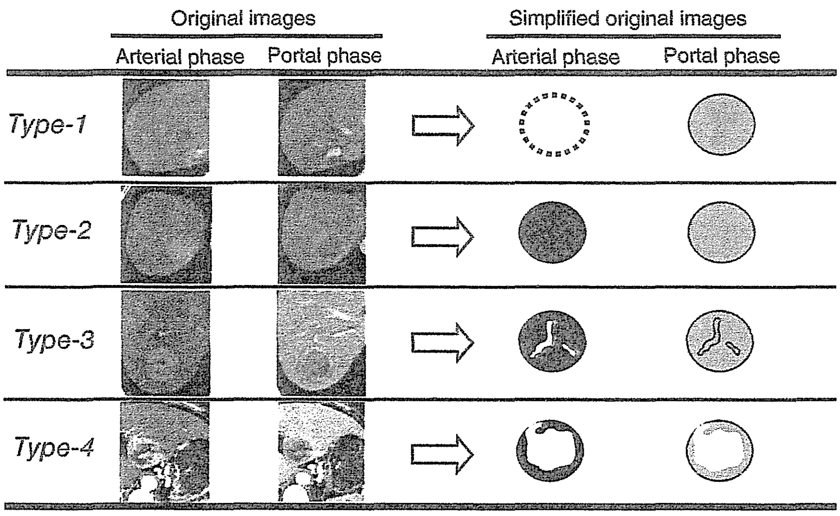

Figure 1 Sample of original dynamic computed tomography images and simplified images for each enhancement pattern.

HCC was based primarily on the definition of Kanai *et al.*[22] and the Liver Cancer Study Group of Japan:[21] small nodular type with indistinct margin (SNIM type, indistinct margins and containing portal tracts); simple nodular type (SN type, round nodule with clear margin); simple nodular type with extranodular growth (SNEG type, similar to the SN type with extranodular growth); confluent multinodular type (CMN type, nodular lesion consisting of a cluster of small and confluent nodules); and infiltrative type (IF type, nodular lesion with irregular and indistinct margins). The histopathological diagnosis of HCC was established by consensus of at least two pathologists and three hepatologists.

Immunohistochemical staining

Part of the liver specimen was soaked in neutral formalin solution immediately after resection and fixed for 24–48 h. Paraffin-embedded fixed specimens were sliced into 2 μm thick sections. After deparaffinization, the sections were immunostained with α-SMA monoclonal antibody (Actin, α-smooth muscle, clone 1A4; Ventana Medical Systems, Tucson, AZ, USA) using an automated system (BenchMark; Ventana Medical Systems). When a round, oval or slender ring-shaped structure was identified in the α-SMA-immunostained specimen, it was regarded as an abnormal new blood vessel irrespective of its site in the liver, provided it was unrelated to the portal area. Therefore, we regarded such ring-shaped α-SMA-stained structures as non-triadal vessels and defined them as "positive neovascularity". We have also reported thick-walled, nuclei-rich, and slender-shaped α-SMA-positive vessels (Fig. 2a "HE staining", Fig. 2b "α-SMA staining") in HCC tissue that were closely related to angiographic hypervascularity.[20] This type of blood vessel was termed "Type-II vessel". To understand the relation between the findings on dynamic CT and immunohistochemical pattern, we analyzed the relationship between Type-4 pattern and the distribution of Type-II vessels.

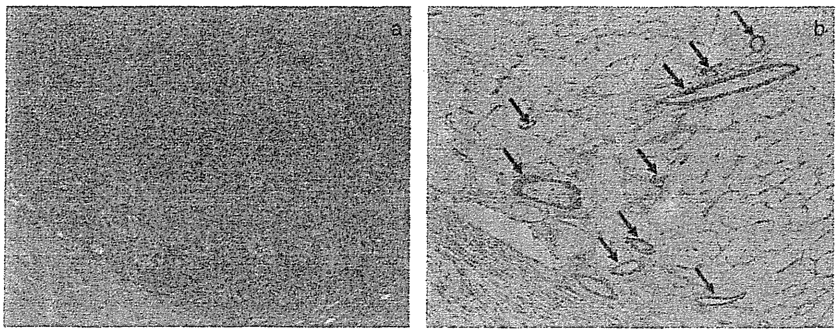

Figure 2 (a) Hematoxylin–eosin staining of hepatocellular carcinoma tissue. (b) Immunohistochemical staining for α-smooth muscle actin (α-SMA) of the same slice shown in (a). Solid arrows: typical Type II vessels. (Original magnifications: [a] ×100; [b] ×100.)

Table 1 Clinical profile and laboratory data of 86 patients with HCC

Sex (M : F)	57:29
Age (years)	62 (35–80)
Background of liver disease	
Hepatitis B surface antigen positive	26
Anti-HCV antibody positive	50
Both negative	10
Status of liver function	
Child–Pugh classification (A/B/C)	83/3/0
Preoperative image diagnosis of HCC	
Tumor diameter (mm)	23 (9.0–50)
Portal vein invasion (yes/no)	0/86
Laboratory data	
Platelet count ($\times 10^4/\mu L$)	13.0 (4.0–30.1)
Albumin (g/dL)	4.0 (3.1–4.3)
Bilirubin (mg/dL)	1.0 (0.3–1.7)
AST (IU/L)	43 (18–386)
Prothrombin time (%)	91 (60–124)
ICG-R15 (%)	19 (3.0–68)
AFP (μg/L)	13 (1.0–5541)
DCP (AU/L)	19 (4.0–1650)

AFP, α-fetoprotein; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ICG-R15, indocyanine green retention rate at 15 min.

Clinical background and laboratory data

Table 1 summarizes the clinical profile and laboratory data of 86 HCC patients in this study. The male : female ratio was 1.97:1. HCV antibody was detected in 58.1% of the patients, and 96.5% patients were classified as Child–Pugh A but none of the patients was classified as Child–Pugh C. Based on preoperative image analysis, the median tumor diameter was 23 mm and none of the patients had portal vein tumor invasion. Furthermore, the median levels of AFP and des-γ-carboxy prothrombin (DCP) were 13 μg/L and 19 AU/L, respectively.

Statistical analysis and ethical considerations

The factors associated with poorly-differentiated HCC, SNEG and CMN were analyzed by the χ^2-test and Fisher's exact test. The independent factors associated with preoperative diagnosis of poorly-differentiated HCC were identified by multivariate logistic regression analysis. The potential predictive factors for poorly-differentiated HCC, SNEG and CMN were age, sex, hepatitis B surface antigen (HBsAg), HCV antibody, platelet count, aspartate transaminase (AST), albumin, bilirubin, AFP, DCP, prothrombin activity, indocyanine green retention rate at 15 min (ICG-R15), and tumor size. Several variables were transformed into categorical data consisting of two or three simple ordinal numbers for univariate and multivariate analyses. All factors that were at least marginally associated with poorly differentiated HCC, SNEG and CMN ($P < 0.10$) in univariate analysis were entered into a multivariate logistic regression analysis. Significant variables were selected by the stepwise method. A two-tailed *P*-value less than 0.05 was considered significant. Data analysis was performed using the SPSS ver. 11.0 software.

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

RESULTS

Distribution of enhancement patterns and proportions of histopathological types

PREOPERATIVE IMAGE ANALYSIS of 86 HCC patients showed the following results: Type-1 in 10 (11%) patients; Type-2 in 41 (48%) patients; Type-3 in 24 (28%) patients; and Type-4 in 11 (13%) patients. Furthermore, the percentages of poorly-differentiated HCC according to the enhancement pattern were zero of 10 (0%) patients with Type-1, three of 41 (7%) with Type-2, three of 24 (13%) with Type-3, and eight of 11 (73%) with Type-4. The percentages of SNEG/CMN according to the enhancement pattern were 12 of 51 nodules (24%) of Type-1 and -2, 13 of 24 (54%) of Type-3, and five of 11 (45%) of Type-4 (Table 2).

Correlation between preoperative features and diagnosis of poorly-differentiated HCC

We also investigated the factors that correlated with preoperative diagnosis of poorly-differentiated HCC. Univariate analysis showed the type of enhancement pattern (Type-4, Type-3 or other enhancement pattern, $P < 0.001$), tumor size (<35 mm/≥35 mm, $P = 0.005$), serum AST level (<40 IU/≥40 IU, $P = 0.069$) and serum DCP level (<30 AU/L/≥30 AU/L, $P = 0.079$) to correlate with preoperative diagnosis of poorly-differentiated HCC. These parameters were entered into multivariate logistic regression analysis. The percentage of poorly-differentiated HCC was significantly higher for large-size HCC (≥35 mm, risk ratio 14.72, 95% confidence interval [CI] 1.15–188.10) and Type-4 enhancement pattern (risk ratio 12.86, 95% CI 1.56–105.94) (Table 3).

Correlation between preoperative features and diagnosis of SNEG and CMN types

We also investigated the factors associated with the development of SNEG and CMN types of HCC. Univari-

Table 2 Distribution of enhancement patterns and frequency of each macroscopic type and poorly-differentiated HCC by histological examination

Enhancement pattern	No. of nodules	Poorly-differentiated HCC	Distribution of macroscopic type according to the enhancement pattern				
			SNIM	SN	SNEG	CMN	IF
Type-1	10/86 (11%)	0/10 (0%)	5/10 (50%)	3/10 (30%)	1/10 (10%)	0/10 (0%)	1/10 (10%)
Type-2	41/86 (48%)	3/41 (7%)	2/41 (5%)	27/41 (66%)	7/41 (17%)	4/41 (10%)	1/41 (2%)
Type-3	24/86 (28%)	3/24 (13%)	0/24 (0%)	11/24 (46%)	10/24 (42%)	3/24 (13%)	0/24 (0%)
Type-4	11/86 (13%)	8/11 (73%)	0/11 (0%)	6/11 (55%)	3/11 (27%)	2/11 (18%)	0/11 (0%)

CMN, confluent multinodular type; HCC, hepatocellular carcinoma; IF, infiltration type; SN, simple nodular type; SNEG, simple nodular type with extranodular growth; SNIM, small nodular type with indistinct margin.

Table 3 Results of multivariate logistic regression analysis for predictive factors of poorly-differentiated hepatocellular carcinoma according to preoperative factors

Factors	Category	Hazard ratio (95% confidence interval)	*P*-value
Tumor size (mm)	1: <35	1	
	2: ≥35	14.72 (1.15–188.10)	0.039
Type of enhancement pattern	1: Type-1 and -2	1	
	2: Type-3	1.19 (0.17–8.43)	0.87
	3: Type-4	12.86 (1.56–105.94)	0.001

ate analysis identified the following three factors that correlated with SNEG/CMN type: age (<65/≥65 years, $P = 0.030$), type of enhancement pattern (Type-4, Type-3 or other enhancement pattern, $P = 0.055$) and tumor size (<35 mm/≥35 mm, $P = 0.060$). These parameters were entered into multivariate logistic regression analysis. The percentage of SNEG/CMN types of HCC was significantly higher for Type-3 enhancement pattern (risk ratio 3.82, 95% CI 1.28–11.44) and age less than 65 years (risk ratio 3.57, 95% CI 1.29–9.90) (Table 4).

Cumulative recurrence rate according to each enhancement pattern

During a median observation period of 4.8 years, 46 (54%) out of 86 patients developed HCC recurrence in all patients. The cumulative recurrence rates after the surgical resection for HCC according to each enhancement pattern were 14% at the end of the first year, 44% at the third year and 54% at the fifth year for patients with Type-1 and -2 enhancement patterns; 13% at the first year, 28% at the third year and 38% at the fifth year

Table 4 Results of multivariate logistic regression analysis for predictive factors of SNEG and CMN types of HCC

Factors	Category	Hazard ratio (95% confidence interval)	*P*-value
Type of enhancement pattern	1: Type-1 and -2	1	
	2: Type-3	3.82 (1.28–11.44)	0.017
	3: Type-4	2.43 (0.60–9.86)	0.213
Age (years)	1: ≥65	1	
	2: <65	3.57 (1.29–9.90)	0.014

CMN, confluent multinodular type; HCC, hepatocellular carcinoma; SNEG, simple nodular type with extranodular growth.

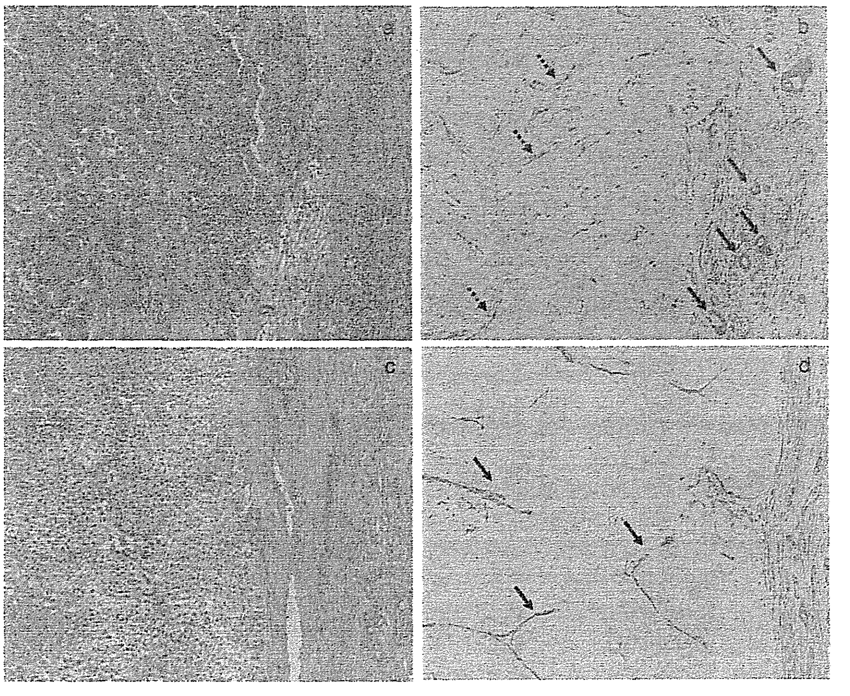

Figure 3 (a) Hematoxylin–eosin staining of hepatocellular carcinoma (HCC) tissue. (b) Immunohistochemical staining for α-smooth muscle actin (α-SMA) of the same slice shown in (a). Solid arrows: typical Type II vessels. Dotted arrows: α-SMA-positive vessels, which are atypical for Type II vessels. Note that the typical Type II vessels tended to be located in the peripheral parts of the HCC. (c) Hematoxylin–eosin staining of HCC tissue. (d) Immunohistochemical staining for α-SMA of the same slice shown in (c). Solid arrows: α-SMA-positive vessels, which are atypical for Type II vessels. HCC tissue exhibited thick trabecular pattern, and α-SMA-positive vessels were recognized in the gaps of the trabecular pattern. In this tumor, few typical Type II vessels were detected, and most α-SMA-positive vessels were atypical for Type II vessels. (Original magnifications: [a] ×100; [b] ×100; [c] ×100; [d] ×100.)

for patients with Type-3 enhancement pattern; and 9% at the first year, 52% at the third year and 84% at the fifth year for patients with Type-4 enhancement pattern. There was no difference with statistical significance among each enhancement pattern ($P = 0.163$).

Cumulative survival rate according to each enhancement pattern

The cumulative survival rates after the surgical resection for HCC according to each enhancement pattern were 100% at the end of the first year, 96% at the third year and 85% at the fifth year for patients with Type-1 and -2 enhancement patterns; 100% at the first year, 100% at the third year and 83% at the fifth year for patients with Type-3 enhancement pattern; and 100% at the first year, 100% at the third year and 86% at the fifth year for patients with Type-4 enhancement pattern. There was no statistical significance on survival rate among each enhancement pattern ($P = 0.758$).

Correlation between immunopathological findings and HCC enhancement patterns

Dynamic CT examination revealed ring-like enhancement pattern (Type-4) in 11 of 86 (13%) patients. We examined the distribution of Type II vessel in 11 nodules of Type-4. Seven of these nodules showed heterogeneous distribution of typical Type II vessels (Fig. 3a,b). The tumor tissue in one of these nodules exhibited a thick trabecular pattern and typical Type II vessels were not present in the tumor (Fig. 3c,d). The other three nodules contained large necrotic areas in the tumor and a homogeneous distribution of Type II vessels.

DISCUSSION

A VARIETY OF potentially curative therapies is currently available for HCC. However, except for surgical resection, a potential risk of tumor dissemination always exists in patients who receive such therapies. Therefore, in order to use the most suitable therapy for the individual patient, it is important to predict the potential risk of HCC before treatment.

Previously reported, to predict poorly differentiated HCC and macroscopic type of SNEG and CMN is especially important for good therapeutic progress.[17–19] In our study, Type-4 enhancement pattern was identified as an independent predictive factor of poorly-differentiated HCC. Patients who show Type-4 enhancement pattern in preoperative dynamic CT study are at higher risk of poorly-differentiated HCC (about 13-fold) than Type-1 and -2 enhancement patterns. On

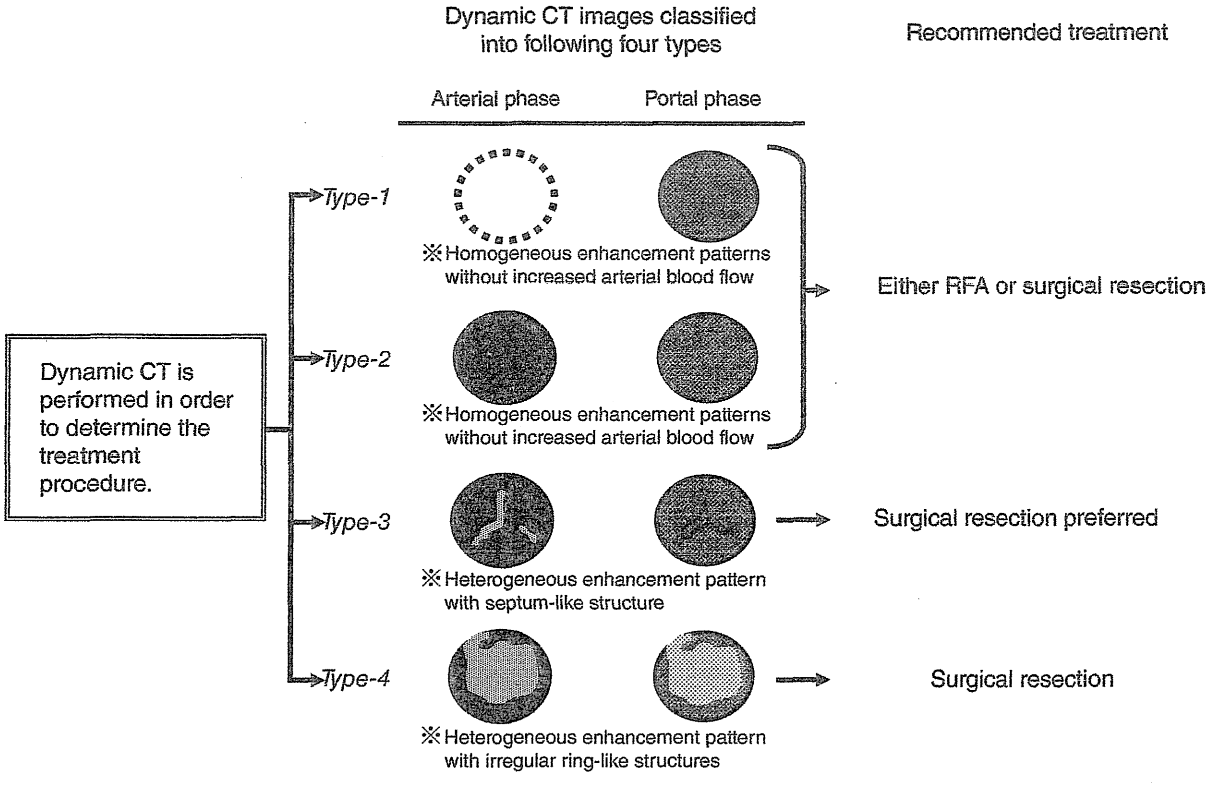

Figure 4 Treatment strategy for hepatocellular carcinoma according to a new classification of dynamic computed tomography (CT) images. RFA, radiofrequency ablation.

the other hand, Type-3 enhancement pattern was an independent predictor of SNEG and CMN, with higher risk (~fourfold) than Type-1 and -2 enhancement patterns. These results could be important for the selection of suitable therapy.

On the other hand, as previously reported, patients with SNEG and CMN have a high complication rate of poorly-differentiated HCC.[22] However, in this study, there was no statistical relation between SNEG/CMN and Type-4 enhancement pattern that had statistical relation with poorly-differentiated HCC. One possible cause which brought this result is that the present study size is too small. Therefore, future studies are needed of larger size to reconsider this point.

Although the present study included only a small sample number of Type-4 enhancement pattern, the results showed that this pattern was associated with α-SMA staining pattern. Based on the results of the present study, at least three explanations were considered for the Type-4 enhancement pattern: (i) non-equal distribution of α-SMA-positive Type II vessels; (ii) the thick trabecular pattern and lack of typical Type II vessels; and (iii) large necrotic tissue in the HCC nodule. It is clear from this examination that the cause of Type-4 enhancement pattern is not dependent on tissue necrosis only but other factors may be involved including Type II vessels. Admittedly, the present results do not fully explain the pathomechanism of Type-4 enhancement pattern. Further studies are needed to determine the main mechanism of this enhancement pattern.

With respect to prognosis after curative surgical resection based on each enhancement pattern, there are no significant differences among the four groups regarding recurrence and survival rate in this study. However, this study group mostly received curative therapy of "surgical resection" for HCC treatment. Therefore, we are currently investigating local recurrence and survival rate after RFA therapy based on this classification.

The present study included HCC only. However, heterogeneous enhancement resembling the Type-4 enhancement pattern is recognized in other hepatic tumors (e.g. cholangiocellular carcinoma [CCC] and fibrolamellar HCC [F-HCC]). It is noteworthy, however, that these tumors are rare in chronic hepatitis or liver cirrhosis compared with HCC. CCC comprises 4.4% of primary liver cancers,[23,24] while F-HCC forms only 0.68% of liver tumors in Japan. Thus, detection of heterogeneous enhancement pattern on dynamic CT images should be considered first to represent HCC with highly malignant potential.

Therefore, we indicate our treatment strategy for HCC according to a new classification of dynamic CT images (Fig. 4). The present results indicate that for patients with Type-4 or Type-3 enhancement patterns on dynamic CT images who have adequate liver reserve to allow any treatment including surgical resection, the above information could be used as an index to prioritize surgical resection. Especially, in patients with Type-4 enhancement pattern, we strongly recommend surgical resection.

In conclusion, the present study demonstrated a strong relationship between Type-4 and -3 enhancement pattern and malignant characteristics of HCC. The management of HCC with Type-4 and -3 enhancement pattern should include a thorough therapeutic approach including surgical resection.

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Original Article

Administration of interferon for two or more years decreases early stage hepatocellular carcinoma recurrence rate after radical ablation: A retrospective study of hepatitis C virus-related liver cancer

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Background: Since hepatocellular carcinoma often recurs after surgical resection or radiofrequency ablation, we analyzed a retrospective large cohort of patients with small hepatocellular carcinoma caused by hepatitis C virus (HCV).

Methods: Among 379 patients with HCV RNA-positive small hepatocellular carcinoma (multiple up to three nodules, 3 cm or less each), 77 received interferon-alpha injection and 302 received no anti-viral therapy.

Results: Four patients (5.2%) attained sustained virological response (SVR). Cumulative recurrence rates in the treated and untreated groups were 41.1% and 57.5% at the end of the third year, and 63.0% and 74.5% at the fifth year, respectively ($P = 0.013$). Fifth year-recurrence rates in treated group were 25.0% in SVR, 85.7% in biochemical response, 71.1% in no response, and 46.7% in patients with continuous administration. When four patients with SVR were excluded, recurrence rates in short-term interferon therapy (<2 years) and long-term therapy (≥2 years) were 46.2% and 39.3% at the third year, and 66.2% and 57.4% at the fifth year, respectively ($P = 0.012$). Multivariate analysis showed that long-term interferon therapy significantly decreased recurrence rate (hazard ratio for interferon <2 years 0.80, interferon ≥2 years 0.60, $P = 0.044$), after adjustment with background covariates including indocyanine green retention rate ($P = 0.018$), alpha-fetoprotein ($P = 0.051$), and tumor treatment ($P = 0.066$).

Conclusion: A long-term administration of low-dose interferon significantly decreased recurrence of hepatocellular carcinoma after surgical resection or radiofrequency ablation.

Key words: hepatitis C, hepatocellular carcinoma, interferon, prevention, recurrence

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) remains one of the most common cancers, and cause of cancer death, worldwide. Since the recurrence rate of HCC is high even after potentially curative therapies with surgical resection or radiofrequency ablation (RFA) therapy, suppression of recurrence is of great importance for prolonging the life of patients with hepatitis C virus (HCV)-related liver disease. This high recurrence rate, after curative therapy, was explained by occult intra-hepatic metastasis of HCC or by multi-centric carcinogenesis in the setting of chronic viral hepatitis or liver cirrhosis.[1,2]

Interferon (IFN) is effective in reducing hepatocellular carcinogenesis rate through suppression of necro-inflammatory process and in eliminating HCV in some patients with chronic hepatitis C and cirrhosis. Although IFN proves to be valuable in suppression of the risk of carcinogenesis in many literatures,[3–5] only several reports mentioned the efficacy of IFN in the suppression of tumor recurrence or in prolongation of survival period after ablation of HCC[6–12]. We once

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demonstrated the preventive activity of HCC recurrence by IFN-beta in a randomized controlled trial,[5] but intravenous type of IFN-beta was not universally available outside Japan in spite of the superiority of tumor suppressive activity to IFN-alpha.[13–17] Some investigators[14–17] showed that IFN acted as an anti-cancer agent in the treatment of HCC *in vivo* and *in vitro*. However, the actual efficacy of IFN in preventing recurrence of HCV-associated HCC in optimally treated patients remains unclear. Since some prospective study failed to demonstrate a beneficial effect of IFN-alpha in cumulative recurrence rate,[11] we analyzed a large cohort of patients for a long period up to 18 years.

To what extent IFN suppresses the recurrence rate of early stage of HCC, we analyzed a large retrospective cohort with and without a long-term administration of IFN-alpha in patients with HCC. The purposes of this study were (i) to evaluate the influence of IFN-alpha on HCC recurrence rate after treatment of an early stage of HCV-related HCC, and (ii) to explore effective ways of IFN administration, if any.

PATIENTS AND METHODS

Study population

A TOTAL OF 729 patients were diagnosed as having HCC associated with HCV-related chronic liver disease from 1990 to 2006 in our hospital. Among them, 379 patients underwent surgical resection or sufficient medical ablation therapy for small HCC (multiple up to three nodules, 3 cm or less each). All were positive for anti-hepatitis C antibody and negative for hepatitis B surface antigen. The consecutive patients were analyzed, who met inclusion criteria of (1) initial diagnosis of HCC (2) early stage of HCC (multiple up to three nodules, 3 cm or less each) (3) potentially curative manner of resection or radiofrequency ablation for HCC, and (4) positive HCV RNA. Exclusion criteria of this study were (1) positive portal vein invasion on imaging of computerized tomography or ultrasonography (2) residual HCC on imaging diagnosis after surgical or medical therapy (3) Child-Pugh score C (4) other etiology of liver disease (hepatitis B, alcoholic, non-alcoholic liver disease, etc.) (5) use of other antiviral agents including interferon-beta (6) use of retinoid derivatives, and (7) concomitant malignant tumor in addition to HCC.

The diagnosis of HCC was established by integrated imagings of ultrasonography, dynamic computerized tomography (CT), magnetic resonance imaging (MRI). To exclude additional small HCC nodules in the liver, computerized tomographic hepatic arteriography (CT-HA) and computerized tomographic arterioportography (CT-AP) were also performed in 356 patients (93.9%). Among the consecutive 379 patients with surgical resection or sufficient radiofrequency ablation for HCC, 77 (20.3%) patients received intermittent IFN-alpha injection two or three times a week for 6 months or longer, mainly after the year of 1995 when this medication became available for use in Japan: Two (3.4%) of 59 patients received IFN therapy during 1990–1994, 21 (21.2%) of 99 patients during 1995–2000, and 54 (24.2%) of 223 patients during 2001–2006, respectively. The other 302 patients did not receive IFN therapy or other anti-viral therapy. None of the patients received any other anti-viral or anti-carcinogenic treatment including nucleoside analogues. We therefore, performed this analytical study as a retrospective cohort study.

Clinical background and laboratory data

Table 1 summarizes the profiles and laboratory data of the IFN group (group A) and the untreated group (group B) at the time of diagnosis of HCC. The median age in the IFN group was lower than that of the untreated group by 3 years, but the other features were not different between the two groups regarding demography, liver function, state of HCC, and treatment of HCC.

Interferon treatment and judgment of the effect

Seventy-seven patients underwent IFN therapy after treatment of HCC. IFN therapy was usually initiated within several months after ablation of HCC, and a median period from HCC treatment to initiation of IFN was 5.6 months.

All the patients received IFN-alpha (natural or recombinant): Seven received interferon plus ribavirin combination therapy, and 68 underwent interferon monotherapy. Ten patients (13.0%) underwent interferon therapy for 6 months or less, 15 patients (19.5%) for 7 to 12 months, 13 patients (16.8%) for 13 to 24 months, 28 (36.4%) for 25 to 60 months, and the remaining 11 (14.3%) for a prolonged period of 61 months or longer. As a whole, a median dose of 242 million units was administered during the median period of 24.2 months. A total of 50.6% of all the patients received IFN for 2 years or longer.

Judgment of IFN effect was classified according to elimination of HCV RNA and alanine aminotransferase (ALT) value at a time of 6 months after the end of the

Table 1 Profiles and laboratory tests of the patients with and without interferon

Groups/characteristic	Group A (interferon)	Group B (none)	*P**
Patients characteristics			
N	77	302	
Age (year) (median, range)	63 (43–77)	66 (39–87)	0.003
Sex (Male/Female)	46/31	191/111	0.57
Positive HBs antigen	0	0	NS
Positive HCV antibody	77 (100%)	302 (100%)	NS
Positive HCV-RNA	77 (100%)	302 (100%)	NS
Cancer characteristics before treatment			
Number of nodules			0.89
Solitary	63	260	
Two	11	33	
Three	3	9	
Size of maximal tumor (median, range)	18 (5–30)	18 (8–30)	0.50
Vascular invasion on imaging	0	0	NS
Cancer therapy			
Surgery	35 (45.5%)	146 (48.3%)	0.65
Radiofrequency ablation	42 (54.5%)	156 (51.7%)	
Laboraory findings (median, range)			
Albumin (g/dl)	3.6 (2.4–4.3)	3.6 (2.4–4.5)	0.80
Bilirubin (mg/dl)	1.0 (0.3–2.5)	1.0 (0.2–3.3)	0.96
Aspartic transaminase (IU)	54 (16–311)	54.5 (13–191)	0.94
Alanine transaminase (IU)	57 (12–273)	54 (11–230)	0.89
Platelet (×1000/cmm)	100 (20–272)	110 (20–256)	0.85
ICG R15 (%)	25 (1–75)	27 (2–78)	0.58
Alpha-fetoprotein (mg/L)	22 (3–1411)	22 (1–4950)	0.28
DCP (AU/L)	19 (11–635)	17 (0–1470)	0.50

*Non-parametric test (χ^2 test or Mann–Whitney *U*-test). DCP, des-gamma-carboxyprothrombin; ICG R15, indocyanine green retention test at 15 minutes.

treatment. Sustained virological response (SVR) was defined as persistent disappearance of HCV RNA after therapy, biochemical response (BR) as normal ALT values (40 IU/L or less) without elimination of HCV RNA for at least 6 months after therapy, and no response (NR) as persistently abnormal or only transient normalization of ALT for less than 6 months.

Follow-up and diagnosis of HCC

Physicians examined the patients every 4 weeks after entry to the study. Liver function tests and hematologic and virologic tests were conducted every month. To diagnose recurrent HCC nodules at an early stage, imaging studies were performed every 3 months, using ultrasonography and computerized tomography. Alfa-fetoprotein and des-gamma-carboxyprothrombin were also assayed bimonthly. When angiography demonstrated a characteristic hypervascular nodule, it was usually a specific finding for HCC in these follow-up patients, and histological confirmation was usually not required in the majority of these HCC patients. Most of the "angiographically-diagnosed HCC" showed intrahepatic multiplicity and pathognomonic findings of capsule formation or nodule-in-nodule appearance, or even portal vein invasion. If angiography did not show any hypervascular stain in a small hepatic nodule, histological study was always performed.

A total of 8 patients could not continue the IFN treatment due to side effects, following studies of tumor recurrence and survival were analyzed on an intention-to-treat basis.

Eight patients were lost to follow-up: 2 in IFN group and 6 in untreated group. Treated and untreated patients were followed at intervals of one month for a median observation period of 4.6 years, ranged from 0.1 to 18.4 years: 5.6 years in interferon group and 4.2 years in untreated group. The date of the last follow-up for this study was 30th August, 2009.

The end point of the study was tumor recurrence after treatment.

Statistical methods

The obtained clinical data were analyzed on an intention-to-treat basis. Standard statistical measures and procedures were used in the analysis. The chi-square, Fisher's exact test, and Mann-Whitney's *U*-tests were used to analyze the differences of background features and biochemical data between the two groups. HCC recurrence rate was calculated from the day of HCC treatment in both groups, using the Kaplan-Meier technique. The differences in recurrence curves were tested using the log-rank test. Cox proportional hazard analysis was performed to evaluate independent predictors of tumor recurrence after treatment. A *P*-value of less than 0.05 with two-tailed analysis was considered significant. Data analysis was performed using the computer program SPSS version 11 (SPSS Inc. Chicago, IL).[18]

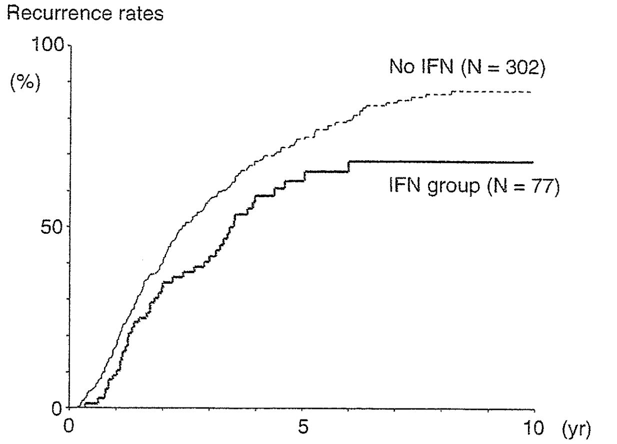

Figure 1 Cumulative recurrence rates of hepatocellular carcinoma in patients with and without interferon therapy.

RESULTS

Effects and toxicity of interferon

SVR WERE FOUND in 4 (5.2%) of 77 patients in IFN-treated group and none in untreated group. BR were found in 7 (9.1%), NR in 36 (46.8%), and undetermined judgment due to continuous administration currently in 30 (39.0%).

Almost all of the patients given IFN therapy showed varied degrees of fever, chills, myalgias, headache, and general malaise after the first injection of IFN. Most of patients revealed a various degree of leukocytopenia and thrombocytopenia. A total of 8 patients (10.4%) withdrew from IFN therapy before development of tumor recurrence. Three patients with depression or psychosis ceased the IFN therapy. The other 5 patients also stopped IFN administration because of varied degree of adverse effects: thrombocytopenia, insomnia, slight degree of hepatic encephalopathy, minor episode of cerebrovascular accident, and generalized fatigue with significant weight loss.

Recurrence rates of hepatocellular carcinoma

During the median observation period of 4.6 years, HCC recurred in 264 patients (69.7%); 45 patients belonged to the IFN group, and the other 219 patients to the untreated group. The cumulative recurrence rate in all patients was 16.2% at the end of the first year following the surgical treatment of HCC, 39.6% at the second year, 54.5% at the third year, 73.0% at the fifth year, 82.8% at the seventh year, and 85.5% at the 10th year. Crude recurrence rates in the IFN group and untreated group were 9.1% and 18.8% at the end of the first year, 33.3% and 42.1% at the second year, 41.1% and 58.1% at the third year, 63.0% and 76.6% at the 5th year, 68.5% and 86.2% at the seventh year, and 68.5% and 93.2% at the 10th year, respectively (Fig. 1). The recurrence rate in the IFN group was significantly lower than that of the untreated group (log-rank test: $P = 0.013$).

In univariate analysis, factors associated with tumor recurrence were explored in all of the 379 patients *en masse*. HCC recurrence was associated with high indocyanine green retention rate at 15 minutes (ICG R15) ($P = 0.004$), low albumin concentration ($P = 0.005$), no IFN therapy ($P = 0.010$), prolonged prothrombin time ($P = 0.041$), and RFA as treatment for HCC ($P = 0.046$).

Multivariate analysis disclosed that recurrence of HCC was independently associated with IFN therapy (hazard ratio 0.66, $P = 0.020$), a high ICG R15 of 20% or more (hazard ratio 1.43, $P = 0.008$), and RFA therapy (hazard ratio 1.32, $P = 0.041$). IFN treatment proved to prevent tumor recurrence after ablation of HCC in those patients with an early stage of HCC (Table 2).

Recurrence rates according to interferon effect

Tumor recurrence rates were evaluated according to judgment of IFN effect in the treated group: SVR ($n = 4$), BR ($n = 7$), NR ($n = 36$), continued IFN administration (N = 30), and untreated group.

Table 2 Independent factors affecting the recurrence of hepatocellular carcinoma after curative treatment

Factors	Category	Hazard ratio (95% CI)	*P*
Interferon therapy	1: No	1	
	2: Yes	0.66 (0.46–0.94)	0.020
ICG R15	1: <20%	1	
	2: ≥20%	1.43 (1.10–1.85)	0.008
Cancer treatment	1: Surgical resection	1	
	2: PRFA	1.32 (1.01–1.72)	0.041

ICG R15, indocyanine green retention rate at 15 minutes; PRFA, percutaneous radiofrequency ablation therapy.

Recurrence rates in the subgroup of SVR, BR, NR, continued administration, and untreated patients were 0%, 0%, 6.7%, 12.5%, and 18.8% at the end of the first year, 25.0%, 28.6%, 37.0%, 25.3%, and 42.1% at the second year, 25.0%, 42.9%, 52.0%, 32.6%, and 58.1% at the third year, 25.0%, 85.7%, 71.1%, 46.7%, and 76.6% at the fifth year, and 25.0%, 100%, 79.3%, 54.3%, and 86.2% at the seventh year, respectively (Fig. 2a). The recurrence rates in a combined group of SVR and continued IFN administration were significantly lower than those in a combined cohort of the other groups (log-rank test, $P = 0.0005$) (Fig. 2b). The recurrence rates of the former and the latter groups were 30.6% and 56.7% at the end of the third year, 43.3% and 75.0% at the fifth year, and 43.3% and 84.7% at the seventh year, respectively.

Recurrence rates according to length of interferon administration

Since HCV RNA eradication (SVR) was found in only four patients, significance of prolonged administration of IFN was assessed in those patients with positive HCV RNA during therapy ($n = 73$).

Recurrence rates in the subgroup with a long IFN therapy of 2 years or more ($n = 39$), a short IFN therapy of less than 2 years ($n = 34$), and in the untreated patients ($n = 302$) were 8.7%, 7.1%, and 18.8% at the end of the first year, 23.9%, 40.2%, and 42.1% at the second year, 39.3%, 46.2%, and 58.1% at the third year, 57.4%, 66.2%, 76.6% at the fifth year, and 66.0%, 77.5%, and 86.2% at the seventh year, and 66.0%, 77.5%, and 93.2%, respectively (Fig. 3). The recurrence rates in the long IFN-therapy group was significantly lower than those with a short therapy group and untreated group (log-rank test, $P = 0.012$).

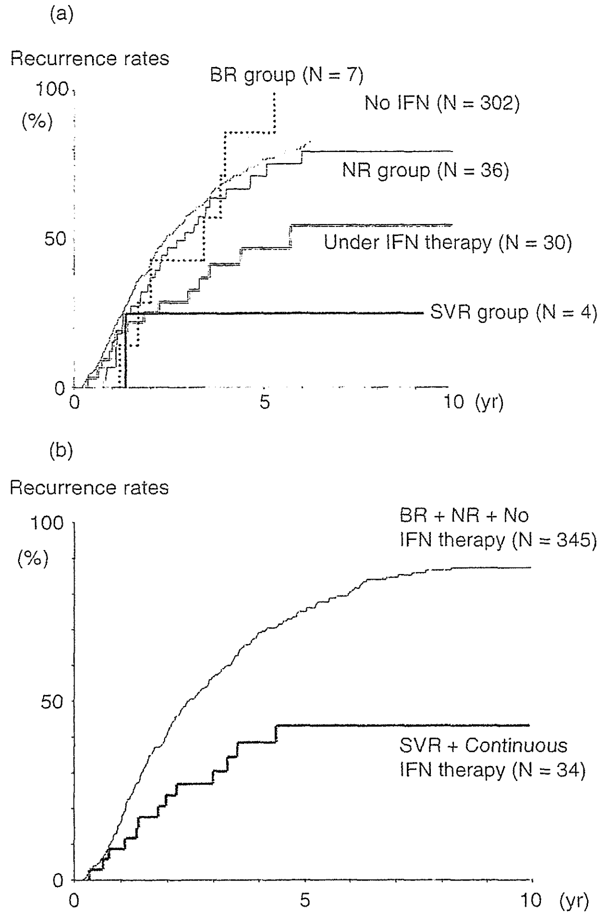

Figure 2 (a) Cumulative recurrence rates of hepatocellular carcinoma according to the effect of interferon. (b) Cumulative recurrence rates of hepatocellular carcinoma in a combined group of sustained virological response and continuous interferon administration and those in a combined group of biochemical response, no response, and no interferon therapy.

To elucidate the impact of a long-term administration of IFN in the prevention of HCC recurrence, multivariate hazard analysis was introduced in the IFN-treated patients without SVR effect ($n = 73$) and the untreated patients ($n = 302$). Multivariate analysis showed that a long-term IFN therapy significantly lowered the recurrence rate in patients with HCV-related HCC: hazard ratios of short-term therapy less than two years and long-term therapy for two years or longer of 2 years or more were 0.80 and 0.60, respectively ($P = 0.044$). The other covariates for recurrence rate included high ICGR15, high AFP value, and initial treatment modality (Table 3).

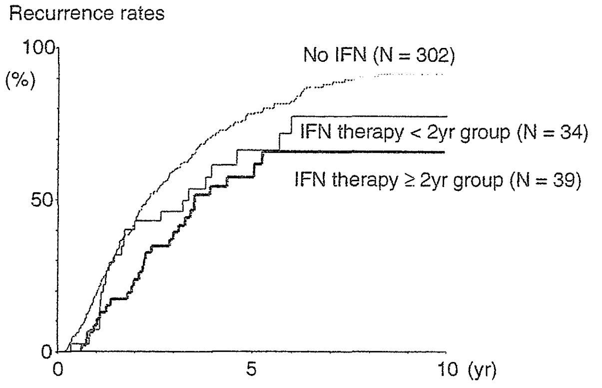

Figure 3 Cumulative recurrence rates of hepatocellular carcinoma in patients without sustained virological response. Recurrence rates were assessed according to the length of interferon administration.

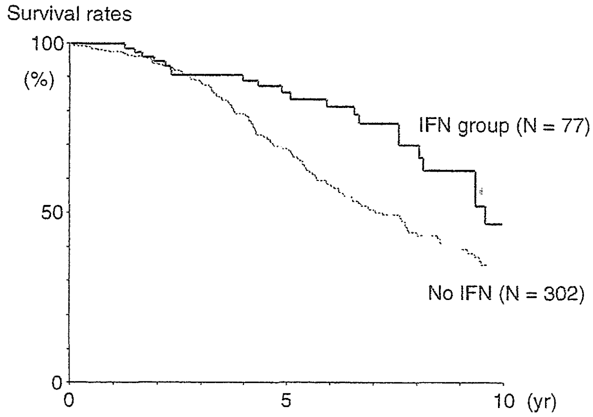

Figure 4 Overall survival rates of patients with or without interferon therapy after potentially curative therapy for hepatocellular carcinoma.

Overall survival rates

A total of 159 patients died during the observation period: 23 (29.9%) in the IFN-treated group and 136 (45.0%) in the untreated group. Crude survival rates of patients after potentially curative therapy for HCC in the IFN-treated and untreated patients were 90.7% and 88.5% at the end of the third year, 85.6% and 68.8% at the fifth year, 76.5% and 50.9% at the seventh year, and 47.0% and 34.7% at the tenth year, respectively (Fig. 4). The survival rates of IFN-treated group were significantly higher than that of those of untreated group (log-rank test, $P = 0.0044$).

Table 3 Independent factors affecting the recurrence of hepatocellular carcinoma after curative treatment, according the length of interferon administration†

Factors	Category	Hazard ratio (95% CI)	*P*
Interferon therapy	1: None	1	0.044
	2: <2 years	0.80 (0.51–1.24)	
	3: ≥2 years	0.60 (0.40–0.91)	
ICG R15	1: <20%	1	
	2: ≥20%	1.37 (1.06–1.77)	0.018
Alpha-fetoprotein	1: <40 mg/L	1	
	2: ≥40 mg/L	1.31 (1.00–1.71)	0.051
Cancer treatment	1: Surgical resection	1	
	2: PRFA	1.28 (0.98–1.65)	0.066

†Four patients with sustained virological response were excluded in the analysis. ICG R15, indocyanine green retention rate at 15 minutes; PRFA, percutaneous radiofrequency ablation therapy.

Multivariate analysis showed overall survival rates were significantly affected by interferon therapy ($P = 0.014$), albumin concentration ($P = 0.015$), platelet count ($P = 0.014$), and ICG R15 ($P = 0.0068$) (Table 4). Hazard ratio for death in those patients with IFN therapy was 0.55 (95% confidence interval 0.34–0.88).

DISCUSSION

ALTHOUGH THIS STUDY was not a prospective, randomized one, there was no significant difference in the background features and laboratory tests except for age, between the treated and untreated groups. This study was based on a long-term observation for a median of 4.6 years, and the number of patient was sufficiently large for sensitivity and reliabil-

Table 4 Independent factors affecting the survival rates of patients with hepatocellular carcinoma after curative treatment

Factors	Category	Hazard ratio (95% C.I.)	*P*
Interferon therapy	1: None	1	
	2: Yes	0.55 (0.39–0.88)	0.014
ICG R15	1: <20%	1	
	2: ≥20%	1.65 (1.15–2.37)	0.0068
Albumin	1: <3.5 g/dl	1	
	2: ≥3.5 g/dl	0.64 (0.44–0.92)	0.015
Platelet count	1: <100,000/mm3	1	
	2: ≥100,000/mm3	0.64 (0.45–0.91)	0.014

ICG R15, indocyanine green retention rate at 15 minutes.

ity for the data regarding recurrence and survival. We also analyzed only those patients with "an early stage" of HCC to minimize the influence of tumor recurrence due to small and undetectable metastatic tumors often found in patients with large or multiple tumors. In the establishment of the diagnosis of early stage of HCC, more than 93% of the patients underwent intensive imaging investigation with CT-HA and CT-AP, together with dynamic CT and dynamic MRI study. Therefore, the diagnosis of a few numbers with small-sized tumor was sufficiently reliable in the study.

This cohort study indicated IFN suppressed the recurrence rate after potentially curative treatment of HCC caused by HCV. Indeed SVR effect after IFN therapy did decrease recurrence rate, majority of patients were not tolerable for a large amount of IFN administration with or without ribavirin because of an old age or advanced liver disease with significant cytopenia. This study demonstrated interferon significantly decreased tumor recurrence rate, irrespective of "anti-viral interferon effect". This study also revealed relatively "rapid" anti-carcinogenic effect compared with the results of a study performed by Mazzaferro *et al.*[11] Most cases of late-phase recurrence are thought to be due to metachronous multicentric, or *de novo*, carcinogenesis. This is quite understandable, because the remaining liver, often cirrhotic, is still at high risk of carcinogenesis.

Our study also emphasizes that long-term, low-dose, intermittent administration of IFN was useful in prevention of tumor recurrence in patients without SVR, with a hazard ratio of 0.60 compared to those with no IFN administration.

The reason why IFN administration suppresses the recurrence rate in HCV-related liver disease remains uncertain. One reason may be anti-tumor activity in the early stage of HCC and another antiviral or anti-necroinflammatory effect for hepatitis. Our data did not disclose the relationship between ALT normalization and prevention of cancer recurrence, since the number of BR group was small ($N = 7$), and since many patients were currently continuing IFN therapy with normal ALT. Human lymphoblastoid IFN alpha has a powerful anti-proliferative effect on human hepatoma cell line PLC/PRF/5, both *in vitro* and *in vivo*, after implantation in nude mice.[19] Lai *et al.*[20] showed IFN induced objective tumor regression in a significant number of patients with inoperable hepatocellular carcinoma in a randomized controlled trial. Considering the short period to recurrence in our study, IFN may have a direct anti-tumor effect on clinically undetectable HCC. Wang *et al.*[21] showed anti-angiogenesis activity of IFN, and Wu *et al.*[22] demonstrated suppression of vascular endothelial growth factor and inhibition of tumor signaling pathways. Moreno *et al.*[23] reported that IFN induced remission of liver fibrosis irrespective of anti-viral effect. Control of necro-inflammatory process may therefore induce a suppression of the growth process of HCC. Tarao *et al.*[24] reported that high aminotransferase activity resulted in an increased HCC recurrence rate. A randomized controlled trial of IFN for patients with cirrhosis showed that IFN therapy decreased the HCC appearance rate in association with disappearance of HCV-RNA[3]. We also demonstrated IFN suppressed the carcinogenesis rate in patients with chronic hepatitis type C[5]. Taking into account that hepatocellular carcinogenesis in HCV-related chronic liver disease is accelerated by a prolonged period of necro-inflammation of hepatocytes, IFN is hypothesized to diminish the HCC appearance rate through suppression of excessive replication and turnover of hepatocytes. Since the entire process of hepatocellular carcinogenesis from initial transformation of a hepatocyte to detectable growth is considered to take at least several years, the influence of IFN on the carcinogenesis rate or recurrence rate might not be evaluated in as short period of three years or less. Aside from the exact mechanism of the prevention of HCC recurrence, our study demonstrated an encouraging result in the medical management of HCC.

Since these results were not generated from a prospective randomized study, we tried to adjust background biases using multivariate analysis between the treated and untreated group, if any. We should realize the significance of the decrease in recurrence rate by IFN therapy with a hazard ratio by 0.66. Cost-effectiveness and individual and social expenses should be evaluated in detail between those patients with reduction of recurrence rate and those with high recurrence rate with additional tumor ablation therapy. Considering that a long-term prospective trial with and without IFN arm seemed very difficult to perform ethically and economically, we should further accumulate these comparative studies and consider the efficacy of weekly injections of pegylated IFN and adequate dose and length of IFN therapy. Identification of suitable cases for IFN therapy and exact mechanisms of suppression of tumor recurrence are of paramount importance for increasing number of patients with HCC.

In conclusion, long-term intermittent IFN therapy reduced HCC recurrence rate in patients with HCV-related HCC.

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CLINICAL RESEARCH STUDY

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Diabetes Enhances Hepatocarcinogenesis in Noncirrhotic, Interferon-treated Hepatitis C Patients

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ABSTRACT

BACKGROUND: This retrospective cohort study assessed the impact of diabetes mellitus on hepatocarcinogenesis and determined the predictors of hepatocarcinogenesis in noncirrhotic, interferon-treated patients with hepatitis C virus infection.

METHODS: A total of 2058 hepatitis C virus-positive, noncirrhotic patients treated with interferon were enrolled. The median follow-up period was 6.7 years. The primary end point was the onset of hepatocellular carcinoma. The cumulative rate of new hepatocellular carcinoma cases was computed by the Kaplan–Meier method and Cox proportional hazard analysis according to diabetic state and response to interferon therapy.

RESULTS: The cumulative rates of hepatocellular carcinoma in diabetic patients (3.2% at 4 years, 8.5% at 8 years, and 24.4% at 12 years) were significantly higher than those of nondiabetic patients (1.3% at 4 years, 2.2% at 8 years, and 5.6% at 12 years, $P<.001$). In patients with a sustained virologic response, diabetes had no significant effect on the rate of hepatocarcinogenesis. In contrast, the rate in patients with a nonsustained virologic response was significantly higher in diabetic than in nondiabetic patients. Multivariate analysis identified lack of sustained virologic response (hazard ratio [HR] 7.28; 95% confidence interval [CI], 3.28-16.15; $P<.001$) and diabetes as independent risk factors for hepatocarcinogenesis (HR 2.00; 95% CI, 1.05-3.84; $P=.036$).

CONCLUSIONS: Our results highlight the enhancing effect of diabetes mellitus on hepatocarcinogenesis in noncirrhotic, interferon-treated patients with hepatitis C virus. The sustained virologic response induced by interferon therapy eliminates the influence of diabetes and markedly reduces the rate of hepatocarcinogenesis in such patients.

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KEYWORDS: Diabetes; Hepatocellular carcinoma; Interferon; Sustained virologic response

Hepatitis C virus is a common cause of chronic liver disease worldwide and a major risk of hepatocellular carcinoma.[1-10] The estimated incidence of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis is 5% to 10% per year, and hepatocellular carcinoma is one of the major causes of death, especially in Asian countries.[10] In recent years, diabetes mellitus has attracted attention as a risk factor of hepatocarcinogenesis. Evidence suggests that in addition to various factors that affect liver fibrosis and hepatocarcinogenesis, diabetes and obesity are independent risk factors for the progression of liver fibrosis and development of hepatocellular carcinoma in chronic hepatitis C.[10-15] The majority of such clinical studies included patients with liver cirrhosis. However, for pathophysiologic reasons, liver cirrhosis increases the probability of impaired glucose tolerance. Therefore, in studies of cirrhotic patients,

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it is difficult to pinpoint the true effects of diabetes on hepatocarcinogenesis. On the other hand, we recently reported that a sustained virologic response to interferon therapy reduces the incidence of type 2 diabetes onset in chronic hepatitis C.[16] Thus, there is a gap in our knowledge on the exact effect of diabetes on hepatocarcinogenesis in interferon-treated patients.

The present retrospective study was designed to determine the effects of diabetes on hepatocarcinogenesis in noncirrhotic, interferon-treated patients with chronic hepatitis C virus infection, including the effects of viral clearance on diabetes-related hepatocarcinogenesis.

CLINICAL SIGNIFICANCE

- The hepatocarcinogenesis rate from first interferon therapy for noncirrhotic patients with chronic hepatitis C was 2 times greater in diabetic cases than in nondiabetic cases.
- Diabetes was an independent predictive factor of hepatocellular carcinoma in interferon-treated, noncirrhotic patients with chronic hepatitis C virus.
- In patients without a sustained virologic response from interferon therapy, the hepatocarcinogenesis rate of diabetic cases was approximately 15 times greater than that of nondiabetic, noncirrhotic patients with chronic hepatitis C and a sustained virologic response.

PATIENTS AND METHODS

Study Population

In this retrospective cohort study, we obtained the medical records of all patients in our database who had received interferon therapy for chronic hepatitis C between 1987 and 2007 at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these patients, 2058 satisfied the following criteria: 1) no evidence of diabetes after termination of interferon; 2) laparoscopy or liver biopsy performed before initiation of interferon therapy confirmed the lack of liver cirrhosis; 3) measurement of serologic type and hepatitis C virus viral load before initiation of interferon therapy; 4) platelet count of $\geq 10 \times 10^4$/mL; 5) negativity for hepatitis B surface antigen, antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay or spot hybridization; 6) no underlying metabolic disease, such as hemochromatosis, alpha-1-antitrypsin deficiency, or Wilson disease; 7) no underlying systemic disease, such as systemic lupus erythematosus or rheumatic arthritis; 8) no evidence of hepatocellular carcinoma on ultrasonography or computed tomography before the initiation of interferon therapy; and 9) follow-up period of $\geq$ 24 weeks.

All patients who did not show a sustained virologic response and persistently high alanine aminotransferase level (normal range: 6-50 IU/L) received liver protection therapy, consisting mainly of glycyrrhizin and ursodeoxycholic acid (300-600 mg/d), during this research.

In all patients, the observation starting point was the time of initiation of the first interferon treatment. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. The study was approved by the institutional review board of the Toranomon Hospital.

Background and Laboratory Data

Table 1 (available online) summarizes the clinical profile and laboratory data of 2058 interferon-treated patients with chronic hepatitis C. The male to female ratio was 1.78:1. Of 2058 patients, 164 (8.0%) were alcoholic (total alcohol intake > 500 kg until the initiation of interferon therapy). Before the initiation of interferon therapy, 104 patients (5.1%) were known diabetics. Furthermore, 71.2% patients had a high viral titer (low viral load; Amplicor < 100 KIU/mL [Cobas Amplicor HCV Monitor Test, version 2.0, Roche Molecular Systems, Inc, Belleville, NJ] or probe < 1 MEq/mL [branched DNA probe assay; version 2,0; Chiron, Daiichi Kagaku, Tokyo], high viral load; Amplicor $\geq$ 100 KIU/mL or probe $\geq$ 1 MEq/mL).

Type of Interferon and Assessment of Response to Interferon Therapy

Among 2058 patients treated with interferon, 1207 (58.6%) received interferon-α, 329 (16.0%) received interferon-β, and the remaining 522 (25.4%) received a combination therapy of interferon and ribavirin. The response to interferon therapy was assessed on the basis of sustained virologic response (sustained virologic response was regarded as elimination of hepatitis C virus-RNA at 6 months after the termination of interferon treatment). After interferon therapy, 52.5% of the patients showed sustained virologic response.

Markers of Hepatitis B and C Viruses

Anti-hepatitis C virus was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II; Abbott Laboratories, North Chicago, IL). Hepatitis C virus-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, version 2.0; Roche, Tokyo, Japan) or the branched DNA probe assay (branched DNA probe assay; version 2.0; Chiron). Hepatitis B surface antigen was tested via radioimmunoassay (Abbott Laboratories, Detroit, MI). The used serum samples were stored at −80°C at the first consultation. Diagnosis of hepatitis C virus infection was based on detection of serum hepatitis C virus antibody and hepatitis C virus RNA.

Histopathologic Examination of the Liver

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim-Silverman needle with an internal diameter of 2 mm (Tohoku University, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin–eosin, Masson's trichrome, silver impregnation, and pe-

Table 1 Characteristics of 2058 Noncirrhotic, Interferon-Treated Patients with Chronic Hepatitis C Virus Infection at the Initiation of Interferon and Efficacy

Parameter	(n = 2058)
Gender (M:F)	1317:741
Age (y)†	50 (15-72)
Histopathologic grade (F1-2:F3)	1916:142
Total ethanol intake (≥500 kg) (yes/no)	164:1894
Follow-up period (d)†	2443 (170-7562)
Albumin (g/dL)†	4.2 (2.3-5.3)
Total bilirubin (mg/dL)†	0.7 (0.1-11.7)
AST (IU/L)†	68 (21-488)
ALT (IU/L)†	77 (5-1212)
γ-GTP (IU/L)†	43 (5-805)
Platelet count ($\times 10^4/\mu$L)†	18.3 (10.0-48.1)
AFP (μg/L)†	4 (1.0-780)
Fasting/casual plasma glucose (mg/dL)†	96 (66-376)/100 (49-415)
Diabetes (yes/no)	104:1954
Total cholesterol (mg/dL)†	172 (102-348)
Triglyceride (mg/dL)†	89 (32-325)
LDL cholesterol (mg/dL)†	105 (39-209)
HDL cholesterol (mg/dL)†	46 (8-107)
IFN (monotherapy/combination therapy)	1536:522
HCV serologic group (1:2)	1310:748
Viral load (low:high)	592:1466
Efficacy of IFN therapy acquired viral elimination* (yes:no)	1081:977

AST = aspartate aminotransferase; ALT = alanine aminotransferase; γ-GTP = gamma-glutamyl transpeptidase; AFP = alpha-fetoprotein; LDL = low-density lipoprotein; HDL = high-density lipoprotein; IFN = interferon; HCV = hepatitis C virus.

*Viral elimination means sustained virologic response.

†Expressed as median (minimum, maximum).

riodic acid-Schiff after diastase digestion. All specimens for examination contained at least 6 portal areas. Chronic hepatitis was diagnosed on the basis of histopathologic assessment according to the scoring system of Desmet et al.[17]

Definition of Diabetes Mellitus

Diabetes was diagnosed by the use of the 2003 criteria of the American Diabetes Association.[18] These criteria include 1) casual plasma glucose ≥ 200 mg/dL; 2) fasting plasma glucose ≥ 126 mg/dL; and 3) 2-hour post-glucose (oral glucose tolerance test) ≥ 200 mg/dL.

Follow-up and Diagnosis Procedure of Hepatocellular Carcinoma

The starting time of follow-up was the point of the initiation of the first interferon treatment. After that, patients were followed up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each visit together with regular checkups. Ultrasonography or computed tomography were performed every 3 to 6 months. The diagnosis of hepatocellular carcinoma was performed by biochemical examination (include alpha-fetoprotein and des-gamma carboxyprothrombin) and triple-phase dynamic computed tomography study. The number of cases lost to follow-up was 147 patients (7.1%) in this group.

Statistical Analysis

The cumulative rate of hepatocarcinogenesis (new cases of hepatocellular carcinoma) was calculated from the point of initiation of the first interferon treatment to the diagnosis of hepatocellular carcinoma using the Kaplan–Meier method. Differences in the development of hepatocellular carcinoma between different groups were tested using the log-rank test. Independent factors associated with the rate of hepatocellular carcinoma were analyzed by the Cox proportional hazard model. The following 19 variables were analyzed for potential covariates for incidence of hepatocellular carcinoma at the time of first interferon treatment initiation at Toranomon Hospital: gender, age, histologic stage of the liver, amount of total ethanol intake, existence of diabetes, viral serologic group, viral load, existence of sustained viral clearance by interferon therapy, serum concentration of albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, alpha-fetoprotein, total cholesterol, triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and platelet count. A P value of less than .05 in a 2-tailed test was considered significant. Data analysis was performed using the Statistical Package for the Social Sciences version 11.0 for Windows (SPSS, Inc, Chicago IL).

RESULTS

Incidence of Hepatocellular Carcinoma in Noncirrhotic, Interferon-Treated Patients with Chronic Hepatitis C

In this cohort, hepatocellular carcinoma developed in 73 patients (3.5%) during a median observation period of 6.7 years. The cumulative rate of newly diagnosed hepatocellular carcinoma was 1.2% at 4 years, 2.6% at 8 years, and 6.8% at 12 years (Figure 1). The hepatocarcinogenesis rate according to interferon therapy was 2.1% at 4 years, 4.4% at 8 years, and 11.6% at 12 years in patients who did not acquire a sustained virologic response, and 0.7% at 4 years, 1.0% at 8 years, and 1.6% at 12 years in patients who acquired a sustained virologic response (Figure 2). The cumulative incidence rate of hepatocellular carcinoma was significantly lower in patients who acquired a sustained virologic response than in those who did not (P <.001).

Effect of Diabetes Mellitus on Hepatocarcinogenesis in Noncirrhotic, Interferon-Treated Patients with Hepatitis C

During the follow-up period, 58 of the 1954 nondiabetic patients (3.0%) developed hepatocellular carcinoma, and 15 of the 104

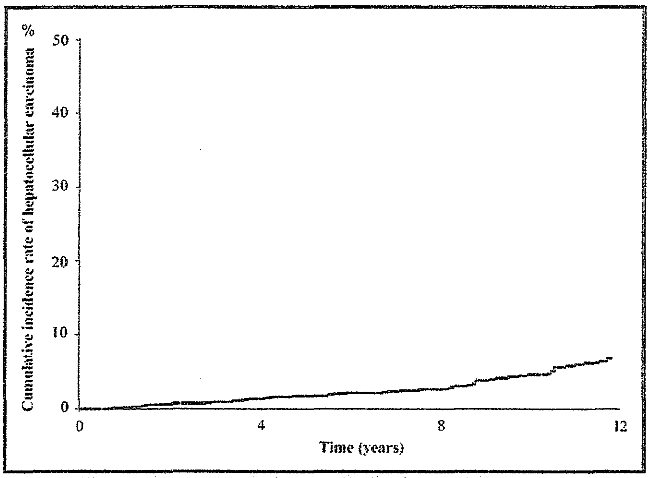

Figure 1 Cumulative rate of development of hepatocellular carcinoma from first interferon therapy in noncirrhotic patients with chronic hepatitis C infection.

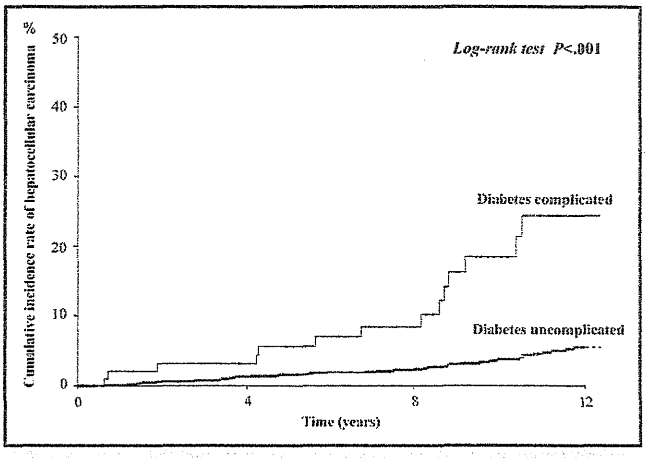

Figure 3 Cumulative rate of development of hepatocellular carcinoma from first interferon therapy in noncirrhotic patients with chronic hepatitis C infection according to the presence or absence of diabetes.

diabetic patients (14.4%) developed hepatocellular carcinoma. The cumulative rate of hepatocellular carcinoma in nondiabetic patients was 1.3% at 4 years, 2.2% at 8 years, and 5.6% at 12 years. For diabetic patients, these rates were 3.2%, 8.5%, and 24.4%, respectively (Figure 3). The cumulative rate of hepatocellular carcinoma was significantly higher in patients with diabetes than those without (P<.001).

Effect of Sustained Virologic Response on Rate of Hepatocarcinogenesis in Noncirrhotic, Interferon-Treated Patients with Hepatitis C According to Presence of Diabetes

In the nonsustained virologic response group (n = 977), 47 (5.2%) of the nondiabetic patients (n = 906) developed hepatocellular carcinoma during the observation period, whereas 14 (19.7%) of diabetic patients (n = 71) developed hepatocellular carcinoma. In the sustained virologic response group (n = 1081), 11 (1.0%) of the nondiabetic patients (n = 1048) developed hepatocellular carcinoma during the observation period, whereas 1 (3.0%) of the diabetic patients (n = 33) developed hepatocellular carcinoma.

Analysis of data according to the efficacy of interferon therapy in diabetic and nondiabetic patients showed that in patients with nonsustained virologic response, the cumulative rate of hepatocellular carcinoma in nondiabetic patients was 1.9% at 4 years, 3.6% at 8 years, and 9.6% at 12 years, whereas in diabetic patients, these rates were 4.7%, 12.1%, and 31.0%, respectively (Figure 4). The cumulative rate of hepatocellular carcinoma was significantly higher in diabetic patients with a nonsustained virologic response than in nondiabetic patients (P<.001). The same analysis in

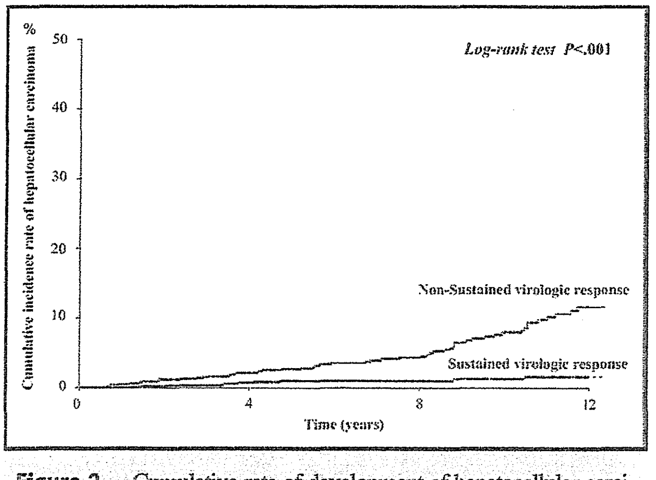

Figure 2 Cumulative rate of development of hepatocellular carcinoma from first interferon therapy in noncirrhotic patients with chronic hepatitis C infection according to effect of interferon therapy.

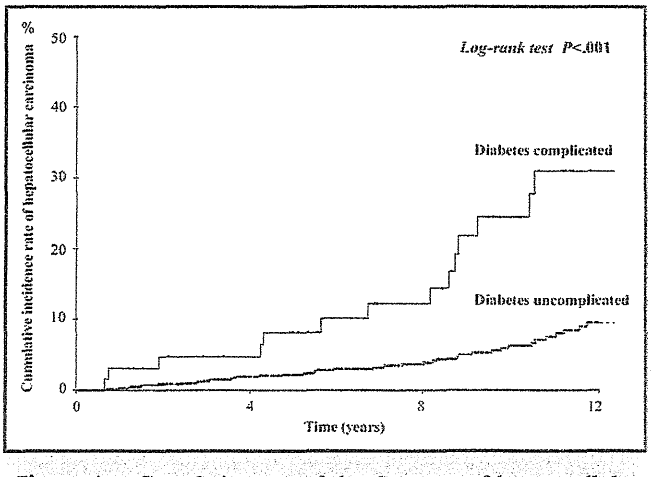

Figure 4 Cumulative rate of development of hepatocellular carcinoma from first interferon therapy in noncirrhotic patients with chronic hepatitis C infection who showed nonsustained virologic response to interferon therapy according to the presence or absence of diabetes.

patients with a sustained virologic response showed a cumulative rate of hepatocellular carcinoma of 0.7%, 1.0%, and 1.7% in nondiabetic patients, and 0.0%, 0.0%, and 0.0% in diabetic patients, respectively (Figure 5). There was no significant difference between diabetic and nondiabetic groups in patients with a sustained virologic response ($P = .249$).

Factors Associated with Rate of Hepatocarcinogenesis

Multivariate Cox proportional hazard analysis revealed the following independent factors for hepatocellular carcinoma development after the initiation of the first interferon therapy in patients who showed a nonsustained virologic response (hazard ratio 7.28; 95% confidence interval [CI], 3.28-16.15; $P < .001$): male (hazard ratio 4.90; 95% CI, 2.47-9.71; $P < .001$), aged ≥ 60 years (hazard ratio 3.28; 95% CI, 1.88-5.74; $P < .001$); aspartate aminotransferase ≥ 50 IU/L (hazard ratio 3.91; 95% CI, 1.81-8.43; $P = .001$); alpha-fetoprotein ≥ 20 mg/L (hazard ratio 2.89; 95% CI, 1.43-5.84; $P = .003$); diabetes (hazard ratio 2.00; 95% CI, 1.05-3.84; $P = .036$); and platelet count $< 17 \times 10^4$/mL (hazard ratio 1.96; 95% CI, 1.11-3.48; $P = .021$) (Table 2, available online).

Rate and Prognosis of Diabetic Patients with Marked Fatty Deposition at First Interferon Initiation

Fourteen of 104 diabetic patients (13.5%) had fatty deposition in hepatic cells of ≥ 30% before the initiation of interferon therapy. Of these 14 patients, 2 were diagnosed with hepatocellular carcinoma during the observation period. One patient underwent liver resection to treat hepatocellular carcinoma, and background liver tissue was liver cirrhosis. One patient did not receive a liver resection; however, this patient's platelet count was approximately $20 \times 10^4/\mu L$ at the time of diagnosis of hepatocellular carcinoma. Thus, severe fibrosis was not suspected in view of this platelet count level.

Rate of Liver Cirrhosis at Hepatocellular Carcinoma Diagnosis

In 23 of 73 patients with hepatocellular carcinoma (31.5%), hepatic resection was performed for treatment. Five of 23 resected patients (21.7%) had liver cirrhosis in background hepatic tissue. The remaining 50 of 73 patients (68.5%) did not receive hepatic resection, and these patients received other nonresection therapy. Because the platelet count level was less than $10 \times 10^4/\mu L$ in 17 of 50 patients without resection (34.0%), liver cirrhosis was suspected. In these patients with histologic or clinical diagnosis of liver cirrhosis at the time of onset of hepatocellular carcinoma, none had a sustained virologic response by interferon therapy.

DISCUSSION

The present study described the incidence of hepatocellular carcinoma after the initiation of interferon therapy in patients with chronic hepatitis C infection. The results indicate that the annual incidence of hepatocellular carcinoma over a prolonged follow-up from first interferon therapy among noncirrhotic patients with hepatitis C virus is 0.3% to 0.5%. The present study was limited by its retrospective design. Moreover, the number of diabetic and nondiabetic patients was markedly different, which might be a potential source of bias. Another limitation of the study was that patients received different types of antiviral therapies for different duration. Thus, we did not evaluate the effect of different interferon regimens but assessed the impact of having or not having a sustained virologic response. This heterogeneity makes it somewhat difficult to interpret the results. On the other hand, the strengths of the present study are the long-term follow-up in a large number of patients treated at the same institution. The present study highlights several new findings with regard to the development of hepatocellular carcinoma after interferon therapy in noncirrhotic patients with hepatitis C virus. First, in patients with a sustained virologic response, diabetes had no significant effect on the rate of hepatocarcinogenesis. Second, in patients with a nonsustained virologic response, the rate of hepatocarcinogenesis was significantly higher in diabetics; diabetes was associated with 2-fold increase in the incidence of hepatocellular carcinoma.

In the present study, no significant difference was noted in the rate of hepatocarcinogenesis in patients with a sustained virologic response with and without diabetes. However, at least 2 studies have described a relationship between diabetes and hepatocellular carcinoma in patients without viral hepatitis.[18,19] In our study, 7.3% of the patients with a nonsustained virologic response were diabetics, compared with approximately 3.0% in the group with a sustained virologic response. These rates were lower than those in the general Japanese population (~15% for men, 9% for women), especially in those with a sustained virologic response. With regard to interferon treatment, previous studies reported that insu-

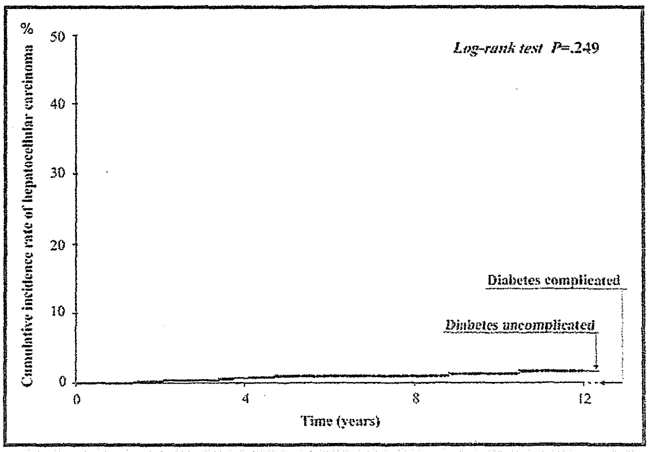

Figure 5 Cumulative rate of development of hepatocellular carcinoma from first interferon therapy in noncirrhotic patients with chronic hepatitis C infection who showed sustained virologic response to interferon therapy according to the presence or absence of diabetes.