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Ⅳ．研究成果の刊行物・別刷

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α-Fetoprotein Levels After Interferon Therapy and Risk of Hepatocarcinogenesis in Chronic Hepatitis C

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The effects of interferon (IFN) treatment and the post-IFN treatment α-fetoprotein (AFP) levels on risk of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C (CHC) are unknown. To determine the relationship between AFP and alanine transaminase (ALT) levels and HCC risk, a cohort consisting of 1,818 patients histologically proven to have CHC treated with IFN were studied. Cumulative incidence and HCC risk were analyzed over a mean follow-up period of 6.1 years using the Kaplan-Meier method and Cox proportional hazard analysis. HCC developed in 179 study subjects. According to multivariate analysis, older age, male gender, advanced fibrosis, severe steatosis, lower serum albumin levels, nonsustained virological response (non-SVR), and higher post-IFN treatment ALT or AFP levels were identified as independent factors significantly associated with HCC development. Cutoff values for ALT and AFP for prediction of future HCC were determined as 40 IU/L and 6.0 ng/mL, respectively, and negative predictive values of these cutoffs were high at 0.960 in each value. The cumulative incidence of HCC was significantly lower in patients whose post-IFN treatment ALT and AFP levels were suppressed to less than the cutoff values even in non-SVR patients. This suppressive effect was also found in patients whose post-IFN treatment ALT and AFP levels were reduced to less than the cutoff values despite abnormal pretreatment levels. *Conclusion*: Post-IFN treatment ALT and AFP levels are significantly associated with hepatocarcinogenesis. Measurement of these values is useful for predicting future HCC risk after IFN treatment. Suppression of these values after IFN therapy reduces HCC risk even in patients without HCV eradication. (HEPATOLOGY 2013;58:1253-1262)

Hepatocellular carcinoma (HCC), one of the most frequent primary liver cancers,[1,2] is the third most common cause of cancer mortality worldwide.[3] Hepatitis C virus (HCV) infection is a common cause of chronic hepatitis, which progresses to HCC in many patients.[4] In the last two decades, interferon (IFN) therapy has been used to treat chronic hepatitis C (CHC) with the goal of altering the natural history of this disease. Although HCV eradication with IFN therapy for CHC has been shown to prevent HCC,[5-9] HCC sometimes develops even after achieving viral eradication.[5] Because the number of sustained virological responders (SVRs) is increasing along with recent advances in the development of effective anti-HCV therapy, it is very important to determine factors responsible for HCC

Abbreviations: AFP, α-fetoprotein; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; CHC, chronic hepatitis C; CT computed tomography; γ-GTP, gamma-glutamyl transpeptidase; HCC, hepatocellular carcinoma; HCV, Hepatitis C virus; IFN, interferon; MRI, magnetic resonance imaging; PEG-, pegylated; RBV, ribavirin; ROC, receiver operator characteristic; SVR, sustained virological response.

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development among IFN-treated patients. However, this information is difficult to determine because of the paucity of large-scale, long-term cohort studies.

The 70-kDa glycoprotein α-fetoprotein (AFP), encoded by a gene located on chromosome 4, is the major serum protein during fetal life.[10] Shortly before birth, AFP is replaced by albumin as the major serum protein,[11,12] and thereafter, serum AFP levels remain extremely low throughout life (<10 ng/mL). Because serum AFP levels are frequently elevated in patients with HCC and germ-cell tumors, measurement of AFP is widely used as a serological marker for these tumors.[8,13] However, AFP levels are sometimes elevated in patients with chronic viral hepatitis and cirrhosis who do not have HCC.[3,19] While one possible explanation for this elevation is liver inflammation, in patients with CHC, the relationship between AFP and markers of liver inflammation such as alanine aminotransferase (ALT) is unclear. Moreover, although several reports suggest that pre-IFN treatment ALT and AFP levels in patients or those in patients who did not undergo subsequent treatment are associated with the development of HCC, it is unclear whether post-IFN treatment ALT and AFP levels are associated with hepatocarcinogenesis in patients with CHC. Hence, to clarify these associations we conducted a large-scale, long-term cohort study of patients with CHC to analyze the influence of ALT and AFP levels before and after IFN therapy on hepatocarcinogenesis in addition to other host and virological factors.

Patients and Methods

Patients. Patients chronically infected with HCV who had histologically proven chronic hepatitis or cirrhosis and had undergone IFN treatment between 1992 and 2010 were enrolled in the cohort. HCC was definitively ruled out by ultrasonography, dynamic computed tomography (CT), and/or magnetic resonance imaging (MRI) on enrollment. Patients were excluded if they had a history of HCC at the time of liver biopsy, autoimmune hepatitis, primary biliary cirrhosis, excessive alcohol consumption (≥50 g/day), hepatitis B surface antigen, or antihuman immunodeficiency virus antibody. Based on these criteria, a total of 2,689 patients were initially enrolled. Of these, 223 (8.3%) patients were excluded from the cohort because of loss to follow-up. In the remaining 2,466 patients, 133 and 515 patients were excluded from this analysis because of short follow-up and retreatment with IFN-based therapy during the follow-up period, respectively. Thus, the cohort comprising 1,818 patients was analyzed in the present study. Written informed consent was obtained from all patients and the Ethical Committee of Musashino Red Cross Hospital approved this study, which was conducted in accordance with the Declaration of Helsinki.

Histological Evaluation. To obtain liver specimens, laparoscopic or ultrasound-guided liver biopsies were performed with 13G or 15G needles, respectively. The median length of specimen was 18 mm (range, 11-41 mm), and the mean number of portal tracts was 17 (range, 9-35). The stage of fibrosis and the grade of inflammatory activity were scored by two pathologists according to the classification of Desmet et al.[24] The percentage of steatosis was quantified by determining the average proportion of hepatocytes affected.

IFN Therapy and Definitions of Response to IFN Therapy. All patients had chronic HCV infection at liver biopsy, which was confirmed by the presence of HCV-RNA in serum. All IFN therapies were initiated within 48 weeks after liver biopsy. Among the 1,818 patients, 535 received IFNα or IFNβ monotherapy for 24 weeks, 244 patients received IFNα ribavirin (RBV) combination therapy for 24 weeks, 299 patients received pegylated (PEG-) IFNα monotherapy for 48 weeks, and 760 patients received PEG-IFNα RBV combination therapy for 48-72 weeks.

Patients negative for serum HCV-RNA 24 weeks after IFN therapy completion were defined as SVRs. Patients who remained positive for HCV-RNA 24 weeks after therapy completion were defined as non-SVRs. HCV-RNA was determined by the qualitative Amplicor or TaqMan HCV assay (Roche Molecular Diagnostics, Tokyo, Japan).

Data Collection and Patient Follow-up. At enrollment, patient characteristics, biochemical,

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Additional Supporting Information may be found in the online version of this article.

Table 1. Characteristics of Patients Enrolled in the Present Study

Factors	Value
Patients, n	1818
Sex, n (%)	
Male	833 (45.8)
Female	985 (54.2)
Age (SD), year	57.1 (12.0)
BMI (SD), kg/m^2	23.1 (3.2)
Fibrosis stage, n (%)	
F1/2	1384 (76.1)
F3/4	434 (23.9)
Activity grade, n (%)	
A0/1	964 (53.0)
A2/3	854 (47.0)
%Severe steatosis (≥10%)	23.7
Albumin (SD), g/dL	4.0 (0.38)
ALT (SD), IU/L	78.3 (71.0)
γ-GTP (SD), IU/L	49.8 (50.6)
T. Bilirubin (SD), mg/dL	0.73 (0.34)
Fasting blood sugar (SD), mg/dL	113.4 (37.8)
LDL-Cholesterol (SD), mg/dL	101.6 (28.9)
T. Cholesterol (SD), mg/dL	176.2 (38.4)
AFP (SD), ng/mL	11.3 (28.3)
WBC counts (SD),/μL	4990 (1516)
Hb (SD), g/dL	14.0 (1.7)
Platelet counts (SD), x10^3/μL	164 (54)
HCV load (SD), KIU/mL	1097 (1263)
HCV genotype, n (%)*	
1a	11 (0.56)
1b	1183 (67.4)
2a	361 (20.6)
2b	180 (10.3)
Others	20 (1.1)
%Core 70 a.a. mutation†	34.2
%ISDR wild or 1 mutation‡	63.9
IFN regimen, n (%)	
IFN mono	758 (35.0)
IFN + RBV	275 (12.7)
PEG-IFN mono	307 (14.2)
PEG-IFN + RBV	758 (38.2)

Unless otherwise indicated, data are given as mean (SD).
*HCV genotype was determined in 1755 patients.
†HCV core mutation was determined in 409 patients with genotype 1b.
‡ISDR was determined in 1264 patients with genotype 1b.
Abbreviations: BMI, body mass index; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; LDL, low-density lipoprotein; AFP, α-fetoprotein; WBC, white blood cell; Hb, hemoglobin; HCV, hepatitis C virus; ISDR, interferon sensitivity determining region; IFN, interferon; RBV, ribavirin; PEG, pegylated.

hematological, virological, and histological data were collected. Age was determined at the time of primary liver biopsy. Patients were examined for HCC by abdominal ultrasonography, dynamic CT, and/or MRI every 3-6 months. Serum ALT and AFP levels were measured every 1-6 months. The surveillance protocols were in accordance with the standard of care in Japan. If HCC was suspected on the basis of the screening examination, additional procedures (e.g., dynamic CT, dynamic MRI, CT during hepatic arteriography, CT during arterial portography, contrast-enhanced ultrasonography, and tumor biopsy) were used to confirm the diagnosis. HCC diagnosis was confirmed by needle biopsy, histology of surgically resected specimens, or characteristic radiological findings. To evaluate the effects of changes in serum ALT and AFP levels during IFN therapy on hepatocarcinogenesis, the average integration values of ALT and AFP in each patient were calculated before and after IFN therapy. Data obtained more than 1 year prior to HCC development were used to exclude AFP elevation caused by HCC itself.

Follow-up was between the date of primary liver biopsy and HCC development or the last medical attendance until June 2011. The mean follow-up period was 6.1 years (range, 1.0-20.8 years).

Statistical Analyses. Categorical data were compared by the chi-square test or Fisher's exact test. Distributions of continuous variables were analyzed with Student *t* test for two groups. All tests of significance were two-tailed and $P < 0.05$ was considered statistically significant. The cumulative incidence curve was determined by the Kaplan-Meier method, and differences among groups were assessed using the log-rank test. Factors associated with HCC risk were determined by the Cox proportional hazard model. As covariates in the multivariate stepwise Cox model, age, sex, stage of liver fibrosis, grade of histological activity, presence of hepatic steatosis, serum albumin levels, γ-glutamyl transpeptidase (γ-GTP) level, fasting blood sugar levels, platelet counts, pre-IFN ALT levels, pre-IFN AFP levels, post-IFN ALT levels, post-IFN AFP levels, and virological response were included. HCC development was the dependent variable. Time zero was defined as the time of primary liver biopsy. The proportional assumption was supported by log[-log(survival)] versus log(time) plots that showed parallel lines. Statistical analyses were performed using the Statistical Package for the Social Sciences software v. 18.0 (SPSS, Chicago, IL).

Results

Patient Characteristics and Factors Associated With Risk of HCC. Table 1 shows patient characteristics at the time of enrollment. During follow-up, HCC developed in 179 patients. The cumulative incidence of HCC for 5 and 10 years was 6.5% and 15.0%, respectively. The final virological response to IFN therapy was determined in all patients. The overall rate of SVRs was 50.2% (913/1818). The cumulative incidence in SVRs was 2.3% and 5.5%, respectively, which was significantly lower than that in non-SVRs (6.9% and 21.9%, respectively; log-rank test, $P < 0.0001$).

Univariate analysis demonstrated factors that increase the risk for HCC development (Table 2). According to

multivariate stepwise Cox analysis, older age, male gender, advanced fibrosis, severe steatosis, lower serum albumin levels, non-SVR, and higher post-IFN treatment ALT and AFP levels, but not pre-IFN treatment ALT and AFP levels, were identified as independent factors that were significantly associated with HCC development (Table 2).

Association of Post-IFN Treatment ALT and AFP Levels With HCC Development in SVRs and Non-SVRs. Because our multivariate analysis identified post-IFN treatment ALT and AFP levels as independent factors associated with HCC risk, we determined the cutoff values of these factors for predicting the development of HCC by receiver operator characteristics (ROC) analysis. The area under the ROC curve for post-IFN treatment ALT and AFP levels were higher than that for pre-IFN treatment ALT and AFP levels, suggesting that quantification of post-IFN treatment ALT and AFP levels rather than pre-IFN treatment levels of these values is useful for predicting HCC (Fig. 1A). From this ROC analysis, ALT <40 IU/L and AFP <6.0 ng/mL were identified as cutoff values. Negative predictive values were extremely high at 0.960 in each value, suggesting patients with ALT and/or AFP levels below these cutoff values are at a lower risk for HCC.

As shown in Fig. 1B, the hazard ratio determined by Cox proportional hazard analysis after adjustment for age, sex, stage of liver fibrosis, degree of liver steatosis, serum albumin levels, and virological response to therapy demonstrated that the hazard ratio for HCC was dependent on post-IFN treatment ALT and AFP levels. These hazard ratios increased predominantly when post-IFN treatment ALT and AFP levels were more than the cutoff values.

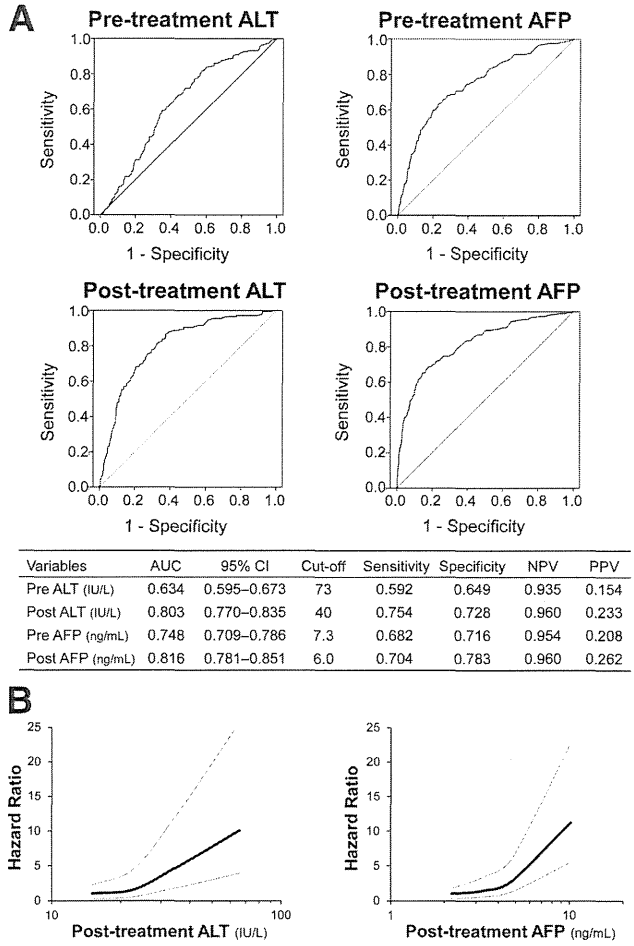
As shown in Fig. 2, the cumulative incidence of HCC was closely related to post-IFN treatment ALT and AST levels and was significantly lower in patients whose post-IFN treatment ALT and AFP levels was suppressed to <40 IU/L and 6.0 ng/mL, respectively. This suppressive effect was also notable in non-SVRs (Fig. 2C,D). Moreover, the cumulative incidence of HCC was significantly higher even in SVRs whose post-IFN treatment ALT and AFP levels were not <40 IU/L and 10 ng/mL, respectively (Fig. 2E,F).

Changes in ALT and AFP Levels With IFN Therapy and HCC Development. In the entire cohort, the mean ALT and AFP levels significantly decreased after IFN therapy (ALT = 78.4 to 36.6 IU/L, 95% confidence interval [CI] = 38.6-45.0, $P < 0.0001$; AFP = 11.3 to 6.9 ng/mL, 95% CI = 3.25-5.69, $P < 0.0001$; paired Student t test), and this significant decrease was found not only in SVRs, but also non-SVRs (Fig. 3A). Because post-IFN treatment ALT and AFP levels rather than pre-IFN treatment levels were

Table 2. Factors Associated With Hepatocellular Carcinoma

Risk Factor	Hazard Ratio (95% CI)	*P* Value
Univariate analysis		
Age (by every 10 year)	1.82 (1.52-2.20)	<0.0001
Sex		
Female	1	
Male	1.61 (1.20-2.17)	<0.0001
Fibrosis stage		
F1/F2	1	
F3/F4	4.90 (3.64-6.61)	<0.0001
Activity grade		
A0/A1	1	
A2/A3	3.38 (2.41-4.74)	<0.0001
Degree of steatosis		
<10%	1	
≥10%	3.84 (2.62-5.63)	<0.0001
Albumin (by every 1 g/dL)	0.18 (0.22-0.25)	<0.0001
Pre-ALT (by every 40 IU/L)	1.04 (0.96-1.08)	0.525
Post-ALT (by every 40 IU/L)	1.68 (1.55-1.81)	<0.0001
γ-GTP (by every 40 IU/L)	1.17 (1.08-1.27)	<0.0001
Fasting blood sugar (by every 100 mg/dL)	1.82 (1.35-2.45)	<0.0001
Pre-AFP (by every 10 ng/mL)	1.07 (1.05-1.09)	<0.0001
Post-AFP (by every 10 ng/mL)	1.08 (1.06-1.12)	<0.0001
Platelet counts (by every $10^4/\mu L$)	0.88 (0.85-0.90)	<0.0001
Genotype		
Non-1	1	
1	2.27 (1.51-3.45)	<0.0001
Core 70 mutation		
Wild	1	
Mutant	2.79 (1.19-6.53)	0.018
ISDR		
More than 1 mutation	1	
Wild or 1 mutation	1.27 (0.87-1.85)	0.216
Virological response		
SVR	1	
Non-SVR	3.66 (2.51-5.35)	<0.0001
Multivariate analysis		
Age (by every 10 year)	2.18 (1.71-2.81)	<0.0001
Sex		
Female	1	
Male	2.66 (1.86-3.80)	<0.0001
Fibrosis stage		
F1/F2	1	
F3/F4	2.27 (1.58-3.27)	<0.0001
Degree of steatosis		
<10%	1	
≥10%	2.29 (1.49-3.50)	<0.0001
Albumin (by every 1 g/dL)	0.35 (0.23-0.55)	<0.0001
Post-ALT (by every 40 IU/L)	1.81 (1.55-2.12)	<0.0001
Post-AFP (by every 10 ng/mL)	1.06 (1.02-1.10)	0.007
Virological response		
SVR	1	
Non-SVR	1.58 (1.01-2.48)	0.044

Hazard ratios for development of hepatocellular carcinoma were calculated by the Cox proportional hazards analysis. ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase; AFP, alpha-fetoprotein; ISDR, interferon sensitivity determining region; SVR, sustained virological responder. As covariates in the multivariate stepwise Cox model, age, sex, stage of liver fibrosis, grade of histological activity, presence of hepatic steatosis, serum albumin levels, γ-GTP level, fasting blood sugar levels, platelet counts, pre-IFN ALT levels, pre-IFN AFP levels, post-IFN ALT levels, post-IFN AFP levels, and virological response were included.



Variables	AUC	95% CI	Cut-off	Sensitivity	Specificity	NPV	PPV
Pre ALT (IU/L)	0.634	0.595–0.673	73	0.592	0.649	0.935	0.154
Post ALT (IU/L)	0.803	0.770–0.835	40	0.754	0.728	0.960	0.233
Pre AFP (ng/mL)	0.748	0.709–0.786	7.3	0.682	0.716	0.954	0.208
Post AFP (ng/mL)	0.816	0.781–0.851	6.0	0.704	0.783	0.960	0.262

Fig. 1. Predictive values for ALT and AFP, and hazard ratios (HRs) according to post-IFN treatment ALT and AFP levels. (A) ROC curve for prediction of HCC. Area under the ROC curve, 95% CI, cutoff value, sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) are shown in the bottom of the figure. (B) Spline curves of HR (solid line) and 95% CI (dotted line) for HCC development according to post-IFN treatment ALT and AFP levels. Curves were fitted using polynomial regression.

significantly associated with the development of HCC in non-SVRs, we determined the effects of changes in ALT and AFP levels by IFN therapy on hepatocarcinogenesis. Even in non-SVRs with equal or higher pre-IFN treatment ALT or AFP levels than the cutoff values, the cumulative incidence of HCC was significantly suppressed in patients whose post-IFN treatment ALT or AFP level was reduced to less than the cutoff values (Fig. 3B). In contrast, persistence of post-IFN treatment ALT or AFP levels to more than the cutoff values after IFN therapy was associated with a significantly higher incidence of HCC (Fig. 3B).

Relation Between AFP and ALT or Histological Findings. Univariate analysis using logistic regression determined factors that were associated with post-IFN treatment ALT or AFP levels (Supporting Table). Although many clinical factors were associated with post-IFN ALT and/or AFP levels, post-IFN ALT and AFP levels were not correlated with each other ($r^2 = 0.050$). Therefore, the cumulative incidence of HCC was significantly higher in patients with higher post-IFN treatment AFP levels, even when patients were stratified by post-IFN treatment ALT levels (Fig. 4A,B).

As shown in Fig. 4C-F, the cumulative incidence of HCC development was significantly lower in patients whose post-IFN treatment AFP level was <6.0 ng/mL in all subgroups stratified by stage of fibrosis and grade of activity. Therefore, reduction in post-IFN treatment AFP levels reduces HCC risk even in patients with advanced fibrosis. Although pre-IFN treatment AFP levels correlated with the advance of histological fibrosis and grade of activity, such correlations became less significant with post-IFN treatment AFP levels (data not shown).

Platelet Counts and Aspartate Aminotransferase-to-Platelet Ratio Index (APRI) in Patients Without Advanced Fibrosis. Because a substantial amount of HCC cases developed in the patients without histologically advanced fibrosis (Fig. 4C), we characterized these individuals using platelet counts and APRI,[25] which are the readily available surrogate markers for liver fibrosis. We first determined the cutoff values of platelet counts and APRI for predicting HCC development by ROC analyses. Accordingly, platelet counts $<150 \times 10^3/\mu L$ and APRI >0.96 were identified as cutoff values, and the areas under the ROC curve for platelet counts and APRI were 0.715 (95% CI: 0.675-0.755) and 0.740 (95% CI: 0.701-0.779), respectively (Supporting Figure). Even in individuals without advanced fibrosis (F1 and F2 patients), the proportion of patients with platelet counts $<150 \times 10^3/\mu L$ or APRI >0.96 was significantly higher in patients with HCC than in those without HCC (platelet counts, 53.0% [35/66] versus 31.3% [387/1238], $P = 0.0002$; APRI, 53.0% [35/66] versus 26.4% [325/1229], $P < 0.0001$). Moreover, the cumulative incidence of HCC development was significantly higher in patients with platelet counts $<150 \times 10^3/\mu L$ or APRI >0.96 in the subgroups without advanced fibrosis (Supporting Figure). Therefore, patients with low platelet counts or high APRI still have a substantial risk for HCC development even though they were diagnosed with mild fibrosis by liver biopsy.

Hepatic Steatosis and Post-IFN ALT and AFP Levels in SVRs. To characterize SVRs without ALT and AFP normalization after IFN therapy, we evaluated the percentage of severe hepatic steatosis in these patients. Accordingly, the percentages of severe hepatic steatosis were significantly higher in SVRs without ALT and AFP normalization than in those with normal

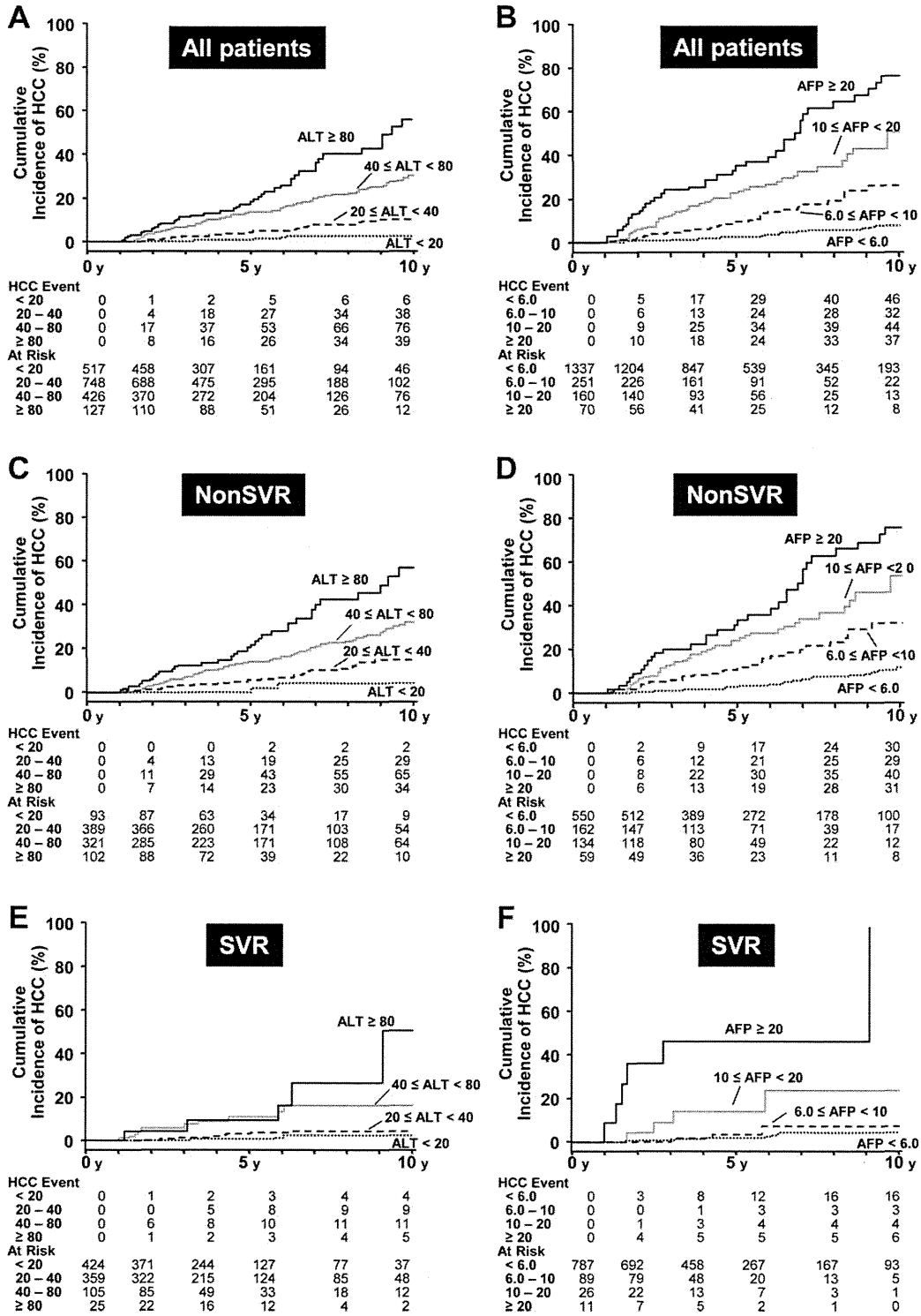
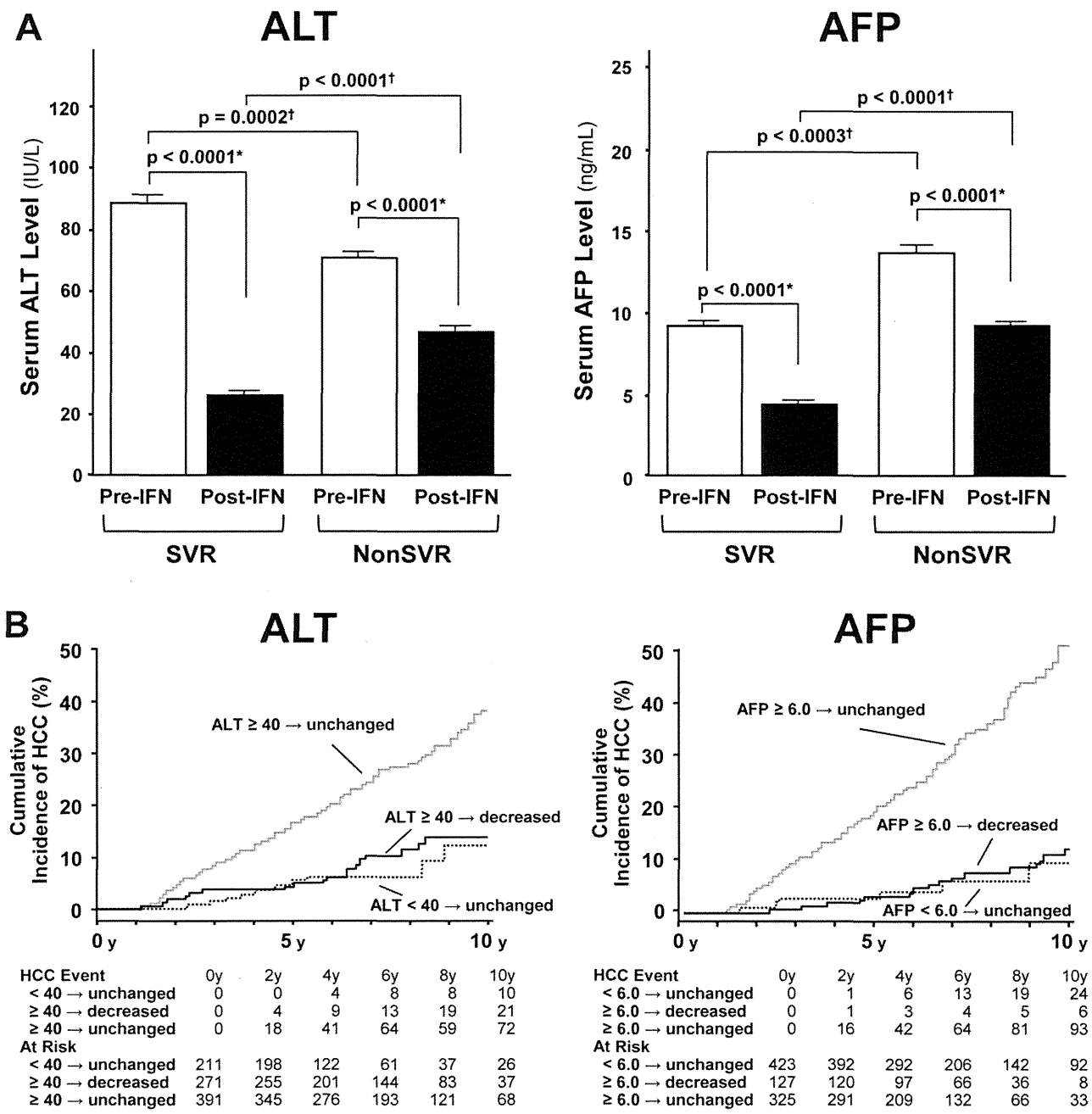


Fig. 2. Cumulative incidence of HCC according to post-IFN treatment ALT and AFP levels. (A) Entire cohort stratified by post-IFN treatment ALT levels (log-rank test: $P < 0.0001$). (B) Entire cohort stratified by post-IFN treatment AFP levels (log-rank test: $P < 0.0001$). (C) Non-SVRs stratified by post-IFN treatment ALT levels (log-rank test: $P < 0.0001$). (D) Non-SVRs stratified by post-IFN treatment AFP levels (log-rank test: $P < 0.0001$). (E) SVRs stratified by post-IFN treatment ALT levels (log-rank test: $P < 0.0001$). (F) SVRs stratified by post-IFN treatment AFP levels (log-rank test: $P < 0.0001$). The number of HCC events and patients at risk at each timepoint are shown below the graphs.

ALT and AFP (ALT, 37.9% [36/95] versus 13.8% [77/557], $P < 0.0001$; AFP, 31.6% [31/98] versus 14.8% [82/554], $P < 0.0001$). Therefore, it is likely that presence of hepatic steatosis is associated with ALT and/or AFP elevation, and it is one of the risks for HCC development even after achieving SVR.



HCC Event	0y	2y	4y	6y	8y	10y
< 40 → unchanged	0	0	4	8	8	10
≥ 40 → decreased	0	4	9	13	19	21
≥ 40 → unchanged	0	18	41	64	59	72
At Risk						
< 40 → unchanged	211	198	122	61	37	26
≥ 40 → decreased	271	255	201	144	83	37
≥ 40 → unchanged	391	345	276	193	121	68

HCC Event	0y	2y	4y	6y	8y	10y
< 6.0 → unchanged	0	1	6	13	19	24
≥ 6.0 → decreased	0	1	3	4	5	6
≥ 6.0 → unchanged	0	16	42	64	81	93
At Risk						
< 6.0 → unchanged	423	392	292	206	142	92
≥ 6.0 → decreased	127	120	97	66	36	8
≥ 6.0 → unchanged	325	291	209	132	66	33

Fig. 3. Changes in pre- and post-IFN treatment ALT and AFP levels, and their effects on HCC development. (A) Mean serum pre- (open columns) and post-IFN treatment (solid columns) ALT and AFP levels in SVRs and non-SVRs. Error bars indicate the standard error. *Paired Student *t* test. †Unpaired Student *t* test. (B) Cumulative incidence of HCC stratified by changes in pre- and post-IFN treatment ALT and AFP levels (log-rank test: $P < 0.0001$). ALT <40 → unchanged, patients with ALT <40 IU/L before IFN therapy unchanged after IFN therapy; ALT ≥40 → decreased, patients with ALT ≥40 IU/L before IFN therapy decreased to ALT <40 IU/L after IFN therapy; ALT ≥40 → unchanged, patients with ALT ≥40 IU/L before IFN therapy unchanged at ALT not <40 IU/L after IFN therapy. AFP <6.0 → unchanged, patients with AFP <6.0 ng/mL before IFN therapy unchanged at AFP <6.0 ng/mL after IFN therapy; AFP ≥6.0 → decreased, patients with AFP ≥6.0 ng/mL before IFN therapy decreased to AFP <6.0 ng/mL after IFN therapy; AFP ≥6.0 → unchanged, patients with AFP ≥6.0 ng/mL before IFN therapy unchanged at AFP ≥6.0 ng/mL after IFN therapy.

Discussion

This large-scale, long-term cohort study establishes important findings, which demonstrate a strict association between hepatocarcinogenesis and post-IFN treatment ALT and AFP levels in patients with CHC. This association was notable in both SVR and non-SVR subgroups, and suppression of these values by IFN therapy reduced the hepatocarcinogenesis risk despite failure of HCV eradication. These data, which

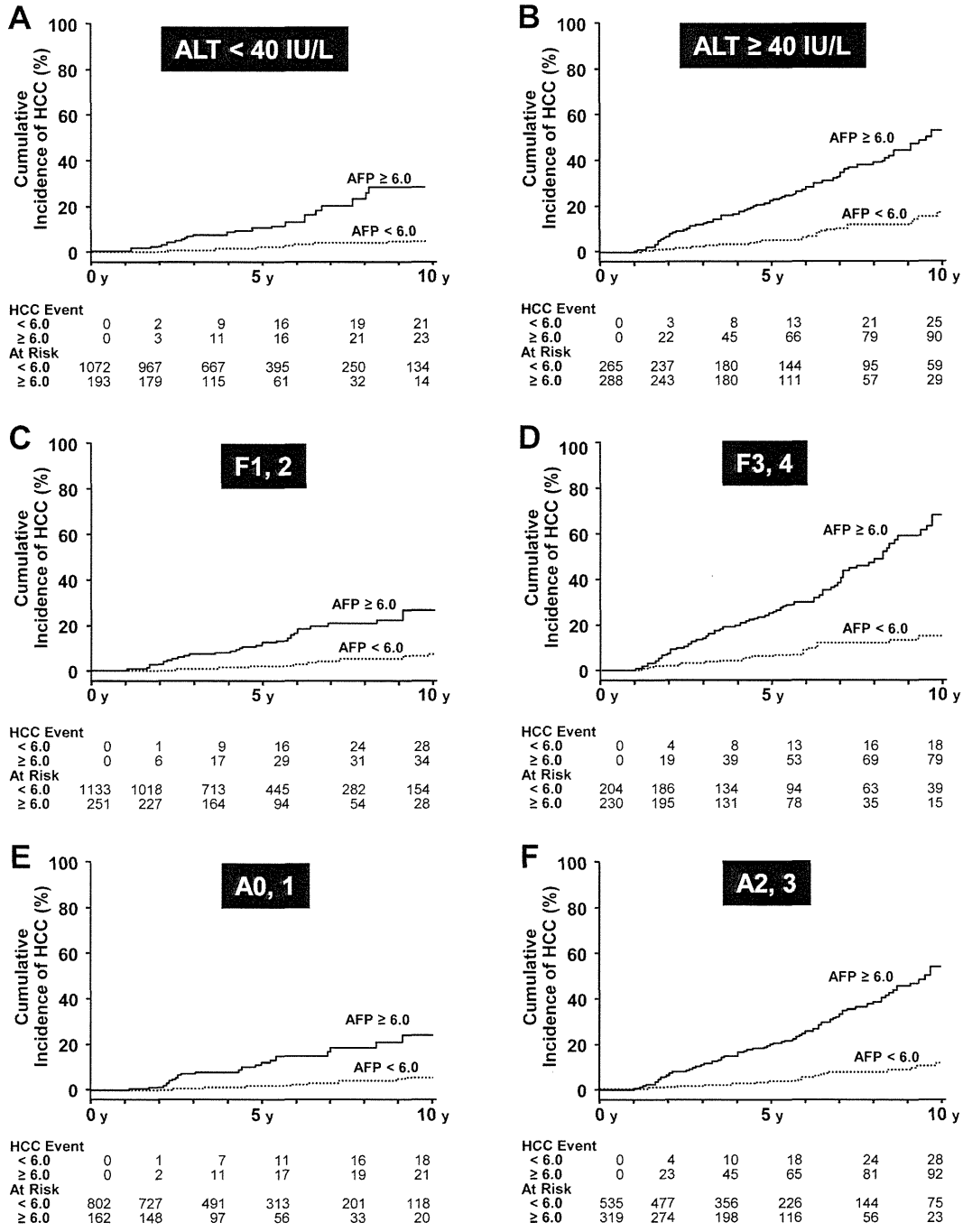


Fig. 4. Relationship between AFP and ALT or histological findings. (A) The cumulative incidence of HCC stratified by post-IFN treatment AFP levels in subgroups according to the post-IFN treatment ALT (log-rank test: $P < 0.0001$ in both subgroups). (B) Cumulative incidence of HCC stratified by post-IFN treatment AFP levels in subgroups according to the histological stage of fibrosis (log-rank test: $P < 0.0001$ in both subgroups). (C) Cumulative incidence of HCC stratified by post-IFN treatment levels of AFP in subgroups according to the histological grade of activity (log-rank test: $P < 0.0001$ in both subgroups).

demonstrate the efficacy of IFN against HCC development associated with suppression of AFP, have clinically important implications for physicians.

Although there have been reports on the association between baseline pretreatment AFP levels and HCC risk,[26-35] little is known regarding the effects of IFN therapy on change in post-IFN treatment AFP and its relation to HCC risk.[36] Although a previous report demonstrated that a decrease in AFP levels in patients receiving IFN therapy reduced the incidence of HCC,[37] this study was performed in a small number of patients (n = 382), and cutoff values, relation to ALT, or histological findings were not determined. Our study, performed in a large well-characterized

cohort, had a greater advantage in that it allowed determination of cutoff values for post-IFN treatment ALT and AFP levels useful for predicting HCC development. Although a higher cutoff value of 20 ng/mL was used to determine the incidence of HCC in the previous study,[36] we propose a lower value for negatively predicting HCC. From our results, those with AFP levels ≥6.0 ng/mL have a substantial HCC risk, even if it is <20 ng/mL. Therefore, post-IFN treatment AFP levels should be <6.0 ng/mL to suppress HCC risk in patients with CHC.

It should be noted that AFP produced by HCC itself was carefully excluded in our study. Serum AFP elevation is frequently observed in patients with advanced CHC in the absence of HCC.[19-23] Although the precise mechanisms accounting for this observation are unknown, Hu et al.[38] found a correlation between AFP and measures of liver disease activity, suggesting that AFP production is enhanced in the presence of necroinflammatory injury of the liver. However, in our study post-IFN treatment ALT and AFP levels were not correlated, and the cumulative incidence of HCC was significantly higher in patients with higher post-IFN treatment AFP levels, even when patients were stratified by post-IFN treatment ALT levels. Moreover, multivariate analysis confirmed that AFP and ALT are independently associated with HCC risk. Therefore, observed elevation in AFP levels in patients with subsequent HCC development is not necessarily caused by necroinflammation of the liver. Alternatively, increased AFP levels have been reported during liver regeneration following hepatic resection and during recovery from massive hepatic necrosis,[39-41] suggesting that elevated AFP levels are a surrogate for proliferative activity of liver cells, which may cause hepatocarcinogenesis in patients with CHC.

Other possible reasons accounting for HCC risk related to AFP are the close association between AFP levels and the stage of liver fibrosis, which is consistent with a previous report.[35] However, we further clarified the fact that correlation between post-IFN treatment AFP levels and liver fibrosis was less notable in patients without subsequent development of HCC (data not shown). Cumulative incidence of HCC was significantly higher in patients with higher post-IFN treatment AFP levels at each stage when patients were stratified by the histological stage of fibrosis (Fig. 4). Therefore, post-IFN treatment AFP is not just a surrogate marker for liver fibrosis, and elevation of post-IFN treatment AFP as a potential risk for hepatocarcinogenesis is not only the result of advanced liver fibrosis. Conversely, suppression of post-IFN treatment AFP levels may reduce HCC risk even in patients with advanced fibrosis.

This study has a few limitations, the first being the heterogeneity of our cohort, which included various treatment regimens with different treatment responses. However, we obtained similar results in a more uniform subgroup of HCV genotype 1b patients treated with PEG-IFNα/RBV (data not shown). The second limitation is the ethnic homogeneity of the Japanese population. Because the baseline incidence of HCC development differs among population groups, longer-term longitudinal studies in larger cohorts with various population subgroups are required to verify the generality of our results.

With the development of potent direct-acting antiviral agents combinations, IFN-free therapy is likely to be approved in the near future. This raises the question of whether posttreatment ALT and/or AFP levels will remain a significant predictor of HCC risk. Moreover, it is uncertain whether the suppressive effect of viral eradication by IFN-free regimens on hepatocarcinogenesis will be identical to that obtained by IFN-based regimens. Therefore, it is extremely interesting to prove these issues in future studies.

In conclusion, post-IFN treatment ALT and AFP levels are strictly associated with hepatocarcinogenesis risk in patients with CHC. Measurement of these values is useful for predicting future HCC risk in IFN-treated patients. Suppression of these values after IFN therapy reduces HCC risk even in patients without HCV eradication, while SVRs with increased ALT and/or AFP levels are at risk for HCC development. The present results have potentially important clinical implications for physicians and may influence their decisions regarding treatment strategy and HCC surveillance for individual patients.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Inhibition of hepatocellular carcinoma by PegIFNα-2a in patients with chronic hepatitis C: a nationwide multicenter cooperative study

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Abstract
Background We investigated whether the administration of maintenance doses of interferon prevented hepatocellular carcinoma (HCC) in patients with chronic hepatitis C.
Methods Study 1: A multicenter, retrospective, cooperative study was carried out to determine whether long-term administration of low-dose peginterferon alpha-2a (PegIFNα-2a) prevented HCC development in patients with chronic hepatitis C. In total, 594 chronic hepatitis C patients without a history of HCC were enrolled and treated with 90 μg PegIFNα-2a administered weekly or bi-weekly for at least 1 year. Study 2: HCC developed in 16 of 99 additional patients without PegIFNα-2a treatment during 3.8 years of observation. A propensity-matched control study was then carried out to compare the incidence of

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HCC between the 59 patients who received low-dose PegIFNα-2a (PegIFNα-2a group) and 59 patients who did not receive PegIFNα-2a treatment (control group), matched for sex, age, platelet count, and total bilirubin levels.

Results Study 1: HCC developed in 49 patients. The risk of HCC was lower in patients with undetectable hepatitis C virus RNA, ≤40 IU/L alanine aminotransferase (ALT), or ≤10 ng/L alpha-fetoprotein (AFP) 24 weeks after the start of therapy. Study 2: The incidence of HCC was significantly lower in the PegIFNα-2a group than in the control group.

Conclusions Low-dose and long-term maintenance administration of PegIFNα-2a decreased the incidence of HCC in patients with normalized ALT and AFP levels at 24 weeks compared with patients without normal ALT and AFP levels.

Keywords Chronic hepatitis C · Hepatocellular carcinoma · Peginterferon

Introduction

Hepatocellular carcinoma (HCC), the sixth most common cancer worldwide, often develops because of long-term hepatitis B or C virus infection [1, 2]. In particular, chronic hepatitis C and hepatic cirrhosis increase the risk of HCC; the annual incidence of tumor development in such patients may be as high as 2–4 % [3–5]. The incidence of HCC decreases in patients who achieve a sustained virological response (SVR) to interferon (IFN) treatment, although the incidence remains high in non-SVR patients [6–9]. A detailed analysis of HCC development revealed that chronic hepatitis C patients aged 65 years or more, especially those with advanced fibrosis of the liver, were at an increased risk of developing HCC [10]. For patients 65 years or older with advanced liver fibrosis, the dose of ribavirin is often reduced or the agent is discontinued, resulting in lower SVR rates in those with discontinuation of ribavirin. Establishing an effective treatment strategy for preventing the development of HCC is important for these high-risk patients.

Factors related to the development of HCC have been analyzed in patients who did not achieve an SVR even after IFN treatment; advanced fibrosis of the liver and high levels of serum alanine aminotransferase (ALT), and alpha-fetoprotein (AFP) are risk factors for HCC development [11, 12]. A randomized controlled trial was conducted in Western countries to determine whether combined peginterferon and ribavirin treatment with weekly administration of 90 μg peginterferon alpha-2a (PegIFNα-2a) could prevent HCC in non-responders. A 3.5-year follow up showed that administration of a maintenance dose of PegIFNα-2a did not reduce tumor incidence in these patients [13]. However, after 8.5 years of observation, the incidence of HCC was decreased among those in the PegIFNα-2a group with cirrhosis [14]. Meanwhile, Bruix et al. [15] reported that maintenance therapy with PegIFNα-2b did not prevent HCC in chronic hepatitis C patients with cirrhosis. In Japan, long-term low-dose administration of natural IFN has been reported to decrease the incidence of HCC [16]. In light of these conflicting results, investigations should be carried out in a large number of patients with chronic hepatitis C to resolve the question of whether IFN treatment prevents the development of HCC.

We carried out a multicenter retrospective cooperative study of patients with chronic hepatitis C to determine whether those treated with 90 μg PegIFNα-2a without ribavirin had a reduced incidence of HCC compared with those not treated with IFN.

Patients and methods

Study 1: analysis of risk factors for HCC in patients treated with long-term low-dose-PegIFNα-2a

In total, at 21 hepatitis centers throughout Japan, 743 patients with hepatitis C who had received 90 μg of PegIFNα-2a therapy weekly or bi-weekly for 1 year or more without having received the full dose (180 μg) since December 2003 were examined retrospectively for the development of HCC. The end of enrollment in this study was the end of December 2008 and the end of follow up was the end of December 2010. Patients with a history of HCC before the start of therapy and those with a therapy period of less than 48 weeks were excluded, leaving 594 patients who had undergone long-term administration of PegIFNα-2a for analysis. At the 21 centers involved in this

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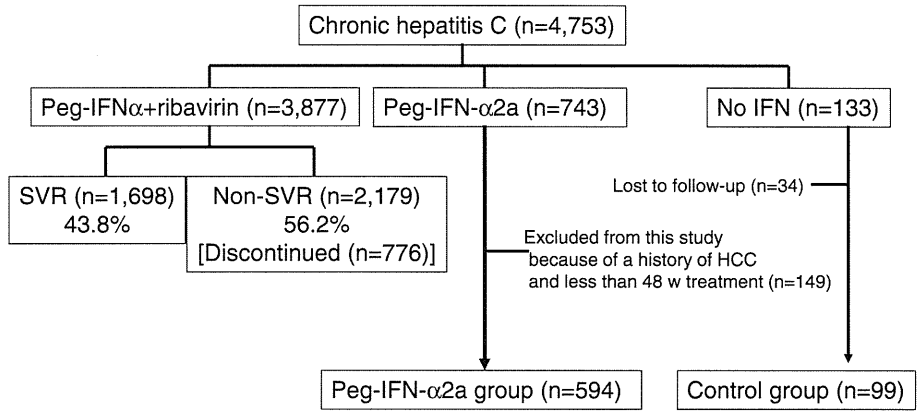

Fig. 1 Flow diagram of the patients' enrollment in the study. *Peg-IFNα* pegylated interferon α, *SVR* sustained viral response, *HCC* hepatocellular carcinoma, *w* week

study, 4,753 patients with chronic hepatitis C had been treated; Peg-IFN and ribavirin combination treatment had been administered to 3,877 patients, 743 patients had received Peg-IFN alone, and 133 patients had not agreed to receive IFN (a flow diagram of the enrollment of patients in this study is shown in Fig. 1). In the patients with Peg-IFN and ribavirin combination treatment, the SVR rate was 43.8 %; SVR was not achieved in 2,179 patients, and in 776 of these patients, the combination therapy was discontinued owing to adverse events or the patient's choice. Patients who failed to achieve an SVR were not included in this study, because the incidence of HCC is known to be reduced even in non-responders to IFN [17].

The backgrounds of the 594 patients studied are shown in Table 1. Findings from the liver biopsies of the patients were classified according to international standards [18]. Long-term PegIFNα-2a treatment is approved by the Japanese Medical Insurance system. Written informed consent was obtained from all patients prior to participation in this study. The study design was approved by the regional ethics committees of the 21 centers involved in this study, including the Musashino Red Cross Hospital, in accordance with the Helsinki Declaration. The 743 patients treated with PegIFNα-2a alone were not indicated for Peg-IFNα and ribavirin combination therapy because of anemia or heart disease. The 133 patients who did not agree to receive IFN served as the control group (see Fig. 1). A large proportion of the 594 study patients had advanced fibrosis of the liver and active inflammation. A dose of 90 μg PegIFNα-2a was administered to 512 and 82 patients weekly and biweekly, respectively, according to the patients' wishes. There were no significant differences between the weekly and biweekly groups in the patients' background data (data not shown).

The median duration of follow up in the PegIFNα-2a group was 1,273 days (range 228–2,768 days) and HCC was observed in 49 of the 594 patients (Table 1). Pre-treatment and on-treatment factors associated with the development of HCC were analyzed by Student's *t*-test, the Mann–Whitney *U*-test, and the χ^2 test (Table 2). Independent factors for the development of HCC were assessed by multivariate analysis using logistic regression. The

Table 1 Background data of patients treated with PegIFNα-2a ($n = 594$)

	$n = 594$
Age (years)	61.7 ± 11.7
Sex (male/female)	258/336
BMI	23.2 ± 3.3
Genotype (1/2)	443/151
Diagnosis (ASC/CH/LC)	4/460/130
History of excess alcohol consumption (≥60 g/day; yes/no)	118/376
Fibrosis (F0, 1, 2/F3, 4)	443/151
Inflammatory activity (A0, 1/A2, 3)	469/125
Diabetes mellitus (no/yes)	499/95
LDL cholesterol (mg/dL)	94.2 ± 31.1
Fasting blood sugar (mg/dL)	106.3 ± 28.5
White blood cell count (/mm^3)	4,360 ± 1,470
Red blood cell count (×10^6/μL)	423.8 ± 56.4
Hemoglobin (g/dL)	13.3 ± 1.8
Platelet count (×10^3/μL)	137 ± 56
Albumin (g/dL)	4.0 ± 0.5
Total bilirubin (mg/dL)	0.8 ± 0.6
AST (IU/L)	65.8 ± 47.8
ALT (IU/L)	72.1 ± 68.0
Gamma-GTP (IU/L)	55.2 ± 51.3
Esophageal varices (no/yes)	344/31
Alpha fetoprotein (ng/L)	6.9 (4.2–13.8)
Once weekly or biweekly PegIFNα-2a	512:82
Baseline HCV RNA (KIU/mL)	1,024 (73–2,130)
Development of HCC (no/yes)	545/49

PegIFN pegylated interferon, *BMI* body mass index, *ASC* asymtomatic carrier, *CH* chronic hepatitis, *LC* liver cirrhosis, *LDL* low-density lipoprotein, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GTP* guanosine triphosphate, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma

Values are means ± SD, with ranges in parentheses