HCV RNA for 6 months or longer after the completion of IFN therpay. Cumulative rates of hepatocarcinogenesis in sustained virological responders and biochemical responders were 1.4 and 2.0% at the end of the 5th year, 1.9 and 3.6% at the 10th year and 1.9 and 7.5% at the 15th year, respectively. The hazard ratio of sustained virological response was 0.10 ($p < 0.001$), and that of biochemical response was 0.12 ($p < 0.001$). Normalization of aminotransferase levels after IFN therapy without loss of serum HCV RNA decreased hepatocarcinogenesis. ***Conclusion:*** IFN significantly decreased the rate of hepatocarcinogenesis in patients with chronic hepatitis C as a whole in Japan, even in those who fail to clear HCV RNA from serum.

Introduction

In most developed countries, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections account for the great majority of hepatocellular carcinoma (HCC), with incidence rates dependent on the regional prevalence of these hepatitis viruses. HCV-associated HCC typically develops through a sequence of events that progress from chronic inflammation through fibrosis and cirrhosis accompanying dysplasia and ultimately to HCC. In our previous cohort study on Japanese patients with HCV-related cirrhosis [1], cumulative rates of developing HCC at 5, 10 and 15 years were 21.5, 53.2 and 75.2%, respectively. According to our observations of untreated patients with chronic hepatitis C [2], rates of hepatocarcino-

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genesis at 5, 10 and 15 years were estimated to be 4.8, 13.6 and 26.0%, respectively. The life expectancy of patients with HCV-related cirrhosis is largely influenced by the development of HCC in the clinical course. As the efficacy of radically curative therapies for HCC remains limited at best, and since a severe organ shortage does not provide with sufficient chances for liver transplantation, the prevention of HCC in patients with chronic liver disease is of great importance at the present.

Interferon (IFN) is effective in eliminating HCV and reducing serum levels of alanine aminotransferase (ALT) in some patients with chronic hepatitis C [3–6]. Reduced incidence of HCC in HCV-associated cirrhosis by IFN has been reported by many investigators including ourselves [7–14]; only a few studies have failed to find its benefit [15, 16]. However, many published studies had shortcomings in the study design, in terms of pooling patients who received IFN in diverse regimens, relatively short periods of follow-up despite a long incubation period of HCC, large numbers of dropouts and retrospective studies with historical controls. Moreover, almost all studies evaluated the activity of IFN to prevent HCC by comparing responders and nonresponders to the treatment. Due to difficulties in studying patients with chronic hepatitis C, a number of nonrandomized studies examined the effect of IFN on the incidence of hepatocarcinogenesis [17–20]. With invariable limitations in study design and interpretation of the results, these studies have disclosed useful information as regards the treatment of patients with chronic HCV infection.

In order to evaluate whether IFN can reduce the rate of carcinogenesis in patients with chronic hepatitis C, we compared 1,654 patients with IFN therapy with 512 patients without treatment in a single clinical center, who were adjusted for background features by the multivariate analysis. Therefore, the principal aims of our study were to show the role of IFN in preventing HCC in chronic hepatitis type C en masse and to establish the extent to which IFN decreases the rate of carcinogenesis as a sequel to chronic hepatitis C in a society.

Patients and Methods

Study Population

A total of 2,166 patients with chronic hepatitis were examined, whose initial sera tested negative for hepatitis B surface antigen by radioimmunoassay (Ausria, Dainabot, Tokyo, Japan) and positive for anti-HCV by the second-generation enzyme-linked immunosorbent assay (Dainabot); anti-HCV was tested in sera that had been stored frozen at −80°C. They included 1,421 men and 745 women aged 14–78 with a median of 50 years. They were all diagnosed with chronic hepatitis by liver biopsy with or without peritoneoscopy between 1970 and 2000 at the Department of Gastroenterology in Toranomon Hospital, Tokyo, Japan. Patients who had possibly developed HCC already at the time of diagnosis of hepatitis were strictly excluded from the study. In order to exclusively investigate hepatocarcinogenesis in HCV-related cirrhosis, patients coinfected with HBV were excluded.

Among the 2,166 patients with HCV-related hepatitis, 1,654 (76.4%) received IFN therapy, mostly since 1987 when IFN was available in Japan; new antivirals or anticarcinogenic treatments of viral cirrhosis, except for IFN, were not introduced in 1987 or thereafter in Japan. The remaining 512 patients did not receive IFN or any other antiviral therapies. This is a retrospective cohort study with historical controls composed of patients before 1987 and those who refused or could not receive IFN for various reasons since 1987.

Background and Laboratory Findings

Table 1 shows demographic profiles and laboratory data for the 1,654 patients treated with IFN and the 512 without receiving IFN since they were diagnosed with chronic hepatitis. There were more males, with a median age 3 years lower in treated than in nontreated patients. There were 299 treated patients (18.1%) with a history of alcohol intake ≥500 kg until the diagnosis of chronic hepatitis (corresponding to daily consumption of 3,000 ml of beer or 300 ml of whiskey for 20 years) and 113 (22.1%) untreated patients ($p < 0.001$). Because IFN was introduced to our hospital in 1987, the observation period was significantly shorter in the treated than in untreated patients (median 10.4 vs. 12.3 years; $p < 0.0001$).

Although all patients tested positive for HCV RNA during their clinical courses, tests for the concentration of HCV RNA in the initial serum was possible in 1,863 (86.5%) patients. HCV genotypes were analyzed by the serological typing method with a commercial kit (Kokusai Diagnostic Corporation, Kobe, Japan) in which the serological group 1 represented genotypes 1a and 1b, and group 2 stood for 2a and 2b genotypes. HCV in the serological group 2 was significantly more frequent in patients with IFN treatment than in those without. Concentration of HCV RNA was determined in the initial sera from 1,873 (86.5%) patients by the competitive polymerase chain reaction (PCR) method with the HCV probe assay (Chiron Corp., Emeryville, Calif., USA) or by PCR with Amplicor HCV Monitor kits (Roche Diagnostics Japan Co., Tokyo, Japan). High concentration of HCV ($\geq 10^6$ copies/ml by the competitive PCR or $\geq 10^6$ equivalents/ml by the HCV probe assay) was significantly more frequent in untreated than in treated patients ($p < 0.0001$). The stage of hepatic fibrosis was not different between the two groups.

Interferon Treatment and Judgment of the Effect

A total of 1,654 patients underwent IFN therapy in one or more treatment courses: 1,358 patients (82.1%) received IFN once, 240 patients (14.5%) twice, and the remaining 56 patients (3.4%) three times or more. Initial treatment was performed with natural or recombinant IFN-α (n = 1,238), natural IFN-β (n = 386) or both (n = 30). Regimens of IFN were variable: 926 (56.0%) patients received IFN 6–9 million units (MU) daily for 8 weeks, followed by 2 or 3 times per week for 16 weeks; 329 (20.0%) received IFN 6–9 MU daily for 2–4 weeks, followed by 3 times per week for 20–22 weeks; 185 (11.2%) underwent a short-course therapy with IFN

Table 1. Patient profiles and laboratory data at the diagnosis of chronic hepatitis

Factors	Interferon therapy		p value
	yes (n = 1,654)	no (n = 512)	
Male	1,110 (67.1%)	311 (60.7%)	0.024
Age, years	50 (16–72)	53 (21–78)	<0.001
History of transfusion	607 (36.7%)	229 (44.7%)	0.001
Family member with liver disease	426 (25.8%)	140 (27.3%)	0.47
Alcohol intake ≥ 500 kg	299 (18.1%)	113 (22.1%)	0.044
Observation period, year	10.4 (0.1–33.6)	12.3 (0.1–33.6)	<0.001
Laboratory data			
ALT, IU/l	63 (4–1,266)	67 (4–704)	0.098
AST, IU/l	106 (9–1,660)	96 (12–832)	0.0001
γ-GTP, IU/ml	62 (6–1,118)	70 (3–850)	0.39
Platelet counts, $\times 1{,}000/mm^3$	169 (27–433)	165 (35–560)	0.091
ICG R_{15}, %	14 (1–90)	16 (1–95)	0.003
AFP, ng/ml	4 (1–90)	5 (1–1,180)	0.42
HCV serological group			
Group 1, genotypes 1a/1b	1,021 (66.1%)	259 (81.4%)	<0.0001
Group 2, genotypes 2a/2b	488 (31.6%)	48 (15.1%)	
Undetermined	36 (2.3%)	11 (3.5%)	
HCV RNA concentration			
High[a]	937 (58.4%)	191 (71.3%)	<0.0001
Low[b]	668 (41.6%)	77 (28.7%)	
Histological stage of hepatitis			
F1, slight fibrosis	1,029 (62.2%)	298 (58.2%)	0.10
F2/F3, moderate/severe fibrosis	625 (37.8%)	214 (41.6%)	

AST = Aspartate aminotransferase; AFP = α-fetoprotein; ICG R_{15} = retention of indocyanine green at 15 min.

[a] HCV RNA concentration $\geq 10^6$ copies/ml by the competitive PCR or $\geq 10^6$ equivalents/ml by the HCV probe assay.

[b] HCV RNA concentrations less than high concentrations.

daily for 4–8 weeks; 128 (7.7%) were administered with intermittent IFN 3 times per week for 24 weeks; 72 (4.4%) had a prolonged course of IFN for 8–36 months; 8 (0.5%) received IFN-β 6 MU daily for 6–18 months, and the remaining 6 (0.4%) were given IFN-α combined with IFN-β for 4 months. The median dose of 624 MU was administered during the median period of 24 weeks. IFN for 24 weeks or longer was given to 83.2% of the patients. IFN therapy was usually initiated within a few months after the diagnosis of chronic hepatitis, and all patients were started on it within 12 months. The median interval between liver biopsy and initiation of IFN was 9 days.

Almost all the patients given IFN showed varied degrees of fever, chills, myalgia, headache and general malaise after the first injection. Most patients developed leukocytopenia and thrombocytopenia in various degrees. A significant thrombocytopenia $\leq 40{,}000/mm^3$ required a reduction of the IFN dose in 39 patients. IFN therapy was discontinued due to psychosis in 35 patients and ophthalmological symptoms in 12 patients. None of the patients developed decompensated liver disease with ascites, encephalopathy, jaundice or variceal bleeding. Although only 88 (5.3%) patients could not continue injection with IFN, studies for carcinogenesis were analyzed on the intention-to-treat basis.

The efficacy of IFN was judged by the clearance of HCV RNA from serum and ALT levels 12 months after the completion of treatment. Sustained virological response (SVR) was defined as persistent disappearance of HCV RNA after therapy, biochemical response (BR) as normal ALT levels without elimination of HCV RNA for at least 6 months after therapy, and no response (NR) as persistently elevated or transiently normalized ALT levels without loss of HCV RNA lasting for less than 6 months.

Follow-Up of Patients and Diagnosis of HCC

Patients were followed up monthly after diagnosis of chronic hepatitis in our outpatient clinic and monitored for hematological, biochemical and virological parameters. With their admission, during and after the treatment with IFN, weekly or biweekly follow-up was performed in almost all patients who received IFN. Imaging diagnosis was made once or twice per year in the majority of patients with ultrasonography or computed tomography. Angiography was performed only when HCC was highly suspected on imaging by ultrasonography or computed tomography.

When angiography pictured a characteristic hypervascular nodule specific for HCC in patients, histological confirmation was not required in the majority of them. Microscopic examinations of liv-

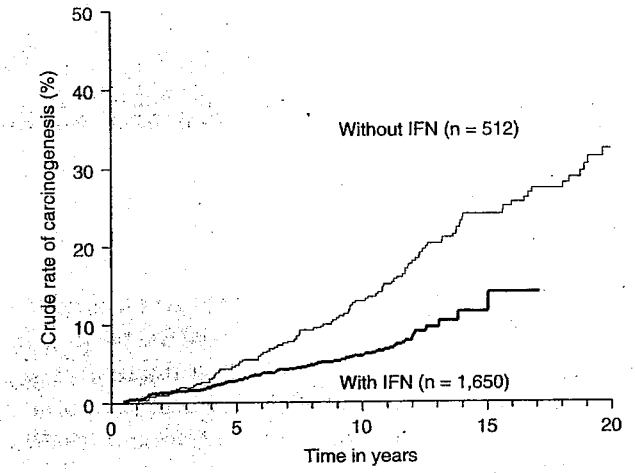

Fig. 1. Crude rates of hepatocarcinogenesis in patients treated with IFN and those untreated. The carcinogenesis rate was significantly lower in treated than in untreated patients (log-rank test, $p < 0.0001$).

er tissues obtained by a fine-needle biopsy were performed in 14 patients whose angiogram could not portray a typical image of HCC. There were 89 patients in whom HCC was confirmed histologically on liver specimens obtained at surgery or autopsy. Detection of serological tumor markers and increase with time were also taken into account in the diagnosis of HCC.

There were 223 (10.3%) patients lost to follow-up, including 164 (9.9%) treated and 59 (11.5%) untreated. Rates of annual dropouts in treated and untreated patients were 0.95 and 0.93%, respectively. In 9 patients, the response to IFN was judged by information on aminotransferase levels determined in other clinics and by persistent HCV RNA, as well as aminotransferase levels at 6 months after the completion of therapy in an additional 3 patients. Therefore, the response to IFN could be judged in all patients including the 12 who were lost to our follow-up early. Since the eventual outcome with respect to the development of HCC was not confirmed in these patients, their data were censored in statistical analyses [21]. Deaths unrelated to liver disease were censored and withdrawn from the analysis. The date of the last follow-up in this study was May 1, 2004, and the median observation period of studied patients was 10.7 years, with a range of 0.1–33.6 years.

Statistical Analysis

Nonparametric Mann-Whitney U test and χ^2 test were used for analysis of background characteristics of patients. The rate of HCC development was calculated by the Kaplan-Meier method [22]; it was based on the duration between diagnosis of chronic hepatitis by liver biopsy and detection of HCC. Differences in slopes of carcinogenesis curves were evaluated by the log-rank test. To gain a robust statistical power for the anticarcinogenic activity of IFN, observation of treated patients was initiated at the commencement of IFN therapy, in lieu of the diagnosis of chronic hepatitis. Independent factors associated with the development of HCC were studied using the stepwise Cox regression analysis [23]. The following 18 variables were analyzed for potential covariates in hepatocarcinogenesis at the time when hepatitis was diagnosed: age, sex, total alcohol intake, family history of liver disease, history of blood transfusion, stage of hepatic fibrosis, aspartic aminotransferase, ALT, albumin, bilirubin, globulin, γ-glutamyl transpeptidase (γ-GTP), platelet count, retention of indocyanine green at 15 min, serological grouping of HCV, HCV RNA level and IFN treatment.

Although continuous variables without conversion of data were evaluated in multivariate analyses, several variables were transformed into categorical data consisting of two or three ordinal numbers in calculating hazard ratios. All factors found to be marginally associated with hepatocarcinogenesis with p values <0.15 were tested by the multivariate Cox proportional hazard model. All analyses of data were performed with the computer program SPSS version 11 [24], and a p value <0.05 was considered significant.

Results

Response to IFN

Response to IFN was judged 12 months after the completion of therapy by both HCV RNA and serial ALT readings. Among the 1,654 patients with IFN treatment, SVR (elimination of HCV RNA) was achieved by 606 (36.6%), BR (ALT normalized for at least 6 months without clearance of HCV RNA from serum) in 266 (16.1%) and NR (elevated or transiently decreased ALT levels without loss of serum HCV RNA) in 782 (47.3%).

Crude Rates of Hepatocarcinogenesis

During the median observation period of 10.7 years, HCC developed in 199 of the 2,166 (9.2%) patients, including 96 of the 1,654 (5.8%) patients treated with IFN and 103 of the 512 (20.1%) patients without IFN (fig. 1). Among the 199 patients with HCC, 140 (70.4%) imaged a typical hypervascular stain on angiography and dynamic computed tomography, while 59 failed to exhibit tumor stains on angiography. HCC in these 59 patients was confirmed histologically on liver specimens obtained at surgery or by fine-needle biopsy.

Crude rates of hepatocarcinogenesis in patients treated with IFN and those untreated were 1.3 and 1.8% at the end of the 3rd year (after the completion of therapy), 2.6 and 4.6% at the end of the 5th year, 5.8 and 12.7% at the 10th year and 13.9 and 23.9% at the 15th year, respectively (fig. 1). The carcinogenesis rate was significantly lower in patients treated with IFN than in untreated patients (log-rank test, $p < 0.0001$).

Impact of IFN on Hepatocarcinogenesis

During the observation period, HCC developed in 96 of the 1,654 (5.8%) patients treated with IFN, including

11 patients (1.8%) with SVR, 10 (3.8%) with BR and 75 (9.6%) with NR to IFN. Rates of hepatocarcinogenesis in patients with SVR, BR and NR were 0.7, 0.8 and 2.0% at the end of the 3rd year, 1.4, 2.0 and 3.8% at the 5th year, 1.6, 2.9 and 6.5% at the 7th year, 1.9, 3.6 and 9.6% at the 10th year and 1.9, 7.5 and 27.6% at the end of 15th year (fig. 2). Hepatocarcinogenesis was significantly less frequent in patients with SVR or BR than in patients with NR and those untreated (log-rank test, $p < 0.0001$).

Factors Influencing Hepatocarcinogenesis

Univariate analysis identified 9 factors significantly associated with carcinogenesis. They were fibrotic stage ($p < 0.001$), age ($p < 0.001$), α-fetoprotein ($p < 0.001$), aspartic aminotransferase ($p = 0.001$), retention of indocyanine green at 15 min ($p = 0.002$), total alcohol intake ($p = 0.002$), γ-GTP ($p = 0.005$) and HCV serotype ($p = 0.045$). IFN therapy ($p = 0.064$), histological activity of hepatitis ($p = 0.069$) and ALT ($p = 0.70$) were marginally associated with carcinogenesis.

In order to prove the role of IFN on carcinogenesis in patients with chronic hepatitis type C en masse, multivariate analysis was performed by non-time-dependent proportional hazard analysis. Fibrotic stage, γ-GTP, gender, IFN therapy, platelet count and age independently influenced the development of HCC in the cohort (table 2). Advanced liver fibrosis in F2/F3 stages imposed a higher risk for carcinogenesis with a hazard ratio of 8.68, 95% confidence interval (CI) 5.08–14.81, compared with the F1 stage. Similarly, higher γ-GTP levels (hazard ratio 2.64), male sex (2.38), low platelet count (2.22) and older age (1.90) posed higher carcinogenesis risks. After adjusting background clinical biases between treated and untreated patients for the 5 significant covariates identified in the multivariate analysis, IFN therapy significantly decreased the hepatocarcinogenesis rate in the entire patients with chronic hepatitis C with a hazard ratio of 0.42 (95% CI 0.29–0.61) in comparison with untreated patients.

Based on the multivariate analysis, curves of carcinogenesis rates were theoretically illustrated in treated and untreated patients with the average histological stage, average γ-GTP value, average ratio of male to female, average platelet count and average age (fig. 3).

Hazard of Hepatocarcinogenesis Stratified by the Response to IFN

Since the carcinogenesis rate in patients with SVR or BR was significantly lower than that of patients with NR or untreated patients by the product limit method, a mul-

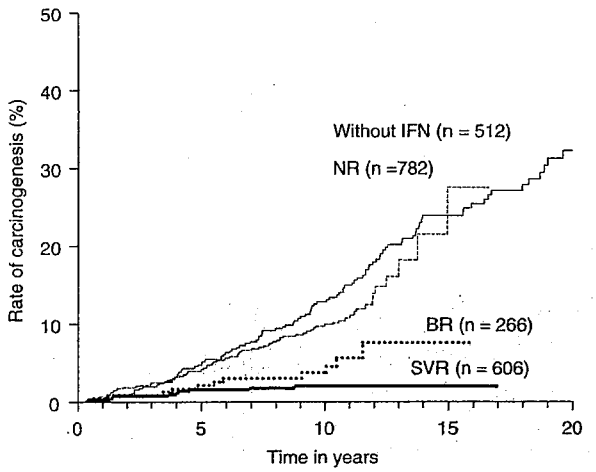

Fig. 2. Rates of hepatocarcinogenesis in patients with SVR, BR and NR to IFN. The rate in patients with NR (persistently elevated ALT or transiently normalized ALT for less than 6 months) was significantly higher than that in patients with SVR or BR.

Table 2. Factors associated with hepatocarcinogenesis in patients with chronic hepatitis C[a]

Factors	HR	95% CI	p value
Fibrosis stage			
F1	1		
F2–F3	8.68	(5.08–14.81)	<0.001
γ-GTP, IU/ml			
<50	1		
≥50	2.64	(1.58–4.42)	<0.001
Gender			
Women	1		
Men	2.38	(1.56–3.70)	<0.001
IFN therapy			
No	1		
Yes	0.42	(0.29–0.61)	<0.001
Platelet count, $\times 10^3/mm^3$			
≥100	1		
<100	2.22	(1.47–3.44)	<0.001
Age, years			
<50	1		
≥50	1.90	(1.27–2.85)	0.002

HR = Hazard ratio.
[a] Evaluated by the Cox proportional hazard analysis.

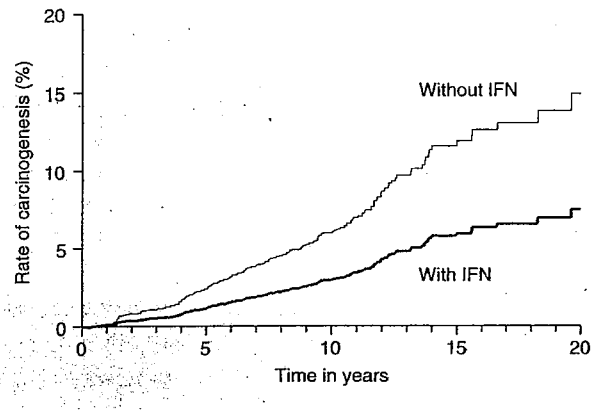

Fig. 3. Theoretical curves of hepatocarcinogenesis in patients treated with IFN and those untreated who have the average histological stage, average γ-GTP value, average ratio of male to female, average platelet count and average age. They are based on the analysis of 1,654 patients treated with IFN and 512 untreated patients.

Table 3. Factors associated with hepatocarcinogenesis in patients with chronic hepatitis C who had distinct responses to IFN therapy[a]

Factors	HR	95% CI	p value
Fibrosis stage			
F1	1		
F2–F3	9.90	(4.19–23.40)	<0.001
Gender			
Women	1		
Men	3.44	(1.89–6.25)	<0.001
γ-GTP, IU/ml			
<50	1		
≥50	2.68	(1.30–5.54)	0.008
Age, years			
<50	1		
≥50	2.56	(1.50–4.38)	0.001
AFP, ng/ml			
<20	1		
≥20	2.32	(1.34–4.02)	0.003
Platelet count, $\times 10^3/mm^3$			
≥100	1		
<100	2.09	(1.14–3.75)	0.013
Response to IFN			
Without IFN	1		<0.001
NR	0.57	(0.13–2.56)	0.46
BR	0.12	(0.04–0.35)	<0.001
SVR	0.10	(0.03–0.30)	<0.001

HR = Hazard ratio; AFP = α-fetoprotein.
[a] Evaluated by the Cox proportional hazard analysis.

tivariate analysis was performed taking into account the response to IFN. Hazard ratios of patients with SVR and BR to IFN therapy were 0.10 (95% CI 0.03–0.30, $p < 0.001$) and 0.12 (95% CI 0.04–0.35, $p < 0.001$), respectively, in comparison with that of untreated patients, when the other 5 factors served as significant covariates (table 3). The hazard ratio of NR at 0.57 (95% CI 0.13–2.56) was less than 1, but fell short of making a significant difference against untreated patients.

Mortality and Causes of Death

During the observation period, 116 of the 2,166 (5.4%) patients died, including 52 of the 1,654 (3.1%) subjects treated with IFN and 64 of the 512 (12.5%) subjects without IFN. Estimated survival rates in the treated and untreated patients were 99.3 and 98.3% at 5 years, 97.8 and 96.0% at 10 years and 93.8 and 86.9% at 15 years, respectively. The survival rate of treated patients was significantly higher than that of untreated patients (log-rank test, $p < 0.0001$).

Discussion

Based on our epidemiological data obtained by long-term observations of patients with chronic hepatitis [2] and patients with cirrhosis [1], the life expectancy of patients with HCV-related chronic liver disease heavily depends on the development of HCC. The possibility of eventually developing HCC in patients with HCV infection and cirrhosis is staggeringly high at 75% [1]. Theoretically, the treatment of chronic HCV infection with IFN can prevent the development of HCC. From the ethical point of view, a prospective randomized trial with control untreated patients is not to be allowed at present when IFN has become the standard radical therapy for chronic hepatitis C; everyone can receive IFN, as expenses are being covered for by the medical insurance in Japan. Another difficulty involves the informed consent in prospective randomized studies. It requires at least 5 years in order that IFN can decrease the incidence of carcinogenesis in chronic hepatitis C, with a statistical difference in the carcinogenesis rate between treated and 'untreated' patients. Since any randomized studies are considered extremely difficult in the future, we attempted to carry out this retrospective study by the multivariate analysis with statistical adjustments for possible covariates.

In the product limit analysis, IFN significantly decreased the crude rate of hepatocarcinogenesis in the

entire cohort of 2,166 patients with chronic hepatitis C. Since there were some background differences between treated and untreated patients, we tried to correct for biases including stage of fibrosis, γ-GTP value, sex, platelet count and age, which significantly affect the carcinogenesis rate. Demographic, histological and biochemical factors having been adjusted, IFN is proven to bring about a significant decrease in the hazard of carcinogenesis in patients with chronic hepatitis C en masse (hazard ratio 0.42, $p < 0.001$ by the non-time-dependent model). Taking into consideration that a significant number of patients without IFN had received anti-inflammatory medicines, which might have contributed to suppression of hepatocarcinogenesis, the actual anticarcinogenic activity of IFN may be higher than the observed. Having published results of a similar study on a cohort of 1,643 patients with a median observation period of 5.4 years in 1999 [18], we could not establish the anticarcinogenic activity of IFN because of a low risk of carcinogenesis in untreated patients (1.2% per year). Nevertheless, we expected a significant statistical difference if we could extend the median observation period to longer than 7 or 10 years in our studied patients. This has been realized in the present study, in which 2,166 patients with and without IFN therapy were observed for a median of more than 10 years. As far as we are aware, it represents the first study that has demonstrated preventive effects of IFN on the carcinogenesis rate in a large cohort of patients in a single center, in correlation with distinct responses to it, such as SVR, BR and NR.

Treatment of patients with chronic HCV infection using IFN-α and ribavirin has led to sustained loss of serum HCV RNA in 40–50% of recipients with HCV genotype 1 and 75–80% with HCV genotype 2 or 3. However, to date, the combination therapy with IFN-α and ribavirin has not been evaluated for its impact on the risk of developing HCC. Monotherapy with IFN-α achieves sustained clearance of serum HCV RNA in only 20–30% of patients; the impact of IFN-α on the development of HCC has been evaluated only in patients who had received IFN-α without ribavirin [17–20, 25–27].

Multivariate analysis definitively demonstrated that IFN lessens the carcinogenesis risk in the patients whose ALT levels decreased after therapy. Furthermore, the anticarcinogenic capacity of IFN was demonstrated not only in the patients with persistent aminotransferase normalization, but also in those with transient normalization of ALT for at least 6 or 12 months. Many authors have already described that the activity of IFN to suppress the development of HCC in patients with HCV RNA clearance (SVR) is similar to that in patients with ALT normalization in the absence of eliminating HCV RNA (BR) [18, 25–27]. Based on these compelling lines of evidence, the anticarcinogenic activity of IFN is ascribed to the suppression of inflammatory and regenerative processes in hepatocytes. Moreno and Muriel [28] reported that IFN reverts liver fibrosis, and therefore, control of the necroinflammatory process can suppress the growth of HCC. Tarao et al. [29] reported that high aminotransferase levels increase the rate of HCC recurrence in patients with cirrhosis. Our results stand in favor of the view that the carcinogenic process in patients with chronic hepatitis C would be enhanced by fluctuating as well as persistently elevated levels of aminotransferases. It does seem that IFN exerts suppressive effects on HCC through reduction or complete remission of inflammatory activity. Recently, a few authors reported that even transient disappearance of HCV RNA during IFN therapy contributed to a low carcinogenesis rate in the clinical course of hepatitis [17, 27]. The significance of transient HCV in decreasing hepatocarcinogenesis should be further explored and confirmed by multicenter clinical studies with rigorous virological assessments.

HCC developed in a few patients with SVR 5 years after the HCV infection had been terminated by IFN, along with normalized ALT levels. These patients would have developed minute HCC in their livers already while receiving IFN which escaped the detection by imaging modalities or screening for serological tumor markers. This would indicate the limitation of IFN in preventing HCC. IFN will not be able to suppress HCC once it has developed, even when it succeeds in eliminating HCV and suppressing necroinflammatory processes in the liver.

With many difficulties in vaccine development, the recent progress in treatment of chronic HCV infection, from IFN monotherapy to combination therapy with ribavirin, is very auspicious. SVR and BR can be achieved in up to 56% of patients with combined IFN and ribavirin [30]. There is evidence that a sustained virological response can lead to decrease in fibrosis and even reversal of cirrhosis [31]. Because HCV-associated HCC occurs almost exclusively in patients with cirrhosis, successful treatment for SVR in patients without cirrhosis is likely to prevent future development of HCC [32]. However, once cirrhosis has been established, a preventive benefit of IFN monotherapy is restricted to the patients who can achieve SVR or BR. In their meta-analysis of 3 randomized and 11 nonrandomized controlled trials, Camma et

al. [33] have reported a low but statistically significant preventive effect.

In conclusion, IFN significantly decreases the rate of hepatocarcinogenesis in patients with chronic hepatitis C, irrespective of the response to it.

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Natural History of Compensated Cirrhosis in the Child-Pugh Class A Compared Between 490 Patients With Hepatitis C and 167 With B Virus Infections

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Natural histories of compensated cirrhosis in the Child-Pugh class A were compared between the 490 patients infected with hepatitis C virus (HCV) and 167 patients with hepatitis B virus (HBV) who were followed for more than 1 year up to 20 years without antiviral treatment. Patients with HCV were older (median age: 59 vs. 45 years), less predominantly male (59.0% vs. 76.0%), transfused more frequently (49.2% vs. 9.0%), and had higher aminotransferase as well as lower albumin levels and fewer platelets ($P < 0.001$ for all). Death was commoner (55.1% vs. 35.9%, $P < 0.001$) and hepatocellular carcinoma developed more often (53.9% vs. 28.7%, $P < 0.001$) in patients with HCV than HBV. In multivariate analysis, low albumin levels (hazard ratio: 1.65), α-fetoprotein (1.55), alcohol consumption (1.49), age >55 years (1.47), and retention of indocyanine green (1.39) were independent risk factors for the survival in patients with HCV, while male gender (4.43), age >45 years (2.24), retention of indocyanine green (2.14), hepatitis B e antigen (2.11), and low platelet counts (1.91) were in those with HBV. Chances for survival was significantly different ($P < 0.001$) among patients with HCV having low (number of factors: 0–1), medium (2–3), and high risks (4–5), as well as in those with HBV having low (0–1), medium (2–4), and high risks (5–6). In conclusion, survival and development of hepatocellular carcinoma, and factors for survival, are considerably different between patients with compensated cirrhosis infected with HCV and HBV, which would need to be taken into consideration in their management and planning treatment strategies. ***J. Med. Virol. 78:459–465, 2006.*** © 2006 Wiley-Liss, Inc.

KEY WORDS: cirrhosis; hepatitis B virus; hepatitis C virus; hepatocellular carcinoma; natural history

INTRODUCTION

Deaths due to hepatocellular carcinoma are increasing and now rank the fourth over the world and in Japan. Up to 80% of hepatocellular carcinomas develop in patients with cirrhosis [Kew and Popper, 1984], most of whom have the end-stage liver disease induced by hepatitis C virus (HCV) or hepatitis B virus (HBV) infection. The prognosis of cirrhotic patients, in terms of survival and the development of hepatocellular carcinoma or decompensation, depends on various host and viral factors [Ikeda et al., 1993; Kato et al., 1994; Fattovich et al., 1997; Niederau et al., 1998; Serfaty et al., 1998; Chiaramonte et al., 1999; Hu and Tong, 1999], and on treatment with interferon [Nishiguchi et al., 1995; Mazzella et al., 1996; Benvegnu et al., 1998].

Survival and development of hepatocellular carcinoma were compared between the 490 patients with compensated cirrhosis in the Child-Pugh class A who were infected with HCV and the 167 with HBV; they were followed for more than 1 year without receiving antiviral treatment. Risk factors for survival were evaluated in patients infected with HCV or HBV separately, and they were classified into groups with low, medium, and high risk, respectively. The prediction of survival in patients with compensated cirrhosis would help in planning strategies for therapeutic invention, including antiviral therapy and liver transplantation.

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MATERIALS AND METHODS

Cirrhotic Patients Infected With HCV or HBV

During 22 years from 1980 to 2001, 1,321 adult patients were diagnosed with liver cirrhosis at the Department of Hepatology, Toranomon Hospital in the Metropolitan Tokyo. Eight hundred two (60.7%) were infected persistently with HCV, 362 (27.4%) with HBV, 26 (2.0%) with both, and 131 (9.9%) with neither. They were aged at a median of 55 years (range: 19–86 years) including 913 (69.1%) men, and 398 of these patients (30.1%) had received antiviral treatment with interferon and/or lamivudine.

Criteria for inclusion in the present study were: (1) histological diagnosis of cirrhosis in Child-Pugh class A [Pugh et al., 1973]; (2) absence of signs for decompensation (ascites, encephalopathy, or gastrointestinal bleeding) or hepatocellular carcinoma at entry; (3) no evidence for coexisting liver disease such as autoimmune hepatitis, alcohol-related liver disease, hemochromatosis, and Wilson's disease (patients with idiopathic portal hypertension, Budd-Chiari syndrome, subacute hepatitis, or chronic aggressive hepatitis accompanied by severe bridging necrosis were also excluded); (4) ongoing infection with either HBV or HCV (patients co-infected with both were excluded); (5) no serological markers for concurrent infection with hepatitis A virus, hepatitis D virus or the human immunodeficiency type-1 virus; (6) no history of antiviral treatment including interferon; and (7) follow-up for at least 1 year after diagnosis of cirrhosis. These inclusion criteria were fulfilled by 490 patients with HCV and 167 with HBV infection (Table I).

Peripheral blood counts, serum biochemistry including liver function tests, α-fetoprotein (AFP), and the percent retention of indocyanine green at 15 min (ICG R_{15}), as well as genotypes of HCV and HBV, were determined on diagnosis of compensated viral cirrhosis in Child-Pugh class A. Patients were examined at regular intervals for liver function, HCV and HBV markers, as well as the development of hepatocellular carcinoma by means of serum AFP, ultrasonography, and computed tomography. They were screened for varices by endoscopy at regular intervals, and received treatment when there was an imminent risk of bleeding. The study design conformed to the 1975 Declaration of Helsinki, and was approved by the Ethic Committee of the institution. Every patient gave an informed consent for this study.

Diagnosis of Hepatitis Virus Infections

Infection with HBV was diagnosed by the detection of hepatitis B surface antigen (HBsAg) with enzyme-linked immunosorbent assay (ELISA) using commercial assay kits (ELISA, F-HBsAg; Sysmex, Kobe, Japan), and that with HCV by ELISA for antibody to HCV (anti-HCV) of the second generation (Ortho HCV2.0 ELISA; Ortho Diagnostic Systems, Raritan, New Jersey). Persistent HCV infection was confirmed by the detection of HCV RNA in serum by the polymerase chain reaction. Hepatitis B e antigen (HBeAg) was determined by ELISA (ELISA, F-HBe; Sysmex).

Genotypes of Hepatitis Viruses

Genotypes of HCV were determined by the polymerase chain reaction with type-specific primers deduced from the 5′-non-structural region by the method reported previously [Chayama et al., 1993]. The six major genotypes of HBV (A–F) were determined serologically by ELISA (HBV GENOTYPE EIA; Institute of Immunology, Tokyo, Japan). The method employs the combination of epitopes on preS2-region products that is specific for each genotype [Usuda et al., 1999, 2000]. Genotype G was determined by the preS2 serotype for genotype D and HBsAg subtype *adw*, and H was recognized by that for genotype C and subtype *adw*, respectively; these combinations were specific for genotypes G and H [Kato et al., 2001, 2004]. Genotyping was possible in all the 490 patients with HCV, while it was feasible in 137 (82.0%) patients with HBV.

Statistical Analyses

Differences in categorical variables were evaluated by the chi-squared test or Fisher's exact test, and those in

TABLE I. Baseline Characteristics of Patients With Cirrhosis Infected With HCV or HBV

Features	HCV (n = 490)	HBV (n = 167)	Differences (*P*-value)
Age (years)	59 (25–82)	45 (20–71)	<0.001
Male	289 (59.0%)	127 (76.0%)	<0.001
Transfusion	241 (49.2%)	15 (9.0%)	<0.001
Total alcohol >500 kg	86 (17.6%)	28 (16.8%)	NS[b]
AST (U/L)	64 (16–1,313)	34 (8–307)	<0.001
ALT (U/L)	58 (9–315)	30 (8–510)	<0.001
Zinc turbidity test	11.9 (0.7–23.5)	8.2 (1.8–22.7)	<0.001
Albumin (g/dl)	3.8 (3.0–5.1)	4.1 (3.2–5.2)	<0.001
Bilirubin (mg/dl)	1.1 (0.4–3.0)	1.0 (0.3–2.6)	<0.001
α-fetoprotein (ng/ml)	14 (2–748)	8 (1–1,520)	<0.001
Platelets ($100 \times 10^3/mm^3$)	9.6 (1.7–39.8)	12.8 (4.8–24.9)	<0.001
ICG R_{15} (%)[a]	28 (2–76)	18 (4–41)	<0.001

[a]Retention of inodcyanine green at 15 min in percent.
[b]Not significant.

continuous variables by the Mann–Whitney's *U*-test. Survival and development of hepatocellular carcinoma were assessed by the Kaplan–Meier life-table method, and differences were evaluated by the log-rank test. Independent risk factors associated with the progression to hepatocellular carcinoma were evaluated by the stepwise Cox regression analysis. Data analysis was performed with use of SPSS statistical software version 10 (SPSS, Inc., Chicago, Illinois). A P-value <0.05 was considered statistically significant.

RESULTS

Clinical Characteristics of Patients With Compensated Cirrhosis in the Child-Pugh Class A Who Were Infected With HCV or HBV

Table I compares demographic and laboratory features between the 490 patients persistently infected with HCV and the 167 with HBV when they were diagnosed with Child-Pugh class-A cirrhosis. There were substantial differences between them. Cirrhotic patients with HCV were significantly older, less often male, had received transfusions more frequently, and had liver function worse than those with HBV. In addition, albumin levels and platelet counts were lower, and the percent retention of indocyanine green at 15 min (ICG R_{15}) was higher in cirrhotic patients infected with HCV than with HBV.

Survival and Development of Hepatocellular Carcinoma in Patients Infected With HCV or HBV

Outcomes were compared between the 490 cirrhotic patients infected with HCV and the 167 with HBV who were followed-up without antiviral therapies for longer than 1 year. There were no differences in the duration of follow-up between them with the median of 8.2 years (range: 1.0–24.0) for HCV and 9.2 years (1.2–23.7) for HBV. During the follow-up ranging to 20 years, death occurred more frequently (55.1% vs. 35.9%, $P < 0.001$) and hepatocellular carcinoma developed more often (53.9% vs. 28.7%, $P < 0.001$) in patients with HCV than HBV. Table II compares the causes of death in patients with HCV and HBV. Hepatocellular carcinoma was the leading cause of death in both, with a significant difference in the development between patients with HCV and HBV (74.1% vs. 58.3%, $P = 0.018$). Causes of death in cirrhotic patients without hepatocellular carcinoma were principally decompensation; they tended to occur less often in patients with HCV than HBV infection (17.8% vs. 28.3%, $P = 0.073$). In surviving patients, hepatocellular carcinoma developed more frequently in those with HCV than HBV (17.8% vs. 28.3%, $P = 0.001$). Death and hepatocellular carcinoma occurred at annual incidence rates of 6.3% and 8.3% in patients with HCV, respectively, and 3.6% and 3.3% in those with HBV.

Causes of death other than hepatocellular carcinoma and decompensation in patients with HCV and HBV were non-hepatic cancers in 4.4% and 3.3%, respectively, and other causes in 3.7% and 10.0%.

Figure 1a compares the survival between cirrhotic patients infected with HCV and HBV. The survival was not different during the initial 6 years. Later on, however, patients infected with HCV fared increasingly worse than those with HBV ($P < 0.001$) with respective survival rates: 52% (n = 172) versus 65% (n = 74) at 10 years; 30% (n = 54) versus 53% (n = 44) at 15 years; and 16% (n = 12) versus 42% (n = 13) at 20 years.

The development of hepatocellular carcinoma is compared between patients with HCV and HBV in Figure 1b. There were no differences in the development of hepatocellular carcinoma during the initial 3 years. Thereafter, however, hepatocellular carcinoma developed increasingly more often in patients with HCV than HBV ($P < 0.001$); their respective frequencies were: 32% (n = 263) versus 22% (n = 98) at 5 years; 60% (n = 98) versus 33% (n = 63) at 10 years; 75% (n = 24) versus 42% (n = 35) at 15 years; and 77% (n = 6) versus 47% (n = 11) at 20 years. The yearly incidence of hepatocellular carcinoma during the first 10 years was 6.0% in patients with HCV and 3.3% in those with HBV.

Factors Influencing the Survival in Cirrhotic Patients With HCV Infection

Variables associated with the survival were evaluated by univariate analysis in cirrhotic patients infected with HCV (Table III). At the diagnosis of cirrhosis, age was significantly higher and total alcohol intake >500 kg less frequent in alive than deceased patients. Albumin,

TABLE II. Causes of Death in Patients With HCV or HBV and Occurrence After the Diagnosis of Compensated Cirrhosis in Child-Pugh Class A

Causes	HCV (n = 270)	HBV (n = 60)	Differences (P-value)
Hepatocellular carcinoma	200 (74.1%)	35 (58.3%)	0.018
Decompensation	48 (17.8%)	17 (28.3%)	NS[b] (0.073)
Liver failure	25 (52%)[a]	9 (53%)[a]	
Bleeding	6 (13%)[a]	3 (18%)[a]	
Infection	17 (35%)[a]	5 (29%)[a]	
Non-hepatic cancer	12 (4.4%)	2 (3.3%)	NS
Others	10 (3.7%)	6 (10.0%)	NS

[a]Percentage of deaths by decompensation is shown.
[b]Not significant.

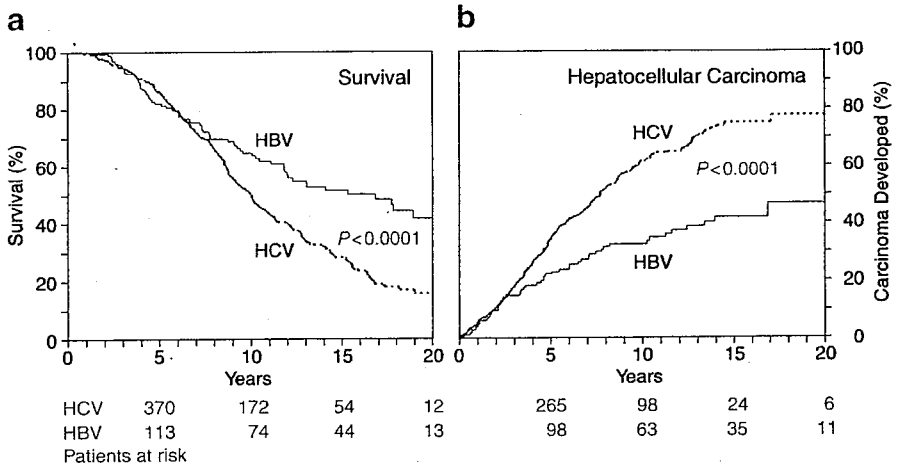

Fig. 1. Kaplan–Meier life tables for survival (**a**) and development of hepatocellular carcinoma (**b**) in patients with compensated cirrhosis in Child-Pugh Class A who were infected with HCV or HBV.

bilirubin levels, AFP, platelet counts, and ICG R_{15} were worse in dead than surviving patients. Five of the seven variables associated with death kept significance in multivariate analysis (Table IV). They were albumin levels <4.0 g/dl (hazard ratio: 1.65), AFP >20 ng/ml (1.55), alcohol intake >500 kg (1.49), age >55 years (1.47), and ICG R_{15} >30% (1.39).

Factors Influencing the Survival in Cirrhotic Patients With HBV Infection

Likewise, variables were evaluated for an association with the survival by univariate analysis in cirrhotic patients infected with HBV (Table V). Age >45 years at the diagnosis of cirrhosis, male gender and HBeAg were significantly more frequent in dead than surviving patients. The other factors associated significantly with death in HBV patients overlapped with those in HCV patients, except for the zinc turbidity test and bilirubin (Tables III and V). Only five of the nine variables associated with death retained significance in multivariate analysis (Table VI). Of them, male gender posed the highest risk (hazard ratio, 4.43) followed by age >45 years (2.24), ICG R_{15} >30% (2.14), HBeAg (2.11), and platelet counts $<100 \times 10^3/mm^3$ (1.91).

Survival of Cirrhotic Patients With Distinct Levels of Risk

Survival was compared among patients with different risk levels (Fig. 2). High, medium, and low risks for patients with HCV were defined by the sum of risk factors (shown in Table IV) at 0–1, 2–3, and 4–5, respectively, while those for patients with HBV (Table VI) were determined by that at 0–1, 2–4, and 5–6; the male gender was scored 2 due to its hazard ratio twice as high as the other factors (Table VI). There were significant differences in the survival among patients having distinct risk levels who were infected with either HCV or HBV.

Influence of HCV and HBV Genotypes on the Survival

The survival was not different between patients infected with HCV of genotypes 1b and non-1. Although

TABLE III. Univariate Analysis for Factors Influencing the Survival of Patients With HCV-Associated Cirrhosis

Factors	Category	Alive (n = 220)	Dead (n = 270)	Differences (*P*-value)
Age	>55 years	156 (70.9%)	174 (64.4%)	0.004
Gender	Male	116 (52.7%)	172 (63.7%)	NS
Transfusion	Received	104 (47.3%)	137 (50.7%)	NS
Total alcohol	>500 kg	27 (12.3%)	60 (22.2%)	0.027
AST (U/L)	>77 U/L	71 (32.3%)	102 (37.8%)	NS
ALT (U/L)	>100 U/L	43 (19.5%)	45 (16.7%)	NS
Zinc turbidity test	>12	105 (47.7%)	131 (48.5%)	NS
Albumin	>4.0 g/dl	83 (37.7%)	114 (42.2%)	<0.001
Bilirubin	>1.5 mg/dl	44 (20.0%)	56 (20.7%)	0.004
α-fetoprotein	>20 ng/ml	72 (32.7%)	115 (42.6%)	0.001
Platelets	$<100 \times 10^3/mm^3$	118 (53.6%)	150 (55.6%)	0.001
ICG R_{15}	>30%	87 (39.5%)	120 (44.4%)	0.001