

Effect of Interferon Therapy on Hepatocellular Carcinogenesis in Patients With Chronic Hepatitis Type C: A Long-Term Observation Study of 1,643 Patients Using Statistical Bias Correction With Proportional Hazard Analysis

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The activity of interferon (IFN) is not elucidated from the viewpoint of cancer prevention in chronic hepatitis C patients *en masse*. The hepatocellular carcinogenesis rate was analyzed statistically in 1,643 patients with chronic hepatitis C: 1,191 patients with IFN therapy and 452 without IFN therapy. Hepatocellular carcinogenesis rates in the treated and untreated groups were 2.1% and 4.8% at the end of the 5th year, and 7.6% and 12.4% at the 10th year, respectively ($P = .0036$). Multivariate analysis showed that IFN slightly decreased the risk of carcinogenesis by 33%, compared with that of untreated patients ($P = .14$), adjusting for the confounding effects of age, fibrotic stage, gender, and γ -glutamyl transpeptidase (GGTP) value. Among 1,191 patients with IFN, 461 patients attained persistent loss of hepatitis C virus (HCV) RNA, and the other 145 patients retained normal alanine transaminase (ALT) values without loss of HCV RNA. The hazard of carcinogenesis in these 606 patients with persistent normal ALT with or without HCV-RNA clearance was significantly lower than that of untreated patients (hazard ratio: 0.32; $P = .012$) and that of the abnormal aminotransferase group. Among patients with chronic hepatitis C, IFN significantly decreased the hepatocellular carcinogenesis rate in those patients with normal or persistent low ALT values. (HEPATOLOGY 1999;29:1124-1130.)

Until recently, hepatitis C virus (HCV) has been reported to be a causative agent of hepatocellular carcinoma (HCC) aside from hepatitis B virus.¹⁻⁴ In two cohort studies from Tokyo⁵ and Osaka⁶ of Japanese patients with cirrhosis, the cumulative appearance rates of HCC at 3, 5, 10, and 15 years were respectively, 12.5%, 19.4%, 44.3%, and 58.2%. HCC occurred

more frequently (75.2% at 15 years) in those patients with only HCV antibodies at enrollment than in those with only hepatitis B surface antigen (27.2%). According to our estimation of the carcinogenesis rates in untreated patients with chronic hepatitis C,⁷ 5-year, 10-year, and 15-year rates were 4.8%, 13.6%, and 26.0%, respectively. Because life expectancy of patients with HCV-related cirrhosis is largely influenced by development of HCC in the clinical course, and because an effective and truly curative therapy for HCC still remains limited at best, primary prevention of HCC in patients with chronic liver disease is of great importance.

Interferon (IFN) is effective in eliminating HCV and in reducing serum alanine transaminase (ALT) in some patients with chronic hepatitis C.⁸⁻¹¹ The response to IFN therapy is related to factors including HCV subtype, serum concentration of HCV, IFN treatment schedule, and liver histology.¹¹⁻¹⁴ A Japanese trial of IFN for patients with HCV-related cirrhosis showed that IFN therapy decreased the HCC appearance rate through the disappearance of HCV RNA.¹⁵ However, there has been no report about the anticarcinogenic activity of IFN in patients with chronic hepatitis type C, comparing a large number of untreated patients. To elucidate whether IFN suppresses the carcinogenesis rate in patients with chronic hepatitis C, we studied a total of 1,191 patients with IFN therapy compared with 452 patients without treatment, adjusting background features using multivariate analysis. One of the principal aims of our study was therefore to show a role of IFN in cancer prevention in chronic hepatitis type C *en masse*: To what extent could IFN decrease the carcinogenesis rate from chronic hepatitis C in society? The other aim was to assess a possible mechanism, if any, of cancer prevention by IFN.

PATIENTS AND METHODS

Study Population. A total of 1,643 patients with chronic hepatitis were examined whose initial sera showed negative hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan) and positive anti-HCV (second-generation anti-HCV, enzyme-linked immunosorbent assay, Dainabot, Japan). Anti-HCV was assayed using stored frozen sera at -80°C . In the study, there were 1,082 men and 561 women, aged 15 to 86 years, with a median age of 51 years. They were diagnosed between 1974 and 1995 at Toranomon Hospital, Tokyo, Japan, as having chronic hepatitis by liver biopsy with or without peritoneoscopy. Although chronic hepatitis varied

Abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; IFN, interferon; ALT, alanine transaminase; CR, complete response; IR, incomplete response; NR, no response; GGTP, γ -glutamyl transpeptidase.

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Received May 18, 1998; accepted January 20, 1999.

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0270-9139/99/2904-0019\$3.00/0

in severity in these patients, those patients with subacute hepatitis or cirrhosis were excluded. Patients with a possible HCC association at the time of diagnosis of hepatitis were strictly excluded from the study. To investigate hepatocellular carcinogenesis from HCV-related cirrhosis, patients with concomitant infection of hepatitis B virus were also excluded.

Among the 1,643 patients with HCV-related hepatitis, 1,191 (72.5%) received IFN therapy (group A), chiefly after 1987, when IFN was available in Japan. Any new antiviral or anticarcinogenic treatment in viral cirrhosis, except for IFN, was not introduced in or after 1987 in Japan. The remaining 452 patients did not undergo IFN therapy or any other antiviral therapy (group B). We therefore performed a retrospective study using a historical control before 1987 and those patients who refused treatment or could not receive treatment for various reasons after 1987.

Background and Laboratory Data of the Patients. Table 1 summarizes the profiles and laboratory data of the 1,191 patients with IFN and

TABLE 1. Patient Profiles and Laboratory Data at the Time of the Diagnosis of Chronic Hepatitis

	IFN Therapy		P
	Yes (group A)	No (group B)	
Demography			
Number of patients	1,191	452	
Sex (M/F)	802/389 (2.06:1)	280/172 (1.62:1)	.040
Age (yr)*	50 (15-86)	53 (21-78)	<.0001
History of blood transfusion	483 (40.6%)	204 (45.1%)	.086
Family history of liver disease	262 (22.0%)	125 (27.7%)	.016
Alcohol intake of 500 kg or more	158 (13.3%)	94 (20.8%)	.00016
Observation period (yr)*	5.1 (0.1-11.3)	8.2 (0.5-22.8)	<.0001
Laboratory data*			
Albumin (g/dL)	4.2 (2.8-5.4)	4.3 (3.1-5.3)	.14
Bilirubin (mg/dL)	0.8 (0.1-4.5)	0.8 (0.3-19.4)	.72
Aspartic aminotransferase (KU)	43 (5-731)	45 (8-1,290)	.44
ALT (KU)	60 (5-830)	52 (5-720)	.0001
GGTP (IU/L)	33 (4-369)	35 (1-630)	.68
Platelet count ($\times 1,000$ /mcl)	171 (43-418)	164 (30-455)	.0050
ICG R15 (%)‡	14 (1-89)	16 (2-61)	.0017
HCV serological group			
Group 1 (genotype 1a, 1b)	558 (66.7%)	156 (76.4%)	.0028
Group 2 (genotype 2a, 2b)	238 (28.4%)	37 (18.1%)	
Undetermined	41 (4.9%)	11 (5.4%)	
HCV concentration			
High†	369 (46.0%)	173 (67.1%)	<.0001
Low†	433 (54.0%)	85 (32.9%)	
Histological stage of hepatitis			
F1 (slight fibrosis)	798 (67.0%)	265 (58.6%)	.0015
F2-F3 (moderate-severe fibrosis)	393 (33.0%)	187 (41.4%)	

*Expressed by median (minimum, maximum).

†High HCV concentration implies 10^6 copies/mL or more by competitive polymerase chain reaction assay or 1 Meq/mL or higher by HCV-probe assay. Low HCV concentration indicates less than 10^6 copies or less than 1 Meq/mL by each assay.

‡ICG R15: indocyanine green retention rate at 15 minutes. The ICG R15 test was performed in 1,232 patients (75.0%) at the initial point of the observation.

TABLE 2. Major Side Effects of IFN Requiring Discontinuation of the Treatment in 1,191 Patients

Psychosis	22 (1.8%)
Ophthalmic symptoms, retinopathy	9 (0.8%)
Thyroid dysfunction	5 (0.4%)
Dermatological manifestation	5 (0.4%)
Generalized eruption without itching	N = 4
Generalized eruption with itching	N = 1
Severe weakness or general malaise	5 (0.4%)
Extrahepatic infection	4 (0.3%)
Other adverse effects	13 (1.1%)
Total	63 (5.3%)

those of the 452 patients without IFN administration at the time of diagnosis of chronic hepatitis.

The male/female ratio in the IFN group was higher in the treatment group than in the no-therapy group. The median age of the treated group was lower than that of the untreated group by 3 years. There were 158 patients (22.0%) with a history of ≥ 500 kg of alcohol intake until the diagnosis of chronic hepatitis (corresponding to a daily intake of 3,000 mL of beer or 300 mL of whiskey for 20 years) in the IFN group, and 94 (20.8%) in the no-therapy group ($P = .00016$). Because IFN was introduced in our hospital in 1987, the observation period was significantly shorter in the treated group than in the untreated one (median, 5.1 years vs. 8.2 years; $P < .0001$). Although all patients showed a positive HCV-RNA result in the clinical courses, serum concentration of HCV RNA using initial sera was analyzed in 1,061 patients (64.6%). HCV subtype was analyzed by the immunoserological typing method with a commercial kit (Kokusai Diagnostic Corporation, Kobe, Japan): serological group 1 indicated genotypes 1a and 1b, and group 2 included 2a and 2b subtypes. The serological grouping of HCV showed that the rate of group 2 was significantly higher in the IFN group than in the untreated patients. The initial serum concentration of HCV RNA was assessed in 1,060 patients using the competitive polymerase chain reaction method or HCV-probe assay (Chiron Corp., Emeryville, CA). A higher concentration of HCV was much more frequently found in group B than in group A. A mild degree of hepatic fibrosis was also found, significantly more so in the IFN group.

IFN Treatment and Judgment of the Effect. A total of 1,191 patients underwent IFN therapy with IFN- α (natural or recombinant), IFN- β (natural), or both: 733 patients (61.5%) received 6 to 9 million units of IFN every day for 8 weeks, followed by twice or three times per week for 16 weeks; 143 patients (12.0%) received 6 to 9 million units of IFN every day for 2 to 4 weeks, followed by three times per week for 20 to 22 weeks; 175 patients (14.7%) underwent short therapy with IFN every day for 4 to 8 weeks; 112 patients (9.4%) received intermittent administration of three times per week for 24 weeks; 14 had a prolonged administration of IFN for 8 to 36 months; 8 (0.7%) received 6 million units of IFN- β every day for 6 to 18 months; and the remaining 6 patients (0.5%) had a combined administration of IFN- α and IFN- β for 4 months. As a whole, a median dose of 624 million units was administered during the median period of 24 weeks. A total of 83.0% of all the patients received IFN for 24 weeks. IFN therapy was usually initiated within a few months after the diagnosis of chronic hepatitis, and every patient began within 12 months. The median interval between liver biopsy and initiation of IFN therapy was 10 days.

Almost all of the patients given IFN therapy showed varied degrees of fever, chills, myalgias, headache, and general malaise after the first injection of IFN. Most of the patients revealed various degrees of leukocytopenia and thrombocytopenia. A significant thrombocytopenia of 40,000 counts/mL or less required a reduction of the IFN dosage in 28 patients. IFN therapy was discontinued because of psychosis in 22 patients and ophthalmic symptoms in 9 patients (Table 2). No patients developed decompensated liver disease with ascites, encephalopathy, jaundice, or variceal bleeding.

Although a total of 63 patients in group A could not proceed with the IFN injection, the following studies of carcinogenesis were analyzed on an intention-to-treat basis.

Judgment of IFN effect was classified according to elimination of HCV RNA and ALT value at 12 months after the end of the treatment. Complete response (CR) was defined as persistent disappearance of HCV RNA after therapy, incomplete response (IR) as normal ALT values without elimination of HCV RNA for at least 6 months after therapy, and no response (NR) as persistently abnormal or only transient normalization of ALT for less than 6 months.

Follow-up and Diagnosis of HCC. Follow-up of the patients was made on a monthly basis after diagnosis of chronic hepatitis by monitoring hematological, biochemical, and virological data. During and after the treatment with IFN, a weekly or biweekly follow-up was performed in almost of the group A patients. Imaging diagnosis was made once or twice per year in a majority of patients with computed tomography or ultrasonography. Angiographic study was performed only when HCC was highly suspected on ultrasonography or computed tomography.

When angiography demonstrated a characteristic hypervascular nodule, it was usually a specific finding for HCC in these follow-up patients, and histological confirmation was usually not required in the majority of these HCC patients. Most of the "angiographically diagnosed HCC" showed intrahepatic multiplicity and pathognomonic findings of capsule formation or nodule-in-nodule appearance, or even portal vein invasion. Clinical trends of tumor markers were also taken into account. If angiographic study could not show any hypervascular stain in a small hepatic nodule, histological study was always performed. Microscopic examination through a fine-needle biopsy was also performed in 8 patients whose angiogram could not demonstrate a typical image of HCC. The other 57 patients also had a pathological confirmation of surgically resected specimens or autopsy.

One hundred seven cases (6.5%) were lost to follow-up: 52 patients (4.4%) in group A and 55 patients (12.2%) in group B. Annual drop-out rates in groups A and B were 0.75% and 1.29% per year, respectively ($P > .05$). Because no patient with HCC development in group A showed carcinogenesis within 14 months after the initiation of IFN, all patients developed HCC after the judgment of IFN effect. Among 1,191 patients with IFN therapy, 6 patients (0.50%) were lost to follow-up in our hospital from 8 to 12 months after initiation of IFN therapy, and the other 6 patients (0.50%) were lost from 13 to 18 months. In 9 patients, IFN effects were judged from information about aminotransferase examined in the other clinics, and the other 3 patients were judged from the evidence of persistent HCV-RNA and aminotransferase activity at 6 months. The IFN effect therefore could be judged eventually in all patients, including the 12 cases lost early to follow-up. Because the eventual outcomes regarding appearance of HCC were not identified in these patients, they were dealt with as censored data in the following statistics.¹⁶ Death unrelated to liver disease was also classified as withdrawal and regarded as a censored case. The date of the last follow-up for this study was April 1, 1998. The median observation period of the total number of patients was 5.4 years, with a range of 0.1 to 22.8 years.

Statistical Analysis. Nonparametric procedures were employed for the analysis of background characteristics of the patients, including the Mann-Whitney U test and χ^2 method. HCC appearance rate was calculated from a period between the diagnosis of chronic hepatitis by liver biopsy and appearance of HCC in both groups, using the Kaplan-Meier technique.¹⁷ The differences in carcinogenesis curves were tested using the log rank test. Independent factors associated with the appearance rate of HCC were studied using the stepwise Cox regression analysis.¹⁸ The following 18 variables were analyzed for potential covariates for liver carcinogenesis at the time of diagnosis of hepatitis: age, sex, total alcohol intake, family history of liver disease, history of blood transfusion, fibrotic staging of hepatitis, aspartic transaminase, ALT, albumin, bilirubin, globulin, γ -glutamyl transpeptidase (GGTP), platelet count, ICG R15, serologi-

cal grouping of HCV, serum concentration of HCV, and IFN administration. In addition to the non-time-dependent analysis, extended Cox proportional hazard analysis was performed using an interaction term of IFN treatment and "waiting time" to the therapy as a time-dependent covariate. The independence of treatment factor from "waiting time" was also confirmed by log-minus-log plot of the proportional hazard model. Although continuous variables without data conversion were used in the following multivariate analyses, several variables were transformed into categorical data consisting of two or three simple ordinal numbers to obtain each hazard ratio. All factors found to be at least marginally associated with liver carcinogenesis ($P < .15$) were tested by the multivariate Cox proportional hazard model. $P < .05$ was considered to be significant.

All data analysis was performed with the computer program SAS.¹⁹

RESULTS

Effect of IFN. First, the judgment of IFN effect was made at the end of the 12th month, according to both HCV-RNA and serial ALT measurements. Among 1,191 patients with IFN treatment, CR (elimination of HCV RNA) was found in 461 patients (38.7%), IR (ALT normalization for at least 6 months without HCV-RNA disappearance) in 145 patients (12.2%), and NR (abnormal or only transient ALT decrease) in 585 patients (49.1%).

Second, the judgment was made at the end of the observation period, according only to ALT values. A persistently normal ALT value was found in 591 patients (49.6%), abnormal but slight ALT elevation within 1.5 times of normal value in 218 patients (18.3%), and high ALT values of 1.5 times or more in 382 patients (32.1%).

Crude Hepatocellular Carcinogenesis Rates. During the observation period of 22.8 years, HCC appeared in 95 patients (5.8%): 28 (2.5%) in the IFN-treated group, and 67 (14.8%) in the untreated group (Fig. 1). Among the 95 patients with HCC, 65 patients demonstrated a typical hypervascular stain on angiography and dynamic computed tomography, but the other 30 patients showed no tumor stain on angiography. All the latter patients were histologically confirmed to have a definitive HCC by surgically resected specimen or fine-needle biopsy. The median α -fetoprotein value was 42 ng/mL (range:

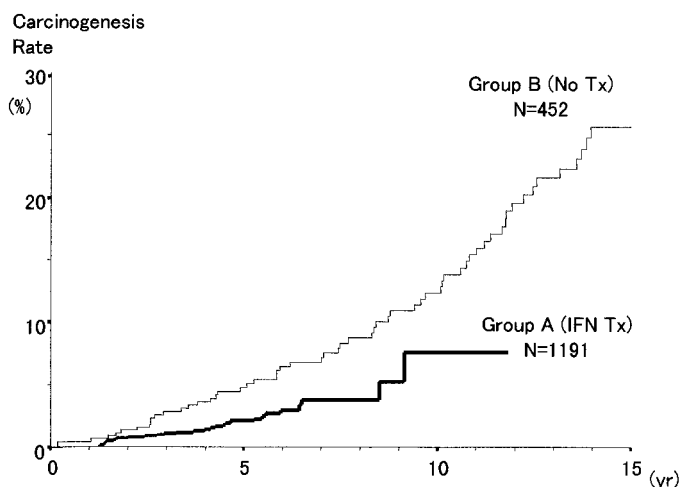


FIG. 1. Crude hepatocellular carcinogenesis rates in treated and untreated groups. The carcinogenesis rate was significantly lower in the IFN-treated group than in the untreated group (log rank test, $P = .020$).

3-24,700 ng/mL), and a higher value of 100 ng/mL or more was found in 35 (37.6%) of 93 patients.

A total of 114 patients developed cirrhosis during the follow-up: 29 patients with IFN and 85 without IFN. Surgically resected specimens showed that at least 18 patients developed HCC without cirrhosis. Although an additional 12 patients with HCC development were similarly not associated with cirrhosis at the time of the diagnosis of HCC, histological confirmation was not performed.

Hepatocellular carcinogenesis rates in groups A and B were 1.1% and 2.8% at the end of the 3rd year, 2.1% and 4.8% at the end of the 5th year, and 7.6% and 12.4% at the 10th year, respectively. The carcinogenesis rate was significantly lower in the IFN-treated group than in the untreated group (log rank test, $P = .0036$).

Carcinogenesis Rates According to IFN Effect. During the observation period in group A, HCC developed in 28 patients (2.5%): 5 patients (1.1%) in the CR group, 2 (1.4%) in the IR group, and 21 (3.6%) in the NR group. Hepatocellular carcinogenesis rates in patients with CR, IR, and NR were 0.7%, 0.7%, and 1.5% at the end of the 3rd year, 1.4%, 1.9%, and 2.9% at the 5th year, 1.4%, 1.9%, and 7.6% at the 7th year, and 1.4%, 1.9%, and 17.5% at the 10th year, respectively (Fig. 2). The carcinogenesis rate in the NR group was significantly higher than in the other groups (log rank test, $P = .025$).

Because the carcinogenesis rates of the CR group and the IR group were almost the same in the above statistics, "responded groups" (CR + IR), "no-response group" (NR group), and the untreated groups were simultaneously compared as to the carcinogenesis. Hepatocellular carcinogenesis rates in the "responded group," the "no-response group," and the untreated group were 0.7%, 1.5%, and 2.8% at the end of the 3rd year, 1.5%, 2.7%, and 4.8% at the 5th year, 1.5%, 6.3%, and 6.8% at the 7th year, and 1.5%, 14.9%, and 12.4% at the 10th year, respectively (Fig. 3). The carcinogenesis rate in the "responded groups" was significantly lower than the "no-response group" and the untreated group (log rank test, $P = .0011$).

Factors Affecting Hepatocellular Carcinogenesis. In the first analysis, factors associated with carcinogenesis were explored only in the untreated-patients group to clarify the risk factors

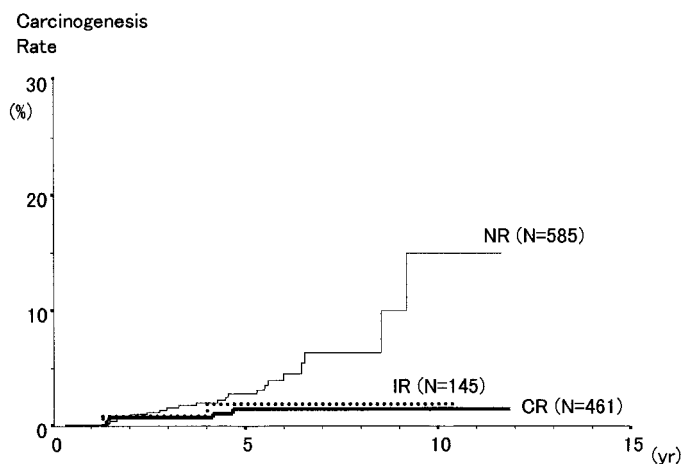


FIG. 2. Hepatocellular carcinogenesis rates of CR, IR, and NR in the treated group. The rate of the NR group (persistently abnormal or only transient normalization of ALT for less than 6 months) was significantly higher than that of the CR and IR groups.

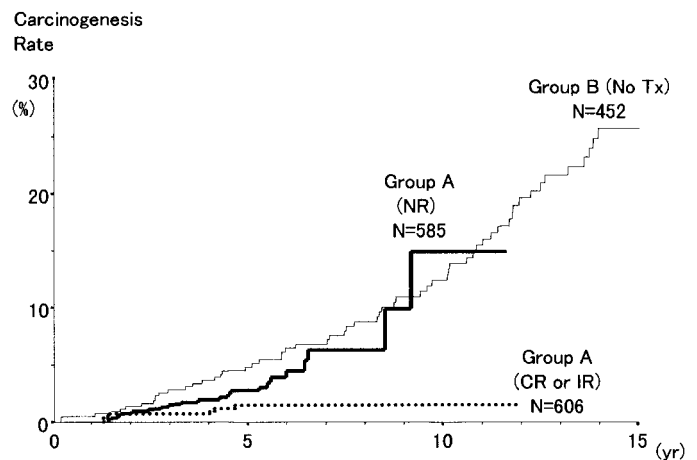


FIG. 3. Hepatocellular carcinogenesis rates of the "responded group" (CR + IR), "no-response group" (NR), and the untreated group. The carcinogenesis rate in the "responded groups" was significantly lower than the "no-response group" and the untreated group.

in the natural clinical course of HCV-related chronic hepatitis. In multivariate analysis, the following four factors influenced carcinogenesis: age ($P < .0001$), histological staging ($P < .0001$), sex ($P = .0065$), and GGTP value ($P = .028$). The analysis showed that older age, moderate to severe fibrotic stages, male sex, and higher GGTP concentration were independently associated with the carcinogenesis rate.

In the second analysis, factors associated with carcinogenesis were explored in all of the 1,643 patients to clarify the role of IFN in carcinogenesis from the patients with chronic hepatitis type C *en masse*. IFN therapy significantly decreased the hepatocellular carcinogenesis rate in the analysis of the product-limit method as shown above (log rank test, $P = .0036$). To further investigate the extent to which IFN therapy contributed to the carcinogenesis rate in non-time-dependent hazard analysis, the IFN therapy factor was added to the predictive model as a covariate. Although IFN lowered the carcinogenesis rate in patients with hepatitis type C *en masse* (hazard ratio = 0.67), the ratio did not show a statistical significance ($P = .14$). Namely, the role of IFN in decreasing carcinogenesis from chronic hepatitis C was not significant in our study with all 1,643 patients, when background biases of patients were statistically corrected with significant covariates in the model (Table 3). The total amount of IFN also did not affect the carcinogenesis rate in the treated group.

Next, further statistical analyses were performed to elucidate whether the IFN treatment factor might be a time-dependent covariate in liver carcinogenesis. When the interaction term (time by IFN treatment) was introduced in a preparatory calculation, multivariate analysis showed that the time-dependent variable was not statistically significant (hazard ratio: 1.25; 95% CI: 0.60-2.51; $P = .53$). Because the median interval between liver biopsy and initiation of IFN therapy was only 10 days in group A, the results of time-dependent analysis and non-time-dependent analysis were essentially identical. Log-minus-log plot (Fig. 4) also demonstrated that the treatment factor was basically time-independent in our analysis and that the analysis did not seem to violate the proportionality assumption. Hazard analysis introducing the interaction term (time by IFN treatment) indicated that IFN treatment decreased the hazard of carcinogen-

TABLE 3. Factors Associated With Hepatocellular Carcinogenesis in Patients With Hepatitis C-Related Hepatitis *en masse* (multivariate Cox proportional hazard analysis)

Factors	[Category]	Hazard Ratio (95% confidence interval)	P
Non-time-dependent model			
Age	1: <49 y.o.	1	<.0001
	2: 50 y.o.-	3.00 (1.78-5.03)	
Histological stage	1: F1 (slight)	1	<.0001
	2: F2-F3 (moderate-severe)	2.97 (2.11-4.16)	
Sex	1: male	1	.0064
	2: female	0.48 (0.28-0.81)	
GGTP value	1: <49 IU/L	1	.015
	2: 50 IU/L-	1.71 (1.11-2.66)	
IFN therapy	1: no	1	.14
	2: yes	0.67 (0.40-1.14)	
Time-dependent model			
Age	1: <49 y.o.	1	<.0001
	2: 50 y.o.-	4.17 (1.97-8.25)	
Histological stage	1: F1 (slight)	1	<.0001
	2: F2-F3 (moderate-severe)	4.17 (2.29-7.61)	
Sex	1: male	1	.007
	2: female	0.32 (0.17-0.62)	
IFN therapy	1: no	1	.10
	2: yes	0.60 (0.32-1.11)	

NOTE. Analysis by non-time-dependent model; IFN therapy was not significantly associated with carcinogenesis rate. Analysis by time-dependent model using interaction term of IFN treatment and "waiting time."

esis rate in patients with chronic hepatitis C by 40% (hazard ratio: 0.60; 95% CI: 0.32-1.11; $P = .10$), but statistical significance was not shown (Table 3).

Hazard of Carcinogenesis According to IFN Effect. Because the carcinogenesis rate of the "responded groups" was significantly lower than that of the "no-response group" and the untreated group in the product-limit method (Fig. 3), multivariate analysis was performed including the factor of IFN

TABLE 4. Factors Associated With Hepatocellular Carcinogenesis in Patients With Hepatitis C-Related Hepatitis, When the Treated Patients Were Classified According to the IFN Effect

Factors	Category	Hazard Ratio (95% CI)	P
Age	1: <49 y.o.	1	<.0001
	2: 50 y.o.-	2.95 (1.75-4.96)	
Histological stage (severity of fibrosis)	1: F1 (slight)	1	<.0001
	2: F2-F3 (moderate-severe)	2.89 (2.05-4.07)	
Sex	1: male	1	.0057
	2: female	0.47 (0.28-0.80)	
GGTP value	1: <49 IU/L	1	.016
	2: 50 IU/L-	1.71 (1.10-2.65)	
IFN therapy	1: no IFN therapy	1	.036
	2: IFN not responsive (NR*)	0.96 (0.55-1.70)	.90
	3: IFN responsive (CR* or IR*)	0.32 (0.13-0.78)	.012

NOTE. The factor of IFN therapy was classified into two groups: "responsive" patients with a history of ALT normalization for at least 6 months after therapy, and "nonresponsive" patients without ALT normalization for 6 months.

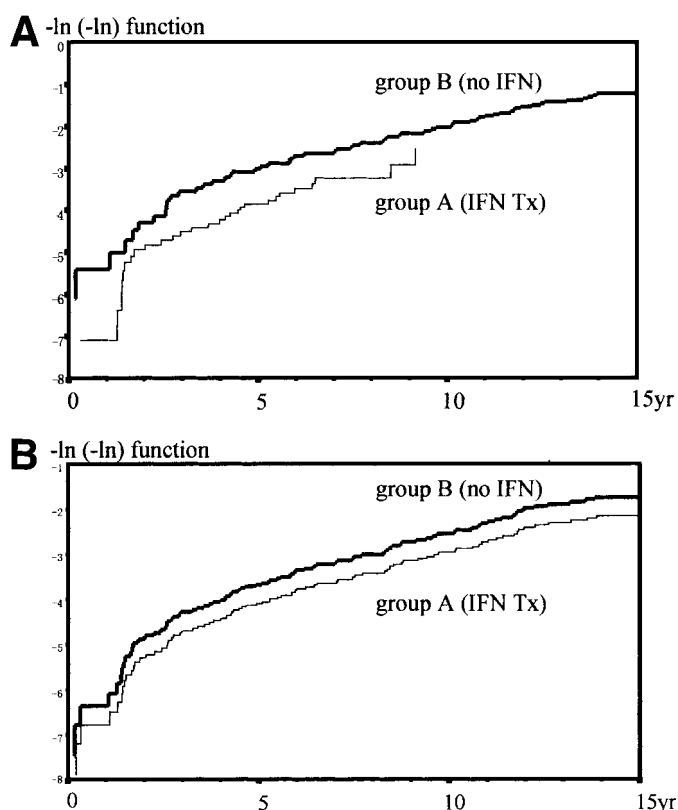


FIG. 4. Confirmation of proportionality assumption of the statistical method by log-minus-log plot. (A) Observed plotting. (B) Theoretical plotting.

response. The hazard ratio of the patients with CR or IR responses by IFN therapy was 0.32 (95% CI: 0.13-0.78; $P = .012$) compared with that of the untreated-patients group, when the other four factors constituted the model as significant covariates (Table 4). Curves of carcinogenesis rates were generated from the multivariate analysis in imaginary treated and untreated groups with average age, average histological stage, average male-female ratio, and average GGTP value (Fig. 5). Because the curves were adjusted with significant covariates assuming a standardized study group, the curves indicated that the "responded" patients showed a significantly lower carcinogenesis rate compared with the untreated-patients group, but that "no-response" patients revealed almost the same rate as the untreated ones.

Mortality and Causes of Death. During the observation period, 41 patients (2.5%) died: 6 in group A and 35 in group B. The estimated 3-year survival rates in groups A and B were 99.9% and 99.5%, 5-year rates were 99.6% and 98.1%, 7-year rates were 99.7% and 97.7%, and 10-year rates were 98.8% and 95.6%, respectively. The survival rate in group A was significantly higher than that of group B (log rank test, $P < .0001$).

DISCUSSION

To recognize the characteristics of the clinical events in liver carcinogenesis, we previously reported epidemiological data concerning long-time occurrence rates of HCC in patients with chronic hepatitis⁷ and in patients with cirrhosis.⁵ In Japan, where highly socialized medicine is practiced with everyone covered by some form of health insurance,

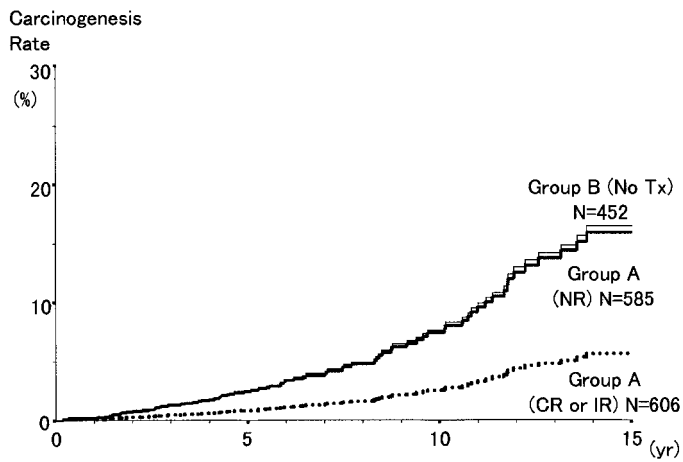


FIG. 5. "Adjusted" hepatocellular carcinogenesis rates in the "responded group," "no-response group," and the untreated group. Cox proportional hazard analysis showed that the carcinogenesis rate in the "responded group" was significantly lower than that of the other groups, when the other significant covariates were substituted with the same average parameters in the three groups. These curves of carcinogenesis rates were generated from the multivariate hazard analysis in imaginary treated and untreated groups with average age, average histological stage, average male-female ratio, and average GGTP value. Because the curves were adjusted with significant covariates assuming a standardized study group, the curves indicated that the "responded" patients showed a significantly lower carcinogenesis rate compared with the untreated-patients group, but that "no-response" patients revealed almost the same rate as the untreated ones.

people can frequently consult physicians, who can perform extensive investigations regardless of cost. As was shown in previous articles,^{5,7} we could therefore perform a reliable and thorough observation study on a monthly basis. Hematological, biochemical, and virological studies were also achieved monthly or bimonthly in every patient. A prospective, randomized trial with untreated control patients is actually impossible in this country, from the ethical viewpoint: IFN is already a standard modality of radical therapy for chronic hepatitis type C, and everyone can undergo the therapy with medical insurance in Japan. Another reason for the difficulty of informed consent in the prospective, randomized study is that it requires at least 5 years to show a statistical difference in the carcinogenesis rate between the treated and "untreated" groups: because of expected low rates of carcinogenesis in chronic hepatitis, there may be a lack of statistical power to detect a preventive effect of IFN. Because any randomized studies are considered to be difficult in the future in developed countries, we attempted to perform this retrospective study with a statistical adjustment using possible covariates explored in multivariate analysis.

In product-limit analysis (Kaplan-Meier method), IFN significantly decreased the hepatocellular carcinogenesis rate in 1,643 patients with chronic hepatitis type C. Because there were some background differences between the treated and the untreated groups, we tried to correct the biases including age, histological staging, sex, and GGTP value, which significantly affected the carcinogenesis rate. After the adjustment of demographic, histological, and biochemical factors between the groups, IFN proved to bring about a slight decrease in hazard of carcinogenesis from hepatitis C patients *en masse* (hazard ratio = 0.67, $P = .14$ by non-time-dependent model; and hazard ratio = 0.60, $P = .10$ by time-dependent model). One of the reasons why statistical significance was not

obtained in the study was because the carcinogenesis rate in the untreated group was too low (1.2% per year) to show the cancer-preventive action of IFN. Another reason was because HCC did develop in several patients with complete elimination of HCV RNA. A significant statistical result surely would be obtained when the median observation period was longer than 7 or 10 years in our subjects. Although several reports suggested a relationship of anti-hepatitis B core antibody or hepatitis B surface antibody with carcinogenesis,²⁰⁻²² we could not show the association because of insufficient available data.

Although a statistical significance was not proven in the study of all the patients with IFN therapy, both univariate and multivariate analyses certainly demonstrated that IFN lowered the carcinogenesis rate in those patients whose ALT decreased after therapy. The anticarcinogenic effect of IFN was found in the patients with persistent aminotransferase normalization, and in the patients with transient ALT normalization for at least 6 months or 12 months. Interestingly, the cancer-suppressive activity of IFN in those patients with HCV-RNA eradication (CR) was similar to that of the patients with ALT normalization without HCV-RNA elimination (IR). From these facts, the anticarcinogenic activity of IFN is deeply indebted to suppression of hepatocellular inflammatory and regenerative processes. Moreno et al.²³ reported that IFN exhibited remission of liver fibrosis, and control of necroinflammatory process can therefore induce a suppression of the growth process of HCC. Tarao et al.²⁴ reported that high aminotransferase activity resulted in an increase of an HCC recurrence rate in patients with cirrhosis. Our results also suggested that the carcinogenic process in the patients with chronic hepatitis C was greatly enhanced by the aminotransferase fluctuation and its persistence. From a viewpoint of suppression of liver carcinogenesis, IFN played a suppressive action of HCC through reduction or complete remission of inflammatory activity.

Because this study analyzed only 1,191 patients with IFN therapy for only 11 years, any differences of carcinogenesis rates might be shown between the patients with CR and IR in the future. Because IFN seemed to behave as an anticarcinogenic agent through an anti-inflammatory mechanism, certain percentages of the patients with an IR effect without HCV-RNA elimination would retrieve an active carcinogenic process with ALT re-elevation and exacerbation of hepatic necroinflammation. On the contrary, the patients with a CR effect with HCV elimination will not repossess any aminotransferase elevation and exacerbation. Because carcinogenesis actually occurred only within 5 years after IFN therapy in the patients with a CR effect, it might occur in the patients with an IR effect even after 5 years or more.

Our study found a total of 5 cases of HCC development after HCV-RNA eradication with normalization of ALT value. Existence of a small invisible HCC, which had still not been shown by various imagings at the time of IFN therapy, was considered responsible for the appearance of HCC after CR to IFN. It is natural to think that HCC cells can autonomously proliferate and grow without promotive activity of hepatocellular inflammation. Certainly, if HCC is never reported to occur after 5 years of CR by IFN therapy in varied reports in the future, then a "latent period" of 5 years should be strictly observed even after the excellent effect of IFN therapy. Indeed, because the rapidity of tumor growth may depend on individual tumor characteristics, much efforts should be paid

to understand the ordinary carcinogenic process in chronic hepatitis C, from epidemiological and detailed clinicopathological studies such as in the current study.

Because a well-controlled, randomized study about anticarcinogenic action of IFN therapy had not been available until now, we retrospectively showed the extent to which IFN had acted as an HCC suppressor and the mechanism with which IFN had behaved as an anticarcinogenic. A randomized, controlled study using as many patients with a 5- to 10-year observation period will be required to elucidate the exact role of IFN in the suppression of HCC in patients with chronic hepatitis type C.

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