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-

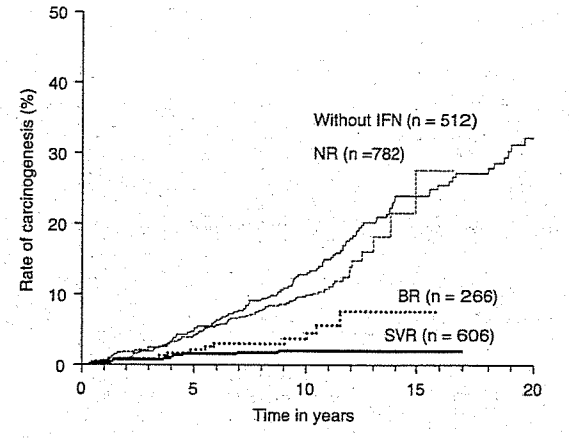

Fig. 2. Rates of hepatocarcinogenesis in patients with SVR, BR and NR to IFN. The rate in patients with NR (persistently elevated ALT or transiently normalized ALT for less than 6 months) was significantly higher than that in patients with SVR or BR.

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.

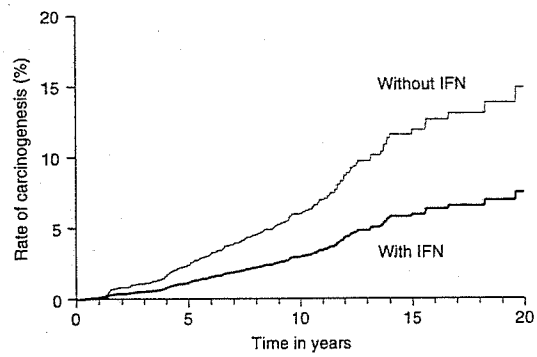

Fig. 3. Theoretical curves of hepatocarcinogenesis in patients treated with IFN and those untreated who have the average histological stage, average γ-GTP value, average ratio of male to female, average platelet count and average age. They are based on the analysis of 1,654 patients treated with IFN 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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A Long-Term Glycyrrhizin Injection Therapy Reduces Hepatocellular Carcinogenesis Rate in Patients with Interferon-Resistant Active Chronic Hepatitis C: A Cohort Study of 1249 Patients

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A Long-Term Glycyrrhizin Injection Therapy Reduces Hepatocellular Carcinogenesis Rate in Patients with Interferon-Resistant Active Chronic Hepatitis C: A Cohort Study of 1249 Patients

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To elucidate the influence of a glycyrrhizin therapy on hepatocarcinogenesis rate in interferon (IFN)-resistant hepatitis C, we retrospectively analyzed 1249 patients with chronic hepatitis with or without cirrhosis. Among 346 patients with high alanine transaminase value (twice or more of upper limit of normal), 244 patients received intravenous glycyrrhizin injection and 102 patients did not, after judgment of IFN resistance. Crude carcinogenesis rates in the treated and untreated group were 13.3%, 26.0% at the 5th year, and 21.5% and 35.5% at the 10th year, respectively ($P = .0210$). Proportional hazard analysis using time-dependent covariates disclosed that glycyrrhizin treatment significantly decreased the hepatocarcinogenesis rate (hazard ratio 0.49, 95% confidence interval 0.27–0.86, $P = .014$) after adjusting the background features with significant covariates. Glycyrrhizin injection therapy significantly decreased the incidence of hepatocellular carcinoma in patients with IFN-resistant active chronic hepatitis C, whose average aminotransferase value was twice or more of upper limit of normal after interferon.

KEY WORDS: chronic hepatitis; hepatitis C virus; glycyrrhizin; hepatocellular carcinogenesis; cancer prevention.

Until recently, hepatitis C virus (HCV) has been reported to be a causative agent of hepatocellular carcinoma (HCC) aside from hepatitis B virus (1–5). In our cohort studies of Japanese patients with HCV-related cirrhosis (5), the cumulative appearance rates of HCC at the 5, 10, and 15 years were 21.5%, 53.2%, and 75.2%, respectively. The carcinogenesis rate was higher in those patients with cirrhosis caused by HCV than in those with hepatitis B virus-related cirrhosis.

Interferon (IFN) is effective in eliminating HCV in some patients with chronic hepatitis C (6–8) and cirrhosis (9–11), and in reducing hepatocellular carcinogenesis rate through suppression of necro-inflammatory process and reduction of serum alanine transaminase (ALT). Kasahara *et al.* (6) reported that sustained normal ALT value after IFN therapy was significantly associated with a decreased hepatocellular carcinogenesis rate in patients with chronic hepatitis C. Our data (7) also demonstrated an anticarcinogenic activity of IFN in patients who attained normal ALT

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level after the therapy compared with IFN-treated patients without normalization of ALT.

Oka *et al.* (12) reported in a randomized controlled trial that a kind of medicinal herb, *Sho-saiko-to*, significantly decreased hepatic carcinogenesis rate in patients with HBsAg-negative cirrhosis. Tarao *et al.* (13) showed that HCC appearance rate was significantly higher in HCV-related cirrhotic patients with a high ALT value of 80 IU/mL or more than that of those with lower ALT value (<80 IU/mL), and also suggested that treatment of cirrhosis and prevention of HCC should be directed to suppress the necro-inflammation of HCV-related hepatitis. A glycyrrhizin-containing product, Stronger Neo-Minophagen C (SNMC; Minophagen Pharmaceutical Co. Ltd., Tokyo, Japan), is widely used in Japan for suppression of hepatitis activity and for prevention of disease progression in patients with hepatitis B virus and HCV-induced chronic hepatitis. Glycyrrhizin has been reported to suppress hepatic inflammation with an effect to improve the elevated ALT levels and histologic findings of the liver (14–17). We reported its favorable effect on hepatocellular carcinogenesis in those patients with chronic hepatitis C who received glycyrrhizin for more than 10 years (18).

To elucidate whether glycyrrhizin suppress the carcinogenesis rate in patients with IFN-resistant chronic hepatitis C, we retrospectively assessed a cohort of 1249 patients without sustained virologic response (SVR) after IFN therapy.

PATIENTS AND METHODS

Study Population. A total of 1249 consecutive Japanese patients with chronic hepatitis C with or without cirrhosis were examined, who did not show an SVR of HCV-RNA under IFN therapy. Sera of the patients showed positive anti-HCV (second-generation anti-HCV kit, enzyme-linked immunosorbent assay, Dainabot, Tokyo, Japan), positive HCV-RNA (nested PCR), and negative hepatitis B surface antigen (HBsAg; radioimmunoassay, Dainabot). Anti-HCV and HCV-RNA were assayed using stored frozen sera at −80°C. There were 778 men and 471 women aged 18–81 years, with a median age of 53 years in the study. They were diagnosed as having liver cirrhosis by peritoneoscopy, liver biopsy, or both between 1987–2002.

All the patients had a history of receiving once or more times of IFN therapy: 1179 patients underwent IFN monotherapy only and the other 70 patients had received an IFN plus ribavirin combination therapy before the entry of this study. A total of 347 patients showed a normal ALT for at least 6 months after cessation of IFN (biochemical responders), and the other 902 patients abnormal ALT at 6 months after the end of IFN therapy. A retrospective cohort study was performed using these 1249 consecutive patients with chronic hepatitis or cirrhosis who failed to show SVR.

Glycyrrhizin Treatment. Glycyrrhizin therapy was performed using intravenous injection of SNMC. The preparation contains 0.2% (4 mg) glycyrrhizinic acid as the main active constituent, 2% (40 mg) glycine, and 0.1% (2 mg) L-cysteine in 20-mL ampoules.

Of 1249 patients with IFN-resistant chronic liver disease, 453 patients underwent glycyrrhizin injection therapy and the remaining 796 patients did not receive the therapy until the end of observation. The purpose of the introduction of the glycyrrhizin injection therapy was to suppress elevated ALT and to prevent disease progression in all the patients. Of the 453 patients, 129 (28.5%) received a daily dose of 40–60 mL of SNMC (80–120 mg as glycyrrhizin) and 324 (71.5%) received 80–100 mL (160–200 mg as glycyrrhizin). A total of 110 patients received the treatment for less than 2 years and 107 patients continued the therapy for 2–4 years, 132 patients for 4–6 years, and the remaining 104 patients for 6 years or longer. When the treatment was regarded as effective from the viewpoint of ALT levels, treatment was usually continued for a period as long as possible. As a result, a median daily dose of 100 mL of SNMC was administered 3 times a week during a median period of 4.3 years (range, 0.1–14.5 years) in the treated group.

Two (0.44%) of 453 treated patients were withdrawn from the glycyrrhizin injection therapy because of side effects: 1 because of hypertension and 1 from skin rash.

Background and Laboratory Data of Patients With and Without Glycyrrhizin Therapy. Table 1 summarizes the profiles and data of the patients at the time of diagnosis of chronic hepatitis with or without cirrhosis. The male/female ratio was not different between the 2 groups. Median age was older by 2 years in the treated group than in the untreated group ($P < .001$). Results of histologic staging of liver disease were classified according to Desmet *et al.* (19). F1stage hepatitis was found significantly more often in the untreated group than in the glycyrrhizin group ($P < .001$, χ^2 test). Both AST and ALT median levels were significantly higher in the treated group than in the untreated group ($P < .001$). HCV subtype was analyzed by the immunoserologic typing method with a commercial kit (Kokusai Diagnostic Corporation, Kobe, Japan): serologic group 1 indicated genotypes 1a and 1b, and group 2 included 2a and 2b subtypes. The rate of HCV serologic group 1 was significantly higher in the glycyrrhizin group than in the untreated group ($P = .032$).

Follow Up. Follow-up of the patients was made monthly after the judgment of IFN-resistance by monitoring hematologic, biochemical, and virologic data. Imaging diagnosis with ultrasonography (US) and/or computed tomography (CT) was made 3 or more times per year in a majority of patients with cirrhosis and once a year in patients without cirrhosis. Angiographic study was performed only when HCC was strongly suspected on US or CT.

When angiography revealed a characteristic hypervascular nodule suggesting a specific finding for HCC, no histologic examination was made in a majority of these patients. An increasing trend of tumor markers was also taken into account in establishment of the diagnosis of HCC. Microscopic examination through a fine needle biopsy was also performed in patients whose angiogram did not show a typical image of HCC.

The number of cases lost to follow-up was 121 (9.7%): 28 patients (6.2%) in the glycyrrhizin group and 93 (11.7%) in the untreated group. Because the outcomes regarding appearance of HCC were not identified in these patients, they were dealt as censored data in the following statistics (20). Death unrelated to HCC was also classified as withdrawal and regarded as a censored case. The median observation period of the total number of patients was 5.7 years with a range of 0.1–16.1 years. Because

TABLE 1. PATIENT PROFILES AND LABORATORY DATA AT TIME OF JUDGMENT OF IFN RESISTANCE

	Glycyrrhizin Group (n = 453)	*Untreated Group* (n = 796)	P
Demographics			
Gender (M/F)	283/170	495/301	.92
Age (y)*	54 (25–81)	52 (18–77)	<.001
Observation period (y)*	8.3 (0.1–16.1)	5.1 (0.1–13.1)	<.001
Liver histology			
F1	146 (32.7%)	502 (64.0%)	<.001
F2	193 (43.3%)	192 (24.5%)	
F3	38 (8.5%)	52 (6.6%)	
F4	69 (15.5%)	38 (4.8%)	
Laboratory data*			
Aspartic transaminase (IU/L)*	81 (19–446)	54 (11–355)	<.001
ALT (IU/L)*	122 (12–630)	83 (10–822)	<.001
HCV serologic group 1 (1a or 1b)	360 (80.2%)	582 (73.7%)	.032
Group 2 (2a or 2b)	73 (16.3%)	165 (20.9%)	
Others	16 (3.6 %)	43 (5.4%)	

*Expressed as median (range).

many patients receiving glycyrrhizin therapy migrated from the untreated group to the treated group, observation period of the untreated group was significantly shorter than that of the treated group (see Table 1). The date of the last follow-up for this study was September 1, 2003.

Statistical Analysis. Nonparametric procedures were employed for the analysis of background characteristics of the patients, including Mann-Whitney U-test and χ^2 method. HCC appearance rates were calculated from the time period between the judgment of IFN ineffectiveness and appearance of HCC in each group, using Kaplan-Meier technique (20). The differences in carcinogenesis curves were tested using the log-rank test. Independent factors associated with the appearance rate of HCC were studied using time-dependent Cox regression analysis (21). An interaction term of IFN treatment and "waiting time" to the therapy was introduced in the analysis as a time-dependent covariate. The independence of treatment factor from "waiting time" was also confirmed by log-minus-log plot of proportional hazard model. Several variables were transformed into categorical data consisting of 2–3 simple ordinal numbers to estimate 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. A P-value of less than .05 was considered to be significant. All data analysis was performed using the computer program SPSS version 11 (22).

TABLE 2. INDEPENDENT RISK FACTORS AFFECTING HEPATOCELLULAR CARCINOGENESIS

Factors	*Category*	*Risk Ratio (95 % CI)*	P
Fibrotic stage			
	F1	1	
	F2–3	2.94 (1.20–7.21)	.018
	F4 (cirrhosis)	9.21 (3.73–22.8)	<.001
Gender			
	1: Female	1	
	2: Male	2.80 (1.35–5.81)	.006
Glycyrrhizin injection			
	1: No	1	
(SNMC)*	2: Yes	0.49 (0.27–0.86)	.014

Time-dependent Cox proportional hazard analysis. *SNMC, Stronger Neo-Minophagen C (herbal medicine containing glycyrrhizin).

RESULTS

Initial Aminotransferase and Carcinogenesis Rates

Patients with and without glycyrrhizin therapy were classified into 6 categories according to average ALT value during the first year after cessation of IFN therapy: group 1, normal ALT; group 2, <1.5 times of upper limit of normal (ULN); group 3, 1.5–2 times ULN; group 4, 2–3 times ULN; group 5, 3–4 times of ULN; and group 6, >4 times ULN. Hepatocellular carcinogenesis rates were 2.5%, 5.0%, 8.1%, 11.8%, 12.0%, and 12.7% at the end of 5 years and 6.6%, 7.2%, 19.6%, 15.1%, 21.0%, and 39.3% at 10 years, respectively (Figure 1). There was a significant statistical difference among the 6 subgroups (log-rank test, $P < .0001$). The higher the average ALT, the higher the carcinogenesis rate was.

Influence of Glycyrrhizin on Carcinogenesis in Patients With High Aminotransferase

Glycyrrhizin therapy was usually performed in patients with a high ALT value and high hepatitis activity. In this retrospective study, average ALT values were significantly different between the treated and untreated groups: group 1, normal average ALT was found in 38 among patients with glycyrrhizin therapy and in 188 among patients without therapy; in group 2, ALT <1.5 times of ULN was found in 42 and 331; in group 3, 1.5–2 times ULN in 84 and 138; in group 4, 2–3 times ULN in 143 and 92; in group 5, 3–4 times in 53 and 29; and in group 6, ALT

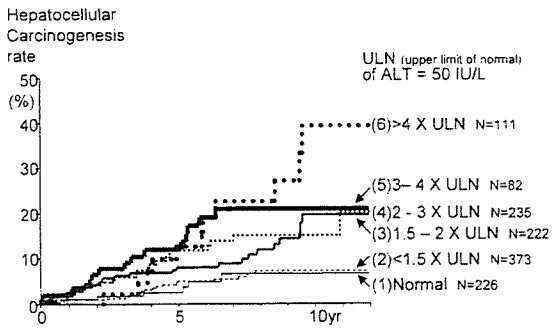

Fig 1. Carcinogenesis rates according to initial ALT values classified into six groups: (1) normal ALT, (2) <1.5 times ULN, (3) 1.5–2 times ULN, (4) 2–3 times ULN, (5) 3–4 times ULN, and (6) >4 times of ULN. The higher the average ALT, the higher the carcinogenesis rate was.

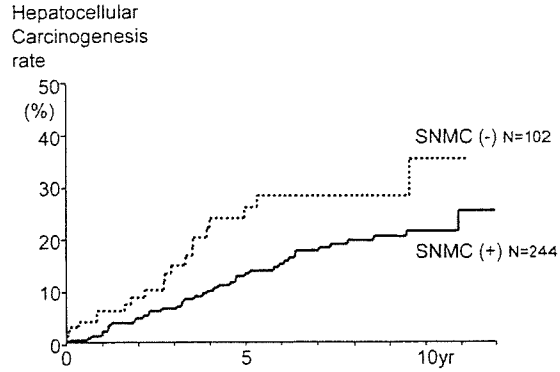

Fig 2. Carcinogenesis rates in patients with high average ALT values of twice or more of ULN, excluding those patients with biochemical responders who continued a normal ALT value at least 6 months just after IFN therapy. The carcinogenesis rate of glycyrrhizin-treated group was significantly lower than that of the untreated group (log-rank test, $P = .0210$).

>4 times ULN in 93 of the glycyrrhizin group and 18 of the untreated group. The rate of a high ALT value of twice or more of ULN in the glycyrrhizin treated group (64.2%, 289/453) was significantly higher than that of the untreated group (16.2%, 129/796).

Of the 418 patients with a high average ALT in both groups, 68 patients showed a normal ALT value for at least 6 months just after IFN therapy (biochemical response). Because biochemical response with normal ALT for a certain period after IFN was likely to affect carcinogenesis rates in those patients, biochemical responders were excluded in the following analyses about the influence of glycyrrhizin on carcinogenesis: after all, 244 patients with glycyrrhizin therapy and the 102 patients without therapy were assessed.

Cumulative hepatocellular carcinogenesis rates were calculated in these 346 patients with a high average ALT values, excluding biochemical responders from both groups. Carcinogenesis rates in the glycyrrhizin group and the untreated group were 6.5% and 13.3% at the end of year 3, 13.3% and 26.0% at the end of year 5, 17.7% and 28.3% at the end of year 7, and 21.5% and 35.5% at year 10, respectively (Figure 2). In the stratified and selected patient group, the carcinogenesis rate of glycyrrhizin-treated group was significantly lower than that of the untreated group (log-rank test, $P = .0210$).

Carcinogenesis Rates According to Hepatitis Staging

Crude carcinogenesis rates were compared between the groups, according to each hepatitis stage. In F1 stage chronic hepatitis, hepatocellular carcinogenesis rates in the glycyrrhizin group ($n = 82$) and the untreated group ($n = 32$) were 1.4% and 4.2% at year 5 and 7.0% and 12.1% at 10 years, respectively (Figure 3A). In F2–3 stage chronic hepatitis, hepatocellular carcinogenesis rates in the glycyrrhizin group ($n = 121$) and the untreated group ($n = 53$) were 14.8% and 28.4% at the end of year 5, and 21.5% and 38.6% at year 10, respectively (Figure 3B). In patients with F4 stage chronic hepatitis (cirrhosis), hepatocellular carcinogenesis rates in the glycyrrhizin group ($n = 38$) and the untreated group ($n = 15$) were 35.2% and 58.0% at the end of year 5, and 57.2% and 58.0% at year 10, respectively (Figure 3C).

In each fibrotic stage of hepatitis, carcinogenesis rates were lower in the glycyrrhizin group than in the untreated group, but statistical significance was not obtained owing to shortage of patient number in these stratified groups.

Aminotransferase Activity Before and After Glycyrrhizin Therapy

ALT values in the patients with glycyrrhizin treatment were serially assessed in those patients who began the therapy after they had shown a high average ALT value (Figure 4). Median value of ALT at the beginning of the glycyrrhizin therapy was 150 IU/L (25th percentile 120, 75th percentile 221), 72 IU/L at month 3, 70 IU/L at month 6, and 64 IU/L (25th percentile 48, 75th percentile 93) at month 12, respectively. ALT value significantly decreased after the initiation of glycyrrhizin injection therapy.

Factors Affecting Carcinogenesis Rates in Active Hepatitis and Cirrhosis

In the selected patients with active hepatitis with an average ALT value of twice ULN or higher, multivariate analysis was performed to explore associating factors with carcinogenesis, using time-dependent Cox proportional hazard model. Time between the judgment of IFN

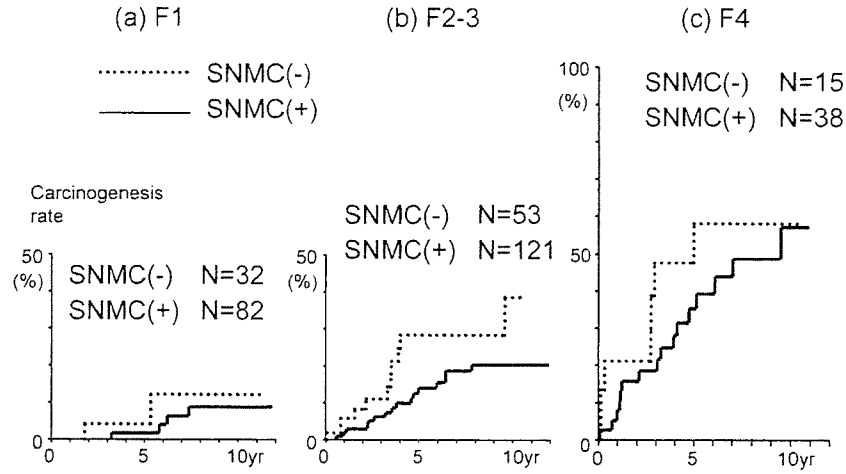

Fig 3. Carcinogenesis rates according to hepatitis staging: (a) F1 stage hepatitis, (b) F2–F3 stage hepatitis, and (c) F4 or cirrhotic stage. In each fibrotic stage of hepatitis, carcinogenesis rates were lower in the glycyrrhizin group than in the untreated group.

ineffectiveness and initiation of glycyrrhizin therapy was set as a time-dependent variable to clarify the significance of glycyrrhizin therapy in the clinical course of HCV-related chronic liver diseases. Patients with biochemical response with a normal ALT value sustained for at least 6 months after IFN therapy were also excluded from the analysis.

In multivariate analysis, following 3 factors influenced the carcinogenesis: fibrotic staging, gender ($P = .006$), and glycyrrhizin therapy ($P = .014$). When a hazard of F1 stage fibrosis for carcinogenesis was set as 1 in the model, hazard ratio of F2–F3 stage fibrosis was calculated as 2.94 ($P = .018$), and that of F4 (cirrhosis) was estimated as 9.21 ($P < .001$). Similarly, the hazard ratio for carcinogenesis of male gender was 2.80, and use of glycyrrhizin independently decreased the carcinogenesis rate in patients with active chronic hepatitis after IFN therapy. Following factors did not affect the HCC appearance rate significantly: age, association of diabetes mellitus, serologic grouping of HCV, HCV-RNA concentration, AST, ALT at the time before IFN therapy, and bilirubin.

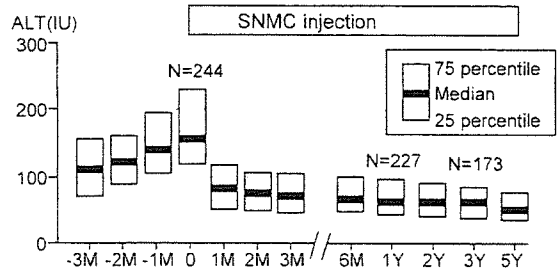

Fig 4. Aminotransferase activity before and after glycyrrhizin therapy. ALT value significantly decreased after the initiation of glycyrrhizin injection therapy.

DISCUSSION

IFN is effective in patients with chronic liver disease caused by HCV, from the viewpoints of anti-inflammatory effect and cancer prevention (6–11). Although the carcinogenesis rate is noticeably reduced when aminotransferase becomes normal with or without HCV-RNA eradication (6–8) after the therapy, the rate of normalization of ALT after IFN therapy is approximately half of patients with high viral load and group 1 HCV-subtype.

This retrospective study was undertaken to evaluate whether long-term glycyrrhizin injection therapy could decrease hepatocellular carcinogenesis rate in patients with IFN-resistant HCV-related chronic hepatitis and cirrhosis. Because it requires at least 5 years to show a statistical difference in carcinogenesis rate from hepatitis or cirrhosis between glycyrrhizin-treated and "untreated" groups, a prospective randomized trial using untreated control patients is difficult from both ethical and medical viewpoints in Japan, where glycyrrhizin injection therapy is covered by standard medical insurance and is already regarded as a usual choice of therapy as a salvaging procedure for IFN-ineffective patients. We, therefore, attempted to carry out this retrospective cohort study to prove an anticarcinogenic activity of glycyrrhizin, with a statistical adjustment using possible covariates explored in multivariate analysis.

Because glycyrrhizin injection therapy was chiefly performed for patients with a high ALT value and because cancer prevention was meaningful in just those patients with a high carcinogenesis risk with high hepatitis activity, we analyzed the role of a long-term glycyrrhizin injection therapy in the patients with a high ALT value. The treated group consisted of significantly more numbers of patients with a high ALT value of twice or more of ULN. When carcinogenesis rates were assessed only in those patients with a high ALT value of twice or more ULN excluding biochemical responders, the rate of the treated group became significantly higher than that of the untreated group ($P = .021$). The cancer preventive effect of glycyrrhizin in IFN-resistant patients was also confirmed by time-dependent Cox proportional analysis that adjusted the background features of the retrospective cohort (hazard ratio = 0.49, $P = .014$). We previously reported a study focused on the anticarcinogenic action of glycyrrhizin for patients with chronic hepatitis C, but the pilot study only demonstrated that 10 years or longer treatment with glycyrrhizin ($n = 84$) could suppress the carcinogenesis rate (18). Current study dealing with a large cohort ($n = 1249$) showed that glycyrrhizin injection therapy significantly decreased carcinogenesis rate irrespective of the length of treatment when comparison was made in a selected patient cohort with high hepatitis activity.

Although a statistically significant difference was not shown for a lack of sufficient patient number in subgroups of chronic hepatitis and cirrhosis, this study also demonstrated that glycyrrhizin was effective not only in chronic hepatitis but also in cirrhosis. Considering that liver cirrhosis generally shows a resistance to IFN treatment, our current study demonstrated encouraging results from the viewpoint of HCC prevention. When IFN therapy was attempted in 7 patients with decompensated cirrhosis by Nevens *et al.* (23), complications sometimes occurred in these patients, including variceal bleeding, aggravation of ascites or encephalopathy, development of pneumonia, and recurrence of spontaneous bacterial peritonitis or gastric ulcer bleeding. Because patients with cirrhosis usually showed lower platelet and leukocyte counts than those with chronic hepatitis and because cirrhotic patients tended to show deterioration with a large dose of IFN, glycyrrhizin therapy proved to be a useful alternative of therapy. Intermittent long-term glycyrrhizin therapy was well tolerated with withdrawal of only 2 patients (0.44%).

Because carcinogenesis is not a single-step event but a complex, multistep process, the exact mechanism of the glycyrrhizin activity in suppression of liver carcinogenesis remains unknown. One of the principal roles of long-term administration of glycyrrhizin in decreasing the carcinogenesis rate is considered to be anti-inflammatory, which blocks the active carcinogenic process of continuous hepatic necro-inflammation and cell damage. In the treated group, median ALT values markedly decreased after initiation of the glycyrrhizin injection, suggesting that pathologic process of hepatocyte necrosis or apoptosis was significantly suppressed by glycyrrhizinic acid. The importance of the action of amino acids, glycine and cysteine contained in SNMC has not been completely explained, but they have been demonstrated to suppress increased aldosterone levels that are induced by glycyrrhizinic acid. Tarao *et al.* (24) reported that high aminotransferase level resulted in an increase of an HCC recurrence rate in patients with HCC. From the viewpoint of these anti-inflammatory activities, SNMC may be considered to only postpone the time of HCC appearance in the clinical course of cirrhosis. Because the entire process of hepatocellular carcinogenesis from the initial transformation of a hepatocyte to a detectable growth of cancer is considered to take at least several years, the influence of glycyrrhizin on the carcinogenesis rate will not be evaluated in a short period. Although several reports suggested a relationship of anti-hepatitis B core antibody or hepatitis B surface antibody with carcinogenesis (25–27), we could not show the association because of insufficient available data.

Because current data were obtained from a retrospective cohort analysis, dose of glycyrrhizin per time, times of injection per week, and duration of therapy varied in each patient in the treated group. To elucidate the cancer preventive effect of glycyrrhizin therapy in active HCV-related liver disease, we should further stratify the treated patients or perform much more detailed statistical procedures. Future studies should, therefore, aim at defining the basic oncogenic mechanisms and roles of long-term administration of glycyrrhizin in carcinogenesis in patients with cirrhosis caused by HCV.

In conclusion, a long-term intermittent glycyrrhizin therapy for a few years or more successfully reduced hepatocellular carcinogenesis in patients with HCV-related chronic liver disease. A randomized control study with a larger number of cases, with or without glycyrrhizin therapy, is expected to confirm the effectiveness of this therapy.

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Prediction model of hepatocarcinogenesis for patients with hepatitis C virus-related cirrhosis. Validation with internal and external cohorts

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See Editorial, pages 1013–1016

Background/Aims: To estimate hepatocarcinogenesis rates in patients with hepatitis C virus (HCV)-related cirrhosis, an accurate prediction table was created.

Methods: A total of 183 patients between 1974 and 1990 were assessed for carcinogenesis rate and risk factors. Predicted carcinogenesis rates were validated using a cohort from the same hospital between 1991 and 2003 ($n=302$) and an external cohort from Tokyo National Hospital between 1975 and 2002 ($n=205$).

Results: The carcinogenesis rates in the primary cohort were 28.9% at the 5th year and 54.0% at the 10th year. A proportional hazard model identified alpha-fetoprotein ($\geq$20 ng/ml, hazard ratio 2.30, 95% confidence interval 1.55–3.42), age ($\geq$55 years, 2.02, 95% CI 1.32–3.08), gender (male, 1.58, 95% CI 1.05–2.38), and platelet count ($<$ 100,000 counts/mm^3, 1.54, 95% CI 1.04–2.28) as independently associated with carcinogenesis. When carcinogenesis rates were simulated in 16 conditions according to four binary variables, the 5th- and 10th-year rates varied from 9 to 64%, and 21–93%, respectively. Actual carcinogenesis rates in the internal and external validation cohorts were similar to those of the simulated curves.

Conclusions: Simulated carcinogenesis rates were applicable to patients with HCV-related cirrhosis. Since, hepatocarcinogenesis rates markedly varied among patients depending on background features, we should consider stratifying them for cancer screening and cancer prevention programs.

Keywords: Cirrhosis; Hepatocellular carcinoma; Carcinogenesis; Hepatitis C virus; Simulation; Proportional hazard model; Validation; Prediction

1. Introduction

There is increasing evidence that chronic hepatitis C virus (HCV) infection is closely associated with the occurrence of hepatocellular carcinoma (HCC) [1–4]. The incidence of patients with HCV-related HCC has increased recently in several parts of the world [5–9]. In Japan, blood transfusion and parenteral drug use became prevalent in 1960s, and patients with HCV-related cirrhosis gradually increased around 1980s. Since, an effective and truly curative therapy for a large and advanced HCC still remains limited at best, evaluation and assessment of carcinogenesis in chronic liver disease and detection at an early stage of HCC are of great importance. Reports of HCC development rates in HCV-cirrhosis differ [10–13], probably due to

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differences of patient characteristics in varied study populations. The lack of reliable data as to the natural history of cirrhosis makes it difficult to evaluate the exact role and cost-effectiveness of interferon therapy.

Platelet count has been used to predict hepatocarcinogenesis [10,13,14], but its usefulness for distinguishing the HCC appearance rate is based on discrimination between chronic hepatitis and cirrhosis [15–18]. Predicting carcinogenesis solely on the basis of platelet count is less valuable in a cohort of patients with cirrhosis, because the liver disease has already advanced to a certain stage with a uniformly low platelet count. When a cohort of patients with HCV-related cirrhosis is analyzed by platelet count, it is usually not possible to discriminate between a super-high-risk group for carcinogenesis and a relatively low-risk group. The availability of a general model that can accurately predict the HCC development rate in HCV-related disease based on readily available data would be helpful in planning the treatment of these patients. Moreover, such a model could be used for the selection and stratification of patients for clinical trials.

In this study, we tried to develop a prediction model for hepatocarcinogenesis rate, using a large cohort with a long observation period. This model was also validated with two independent patient cohorts for generalization and clinical application.

2. Patients and methods

2.1. Study population

Among 457 consecutive patients diagnosed with liver cirrhosis between 1974 and 1990 at Toranomon Hospital, Tokyo, 258 patients had positive anti-HCV antibody (second-generation anti-HCV, enzyme-linked immunosorbent assay, Dainabot, Japan), positive HCV-RNA, and negative hepatitis B surface antigen (HBsAg, radioimmunoassay, Dainabot, Tokyo, Japan). Among them, 75 patients met either of the following exclusion criteria: (1) possible association with HCC, (2) association of hemochromatosis, autoimmune liver disease, primary biliary cirrhosis, alpha-1-antitrypsin deficiency, or Wilson disease, (3) daily drinking habit of 75 g or more, (4) alpha-fetoprotein (AFP) of 400 ng/ml or higher, (5) advanced and decompensated stage of cirrhosis with encephalopathy and refractory ascites, or (6) a short follow-up period of 6 months or less. We excluded those patients with Child–Pugh [19] stage C, because of substantial difference in carcinogenesis [20,21]. Consequently, 183 patients were retrospectively analyzed for HCC appearance rate.

2.2. Background and laboratory data

Table 1 summarizes the profiles and data of the 183 patients at the time of diagnosis. The group consisted of 92 men and 91 women aged from 28 to 80 (median, 55 years). The diagnosis of cirrhosis was made by peritoneoscopy, biopsy or both in 118 patients, and by clinical symptoms with ultrasonographic findings in 55 patients. When the ultrasonography (US) showed a typical irregular-surfaced liver with coarse internal architecture in addition to overt ascites or esophageal varices demonstrated by fiberscopic examination, we regarded the disease as cirrhosis. Although 12.7% of patients (23/181) showed normal aminotransferases at the time of the diagnosis of cirrhosis, all of those patients had been followed up as having chronic hepatitis with fluctuated aminotransferases.

Table 1
Patient profiles and laboratory data at the time of diagnosis of cirrhosis (primary cohort of Toranomon Hospital between 1974 and 1990, $n = 183$)

Demography and backgrounds		
Total number	183	
Sex (M/F)	92/91	
Age, median (range)	55 (28–80)	
Diagnostic method		
Peritoneoscopy and/or biopsy	118 (64.5%)	
Clinical (ultrasonography plus varices or ascites)	65 (35.5%)	
History of blood transfusion	82 (44.8%)	
Diabetes mellitus	23 (12.6%)	
Previous medical history of chronic hepatitis	34 (18.6%)	
Interferon therapy during observation	24 (12.0%)	
Refractory ascites and/or encephalopathy	0	
Hepatitis B surface antigen, positive	0 (100%)	
Anti-hepatitis C virus, positive	183 (100%)	
Hepatitis C virus RNA, positive	183 (100%)	
Child–Pugh score A	136 (74.3%)	
Child–Pugh score B	47 (25.7%)	
Observation period (year) median (range)	10.5 (0.5–26.0)	
Laboratory data	Median (range)	Valid data
Albumin (normal, 3.9–5.1 g/dl)	3.9 (2.5–5.1)	183
Bilirubin (normal, 0.3–1.1 mg/dl)	1.1 (0.4–4.4)	183
Aspartic transaminase (normal, $\leq$38 IU/L[a])	69 (17–372)	181
Alanine transaminase (normal, $\leq$50 IU/L[a])	56 (9–282)	181
Platelet (normal, 149–315$\times 1000^3$/mm^3)	95 (33–213)	183
ICG R15[b] (normal, $\leq$10%)	27 (6–81)	173
Prothrombin time (normal, $\geq$70%)	79 (54–100)	183
Gamma-globulin (normal, $<$1.5 g/dl)	1.9 (1.0–3.5)	174
Alpha-fetoprotein (normal, $<$5 mg/L)	16.5 (3–256)	166
HCV genotype[c]		
1b	107 (69.9%)	153
2a/2b	39 (25.5%)	
Combined/others	7 (4.6%)	
Not examined	30	

[a] Numbers of normal aspartic and alanine transaminases were 25 (13.8%) and 69 (38.1%), respectively. Both transaminases were normal at the time of the diagnosis of cirrhosis in 23 patients (12.7%).
[b] ICG R15: indocyanine green retention rate at 15 min.
[c] HCV genotyping was classified according to Simmonds et al. [22].

HCV-RNA measurement and HCV genotyping [22] are analyzed with nested polymerase chain reaction using initial sera stored at -80 °C.

2.3. Follow-up of patients and diagnosis of hepatocellular carcinoma

Patients were followed-up monthly following the diagnosis of cirrhosis by monitoring hematological and biochemical data. Diagnostic imaging by US was taken approximately once a year in each patient. After 1987, imaging procedures with US or computerized tomography (CT) were performed twice or more per year in the majority of patients for early detection of HCC. HCC was diagnosed by typical hypervascular characteristics on angiography. When combined use of imagings could not demonstrate a typical image of HCC (13/107, 12.1%), a fine needle biopsy was obtained for microscopic examination.

Twenty-four patients (13.1%) received interferon during the follow-up period. Since the therapy could affect the natural clinical course of viral hepatitis, they were treated as censored at the time of the initiation of interferon in the analysis. Sixteen (8.7%) cases were lost to follow-up, and median observation period was 10.5 years (range, 7.0–14.9). Those patients lost to follow-up were treated as censored data in the following statistics.

Any death unrelated to liver disease and cirrhosis-related liver failure were also classified as withdrawal and regarded as a censored case.

2.4. Statistical analysis and predictive model for carcinogenesis

The HCC development rate was analyzed using Kaplan–Meier technique [23] and differences in curves were tested using the log-rank test. The independent risk factors associated with the rate of HCC development were studied using stepwise method of non-time-dependent Cox regression analysis [24]. Potential risk factors assessed for liver carcinogenesis included the following 16 variables: age, sex, HCV genotype, association of diabetes mellitus, total alcohol intake (cumulative alcohol intake $\geq$200 kg), family history of liver disease, history of blood transfusion, association of ascites, serum aspartic transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT), globulin, platelet count, AFP, indocyanine green retention rate at 15 min (ICG R15), and Child–Pugh score [19]. Each variable was transformed into categorical data consisting of two simple ordinal numbers (zero or one) for univariate and multivariate analyses. Although, proper transformation of variables were recommended in this kind of study [25], logarithmic transformation was not employed even for variables with non-symmetric distribution, because simple dichotomization also seemed reliable and robust statistically and because the simplicity was considered to bring about eventual clinical usefulness. Although, a cut-off value of 20 ng/ml proved to be an important point in our previous studies about prediction of liver cancer development in cirrhosis [10,26], other threshold values of dichotomizations were chosen from near figures to median values. In running the proportional regression analysis, care was taken to avoid overfitting the model by studying no more than one variable for every 10 events of carcinogenesis. Goodness-of-fit test together with log-minus–log plot was performed to confirm the proportionality assumption in the model. Since, missing data was not replaced, reduced numbers of cases were use in multivariate analysis. A P-value of less than 0.05 was considered to be significant.

The prognostic model was generated using Cox's regression procedure from the database of the 183 cirrhotic patients in Toranomon Hospital from 1974 to 1990. Using a final model for prediction of HCC appearance, carcinogenesis rate was predicted by substituting the corresponding ordinal numbers (zero or one) for every significant covariate in a given condition of the patients. Simulated carcinogenesis rates were computed for each state consisting of all statistically significant variables.

An internal and external cohorts of patients with HCV-positive cirrhosis verified the predicted carcinogenesis rates and curves: a cohort of 302 patients with HCV-cirrhosis diagnosed at Toranomon Hospital between 1991 and 2003 (internal validation group), and a cohort of 205 patients diagnosed at Tokyo National Hospital, Tokyo, Japan, between 1975 and 2002 (external validation group). The actual survival rates were calculated by the Kaplan–Meier technique in each risk group from the two validation cohorts, and evaluated by log-rank test according to the procedures of Christensen et al. [27].

Data analysis was performed with SAS version 9.1.3 software (SAS Institute, Inc., NC, USA).

The Human Ethics Review Committee of Toranomon Hospital approved the study protocol.

3. Results

3.1. Rate of hepatocellular carcinogenesis and risk factors

During the observation period, 107 (58.5%) out of 183 patients with HCV-related cirrhosis developed HCC. The cumulative HCC appearance rates of all patients were 15.0% at the end of the 3rd year, 28.9% at the 5th year, 37.8% at the 7th year, and 54.0% at the 10th year. Crude HCC development curve was drawn together with those of internal and external validation cohorts (Fig. 1).

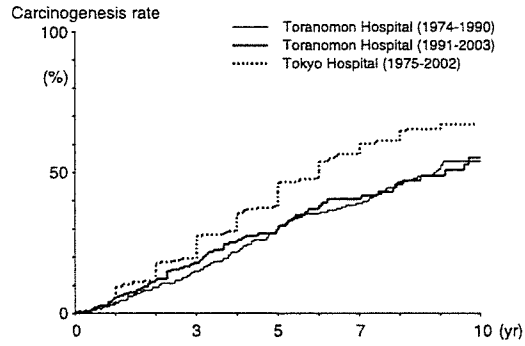

Fig. 1. Cumulative hepatocellular carcinogenesis rates in 183 patients who were diagnosed with HCV-related cirrhosis at Toranomon Hospital between 1974 and 1990. The 5th and 10th year rates were 28.9 and 54.0%, respectively (solid thin line). HCC appearance curves were also drawn in the internal (solid thick line) and external (dotted thick line) validation cohorts. The cancer appearance rate of Tokyo Hospital was significantly higher than those of the other two cohorts from Toranomon Hospital (P=0.0015, log-rank test).

Carcinogenesis rate in Tokyo Hospital was significantly higher than that of Toranomon Hospital (log-rank test P = 0.0015). The risk factors for carcinogenesis were explored using non-time dependent proportional hazard analysis. In the final step of multivariate analysis, AFP (P<0.001), age (P=0.001), sex (P=0.030), and platelet count (P=0.031), were identified as independent significant predictors of future HCC appearance (Table 2). The hazard ratio of patients with AFP value of $\geq$20 ng/ml was 2.30 compared with those with lower AFP value, and the hazard ratio of patients of $\geq$55 years of age was 2.02 compared with younger patients. Child–Pugh score did not affect the carcinogenesis rate independently.

As for 23 patients with normal aminotransferases initially, 5- and 10-years carcinogenesis rates were 27.3 and 39.4%, respectively.

3.2. Simulation of carcinogenesis rates in patients with each prognostic factor

Simulated carcinogenesis curves were generated in each patient group with the Cox proportional hazard model by substituting the corresponding value for each parameter. Based on the four significant covariates, a total of 16 carcinogenesis curves were drawn, and simulated carcinogenesis rates were also estimated in the subgroups. To facilitate the practical use of the prediction model for carcinogenesis rate, we tabulated the results of estimated HCC appearance rates at the end of the 5th and 10th year (Table 3), in which calculated rates for a patient could be easily found for a given set of patient parameters (AFP, age, platelet and gender).

The model showed that when a patient is a male younger than 55 years, with a platelet count less than 100,000/mm^3 and an AFP value less than 20 ng/ml, the estimated hepatocarcinogenesis rates are 19% at the end of the 5th

Table 2
Factors associated with hepatocarcinogenesis (compensated cirrhosis, $n=183$, 1974–1990 cohort of Toranomon Hospital)

Factors	Category	No. of primary cohort	*B*	SE	Hazard ratio (95% CI)	*P*
Alpha-fetoprotein	0: <20 (ng/ml)	97			1	
	1: ≥20 (ng/ml)	69	0.83	0.20	2.30 (1.55–3.42)	<0.001
Age	0: <55 (year)	80			1	
	1: ≥55 (year)	103	0.74	0.22	2.02 (1.32–3.08)	0.001
Sex	0: Female	91			1	
	1: Male	92	0.46	0.21	1.58 (1.05–2.38)	0.030
Platelet count	0: ≥100,000/mm^3	87			1	
	1: <100,000/mm^3	96	0.43	0.20	1.54 (1.04–2.28)	0.031

year and 43% at the 10th year. The highest carcinogenesis rates were computed for males 55 years or older with a low platelet count and a high AFP value (64% at the 5th year, 93% at the 10th year), while the lowest estimated rates were found in females younger than 55 years with a high platelet count and a low AFP value (9% at the 5th year, 21% at the 10th year).

3.3. Validation of the prediction values of carcinogenesis rate

The reliability of the estimated HCC development rates was validated using internal (Toranomon Hospital, 1991–2003) and external (Tokyo National Hospital, 1975–2002) cohorts consisting of patients with HCV-related cirrhosis. Table 4 shows brief characteristics of patients in the two cohorts.

Since, HCC development curves were coarse and unreliable when a subgroup consisted of fewer patient number than 15, six figures of carcinogenesis curves were shown in principal subgroups consisting of ≥20 patients in each validation cohort (Fig. 2). When the parameters for all of the four significant covariates were at their worst (male ≥55 years, AFP≥20 ng/ml, and platelet count<100,000/mm^3), the simulated carcinogenesis rates were 64% at the end of the 5th year and 93% at the 10th year. On the other hand, the actual carcinogenesis rates in the internal and external validation cohorts were 54.9 and 61.5% at the 5th year, and 100 and 100% at the 10th year, respectively. The latter curves corresponded significantly with the simulation-generated carcinogenesis rate (Fig. 2a). Similarly, the other five simulated carcinogenesis curves were compared with both internal and external validation cohorts (Fig. 2b–f). Although the remaining 10 curves were not shown because of lack of sufficient patient number in the subgroup, actual carcinogenesis curves for the internal and external cohorts showed very analogous rates to the simulated ones, indicating that the simulation effectively predicted the future carcinogenesis rates. When we compared actual carcinogenesis rates in the validation groups with their calculated simulation values, 74.0% (375/507) and 70.4% (357/507) of the validation values for their 5th and 10th rates were coincident with those of predicted ones and stayed in an interval between +10% and −10% of simulated values. Although those patients in a large cohort consisting of 15 patients or more (e.g. Fig. 2a–f) usually showed a reliable and consistent values with simulated ones, those in a small cohort often revealed a labile and different values from simulated ones.

When a combined patient group of the three cohorts was analyzed, the same factors proved to affect the HCC appearance rate significantly: AFP (hazard ratio 2.19, $P<0.001$), age (1.96, $P<0.001$), sex (1.80, $P<0.001$), and platelet count (1.51, $P=0.009$). Hazard ratios with 95% confidence interval and *P*-values were also calculated in the individual validation groups (Table 5).

In addition, we evaluated the 'group factor' (study group, internal, and external validation groups) as a covariate in ordinary proportional hazard analysis for a combined patient group. Although, the internal and validation groups showed a slightly low (0.90) and high (1.26) hazard ratios for HCC development compared with that of the study group, the other four factors proved to show higher hazard ratios in the model (Table 6).

Table 3
Simulated carcinogenesis rates in stratified patient groups according to gender, age, platelet count, and alpha-fetoprotein value

Gender	Age (years)	Platelet	Alpha-feto-protein (ng/ml)	Simulated carcinogenesis rate (%)	
				5-year	10-year
Men					
	<55	<100,000/mm^3	<20	19	43
			≥20	42	77
		≥100,000/mm^3	<20	13	31
			≥20	32	65
	≥55	<100,000/mm^3	<20	32	65
			≥20	64	93
		≥100,000/mm^3	<20	23	50
			≥20	50	83
Women					
	<55	<100,000/mm^3	<20	13	30
			≥20	30	61
		≥100,000/mm^3	<20	9	21
			≥20	22	47
	≥55	<100,000/mm^3	<20	22	49
			≥20	49	83
		≥100,000/mm^3	<20	16	37
			≥20	37	69

Table 4
Patient profiles and laboratory data of two cohorts for validation: an internal cohort (Toranomon Hospital from 1991 to 2003, $n=302$) and an external cohort (Tokyo National Hospital, $n=205$)

	Internal cohort (Toranomon Hospital, 1991–2003)		External cohort (Tokyo National Hospital, 1975–2002)	
Demography and backgrounds				
Total number	302		205	
Sex (M/F)	166/136		111/94	
Age (year)[a]	59 (28–80)		62 (13–83)	
Diagnostic method				
Peritoneoscopy and/or biopsy	128		115	
Clinical diagnosis	174		90	
Interferon therapy				
Yes	105 (34.8%)		12 (5.9%)	
No	197		193	
Observation period (year)[a]	5.3 (0.5–13.9)		7.5 (0.5–30.8)	
Laboratory examination	Internal cohort (Toranomon Hospital, 1991–2003)	Valid data	External cohort (Tokyo National Hospital, 1975–2002)	Valid data
Platelet ($\times 1000^3$/mm^3)[a]	91.5 (25–223)	302	100 (19–310)	205
Alpha-fetoprotein (ng/ml)[a]	14 (1–380)	296	15 (2–365)	205

[a] Expressed by median (range).

3.4. Estimation of carcinogenesis rates by number of unfavorable risk factors

The prognostic model showed that the HCC development rate was significantly affected by the following four unfavorable factors: high AFP ($\geq$20 ng/ml), older age ($\geq$55 years), low platelet count ($<$100,000/mm^3), and male sex. Although, limitation of predictability could not be avoided because of different values of hazard ratios, we attempted to make more convenient HCC prediction curves. Five carcinogenesis curves were generated according to the number of unfavorable risk factors among the four significant covariates: no factors, one, two, three, and four unfavorable factors. When no unfavorable factor was found in a cohort of HCV-cirrhosis, the hepatocarcinogenesis rates were 9% at the end of the 5th year and 21% at the 10th year. Similarly, when one, two, three and four factors were found in a cohort, the carcinogenesis rates were 16, 28, 46, and 64% at the 5th year, and 35, 55, 78, and 93% at the 10th year, respectively (log-rank test, $P=0.0001$).

To validate the reliability of the concise prediction curves, the actual carcinogenesis curves were generated by the product-limit method for the 1991–2003 internal cohort of our hospital (Fig. 3). All actual carcinogenesis curves fitted well with the simulated curves, except for the subgroup with 'no unfavorable factors': none of 11 patients in this subgroup developed HCC during a median observation period of 10.0 years (25 percentile 8.1 years, 75 percentile 10.8 years).

4. Discussion

Ten-year-rate of HCC development has been reported as 50–80% in some cohorts of HCV-positive cirrhosis [10–13,28], and the cohorts in our hospital showed 54–55%, and Tokyo Hospital 68%. However, the reasons for the significant differences found in the rates among various hospitals have not been fully elucidated until recently. Many risk factors have been identified as important for the development of HCC in patients with hepatitis or cirrhosis [10,13,29,30], but of even greater interest is the precise prediction of HCC. In order to establish a reliable method for predicting carcinogenesis risk in a variety of patients with HCV-positive cirrhosis (compensated and decompensated), we investigated a large cohort of patients with few dropout cases, using a multivariate proportional model.

In the final step of multivariate analysis, AFP, age, platelet and gender were independently associated with HCC development in the primary cohort of our hospital. A total of 16 simulated carcinogenesis curves were drawn according to the four binary factors. Surprisingly, the estimated carcinogenesis curves significantly differed from each other among the stratified subgroups in our hospital, depending on demographic and background characteristics. In the case of a patient with HCV-cirrhosis, the combination of age, gender, AFP and platelet count could give important prognostic information about future carcinogenesis risk. When HCC appearance rates were simulated under 16 conditions according to the four binary variables identified by multivariate analysis, the 5th year rate varied from 9 to 64%, and 10th year rates from 21 to 93%. On the other hand, aminotransferase level and Child–Pugh score were poor predictors of carcinogenesis in patients with HCV-cirrhosis.

We recognized that the HCC development rate should be evaluated more specifically for each subgroup than for the entire cohort of HCV-positive cirrhosis patients. Integration of the four predictive factors could provide useful information about HCV-related carcinogenesis in actual clinical practice. The reported diversity of carcinogenesis

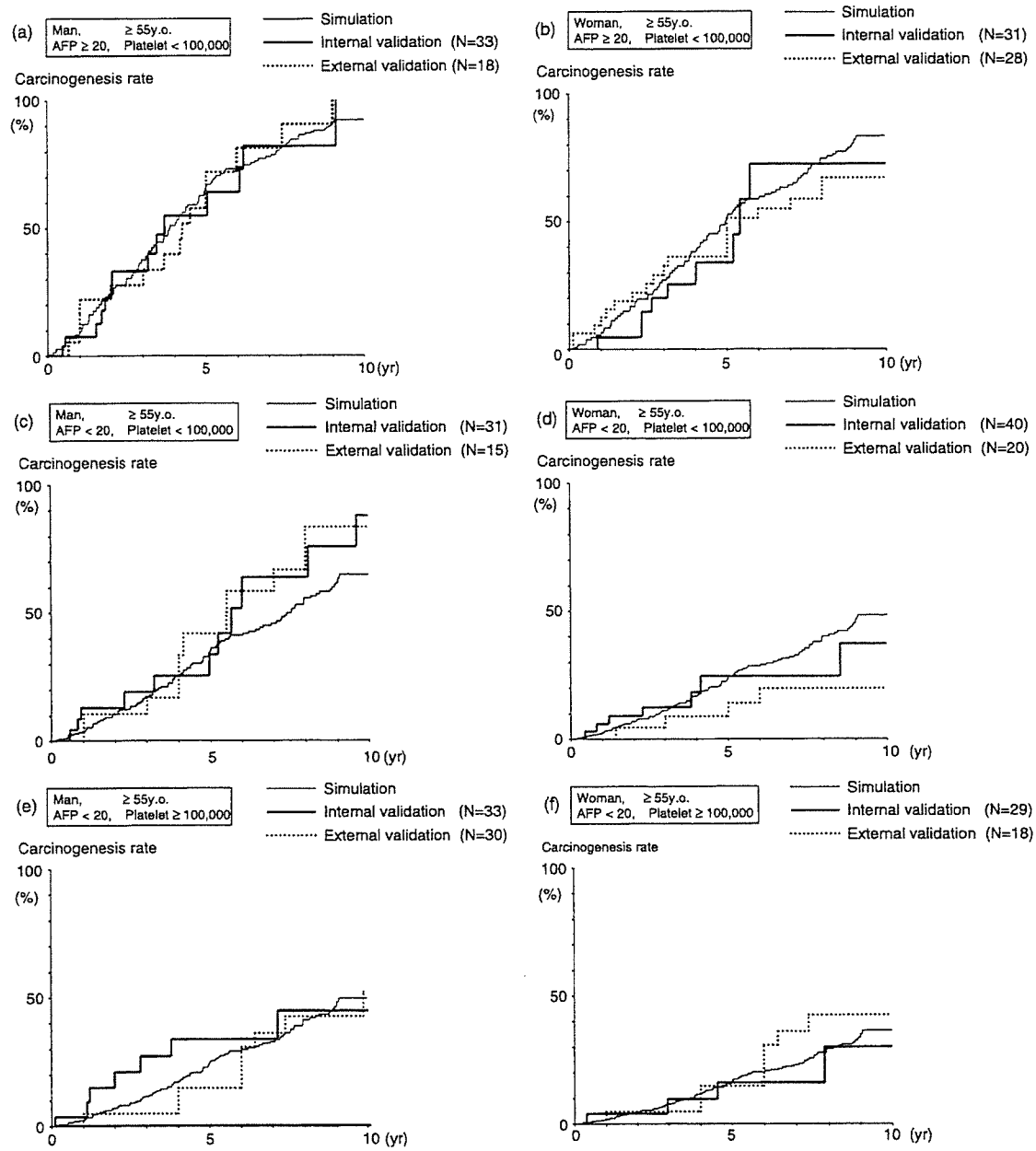

Fig. 2. Simulated carcinogenesis curves with actual carcinogenesis rates of internal and external validation cohorts, according to four significant predictors (gender, age, alpha-fetoprotein [AFP], and platelet count). *Thin solid lines*: simulated carcinogenesis curves, *bald lines*: actual curves of internal cohort (Toranomon Hospital, 1991–2003), *bald dotted lines*: actual curves of external cohort (Tokyo National Hospital, 1975–2002). (a) Carcinogenesis curves for subgroup of man, age ≥ 55 years, AFP ≥ 20 ng/ml, and platelet count < 100,000/mm^3. (b) Subgroup of woman, age ≥ 55 years, AFP ≥ 20 ng/ml, and platelet count < 100,000/mm^3. (c) Subgroup of man, age ≥ 55 years, AFP < 20 ng/ml, and platelet count < 100,000/mm^3. (d) Subgroup of woman, age ≥ 55 years, AFP < 20 ng/ml, and platelet count < 100,000/mm^3. (e) Subgroup of man, age ≥ 55 years, AFP < 20 ng/ml, and platelet count ≥ 100,000/mm^3. (f) Subgroup of woman, age ≥ 55 years, AFP < 20 ng/ml, and platelet count ≥ 100,000/mm^3.

rates also explains the inconsistency of estimated carcinogenesis rates from untreated cirrhosis caused by HCV. One of the reasons why carcinogenesis rates differed between the two hospitals seemed to originate from the difference of age of the patient populations. Current study did aim at precise prediction of carcinogenesis rate of each cirrhotic patient in different hospital and different period of time.

Validation of such a model is essential before these tools can gain widespread clinical use [31]. The best way to validate these models is to assess their performance in sets

Table 5
Significance of four factors associated with hepatocarcinogenesis in the internal validation group ($n=302$) and external validation group ($n=205$, 1975–2002 cohort of Tokyo National Hospital)

Factors	Internal validation cohort (1991–2003 Toranomon Hospital)		External validation cohort (1975–2002 Tokyo National Hospital)	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Alpha-fetoprotein	1 2.13 (1.21–3.78)	0.009	1 2.23 (1.55–3.23)	<0.001
Age	1 3.36 (1.56–7.23)	0.002	1 1.55 (0.96–2.48)	0.071
Sex	1 1.78 (0.99–3.19)	0.040	1 2.01 (1.38–2.92)	<0.001
Platelet	1 1.49 (0.83–2.67)	0.18	1 1.40 (0.97–2.02)	0.070

of patients who are independent in place and time [32]. This external validity is particularly important when models are used to predict outcomes in daily practice, because it is well known that prognostic models do not perform as well in patients outside the clinical context in which they are developed [33]. This study shows that our prognostic model accurately predicts carcinogenesis rates for patients with HCV-cirrhosis from a chronologically different group and a geographically different referral center, and therefore supports the generalization and reliability of the model. The two validation cohorts (302 and 205 patients) were classified into 16 groups according to their risk factors, and the values for the actual and model-predicted survival of each risk group were compared graphically using actual Kaplan–Meier curves. The model provided a very good fit with the carcinogenesis data of each risk group in the validation cohorts (Fig. 2a–f).

We could not draw meaningful and reliable carcinogenesis curves in the remaining 10 risk groups, because of small patient numbers. The significance of current study might be the prediction of hepatocarcinogenesis in these small patient groups.

We also tried to predict carcinogenesis risk using a simplified process in the same patient group, using few unfavorable risk factors instead of individual items of the risk factors. The clinical characteristics of the 302 patients in the internal validation cohort, for whom complete information was available, are summarized in Table 4, together with the characteristics of the 183 patients used to develop the model. Since, both groups of patients were very similar in terms of their risk variables, the estimated carcinogenesis curves showed good agreement: all actual carcinogenesis curves fitted well with the simulated curves, except for a subgroup with 'no unfavorable factors'. The reason for the inconsistency was that none of the 11 patients in the subgroup developed HCC, and because the 'best' subgroup might include a significant number of patients with far better liver function tests for cirrhosis. Since, the external validation cohort included older patients with low platelet counts, the differences in the proportion of unfavorable risk factors would produce contradictory results in this kind of analysis when only using few risk factors.

For pragmatic purposes, a good prognostic model, in addition to being generalizable, needs to be based on readily accessible variables and can be calculated easily at the bedside [34]. Our model employs four variables that are readily available for every patient with cirrhosis, and includes the responses to four yes/no questions. With the help of a pocket table (Table 3), a calculator is even not needed to determine the carcinogenesis risk of a given patient and their estimated median carcinogenesis rate. Since, there is considerable diversity in carcinogenesis risk among individual patients with HCV-cirrhosis, these results will be useful for stratification of patients in future cancer prevention trials. Even though predictability of carcinogenesis risk in individual patients is limited in this kind of statistics [35], this study will be helpful to realize the diversity of carcinogenesis rate in the same 'HCV-related cirrhosis'.

In conclusion, our four-variable model is a simple and useful tool for predicting carcinogenesis rates in patients with cirrhosis caused by HCV. Prediction models for HCC

Table 6
Multivariate analysis for a combined patient group of study cohort, internal validation cohort, and external validation cohort

Factors	Category	Hazard ratio (95% confidence interval)	P
Alpha-fetoprotein	0: <20 (ng/ml)	1	
	1: ≥20 (ng/ml)	2.22 (1.77–2.79)	<0.001
Age	0: <55 (year)	1	
	1: ≥55 (year)	1.90 (1.44–2.51)	<0.001
Sex	0: Female	1	
	1: Male	1.90 (1.50–2.40)	<0.001
Platelet count	0: ≥100,000/mm^3	1	
	1: <100,000/mm^3	1.46 (1.16–1.84)	0.001
Patient groups	0: Study cohort	1	
	1: Internal validation cohort	0.90 (0.66–1.23)	0.52
	2: External validation cohort	1.26 (1.04–1.57)	0.023