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Post-treatment Levels of α-Fetoprotein Predict Incidence of Hepatocellular Carcinoma After Interferon Therapy

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BACKGROUND & AIMS: **In patients with chronic hepatitis C virus (HCV) infection, lack of sustained virologic response (SVR) 24 weeks after the end of interferon therapy is a significant risk factor for hepatocellular carcinoma (HCC). Although many pretreatment factors are known to affect HCC incidence, less is known about post-treatment factors—many change during the course of interferon therapy.**

METHODS: **We performed a prospective study, collecting data from 2659 patients with chronic hepatitis C without a history of HCC who had been treated with pegylated interferon (Peg-IFN) plus ribavirin from 2002 through 2008 at hospitals in Japan. Biopsy specimens were collected before treatment; all patients received Peg-IFN plus ribavirin for 48 to 72 weeks (HCV genotype 1) or 24 weeks (HCV genotype 2). Hematologic, biochemical, and virologic data were collected every 4 weeks during treatment and every 6 months after treatment. HCC was diagnosed based on angiography, computed tomography, and/or magnetic resonance imaging findings.**

RESULTS: **HCC developed in 104 patients during a mean observation period of 40 months. Older age, male sex, lower platelet counts and higher levels of α-fetoprotein at baseline, and lack of an SVR were significant risk factors for HCC. The cumulative incidence of HCC was significantly lower in patients without SVRs who relapsed than those with no response to treatment. Levels of α-fetoprotein 24 weeks after the end of treatment (AFP24) were significantly lower than levels of α-fetoprotein at baseline in patients with SVRs and those who relapsed, but not in nonresponders. Post-treatment risk factors for HCC among patients with SVRs included higher AFP24 level and older age; among those without SVRs, risk factors included higher AFP24 level, integrated level of alanine aminotransferase, older age, and male sex. AFP24 (≥10 ng/mL, 10–5 ng/mL, and then <5 ng/mL) was a better predictor of HCC incidence than pretreatment level of AFP among patients with and without SVRs.**

Abbreviations used in this paper: AFP, α-fetoprotein; AFP24, α-fetoprotein levels at 24 weeks after the end of treatment; ALT, alanine aminotransferase; ALT24, alanine aminotransferase levels at 24 weeks after the end of treatment; CH-C, chronic hepatitis C; CT, computed tomography; EOT, end of treatment; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; i-ALT, integrated alanine aminotransferase value after the end of treatment; IFN, interferon; NR, nonresponse; Peg-IFN, pegylated interferon; PreAFP, α-fetoprotein levels at baseline; PreALT, alanine aminotransferase levels at baseline; SVR, sustained virologic response.

1542-3565/$36.00
http://dx.doi.org/10.1016/j.cgh.2013.11.033

CONCLUSIONS: **In patients with chronic HCV infection, levels of α-fetoprotein decrease during interferon therapy. High post-treatment levels of α-fetoprotein predict HCC, regardless of whether patients achieve an SVR. University Hospital Medical Information Network Clinical Trials Registry: C000000196, C000000197.**

Keywords: ALT; Liver Cancer; Risk Factor; Response to Therapy; Outcome.

Many reports have shown that hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) was suppressed by interferon (IFN) therapy in patients who attained HCV eradication.[1,2] Generally, for patients showing HCV eradication by IFN therapy, the risk for HCC incidence has been shown to be low, but 1.3% to 4.7% of patients developed HCC at 5 years.[3–5] Conversely, for patients without HCV eradication by IFN therapy, although the risk for HCC incidence is high, many patients remain free of HCC incidence for long periods.[2] Therefore, the risk factors for HCC incidence should be evaluated separately between the 2 groups with distinctly different risk levels for HCC incidence, that is, patients who attained HCV eradication and those who did not.

Currently, many studies have assessed factors associated with HCC incidence among pretreatment factors but not post-treatment factors. However, IFN therapy for patients with chronic HCV infection mainly aims for HCV eradication but also may have immunologic and anti-inflammatory effects and antineoplastic activity. Therefore, there is the potential for a change in biochemical parameters. Notably, serum alanine aminotransferase (ALT) or α-fetoprotein (AFP) levels and liver fibrosis have been reported to change after IFN therapy.[5–8] Such synthetic effects can be involved in the suppression of HCC incidence. However, the relationship among the factors changed by IFN therapy and HCC incidence has not been fully examined.

In the present study, the changes in factors caused by pegylated IFN (Peg-IFN) plus ribavirin therapy were analyzed, and the relationship between post-treatment factors and HCC incidence among the 2 patient groups, those who attained HCV eradication and those who did not, was examined in a large-scale cohort of patients with chronic hepatitis C (CH-C).

Patients and Methods

Patients

The current study was a prospective multicenter study conducted by Osaka University Hospital and other institutions participating in the Osaka Liver Forum. A ztotal of 2659 CH-C patients without a history of HCC who had been treated with Peg-IFN plus ribavirin therapy between December 2002 and December 2008 were enrolled in this study. Eligible patients did not have decompensated cirrhosis or other forms of liver disease (alcohol liver disease, autoimmune hepatitis), co-infection with hepatitis B, or human immunodeficiency virus. After enrollment, 26 patients who developed HCC within the first 12 months from the start of therapy were excluded because of the possibility of microscopic HCC having been present before treatment. In addition, 33 patients were excluded because their virologic response to Peg-IFN plus ribavirin therapy was not assessed. Finally, 2600 CH-C patients were assessed for HCC incidence. This study was conducted according to the ethical guidelines of the Declaration of Helsinki amended in 2002, and was approved by the ethics commission of Osaka University Hospital (University Hospital Medical Information Network Clinical Trials Registry: C000000196, C000000197).

Histologic Evaluation

Pretreatment liver biopsies were performed within 6 months before the start of therapy. Experienced liver pathologists who had no clinical, biochemical, or virologic information about the samples performed the histopathologic interpretation of the specimens. The histologic appearances, activity, and fibrosis were evaluated according to METAVIR histologic scores.[9]

Treatment and Definition of Virologic Response

All patients received Peg-IFN alpha-2b (Pegintron; Merck & Co, Inc, Whitehouse Station, NJ) plus ribavirin (Rebetol; Merck & Co, Inc). Peg-IFN was administered once a week at a dose of 1.5 μg/kg, and ribavirin was administered at a total dose of 600 to 1000 mg/d based on body weight, according to the standard treatment protocol for Japanese patients. In principle, treatment duration was 48 to 72 weeks for HCV genotype 1, and 24 weeks for HCV genotype 2. The serum HCV RNA level was analyzed qualitatively using the COBAS AMPLICOR HCV test, version 2.0 (lower limit of detection, 50 IU/L; Roche Diagnostics, Branchburg, NJ). A sustained virologic response (SVR) was defined as an undetectable serum HCV RNA level at 24 weeks after the end of treatment (EOT). Relapse was defined as an undetectable serum HCV RNA level at the EOT but a detectable level after the EOT. Nonresponse (NR) was defined as a detectable HCV RNA level during therapy; the treatment generally was stopped at 24 weeks. The patients who discontinued the treatment because of an adverse event also were assessed in the same way.

Hepatocellular Carcinoma Surveillance and Data Collection

At the start of Peg-IFN plus ribavirin therapy, all patients were assessed by hepatic ultrasonography and/or computed tomography (CT) to confirm the absence of HCC. Hematologic, biochemical, and virologic data were collected every 4 weeks during treatment and every 6 months after treatment. Serum ALT levels after completion of the therapy were indicated as the average integrated values, which were calculated from the area of a trapezoid, with the ALT value divided by the observation period. For HCC surveillance, hepatic ultrasonography, CT, and/or magnetic resonance imaging was performed every 3 to 6 months during the follow-up period. When new space-occupying lesions were detected or suspected, they were examined by hepatic angiography. HCC was diagnosed by the presence of typical hypervascular characteristics on the angiography, in addition to CT and/or magnetic resonance imaging findings. If no typical image of HCC was observed, a fine-needle aspiration biopsy was performed with the patient's consent, or the patient was followed up carefully until a diagnosis was possible by definite observation using CT or angiography.

Follow-up Period

The follow-up period started from the date of the start of Peg-IFN plus ribavirin therapy. The end points were the date when new HCC developed or that of the last follow-up imaging test. For patients who did not attain SVR by Peg-IFN plus ribavirin therapy and had to be re-treated with another antiviral therapy, observation was discontinued at the date of the start of re-treatment. After completion of the Peg-IFN plus ribavirin therapy, liver-supporting therapy using ursodeoxycholic acid or glycyrrhizinate was allowed. The mean observation period was 40.0 ± 16.3 months. The cumulative incidence of HCC was assessed from the date of the start of Peg-IFN plus ribavirin therapy for the pretreatment analysis and from the date of the end of this therapy for the post-treatment analysis.

Statistical Analysis

Baseline continuous variables were expressed as means ± standard deviation and categoric variables were expressed as frequencies. Differences between the 2 groups (SVR vs non-SVR) were assessed by the chi-square test or the Mann–Whitney U test, and differences among 3 groups (SVR vs relapse vs NR) were assessed by analysis of variance and the Tukey post hoc test. The paired t test was used to analyze the difference between continuous variables before and after treatment. The variables of age, sex, white blood cells, hemoglobin levels, platelet counts, total bilirubin levels, albumin levels, ALT levels, AFP levels, and virologic response to the therapy were examined as correlates of HCC development. The Kaplan–Meier method was used to assess the cumulative incidence of HCC, and the groups were compared using the log-rank test. The Cox proportional-hazards model was used to identify the significant risk factors associated with HCC development. The factors selected as significant by simple Cox regression were evaluated by multiple Cox regression. The likelihood ratio test was used to compare the fitness of model for HCC incidence. A P value less than .05 was considered significant. Statistical analysis was conducted with SPSS version 19.0J (IBM, Armonk, NY).

All authors had access to the study data and reviewed and approved the final manuscript.

Results

Patient Characteristics at Baseline and 24 Weeks After the End of Treatment According to Antiviral Effect

The characteristics of the patients at baseline and 24 weeks after the EOT of Peg-IFN plus ribavirin therapy are summarized in Table 1. Of the 2600 patients, 1425 (55%) attained SVR, whereas 1175 showed non-SVR (relapse, n = 607; NR, n = 558) with Peg-IFN plus ribavirin therapy. The patients with METAVIR fibrosis stages 3 to 4 were grouped as advanced liver fibrosis because those with cirrhosis (METAVIR fibrosis stage 4) were in a minority in this study (2%, 47 of 1852 patients who received liver biopsy). The factors at baseline with a significant difference between the SVR and non-SVR groups are shown in Table 1.

The changes of the continuous hematologic and biochemical parameters between baseline and 24 weeks after the EOT were analyzed among the patients with corresponding continuous variables by paired t test. The mean AFP levels at 24 weeks after the EOT (AFP24) were significantly lower compared with AFP levels at baseline (PreAFP) in SVR patients, but not for non-SVR patients. After dividing non-SVR patients into relapse and NR groups, the mean AFP24 level was significantly lower compared with PreAFP in relapsers, but not for NR patients. The mean AFP24 levels were significantly lower in SVR patients and relapsers than in NR patients ($P < .001$ and $P < .001$, respectively), and the percentages of patients with AFP24 less than 5 ng/mL, which is the upper limit of normal range, were higher in the order of SVR, relapse, and NR. Alternatively, the mean ALT levels at 24 weeks after the EOT (ALT24) were significantly lower compared with ALT levels at baseline (PreALT) irrespective of the virologic response. The mean ALT24 levels were significantly lower in the order of SVR, relapse, and NR (SVR and relapse compared with NR, $P < .001$; SVR compared with relapse, $P < .001$), and the respective percentages of patients with ALT24 of 30 IU/L or less were higher in the same manner.

Table 1. Patients' Characteristics at Baseline and 24 Weeks After the Antiviral Treatment According to Antiviral Effect

Factor	SVR		Non-SVR		Non-SVR			
					Relapse		NR	
Factor	Baseline	24 wks after EOT	Baseline	24 wks after EOT	Baseline	24 wks after EOT	Baseline	24 wks after EOT
Age, *y*	54.5 ± 11.5[a]		58.8 ± 9.4		58.6 ± 9.0		59.0 ± 9.8	
Sex, male/female	727/698[b]		519/656		261/346		254/304	
HCV serotype, 1/2/unknown	955/451/19[a]		1049/110/16		512/86/9		529/23/6	
Liver histology[c]								
Activity, A0–1/2–3	573/446[d]		426/407		229/207		194/196	
Fibrosis, F0–2/3–4	902/118[a]		659/174		353/81		299/93	
White blood cells/mm^3	5317 ± 1626[a]	5251 ± 1614	4922 ± 1503	4613 ± 1535[e]	4994 ± 1446	4731 ± 1593[e]	4849 ± 1566	4489 ± 1470[e]
Hemoglobin level, *g/dL*	14.0 ± 1.4[a]	13.8 ± 1.4[e]	13.8 ± 1.4	13.4 ± 1.5[e]	13.8 ± 1.4	13.4 ± 1.5[e]	13.7 ± 1.4	13.5 ± 1.5[e]
Platelet level, $\times 10^4/mm^3$	17.7 ± 5.6[a]	19.0 ± 5.7[e]	15.6 ± 5.7	15.7 ± 5.9	16.2 ± 5.8	16.7 ± 6.0[f]	14.8 ± 5.5	14.6 ± 5.6
Total bilirubin level, *mg/dL*	0.81 ± 0.32[b]	0.74 ± 0.31[e]	0.86 ± 0.34	0.78 ± 0.34[e]	0.86 ± 0.32	0.76 ± 0.33[e]	0.85 ± 0.36	0.80 ± 0.35[e]
Serum albumin level, *g/dL*	4.1 ± 0.4[a]	4.4 ± 0.3[e]	4.0 ± 0.4	4.1 ± 0.4[e]	4.0 ± 0.4	4.2 ± 0.3[e]	3.9 ± 0.4	4.1 ± 0.4[e]
ALT level, *IU/L*	79 ± 78	20 ± 17[e]	75 ± 65	50 ± 39[e]	70 ± 56	44 ± 36[e]	78 ± 61	56 ± 40[e]
ALT ≤30 IU/L	19%	89%	17%	34%	20%	44%	14%	24%
ALT level >30 to ≤60 IU/L	34%	9%	38%	41%	37%	39%	38%	44%
ALT level >60 IU/L	47%	2%	45%	25%	43%	17%	48%	32%
AFP, *ng/mL*	8.4 ± 13.7[a]	3.7 ± 3.1[e]	21.0 ± 82.9	17.5 ± 119.6	11.3 ± 24.0	6.1 ± 14.7[e]	30.8 ± 114.6	29.3 ± 168.7
AFP level <5 ng/mL	51%	79%	34%	49%	47%	67%	21%	32%
AFP level ≥5 to <10 ng/mL	31%	19%	31%	29%	31%	24%	31%	32%
AFP level ≥10 ng/mL	18%	2%	35%	22%	22%	9%	48%	36%

[c]METAVIR, 748 missing.
The values at baseline were compared between SVR and non-SVR by the chi-square test or the Mann–Whitney *U* test: [a]$P < .001$, [b]$P < .01$, [d]$P = .03$.
The values were compared between 24 weeks after EOT and at baseline by paired *t* test: [e]$P < .001$, [f]$P < .01$.

Risk Factors for Hepatocellular Carcinoma Incidence Before Interferon Therapy and the Cumulative Incidence of Hepatocellular Carcinoma According to Antiviral Effects

HCC developed in 104 patients during the follow-up period (SVR, n = 23; non-SVR, n = 81). The significant risk factors of HCC incidence were older age, being male, lower platelet counts at baseline, higher PreAFP levels, and non-SVR to Peg-IFN plus ribavirin therapy according to multiple Cox regression analysis (Table 2). The cumulative incidence of HCC was significantly lower in SVR patients than in non-SVR patients (Figure 1*A*), and in SVR patients and relapsers than in NR patients (Figure 1*B*).

Hepatocellular Carcinoma Incidence According to α-Fetoprotein and Alanine Aminotransferase Levels at 24 Weeks After the End of Treatment

Because the AFP and ALT levels have been reported to be associated with the risk of HCC incidence,[10,11] HCC incidence was assessed according to AFP24 levels and ALT24 levels (Supplementary Tables 1). Among SVR patients, HCC incidence was significantly higher with a higher level of AFP24 ($P < .001$). Among non-SVR patients, HCC incidence was significantly higher with a higher level of AFP24 ($P < .001$) and ALT24 ($P = .002$). After dividing non-SVR patients into relapse and NR groups, the same tendency of HCC incidence increasing with AFP24 and ALT24 increases was observed. HCC incidence was less than 1% in the group with an AFP24 less than 5 ng/mL and an ALT24 of 30 IU/L or less, irrespective of the virologic response (SVR, 0.7%; relapse, 0.8%; NR, 0%).

Risk Factors for Hepatocellular Carcinoma Incidence After Interferon Therapy in Sustained Virologic Response Patients and Non–Sustained Virologic Response Patients

The significant risk factors of HCC incidence were analyzed for patients with and without SVR using host factors and biomarkers at 24 weeks after the EOT using multiple Cox regression analysis (Tables 3 and 4). For ALT, integrated ALT values after the EOT (i-ALT) were used for this analysis because ALT levels can change in response to liver-supporting therapy. The SVR patients showed higher AFP24 levels and older age as the factors associated with HCC incidence (Table 3). Among non-SVR patients, significant risk factors of HCC incidence were older age, being male, higher i-ALT levels, and

Table 2. Risk Factors for HCC Incidence Among the Pretreatment Factors Plus Antiviral Effect (Cox Proportional-Hazards Model)

		Univariate analysis			Multivariate analysis		
Factor	Category	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age, *y*	0: <55	1			1		
	1: 55–64	5.162	2.331–11.434	<.001	4.898	1.437–16.694	.011
	2: ≥65	9.798	4.446–21.590	<.001	9.286	2.765–31.182	<.001
Sex	0: female	1	1.383–3.104	<.001	1	2.335–8.802	<.001
	1: male	2.072			4.534		
Liver fibrosis[a]	0: F0–F2	1	2.037–5.080	<.001	1	0.592–2.147	.716
	1: F3–F4	3.217			1.127		
White blood cells at baseline	0: ≥5000/mm^3	1	0.788–1.711	.450			
	1: <5000/mm^3	1.161					
Hemoglobin at baseline	0: ≥14 g/dL	1	0.554–1.196	.295			
	1: <14 g/dL	0.814					
Platelets at baseline	0: ≥15 × 10^4/mm^3	1	2.003–4.729	<.001	1	1.180–5.105	.016
	1: <15 × 10^4/mm^3	3.078			2.454		
Total bilirubin at baseline	0: <0.8 mg/dL	1	1.409–4.107	.001	1	0.770–2.725	.251
	1: ≥0.8 mg/dL	2.406			1.448		
Serum albumin at baseline	0: ≥4.0 g/dL	1	1.164–3.003	.010	1	0.368–1.228	.196
	1: <4.0 g/dL	1.870			0.672		
PreALT	0: ≤30 IU/L	1			1		
	1: 31–60 IU/L	3.318	1.171–9.404	.024	4.419	0.581–33.577	.151
	2: >60 IU/L	5.564	2.027–15.271	.001	2.845	0.371–21.782	.314
PreAFP	0: <5 ng/mL	1			1		
	1: AFP, ≥5 to <10 ng/mL	3.412	1.434–8.118	.006	2.089	0.761–5.730	.153
	2: ≥10 ng/mL	16.324	7.491–35.574	<.001	5.473	2.102–14.252	<.001
PEG-IFN/RBV antiviral effect	0: non-SVR	1	0.163–0.412	<.001	1	0.183–0.737	.005
	1: SVR	0.259			0.368		

CI, confidence interval; RBV, ribavirin.
[a]METAVIR.

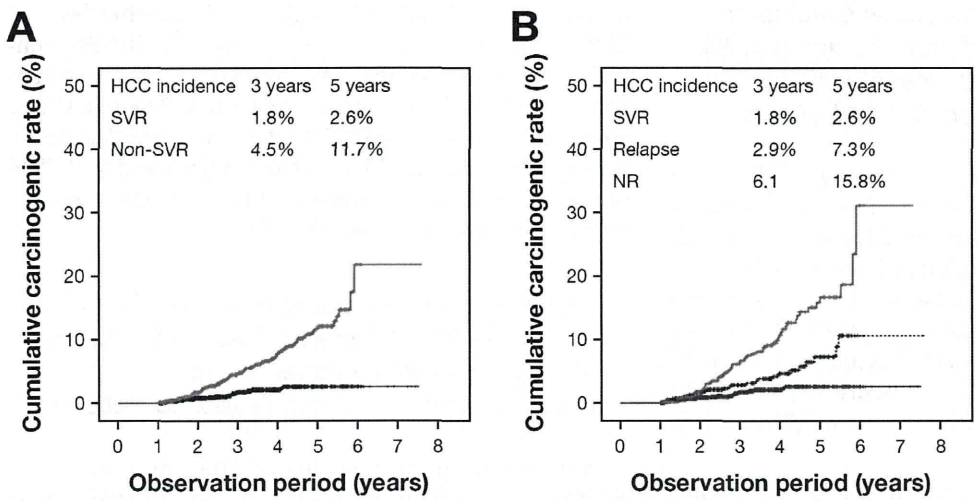

Figure 1. Cumulative incidence of HCC according to the antiviral effect of Peg-IFN plus ribavirin combination therapy. (*A*) The cumulative incidence of HCC was significantly lower in SVR patients (*black line*) than that in non-SVR patients (*gray line*). $P < .001$, SVR vs non-SVR. (*B*) The cumulative incidence of HCC was significantly lower in SVR patients (*black line*) and relapsers (*black dashed line*) than that in NR patients (*gray line*). $P < .001$, SVR vs NR; $P = .002$, SVR vs relapse; $P = .001$, relapse vs NR.

higher AFP24 levels (Table 4). As for stratified analysis for HCC incidence, the cumulative incidence of HCC was higher with higher AFP24 levels in both SVR (Figure 2*A*) and non-SVR patients (Figure 2*B*), and with higher i-ALT levels in all non-SVR patients (Figure 2*C*), and non-SVR patients according to AFP24 levels (Figure 2*D*).

Cumulative Incidence of Hepatocellular Carcinoma According to the Change in α-Fetoprotein Levels

The association between the change in serum AFP levels and the cumulative incidence of HCC was assessed in all patients, in stratified analysis according to SVR and non-SVR (Supplementary Figure 1). For those patients with a PreAFP level of 5 ng/mL or greater, the cumulative incidence of HCC was significantly lower among the patients with an AFP24 level less than 5 ng/mL than the patients with an AFP24 level of 5 ng/mL or greater in each group (all patients, $P < .001$; SVR, $P = .046$; non-SVR, $P = .003$). For those patients with an AFP24 level less than 5 ng/mL, no significant differences were found in the cumulative incidence of HCC between the patients with a PreAFP level of 5 ng/mL or greater and the patients with a PreAFP level less than 5 ng/mL in each group (all patients, $P = .074$; SVR, $P = .299$; non-SVR, $P = .139$).

Table 3. Risk Factors for HCC Incidence Among the Post-treatment Factors According to Antiviral Effect (Cox Proportional-Hazards Model) in Patients With SVR

		Univariate analysis			Multivariate analysis		
Factor	Category	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age, *y*	0: <55	1			1		
	1: 55–64	5.924	1.326–26.471	.020	3.007	0.638–14.181	.164
	2: ≥65	9.649	2.085–44.659	.004	5.814	1.124–30.070	.036
Sex	0: female	1	0.908–5.366	.081			
	1: male	2.207					
White blood cell count at 24 wk after EOT	0: ≥5000/mm^3	1	0.240–1.362	.207			
	1: <5000/mm^3	0.571					
Hemoglobin level at 24 wk after EOT	0: ≥14 g/dL	1	0.359–1.910	.658			
	1: <14 g/dL	0.828					
Platelet count at 24 wk after EOT	0: ≥15 × 10^4/mm^3	1	0.943–5.312	.068			
	1: <15 × 10^4/mm^3	2.238					
Total bilirubin level at 24 wk after EOT	0: <0.8 mg/dL	1	0.386–2.311	.901			
	1: ≥0.8 mg/dL	0.945					
Serum albumin level at 24 wk after EOT	0: ≥4.0 g/dL	1	0.690–8.231	.170			
	1: <4.0 g/dL	2.382					
i-ALT	0: >30 IU/L	1	0.228–1.973	.468			
	1: ≤30 IU/L	0.671					
AFP24	0: <5 ng/mL	1	2.588–22.159	<.001	1	2.738–23.942	<.001
	1: ≥5 ng/mL	7.573			8.096		

CI, confidence interval.

Table 4. Risk Factors for HCC Incidence Among the Post-treatment Factors According to Antiviral Effect (Cox Proportional-Hazards Model) in Patients Without SVR

		Univariate analysis			Multivariate analysis		
Factor	Category	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age, *y*	0: <55	1			1		
	1: 55–64	4.267	1.669–10.911	.002	3.546	1.310–9.596	.013
	2: ≥65	7.128	2.819–18.025	<.001	6.327	2.355–17.00	<.001
Sex	0: female	1	1.418–3.525	.001	1	1.760–5.787	<.001
	1: male	2.236			3.192		
White blood cell count at 24 wks after EOT	0: ≥5000/mm^3	1	0.632–1.617	.963			
	1: <5000/mm^3	1.011					
Hemoglobin level at 24 wks after EOT	0: ≥14 g/dL	1	0.556–1.369	.553			
	1: <14 g/dL	0.873					
Platelet count at 24 wks after EOT	0: ≥15 × 10^4/mm^3	1	1.487–3.920	<.001	1	0.591–2.063	.756
	1: <15 × 10^4/mm^3	2.414			1.104		
Total bilirubin level at 24 wks after EOT	0: <0.8 mg/dL	1	1.075–2.901	.025	1	0.466–1.489	.537
	1: ≥0.8 mg/dL	1.766			0.833		
Serum albumin level at 24 wks after EOT	0: ≥4.0 g/dL	1	1.710–4.579	<.001	1	0.961–3.140	.068
	1: <4.0 g/dL	2.799			1.737		
i-ALT	0: >60 IU/L	1			1		
	1: 31–60 IU/L	0.531	0.339–0.831	.006	0.728	0.388–1.365	.322
	2: ≤30 IU/L	0.115	0.041–0.324	<.001	0.181	0.040–0.827	.027
AFP24	0: <5 ng/mL	1			1		
	1: ≤5 to <10 ng/mL	4.340	1.949–9.663	<.001	3.347	1.371–8.171	.008
	2: ≥10 ng/mL	6.785	3.111–14.797	<.001	4.855	1.814–12.996	.002
PEG-IFN/RBV antiviral effect	0: NR	1	0.349–0.901	.017	1	0.676–2.699	.394
	1: relapse	0.561			1.351		

CI, confidence interval; RBV, ribavirin.

Fitness of Model for Hepatocellular Carcinoma Incidence

Finally, we assessed which was a more applicable model for HCC incidence among 2 models: the pretreatment factor model or the post-treatment factor model. The variables of age, sex, platelet counts, ALT levels, AFP levels, and virologic response were examined for all patients. The post-treatment model was shown to be significantly better fitted for HCC incidence than the pretreatment model ($P = .0008$) (Table 5). When the AFP levels were compared between pretreatment (PreAFP) and post-treatment (AFP24) for all patients, the AFP24 level was shown to be more applicable for HCC incidence than the PreAFP level ($P < .0001$). Furthermore, even in the stratified analysis according to the virologic response, AFP24 levels were more applicable than PreAFP levels in both groups (SVR, $P = .03$; non-SVR, $P = .001$) (Table 5).

Discussion

In the present study, the risk for HCC incidence was significantly lower in SVR patients than in non-SVR patients (at 5 years, 2.6% vs 11.7%), as previously reported.[2,12,13] However, to date, the risk factors for HCC incidence in each virologic response or the relationship between HCC incidence and the factors changed by IFN therapy had not been fully examined. Then, we examined the relationship between HCC incidence and post-treatment factors based on the antiviral effects with a large-scale cohort undergoing Peg-IFN plus ribavirin therapy.

For SVR patients, AFP24 and ALT24 levels significantly decreased compared with PreAFP and PreALT levels. HCC incidence significantly increased with higher AFP24 levels but not with higher ALT24 levels. Moreover, the multiple Cox regression showed that AFP24 levels as well as age were significant risk factors for HCC incidence. These results suggested that HCC incidence in SVR patients is accompanied by an AFP increase but not an ALT increase. Although AFP can be a comprehensive surrogate marker for HCC incidence in relation to various factors, such as liver inflammation and fibrosis, our data suggest that AFP can be a marker for HCC incidence independent of liver inflammation. In clinical practice, even if HCV was eradicated and the serum ALT level was normal, careful surveillance for HCC was needed for patients with an AFP24 of 5 ng/mL or greater.

As previously reported,[13] the cumulative incidence of HCC was significantly lower in relapsers than in NR patients in this study. However, the reason why HCC incidence was reduced among relapsers who showed transient HCV disappearance in sera has been unclear.[13] In this study, multiple Cox regression for HCC incidence among non-SVR patients using post-treatment factors, which included AFP24 levels and i-ALT levels after the EOT, showed that AFP24 and i-ALT levels were significant risk factors for HCC incidence but not factors of

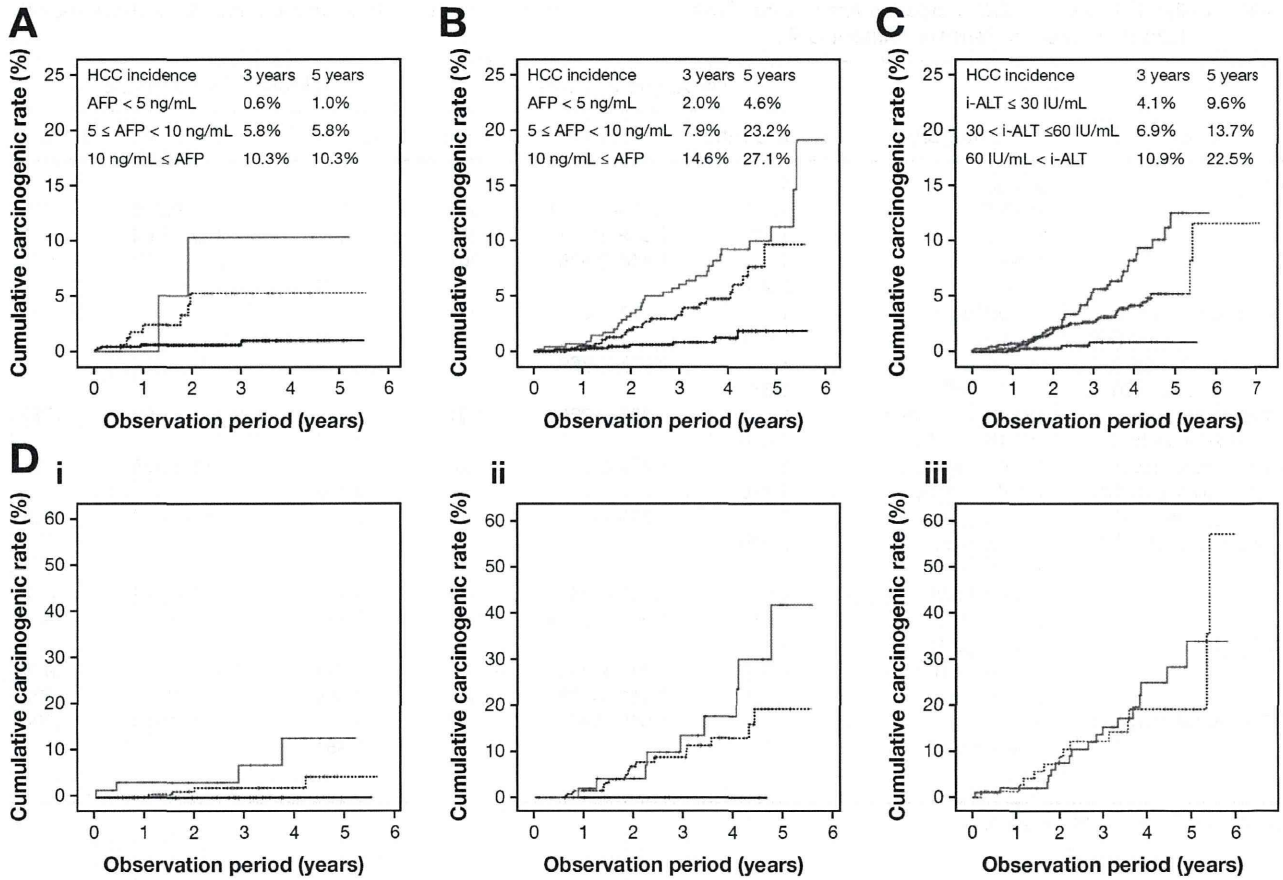

Figure 2. Cumulative incidence of HCC according to AFP levels at 24 weeks after the end of treatment and integrated ALT levels after the end of treatment. The cumulative incidence of HCC was higher with higher AFP24 levels in both SVR (*A*) and non-SVR patients (*B*). AFP24, *black line*, AFP24 < 5 ng/mL; *black dashed line*, 5 ng/mL ≤ AFP24 < 10 ng/mL; *gray line*, 10 ng/mL ≤ AFP24. The cumulative incidence of HCC was higher with higher i-ALT levels in all non-SVR patients (*C*) and in stratified analysis according to AFP24. (*D*) Patients with AFP24 < 5 ng/mL (*i*); AFP24 between 5 to 10 ng/mL (*ii*), AFP24 ≥ 10 ng/mL (*iii*). i-ALT, *black line*, i-ALT ≤ 30 IU/L; *black dashed line*, 30 IU/L < i-ALT ≤ 60 IU/L; *gray line*, 60 IU/L < i-ALT.

virologic response (relapse or NR). After dividing non-SVR patients into relapse and NR groups, if they had an AFP24 level less than 5 IU/L and an ALT24 level of 30 IU/L, HCC incidences were 0.8% (1 of 133) among relapsers and none (0 of 42) among NR patients (Supplementary Table 1), suggesting that the patients with an AFP24 level less than 5 ng/mL and an ALT24 level of 30 IU/L or less have a low potential of HCC

Table 5. The Fitness for HCC Incidence Among the Pretreatment and Post-treatment Factors (the Likelihood Ratio Test)

	Model			Likelihood ratio test	
Patients	Pretreatment factor	Post-treatment factor	Log-transformed likelihood ratio	χ^2 statistics	*P* value
All patients	Age, sex, platelets at baseline, PreALT, PreAFP, PEG-IFN/RBV antiviral effect	Age, sex, platelets at 24 wks after EOT, i-ALT, AFP24, PEG-IFN/RBV antiviral effect	5.641	11.28	.0008
All patients	PreAFP	AFP24	13.28	26.55	<.001
SVR patients	PreAFP	AFP24	2.33	4.66	.03
Non-SVR patients	PreAFP	AFP24	3.69	7.38	.007

NOTE. The likelihood ratio (its logarithm) is calculated as the ratio of the likelihood from the fitted model with post-treatment factors (the numerator) to one with pretreatment factors (the denominator). If the ratio is larger than 1 and it is statistically significant, it suggests that the fitted model with post-treatment factors is a better predictive model compared with the fitted model with pretreatment factors.
RBV, ribavirin.

incidence in both groups. A significant decrease in AFP levels after the treatment was observed only in relapsers but not in NR patients (Table 1). In addition, a decrease in ALT levels after the treatment was more prominent in relapsers than in NR patients (Table 1). Our data suggest that the suppressive effect on HCC incidence in relapsers could be mediated by a decrease in AFP and ALT levels. The relapse factor, which is a confounding factor for the decrease in AFP and ALT levels, could fail to be selected as a significant factor associated with HCC incidence in multiple Cox regression.

In the present study, AFP levels decreased through therapy, and the patients with AFP24 levels less than 5 ng/mL had a low potential of HCC incidence regardless of HCV eradication (Figure 2*A* and *B*). Our findings suggest that AFP24 levels can be a good surrogate marker for HCC incidence irrespective of the virologic response. However, in the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) trial in which Peg-IFN was administered to patients with NR to Peg-IFN plus ribavirin therapy, no significant suppressive effect of Peg-IFN on HCC incidence was observed among patients with CH-C.[14] Whether HCC incidence decreased among patients with lower post-treatment AFP levels in a HALT-C trial is critically interesting. From now on, the CH-C patients will be treated with an IFN-free regimen using direct-acting antivirals. However, it is unknown whether the AFP levels and HCC incidence will decrease in patients treated with an IFN-free regimen. Further examination is needed to clarify this issue.

In addition to age, sex, platelet counts, and AFP24 levels, the i-ALT levels after the EOT were associated significantly with HCC incidence among non-SVR patients. It should be noted that although the AFP and ALT values generally are correlated with each other in patients with CH-C,[15] the present study showed that higher AFP24 levels and higher i-ALT levels after the EOT were associated independently with a higher incidence of HCC. In previous studies, the HCC incidence was reduced significantly if the ALT level was kept below 80 IU/L.[11] However, HCC incidence was significantly higher in patients with i-ALT levels greater than 60 IU/L and lower in those with a serum i-ALT level of 30 IU/L or less in this study. Therefore, keeping the ALT levels below 30 IU/L may suppress the risk of HCC incidence. However, the factor of liver-supporting therapy was not significant in post-treatment simple Cox regression for HCC incidence in this study ($P = .72$; 95% CI, 0.578–1.461). The utility of keeping the ALT level low by using liver-supporting therapy to prevent HCC development needs to be clarified in future investigations.

In this study, the utility of re-evaluation after IFN therapy for the risk factor associated with HCC incidence also was assessed (Table 5). In the re-evaluation, the post-treatment model was shown to be more applicable for predicting HCC incidence than the pretreatment model. Moreover, the AFP24 level was more applicable for HCC incidence than the PreAFP level in all patients, in stratified analysis according to SVR patients and non-SVR patients. These results suggest that the post-treatment AFP24 level is very important for the surveillance of HCC after IFN therapy.

The limitation of this study is as described later. It is well known that HCC occurs more often in patients with cirrhosis. It would be very interesting to determine whether the results obtained from this study are as valid for patients with cirrhosis or not. However, the patients with cirrhosis were in the minority since this study was conducted predominantly for patients with CH. Therefore, these data may not be applicable to other populations, such as HALT-C. Further examination is needed to clarify this issue.

In conclusion, we suggest that the AFP24 value was associated strongly with HCC incidence irrespective of virologic response. Extra attention to the possibility of HCC incidence should be required, even for SVR patients, if their AFP24 levels are high. Among non-SVR patients, those with higher AFP24 levels, and ALT levels after IFN therapy, special caution is needed for HCC incidence.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://dx.doi.org/10.1016/j.cgh.2013.11.033.

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