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Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C after sustained response to interferon

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Editorial on page 220

Background. Interferon (IFN) is expected to prevent the progression of hepatitis C virus infection to cirrhosis and the development of hepatocellular carcinoma (HCC), but there have been several reports of the development of HCC after a sustained response to IFN. Our aim was to elucidate the incidence and clinical features of, and risk factors for, HCC in sustained responders to IFN, taken for the treatment of chronic hepatitis C. ***Methods.*** We designed a retrospective cohort study conducted at 16 major Hospitals. The subjects were a total of 1056 patients showing sustained responses, 29 of whom developed HCC. ***Results.*** The incidence of HCC per 100 person-years was 0.56 (95% confidence interval, 0.35–0.76) in sustained responders. By the Cox proportional hazard model, we found that older age, higher serum aspartate aminotransferase level, and lower platelet count before IFN therapy were independent risk factors associated with the development of HCC. A risk index of HCC development, based on the coefficients of these risk factors, was used to classify patients into three groups, with low, intermediate, and high risk. The incidence rates of HCC for these three groups were 0.11, 0.44, and 1.98 per 100 person-years, respectively. The median period to the development of HCC was 4.6 years (range, 1.4–9.0 years), and there were no other specific clinical features of the HCC that developed in these patients. ***Conclusions.*** This study suggests that the risk of development of HCC is not completely eliminated in sustained responders to IFN. These findings may be useful in determining a follow-up strategy after a sustained response to IFN.

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Introduction

Hepatitis C virus (HCV) infection is one of the most common causes of chronic hepatitis, and it is also a major risk factor for hepatocellular carcinoma (HCC).[1,2] Chronic hepatitis C is often asymptomatic and mild, but may slowly progress to liver cirrhosis and eventually to HCC.[3–5] Therefore, it has been assumed that eradication of HCV would provide the most effective means of preventing HCC.

Currently, interferon (IFN) represents the mainstay of treatment for chronic hepatitis C.[5–9] IFN therapy can lead to a decrease in serum transaminase activity, and to the disappearance of serum HCV RNA in patients with chronic hepatitis C. These patients appear to benefit by the prevention of progression to cirrhosis and HCC.[5,7,10–14] However, HCC can still occur in patients who are treated successfully with IFN, i.e., those showing a sustained response to the therapy.[5,10–25] The incidence and clinical features of HCC, and the risk factors for carcinogenesis, have not yet been investigated, although they have been documented in individuals and in small numbers of patients.[5,10–25] We investigated a large cohort of patients showing a sustained response to IFN therapy given for chronic hepatitis C. Our aims were to assess the incidence of HCC in these patients and to discover the clinical variables that may be associated with the development of HCC. Our study also focused on the clinical features of HCC. We designed a multicenter retrospective cohort study, because a single-institution study would have provided inadequate numbers of sustained responders who developed HCC.

Patients and methods

Patients

This study was conducted at 16 major hospitals belonging to the Japanese Society of Gastroenterology, Kyushu Division. A large cohort of sustained responders to IFN therapy given for chronic hepatitis C, in whom HCC had, or had not, been detected, was assembled consecutively by means of data collection instruments. All sustained responders included in the study were positive for HCV RNA before IFN therapy, and were followed up for more than 1 year after termination of IFN therapy, during the period July 1988 to August 2001. Sustained response was defined as the presence of HCV RNA negativity (determined by using qualitative HCV RNA assay) more than 6 months after the termination of IFN therapy. Diagnosis of HCC was based either on histological examination or on typical computed tomographic and/or angiographic findings at each institution. Patients were excluded if HCC was detected within 1 year after the termination of IFN therapy, because in such cases it was highly likely that the cancer had been present at the end of the IFN therapy. In Japan, at the time of the study, the standard schedule was 6–10 MU IFN-α every day for the first 2–4 weeks and then the same dose given three time a week for the following 20–22 weeks, or 6 MU IFN-β every day for 6–8 weeks.

During the study period at the 16 hospitals, a total of 3504 patients with chronic hepatitis C had received IFN therapy and had been followed up for more than 1 year thereafter, and a sustained response was obtained in 1091 (31.1%) of them. Among the sustained responders, 30 patients (2.7%) developed HCC. By means of the data collection instrument, we requested individual clinical data before IFN therapy for all sustained responders, as well as clinical data at the time of diagnosis of HCC for patients who had developed HCC. The clinical data for all 1091 sustained responders identified were obtained from the 16 hospitals (8 university hospitals and 8 regional hospitals) listed in the appendix. Of these patients, 35 were excluded from the analysis because of the development of HCC within 1 year after IFN therapy (1 patient) or insufficient clinical records before commencement of IFN therapy (34 patients). The final study population comprised a total of 1056 patients showing sustained response to IFN therapy given for chronic hepatitis C, 29 of whom had developed HCC.

Methods

To identify risk factors for the development of HCC in sustained responders to IFN therapy, we used univariate analysis and multivariate analysis to investigate 23 variables before IFN therapy for their relationship to the development of HCC. These variables were chosen by considering possible factors involved in the development of HCC, as indicated by previous investigations,[1–5,10–25] or suggested from our own clinical experience. Each variable, which was classified as host-related or treatment-related, was divided into one of two subgroups on the basis of clinically meaningful values. HCV RNA load was determined quantitatively by competitive reverse-transcription polymerase chain reaction (RT-PCR), branched-DNA probe assay, or Amplicor-HCV monitor assay.[26–28] When the serum HCV RNA level was more than 10^6 equivalents/ml by branched DNA assay, more than 10^6 copies/ml by competitive RT-PCR, or more than 10^5 copies/ml by Amplicor-HCV monitor assay, it was designated as a high viral load; an HCV RNA level of 10^5 copies/ml by the Amplicor-HCV monitor assay has already been demonstrated to correspond to approximately 10^6 equivalents/ml by the branched DNA probe assay or 10^6 copies/ml by competitive RT-PCR.[26–28] HCV subtype was classified by either the method of Okamoto et al.,[29] or Tanaka et al.'s method.[30] Genotypes 1a and 1b corresponded to serological group 1, and genotypes 2a and 2b corresponded to serological group 2, according to the Simmonds et al.[31] classification.[31] The data from liver biopsies that were done within 6 months before IFN therapy were included in this study. Assessments of the staging of liver fibrosis and the grade of inflammatory activity were based on the classification of Desmet and colleagues,[32] in which staging is defined as follows: F0 (no fibrosis), F1 (fibrous portal expansion), F2 (bridging fibrosis), F3 (bridging fibrosis with architectural distortion), and F4 (cirrhosis), and grading is defined as follows: A0 (no activity), A1 (mild activity), A2 (moderate activity), and A3 (severe activity).

To elucidate the clinical features of HCC that developed in sustained responders, 17 variables at the time of diagnosis of HCC were investigated. Number of tumors, maximum tumor size, portal vein invasion, hepatic vein invasion, and bile duct invasion were examined by ultrasonography, computed tomography, and/or angiography. The period to the development of HCC was measured from the day of termination of IFN therapy to the day when HCC was first diagnosed by imaging modalities, such as ultrasonography or computed tomography. The follow-up period for the detection of HCC after termination of IFN therapy was defined as the interval during which checks for HCC were done using tumor markers and/or imaging modalities.

Statistical analysis

Follow up ended with the last recorded visit before August 31, 2001. Incidences were calculated in person-

Table 1. Patient characteristics of 1056 sustained responders to interferon therapy given for chronic hepatitis C

		Number of patients
Host-related variables		
Age (years)	Median (range)	50 (11–76)
Sex	Male	711 (67%)
History of blood transfusion	Positive	266 (27%)
Alcohol abuse[a]	Positive	78 (8%)
Smoking habit[b]	Positive	248 (38%)
HCV viral load	High ($\geq 10^6$)	159 (21%)
HCV serologic group	Group 1	372
	Group 2	466
Hepatitis B surface antigen	Positive	17 (2%)
Treatment-related variables		
Interferon type	α	829 (79%)
	β	166 (16%)
	α + β	61 (6%)
Total amount of interferon (MU)	Median (range)	480 (42–1740)
Treatment period (weeks)	Median (range)	22 (2–56)
Prior interferon therapy	Positive	87

HCV, hepatitis C virus
[a] Alcohol intake, ≥80 g/day × 5 years
[b] Smoking habit, ≥20 cigarettes/day for ≥10 years

years; incidence curves of HCC were calculated by the Kaplan-Meier method; and differences in survival were evaluated by log rank tests. Hazard ratios and trend *P* values were calculated by treating the categories as ordinal variables. The Cox proportional hazard model was used to determine the most significant variables related to the development of HCC. All patients were then assigned a risk index value for the development of HCC, as follows: the value of each factor in the final model was multiplied by its corresponding regression coefficient, and these values were totaled to obtain the risk index for each patient. Stratification of the patients was conducted on the basis of this risk index. All *P* values were two-tailed and were considered significant when less than 0.05.

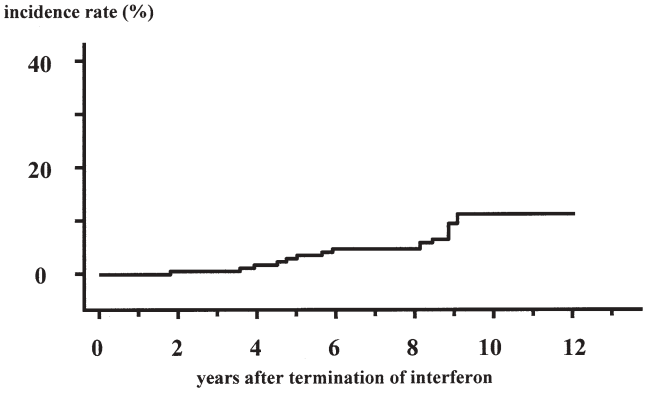

Fig. 1. Cumulative incidence of hepatocellular carcinoma in 1056 sustained responders to interferon therapy given for chronic hepatitis C

Results

Patient characteristics

Table 1 summarizes the patient characteristics of the 1056 sustained responders to IFN therapy given for chronic hepatitis C. The median age was 50 years (range, 11–76) years, and there were 711 men and 345 women (sex ratio, 2.1 : 1). Hepatitis B surface antigen was positive in 17 patients (2%). The HCV serological group was group 1 in 372 patients and group 2 in 466 patients, and thus a higher proportion of patients were in serological group 2. A total of 829 patients (79%) received IFN-α, 166 patients (16%) received IFN-β, and 61 patients (6%) received both. The median dose and duration of IFN administration were 480 MU and 22 weeks, respectively. No patients received peginterferon or combination therapy with ribavirin, and 87 patients (8%) received more than two cycles of IFN therapy.

Incidence of HCC

Twenty-nine of the 1056 sustained responders developed HCC, with a median follow-up period of 4.7 years. The incidence of HCC per 100 person-years was 0.56 (95% confidence interval, 0.35–0.76), and the incidences of HCC at 3, 5, 7, and 10 years after the termination of IFN therapy were 0.5%, 3.3%, 4.9%, and 11.1%, respectively (Fig. 1).

Univariate analyses

On univariate analysis (Table 2), age more than 60 years, positive smoking habit, platelet count less than $15 \times 10^4/mm^3$, aspartate aminotransferase (AST) more than 100 IU/l, prothrombin time less than 80%, and higher fibrosis stage (incidence of HCC per 100 person-years: F0, 0.00; F1, 0.27; F2, 0.47; F3, 0.62; F4, 1.31) were significant risk factors associated with the development of HCC. Alcohol abuse, total bilirubin, albumin, alanine aminotransferase, virological variables (viral load, serological group), tumor markers (alpha-fetoprotein, protein induced by vitamin K absence or antagonist-II), and treatment-related variables (treatment period, IFN type, total amount of IFN) were not significant risk factors.

Multivariate analyses

All variables whose P values were less than 0.20 on the univariate analyses were entered into the multivariate analyses (Table 3). However, history of blood transfusion, smoking habit, prothrombin time, and indocyanine green retention rate at 15 min (ICG R15) were not included in the model because inadequate data were available. Multivariate regression analysis, which assessed the independent predictive importance of each variable studied for the development of HCC, showed that older age, higher serum AST level, and lower platelet count were significantly related to the development of HCC.

Risk groups based on the regression model

For the clinical application of these findings, a risk index was calculated based on the regression coefficients derived from the three variables identified by multivariate analysis. The index equation was as follows: $1.14 \times (0,$ age ≤ 60 years; 1, age > 60 years) $+ 1.13 \times (0,$ AST $\leq$ 100 IU/l; 1, AST $>$ 100 IU/l) $+ 1.02 \times (0,$ platelet count $\geq 15 \times 10^4/mm^3$; 1, platelet count $< 15 \times 10^4/mm^3$). The risk index was $\ln[hi(t)/h_0(t)]$, where $hi(t)/h_0(t)$ was the relative risk of the development of HCC for the i-th patient. The index values ranged from 0.00 to 3.29. The patients were then classified into three groups according to the risk index, as follows: low risk, risk index less than 1.00 (equivalent to patients with none of the three risk factors); intermediate risk, risk index from 1.00 to 2.00 (equivalent to patients with one of the three risk factors); and high risk, risk index greater than 2.00 (equivalent to patients with two or more of the three risk factors). The incidence curves for the three groups are shown in Fig. 2. The incidence rates of HCC per 100 person-years (95% confidence interval) in the low-, intermediate-, and high-risk groups were 0.11 (0.00–0.26), 0.44 (0.11–0.77), and 1.98 (1.09–2.87), respectively. There was a significant difference in survival time among the three groups ($P < 0.0001$).

Clinical features of HCC

The characteristics of the 29 patients in whom HCC developed after sustained response are shown in Table 4. All patients were HCV RNA-negative (determined by using qualitative HCV RNA assay), at the time of diagnosis of HCC. Twenty-five patients (86%) were aged 60 years or more, and 24 patients (83%) were men. Among the 13 patients in whom liver biopsy was done at the time of diagnosis of HCC, A0, A1, and A2 histological activity was observed in 5 (38%), 6 (46%), and 2 (15%) patients, respectively. F0, F1, F2, F3, and F4 histological stages were observed in 1 (8%), 1 (8%), 7 (54%), 2 (15%), and 2 (15%) patients, respectively. The median period from the termination of IFN therapy to the development of HCC was 4.6 years (range, 1.4–9.0 years), and there were 11 patients (38%) in whom HCC was detected more than 5 years after the termination of IFN therapy. The periods and methods of medical follow-up examination after the end of IFN therapy varied among the patients, and 8 patients did not receive a sufficient post-treatment medical examination. Among them, HCC of 5 cm or more in size was detected in 5 patients (63%).

Discussion

IFN is already widely used as a standard therapeutic modality for chronic hepatitis C.[5–9] It is generally assumed that eradication of HCV by IFN halts the progression of the disease and prevents clinical complications, including the development of HCC.[5,7,10–14] However, there have been reports of several patients in whom HCC developed after successful IFN therapy.[5,10–25] The incidence and clinical features of HCC, the risk factors for the disease, and the mechanism of carcinogenesis in these patients have not been fully elucidated, because the development of HCC is very rare in sustained responders to IFN therapy. This prompted us to perform a multicenter retrospective cohort study to gather clinical data on such patients.

Of all 1056 sustained responders to IFN therapy in the 16 hospitals in the study, 29 developed HCC, with a median period to development of 4.7 years, and the incidence of HCC was 0.56 (95% confidence interval, 0.35–0.76) per 100 person-years. This value was consistent with the results of previous studies of small numbers of sustained responders to IFN who developed HCC.[5,11–14,20,21–25] This rate was considerably lower than that in IFN-refractory patients or HCV-positive pa-

Table 2. Univariate analysis of 1056 sustained responders in relation to development of HCC

Variables		No. of patients	No. of patients developing HCC	Incidence (95% CI) (/100 person-years)	Hazard ratio (95% CI)	*P* value (log rank)
Host-related variables						
Age	≤60 years	840	13	0.32 (0.14–0.49)	—	
	>60 years	216	16	1.43 (0.73–2.13)	4.23 (2.04–8.80)	0.001
Sex	Male	711	24	0.67 (0.40–0.94)	—	
	Female	345	5	0.30 (0.04–0.57)	0.47 (0.18–1.23)	0.12
History of blood transfusion	Positive	266	11	0.80 (0.33–1.28)	—	
	Negative	723	16	0.45 (0.23–0.67)	0.60 (0.28–1.30)	0.19
Alcohol abuse[a]	Positive	78	2	0.53 (0.00–1.26)	—	
	Negative	946	26	0.56 (0.34–0.77)	1.05 (0.25–4.42)	0.95
Smoking habit[b]	Positive	248	14	1.16 (0.55–1.77)	—	
	Negative	405	7	0.36 (0.09–0.62)	0.30 (0.12–0.75)	0.009
HCV viral load	High ($\geq 10^6$)	159	1	0.15 (0.00–0.45)	—	
	Low ($<10^6$)	593	11	0.42 (0.17–0.66)	2.68 (0.35–20.77)	0.35
HCV serological group	Group 1	372	5	0.27 (0.03–0.52)	—	
	Group 2	466	10	0.47 (0.18–0.76)	1.78 (0.60–5.26)	0.30
Hepatitis B surface antigen	Positive	17	0	0.00		
	Negative	1008	27	0.54 (0.34–0.75)	[c]	0.56
Platelet count ($\times 10^4/mm^3$)	≥15	568	7	0.27 (0.07–0.46)	—	
	<15	358	21	1.15 (0.66–1.65)	3.95 (1.68–9.30)	0.002
Total bilirubin (mg/dl)	≥1.0	207	8	0.75 (0.23–1.27)	—	
	<1.0	824	21	0.52 (0.30–0.75)	0.37 (0.32–1.65)	0.45
Albumin (g/dl)	>4.0	564	17	0.59 (0.31–0.87)	—	
	≤4.0	396	8	0.42 (0.13–0.72)	0.78 (0.34–1.80)	0.56
Aspartate aminotransferase (IU/l)	>100	196	13	1.26 (0.57–1.94)	—	
	≤100	844	16	0.39 (0.20–0.58)	0.35 (0.17–0.73)	0.005
Alanine aminotransferase (IU/l)	>100	459	17	0.73 (0.38–1.07)	—	
	≤100	591	12	0.42 (0.18–0.66)	0.63 (0.30–1.32)	0.22
Prothrombin time (%)	≥80	493	9	0.39 (0.14–0.65)	—	
	<80	158	10	1.19 (0.45–1.93)	2.72 (1.10–6.74)	0.03
ICG R15 (%)	≥10	322	9	0.52 (0.18–0.86)	—	
	<10	274	1	0.08 (0.00–0.23)	0.18 (0.02–1.44)	0.11
Alpha-fetoprotein (ng/ml)	>20	66	2	0.58 (0.00–1.39)	—	
	≤20	554	16	0.58 (0.30–0.87)	1.10 (0.25–4.81)	0.78
PIVKA–II (AU/ml)	>0.063	42	0	0.00		
	≤0.063	235	8	0.66 (0.20–1.12)	[c]	0.63
Histological activity grade	A0 (No)	12	0	0.00		
	A1 (Mild)	309	6	0.40 (0.08–0.73)		
	A2 (Moderate)	359	11	0.64 (0.26–1.01)		
	A3 (Severe)	169	5	0.61 (0.07–1.14)	1.28 (0.74–2.21)	0.39
Histological fibrosis stage	F0 (No)	26	0	0.00		
	F1 (Mild)	405	5	0.27 (0.03–0.50)		
	F2 (Moderate)	301	7	0.47 (0.12–0.82)		
	F3 (Severe)	170	6	0.62 (0.12–1.11)		
	F4 (Cirrhosis)	97	4	1.31 (0.03–2.60)	1.56 (1.03–2.36)	0.03
Treatment-related variables						
Treatment period (weeks)	≥24	472	17	0.73 (0.38–1.08)		
	<24	584	12	0.41 (0.18–0.65)	0.56 (0.27–1.16)	0.11
Interferon type	α	829	25	0.61 (0.37–0.85)	—	
	β	166	4	0.55 (0.01–1.10)	0.99 (0.34–2.86)	0.98
	α + β	61	0	0.00	[c]	
Total amount of interferon (MU)	>500	491	10	0.42 (0.16–0.68)	—	
	≤500	534	16	0.60 (0.31–0.89)	1.34 (0.61–2.95)	0.47
Prior interferon therapy	Positive	87	2	0.46 (0.00–1.10)	—	
	Negative	955	27	0.57 (0.36–0.79)	1.17 (0.28–5.00)	0.82

HCC, hepatocellular carcinoma; CI, confidence interval; HCV, hepatitis C virus; ICG R15, indocyanine green retention rate at 15 min; PIVKA II, protein induced by vitamin K absence or antagonist-II; —; reference category

[a] Alcohol intake ≥80 g/day + 5 years

[b] Smoking habit, ≥20 cigarettes/day for ≥10 years

[c] not estimated

tients who did not receive IFN therapy, which has been reported to be 1.4%–7% yearly,[4–7,10–13,21–24] and it was obvious that IFN therapy decreased the risk of HCC in sustained responders. However, the incidence of HCC gradually increased over a period of at least 9 years after the termination of IFN therapy (Fig. 1). This suggests that the risk of HCC is not completely eliminated in patients who have a sustained response to IFN therapy,

at least for up to 9 years following cessation of the treatment.

Identification of the risk factors for the development of HCC in sustained responders is important, so that high-risk patients can be screened carefully for early detection of HCC and given potentially curative treatments such as hepatic resection; such patients generally have a good hepatic reserve after the elimination of HCV. Among the variables we investigated, multivariate analysis showed age to be an independent risk factor. As the patient ages, the period of HCV infection becomes longer, and the liver becomes more severely cirrhotic. Therefore, advanced age may simply represent the progression of associated liver disease. These findings are compatible with previous reports of the development of HCC in patients with chronic hepatitis C.[11–14,20–22]

Serum AST level and platelet counts were also independent risk factors in the present study. Some studies have reported that increased AST level and decreased platelet count are correlated with the progression of liver fibrosis,[33–34] which has been reported to be one of the most important risk factors for the development of HCC in patients with chronic hepatitis C.[5,11–13,21] Progression of liver fibrosis may reduce the clearance of AST,[35] leading to increased serum AST levels.[36] This progression is also associated with decreased production of thrombopoietin by hepatocytes[37] and progressive hypersplenism with worsening portal hypertension;[38] and, hence, reduced platelet production and increased platelet destruction. Moreover, in the present study, these factors were strongly associated with histological stage (Pearson's correlation coefficient; $P < 0.0001$). Therefore, increased AST level and decreased platelet count may reflect more progressive liver fibrosis.

For the clinical application of these findings, we proposed a risk index based on the independent risk factors. Patients were classified into three groups, with low, intermediate, and high risk ($P < 0.0001$ for difference in survival time among the three groups; Fig. 2). This index can be easily calculated, because it is based on variables obtained during routine laboratory examinations before IFN therapy is begun. This index, therefore, may be helpful in assessing the risk of development of HCC after sustained response to IFN therapy, although it is also important to validate this risk index by applying it to other populations of patients. Patients in the high-risk group (incidence rate, 1.98 per 100 person-years) may benefit from regular diagnostic imaging for the early detection of HCC.

In the analysis of the clinical features of HCC there were no specific findings. The period to the development of HCC after IFN therapy (median, 4.6 years; 1.4–9.0 range, years) was variable. HCC developed even in two patients whose liver showed improvement to mild fibrosis (stage F0 or F1) and in five patients whose liver improved to no activity (A0) after IFN therapy. The follow-up periods and methods for the detection of HCC after the termination of IFN therapy varied among the patients, and in some patients HCC was detected at far more advanced stages than in others, because of insufficient follow up after IFN therapy. This finding may suggest the need for regular follow up by diagnostic imaging, even after sustained response to IFN therapy for chronic hepatitis C, especially in the high-risk group.

Our study involved some uncertainties. First, because the study was retrospective, many data items were missing from the replies to the data collection instrument, and we had to ignore unmeasured or unrecorded data when conducting the statistical analyses. In the multivariate analysis, therefore, only variables whose *P* values were less than 0.20 on the univariate analysis were entered. Also, history of blood transfusion, smoking habit, prothrombin time, and ICG R15, whose *P* values were lower than 0.20, had to be excluded from the model because of missing data; these factors were potentially significant on multivariate analysis. Secondly, we sought information on serum hepatitis B virus DNA

Table 3. Significant risk factors identified in 1056 sustained responders, as determined by multivariate analysis with the Cox proportional hazard model

Variable	Hazard ratio (95% confidence interval)	*P* value
Age	3.13 (1.32–7.42)	0.01
Aspartate aminotransferase	3.10 (1.31–7.31)	0.01
Platelet count	2.78 (1.07–7.20)	0.04

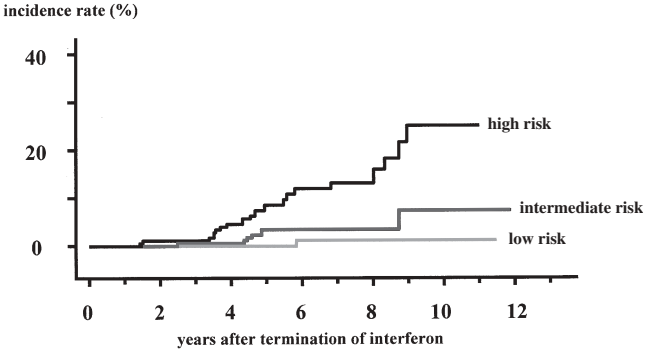

Fig. 2. Cumulative incidence of hepatocellular carcinoma for the three groups determined by a risk index based on the results of multivariate analysis. Low risk (risk index < 1.00); intermediate risk (risk index from 1.10 to 2.00); high risk (risk index, ≥2.00)

Table 4. Clinical features at the time of diagnosis of HCC in 29 patients who developed hepatocellular carcinoma after sustained response to interferon therapy given for chronic hepatitis C

Age (years)	Sex	HCV RNA	HBs Ag	Histological fibrosis stage	Histological activity grade	AFP (ng/ml)	PIVKA II (AU/ml)	Number of tumors
64	Male	Negative	Negative	NA	NA	2	0	4
60	Male	Negative	Negative	NA	NA	51	0.211	1
38	Male	Negative	Negative	NA	NA	4.3	NA	>5
67	Male	Negative	Negative	F4	A0	4.2	0.033	1
75	Male	Negative	Negative	F1	A1	5	0.054	1
65	Female	Negative	Negative	NA	NA	5	0.029	1
62	Male	Negative	Negative	NA	NA	3	NA	1
61	Male	Negative	Negative	F2	A0	3	0.001	1
64	Male	Negative	Negative	F2	A0	4	0.426	1
70	Male	Negative	Negative	NA	NA	46 000	NA	1
64	Male	Negative	Negative	NA	NA	146	0.049	1
54	Female	Negative	Negative	F3	A1	2165	6690	1
65	Male	Negative	Negative	F4	A2	25.9	0.015	>5
61	Male	Negative	Negative	F2	A1	4	1.79	1
64	Male	Negative	Negative	F2	A0	NA	NA	1
63	Male	Negative	Negative	NA	NA	135.3	0.06	1
67	Male	Negative	Negative	NA	NA	3.5	0.013	1
75	Male	Negative	Negative	NA	NA	2	NA	1
62	Male	Negative	Negative	F2	A1	1026	13.32	1
62	Male	Negative	Negative	F2	A1	2.3	1.79	1
68	Female	Negative	Negative	F3	A2	9.1	0.016	1
59	Male	Negative	Negative	F0	A0	29	0.029	1
70	Male	Negative	Negative	NA	NA	488.3	601 371	1
54	Male	Negative	Negative	NA	NA	258	2.1	1
68	Female	Negative	Negative	NA	NA	2.8	0.023	1
60	Male	Negative	Negative	F2	A1	3.2	0.023	1
70	Male	Negative	Negative	NA	NA	5463	6.566	2
70	Female	Negative	Negative	NA	NA	464.2	NA	1
77	Male	Negative	Negative	NA	NA	72	0.136	2

NA, not available; HBs Ag, hepatitis B surface antigen; AFP, alpha-fetoprotein; PIVKA II, protein induced by vitamin K absence or antagonist-II; Vp, portal vein invasion; Vv, hepatic vein invasion; B, bile duct invasion; US, ultrasonography; CT, computed tomography

in sustained responders in whom HCC developed after successful IFN therapy, but data could be obtained for only two patients, who were negative for hepatitis B virus DNA. We cannot rule out the presence of occult hepatitis B virus in the other patients, although all patients were negative for hepatitis B antigen. In spite of these uncertainties, this study represents a comprehensive analysis of HCC developing after sustained response to IFN therapy, because we were able to collect clinical data for a large number of sustained responders at 16 major hospitals.

In this study, we encountered 29 patients in whom HCC developed after successful IFN therapy, but the reason why HCC developed in these sustained responders is unclear. The existence of a small undetected HCC at the time of IFN therapy may have been responsible for the appearance of HCC after the sustained response to IFN therapy. However, in 11 patients (38%), HCC was detected more than 5 years after IFN therapy, and the incidence of HCC gradually increased for at least 9 years after IFN therapy. Considering the late onset of HCC in these patients, we cannot neglect the possibility of the de-novo development of HCC after the eradication of HCV. HCV is a single-stranded RNA virus without a DNA intermediate in its replicative cycle, so that the integration of HCV nucleic acid sequences into the host genome seems unlikely. Therefore, it is difficult to believe that HCV itself is a causative factor of HCC in the absence of chronic inflammation, liver cell necrosis and regeneration, and extensive fibrosis. It is probable that carcinogenesis is not a single-step event, but a complex multistep process. Future studies should aim to define the basic oncogenic mechanisms by which sustained responders to IFN develop HCC. Exploration of these mechanisms may point the way toward new strategies for the prevention of HCC.

In conclusion, some patients showing a sustained response to IFN therapy given for chronic hepatitis C demonstrated potential for the development of HCC for up to 9 years following cessation of the treatment. This suggests that the risk of HCC in sustained responders is not completely eliminated. The establishment of risk factors and an index for the development of HCC may be useful in determining follow-up strategy in patients after a sustained response to IFN therapy given for chronic hepatitis.

Table 4. *Continued*

Maximum tumor size (mm)	Vp	Vv	B	Differentiation of HCC	Period to development Of HCC (years)	Medical follow-up period (months)	Diagnostic modality
18	0	0	0	Moderately	1.43	3	US
16	0	0	0	NA	1.51	1	US
>20	3	0	0	NA	1.79	None	US
15	0	0	0	Moderately	2.52	1	US
25	0	0	0	Moderately	3.32	2	CT
20	0	0	0	Well	3.39	3	US
34	2	1	2	Well	3.54	2	US
20	0	0	0	Well	3.59	3	Laparoscopy
40	0	0	0	NA	3.70	None	US
50	2	2	2	NA	3.89	None	US
30	0	0	0	Well	4.35	1	US
110	0	0	0	Poorly	4.38	6	US
15	0	0	0	Well	4.48	6	US
50	1	0	1	Moderately	4.58	12	US
80	0	0	0	Moderately	4.60	None	CT
NA	0	0	0	NA	4.70	6	US
44	0	0	0	NA	4.88	6	US
28	0	0	0	NA	4.97	3	US
60	1	1	1	Moderately	5.52	None	US
50	1	0	1	Moderately	5.58	6	US
51	0	0	0	Combined type	5.80	3	US
40	0	0	0	Moderately	5.86	None	US
>20	2	0	0	NA	6.61	3	US
150	3	0	0	Poorly	6.86	None	US
15	0	0	0	NA	8.05	3	US
15	0	0	0	Well	8.39	6	US
60	0	0	0	Well	8.78	None	US
16	0	0	0	NA	8.79	3	US
42	0	0	0	NA	8.98	1	CT

Appendix

In addition to the study authors' hospitals (the four institutions listed on the title page), data were supplied by the following hospitals and clinics in the Kyushu Division of the Japanese Society of Gastroenterology: Shinnittetsu Yahata Memorial Hospital; Yame General Hospital; First Department of Internal Medicine, Ryukyu University School of Medicine; Second Department of Internal Medicine, Kagoshima University School of Medicine; Hayato Town Medical Association Medical Center; Department of Internal Medicine, Saga Medical School; Department of Medicine and Biosystemic Science, Kyushu University School of Medicine; Nishinihon Hospital; Kagoshima Kouseiren Hospital; Miyata Memorial Hospital; Second Department of Internal Medicine, Nagasaki University School of Medicine; and Yonabaru Central Hospital.

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