# 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.

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*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.


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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 Table 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.

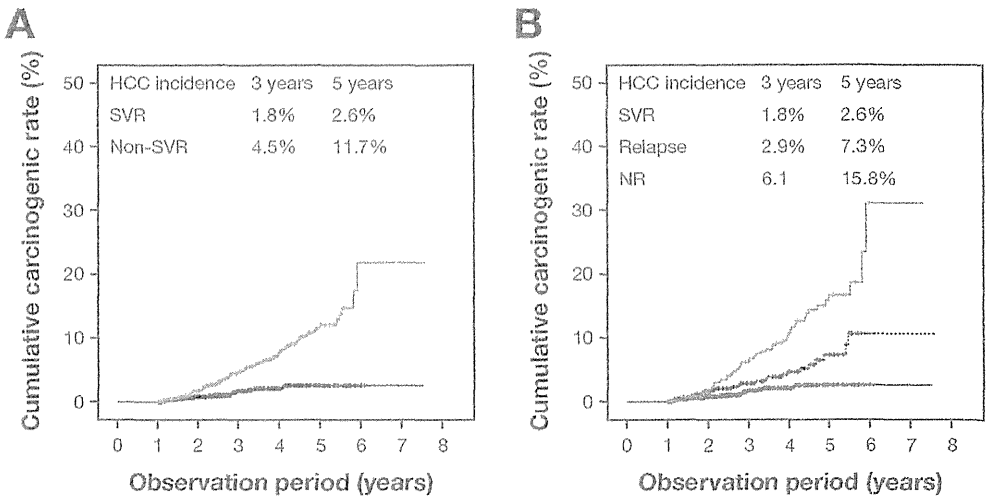


**Figure 1.** Cumulative incidence of HCC according to the antiviral effect of Peg-IFN plus ribavirin combination therapy. (*A*) The cumulative incidence of HCC was significantly lower in SVR patients (*black line*) than that in non-SVR patients (*gray line*). $P < .001$, SVR vs non-SVR. (*B*) The cumulative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

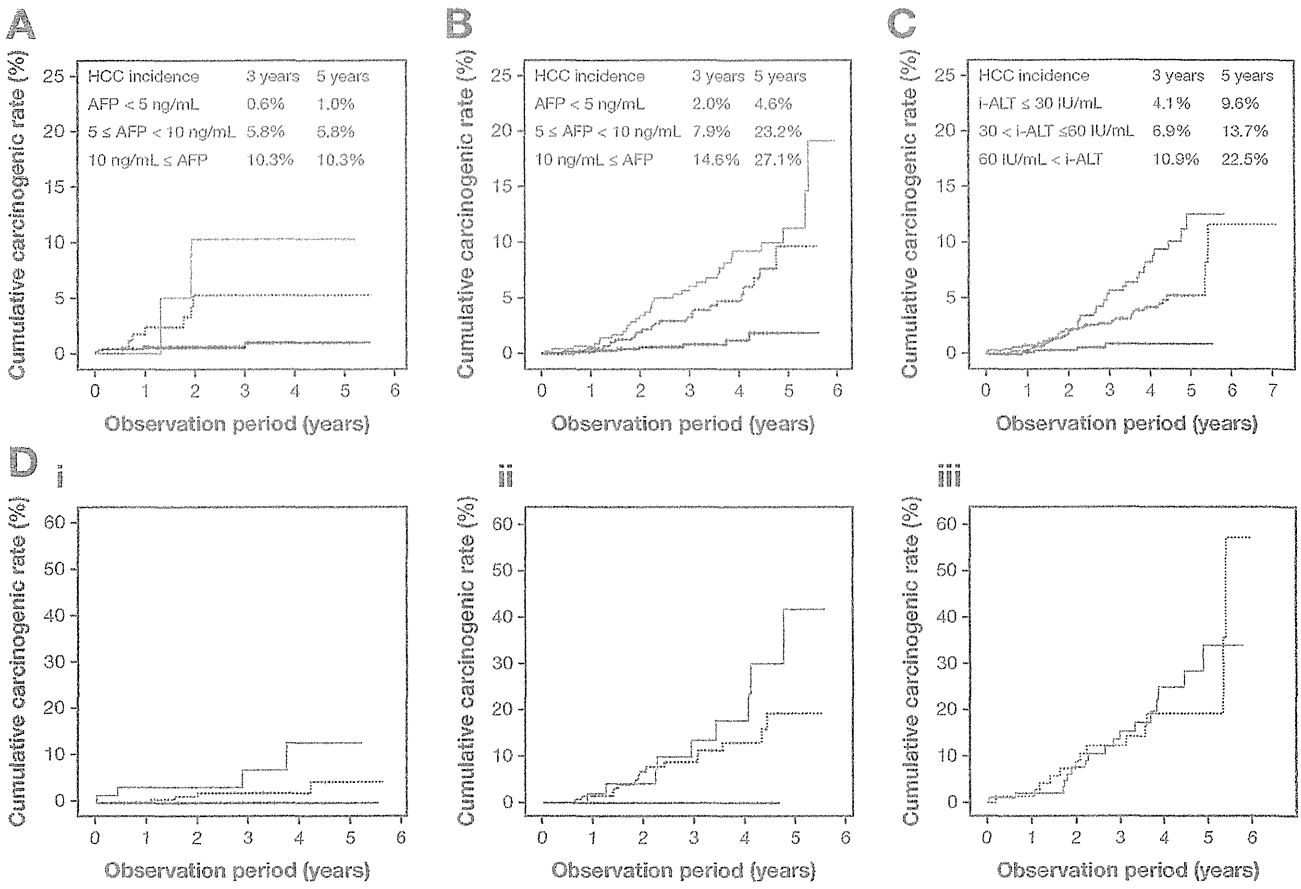


**Figure 2.** Cumulative incidence of HCC according to AFP levels at 24 weeks after the end of treatment and integrated ALT levels after the end of treatment. The cumulative incidence of HCC was higher with higher AFP24 levels in both SVR (*A*) and non-SVR patients (*B*). AFP24, *black line*, AFP24 < 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 | $\chi^2$ 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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# Liver stiffness measurement by acoustic radiation force impulse is useful in predicting the presence of esophageal varices or high-risk esophageal varices among patients with HCV-related cirrhosis

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**Abstract**
*Background* Screening and periodic surveillance for esophageal varices (EVs) by esophagogastroduodenoscopy (EGD) are recommended for cirrhotic patients. We investigated non-invasive liver stiffness measurement using acoustic radiation force impulse (ARFI) for the diagnosis of EV presence and high-risk EVs among patients with HCV-related cirrhosis.
*Methods* Among 181 consecutive patients with HCV-related cirrhosis, we studied 135 patients who had received EGD and ARFI. Serum fibrosis markers [platelet count, FIB-4, and aspartate aminotransferase-to-platelet ratio index (APRI)] were measured in a training set of 92 patients and compared with ARFI in the diagnostic performance for EV presence and high-risk EVs. Furthermore, the obtained optimal cutoff values of ARFI were prospectively examined in a validation set of 43 patients.
*Results* In the training set, the ARFI value increased with the EV grade ($p < 0.001$). The ARFI value for high-risk EVs was significantly higher than that for low-risk EVs ($p < 0.001$). AUROC values for diagnosis of EV presence and high-risk EVs by ARFI were 0.890 and 0.868, which had the highest diagnostic performance among factors including serum fibrosis markers. The optimal cutoff value of ARFI for EV presence was 2.05 m/s with good sensitivity (83 %), specificity (76 %), PPV (78 %), and NPV (81 %), and that for high-risk EVs was 2.39 m/s with good sensitivity (81 %), specificity (82 %), PPV (69 %), and NPV (89 %). These cutoff values obtained in the training cohort also showed excellent performance in the validation set.
*Conclusions* Liver stiffness measurement by ARFI is useful in predicting EV presence or high-risk EVs among patients with HCV-related cirrhosis.

**Keywords** Acoustic radiation force impulse · Esophageal varices · HCV-related cirrhosis · Portal hypertension · Liver stiffness

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## Introduction

Esophageal varices (EVs) resulting from portal hypertension are present in approximately 50 % of patients with cirrhosis [1], and variceal bleeding is life-threatening with a 14 % mortality for hospitalized patients [2, 3]. The risk of bleeding has been shown to be related to the size of the varices, the presence of red signs, and the stage of liver insufficiency as evaluated by the Child–Pugh score [2, 4, 5]. Patients with high-risk EVs require prophylactic treatment to prevent variceal bleeding [1, 6]. Among patients with cirrhosis, the rate of EV incidence was reported to be 5 % at 1 year, 17 % at 2 years, and 28 % at 3 years, and the rate of EV progression from a small to a large size was found to be 12 % at 1 year, 25 % at 2 years, and 31 % at 3 years [7]. Therefore, cirrhotic patients should undergo periodic surveillance for EVs.

Esophagogastroduodenoscopy (EGD) is the gold standard for the diagnosis of EVs. American Association for the Study of Liver Diseases (AASLD) guidelines and Baveno V consensus strongly recommend screening EGD for all patients who are diagnosed with cirrhosis [1, 6]; the recommended intervals are 2–3 years for patients without varices and 1–2 years for those with small varices [6]. However, EGD causes psychological distress for patients when not performed under sedation, resulting in poor acceptance by patients. Furthermore, repeated EGD examinations can lead to complications and entail high costs. Therefore, instead of EGD, the development of non-invasive methods which can be useful in evaluating EVs is very important.

Several non-invasive markers such as platelet count, FIB-4 index, aspartate aminotransferase-to-platelet ratio index (APRI), and FibroTest have been examined as predictors of the accumulation of fibrosis and have been reported to predict the presence of EVs and large EVs in cirrhotic patients [8–10]. However, their performance values were not sufficiently accurate to support the use of these markers as alternatives to EGD. On the other hand, the platelet count/spleen diameter ratio (PSR) with a cutoff value of 909 has shown to have high sensitivity (100 %) and specificity (93 %) [11]. However, the PSR cutoff value (909) has not shown good accuracy for the prediction of EVs in a recent study [12].

As a non-invasive and ultrasound-based method, transient elastography (TE) has shown excellent diagnostic accuracy for the estimation of liver fibrosis [13, 14]. In addition, TE is useful for predicting portal hypertension [15]. However, TE measurements are difficult to perform with obese patients and when ascites is present, and the interfering structures such as blood vessels can not to be avoided in TE measurement [16]. On the other hand, acoustic radiation force impulse (ARFI), a new ultrasound imaging modality for evaluation of liver stiffness, can be performed for these patients, and a high level of accuracy of ARFI for predicting liver fibrosis has been reported over the last few years [17–19].

The aim of this study was to investigate whether ARFI can be useful in selecting patients with hepatitis C virus (HCV)-related cirrhosis who need screening EGD for the diagnosis of EV and who need EGD at short intervals for the diagnosis of the progression to high-risk EVs.

## Patients and methods

The 181 consecutive patients with HCV-related cirrhosis visited in our hospital between April 2009 and January 2013. Among them, 40 patients did not undergo ARFI and 6 patients did not undergo EGD. Finally, 135 patients who had undergone both EGD and ARFI were enrolled in this study. ARFI was measured less than 6 months before or after the EGD examination. HCV infection was diagnosed by a real-time PCR method. Diagnosis of cirrhosis was done by histologic examination or combined physical, laboratory, and radiologic findings. Patients with a history of endoscopic treatment for EVs, portal thrombosis, β-blocker use, post-liver transplantation, co-infection with HBV, or other causes of liver disease [autoimmune hepatitis, alcoholic liver disease, non-alcoholic steatohepatitis (NASH), primary biliary cirrhosis, etc.] were excluded from the study.

The patient characteristics and the following biochemical tests were recorded at the time of ARFI: platelet count, prothrombin time, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, and albumin. Data were collected on the presence or absence of ascites, hepatic encephalopathy, and hepatocellular carcinoma (HCC). The Child–Pugh scores were also determined.

The training set comprised 92 patients who had undergone EGD and ARFI measurements between April 2009 and September 2012. The validation set comprised 43 patients who had undergone EGD and ARFI measurements from October 2012 to January 2013. We examined the diagnostic performance of ARFI for the presence of EVs and high-risk EVs and conducted comparative analysis with several serum non-invasive markers in the training set. The obtained optimal cutoff values of ARFI were prospectively examined in the validation set.

This study was approved by the institution's ethics committee and was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki amended in 2008. Written informed consent was obtained from all study patients.

### Non-invasive serum markers

The relationships were examined for three established serum markers of liver fibrosis, i.e., platelet count, FIB-4, and APRI, and the presence of EVs or the risk of variceal bleeding. Serum liver fibrosis scores were calculated according to previously published formulas: FIB-4 = [age (years) × AST (IU/L)]/[platelet count ($10^9$/L) × ALT (IU/L)$^{1/2}$]; APRI = [(AST/ULN) × 100]/platelet count $10^9$/L (ULN = the upper limit of normal) [20, 21].

### Liver stiffness measurements

Liver stiffness was measured by ARFI, using the Siemens ACUSON S-2000 ultrasound system. ARFI is based on the measurement of the acoustic shear wave induced by an ultrasonic push pulse to assess the elastic properties of target tissues [22]. During real-time B-mode imaging, a region of interest (ROI) was set in the right hepatic lobe at a depth of 2 cm below the liver capsule. Patients were

examined in a supine position with short intervals of breath-holding. The operator performing the ARFI measurements was blinded to the EGD results. The velocity of the shear wave was quantified, and the results are expressed in meters per second (m/s). The mean values of 10 valid measurements for each patient were used for further analyses.

### Endoscopic evaluation of EVs

EGD examination was performed by endoscopists who were blinded to the AFRI results. Consensus was reached for discrepancies in the diagnosis of varices during endoscopic conferences. EVs were recorded according to the general rules of the Japanese Society for Portal Hypertension [23]. The grade of EVs was classified as follows: F0, lesions assuming no varicose appearance; F1, straight small-calibered varices; F2, moderately enlarged, beady varices; F3, markedly enlarged, nodular, or tumor-shaped varices. Red color (RC) signs included red wale marking, cherry red spot, or hematocytic spot. We categorized varices as presence (grade $\geq$ F1) or absence (grade = F0). Patients were then divided into two groups according to the risk of EV bleeding; high-risk EVs (grade $\geq$ F2 or F1 with RC signs) and low-risk EVs (F0 or F1 without RC signs).

### Statistical analysis

Quantitative demographic data are expressed as mean ± standard deviation. The non-parametric Mann–Whitney test was used to compare various subgroups. The relationship between the ARFI value and the grade of varices was assessed by the Jonckheere–Terpstra test. The diagnostic performance of ARFI, platelet count, APRI, and FIB-4 was assessed by using curves of receiver operating characteristics (ROC) and analysis of the area under the ROC (AUROC) curve. The optimal cutoff values were determined to maximize the sum of sensitivity (Se) and specificity (Sp). Se, Sp, positive predictive value (PPV), and negative predictive value (NPV) were calculated. AUROCs were compared using the DeLong test. The 95 % confidence intervals (95 % CIs) were calculated for each predictive test and a $p$ value less than 0.05 was regarded as significant for each statistical test. All statistical analyses were performed with SPSS software, version 19 (SPSS, Chicago, IL, USA) and MedCalc software (MedCalc, Ostend, Belgium).

## Results

The characteristics of the patients in the training and validation sets are presented in Table 1. ARFI was successfully performed in all patients. In the training set, EVs were found on conducting EGD in 47 patients (51.1 %): 19 with F1, 22 with F2, and 6 with F3. RC signs were present in 24 patients (26.1 %). There were 31 (33.7 %) and 61 (66.3 %) patients with high-risk and low-risk EVs, respectively. In the validation set, EVs were present on EGD in 27 (62.8 %) patients. High-risk EVs were present in 12 patients (27.9 %). The median ARFI values of the training set and the validation set were 2.12 m/s [interquartile range (IQR) 1.56–2.69] and 2.28 m/s (IQR 1.69–2.61).

Table 1 Baseline characteristics of patients in training and validation sets

| Factor | Training set | Validation set |
|---|---|---|
| Number | 93 | 43 |
| Age (year) | 68.8 ± 9.3 | 72.6 ± 6.9 |
| Sex (male/female) | 48/45 | 26/17 |
| BMI (kg/m$^2$) | 22.7 ± 3.2 | 22.3 ± 3.2 |
| Child–Pugh class | | |
| A | 60 (64.5 %) | 31 (72.1 %) |
| B | 31 (33.3 %) | 11 (25.6 %) |
| C | 2 (2.2 %) | 1 (2.3 %) |
| HCC | | |
| No | 47 (50.5 %) | 12 (27.9 %) |
| Yes | 46 (49.5 %) | 31 (72.1 %) |
| Ascites | | |
| No | 82 (88.2 %) | 35 (81.4 %) |
| Yes | 11 (11.8 %) | 8 (18.6 %) |
| Platelet ($\times 10^4$/mm$^3$) | 9.63 ± 4.94 | 9.85 ± 4.30 |
| AST (IU/L) | 54.0 ± 29.9 | 38.6 ± 22.6 |
| ALT (IU/L) | 48.9 ± 32.9 | 53.4 ± 38.6 |
| Albumin (g/dl) | 3.59 ± 0.59 | 3.67 ± 0.56 |
| Total bilirubin (mg/dl) | 1.06 ± 0.86 | 1.02 ± 0.61 |

Results are given as mean ± standard deviation or $n$ (%)

*BMI* body mass index, *HCC* hepatocellular carcinoma, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

### ARFI measurements and EV grades

The median ARFI values for patients without EVs and with EVs were 1.59 m/s (IQR 1.27–2.07) and 2.63 m/s (IQR 2.14–3.03) and the ARFI value for patients with EVs was significantly higher than that without EV ($p < 0.001$) (Fig. 1a). The median ARFI values according to the grade of EVs were as follows: F0, 1.59 m/s (IQR 1.27–2.07); F1, 2.23 m/s (IQR 1.95–2.66); F2, 2.74 m/s (IQR 2.47–3.12); F3, 3.02 m/s (IQR 2.89–3.16) (Fig. 1b). The ARFI value increased with the grade of EVs ($p < 0.001$). The median ARFI value for low-risk and high-risk EVs were 1.74 m/s (IQR 1.40–2.24) and 2.84 m/s (IQR 2.48–3.15), and the

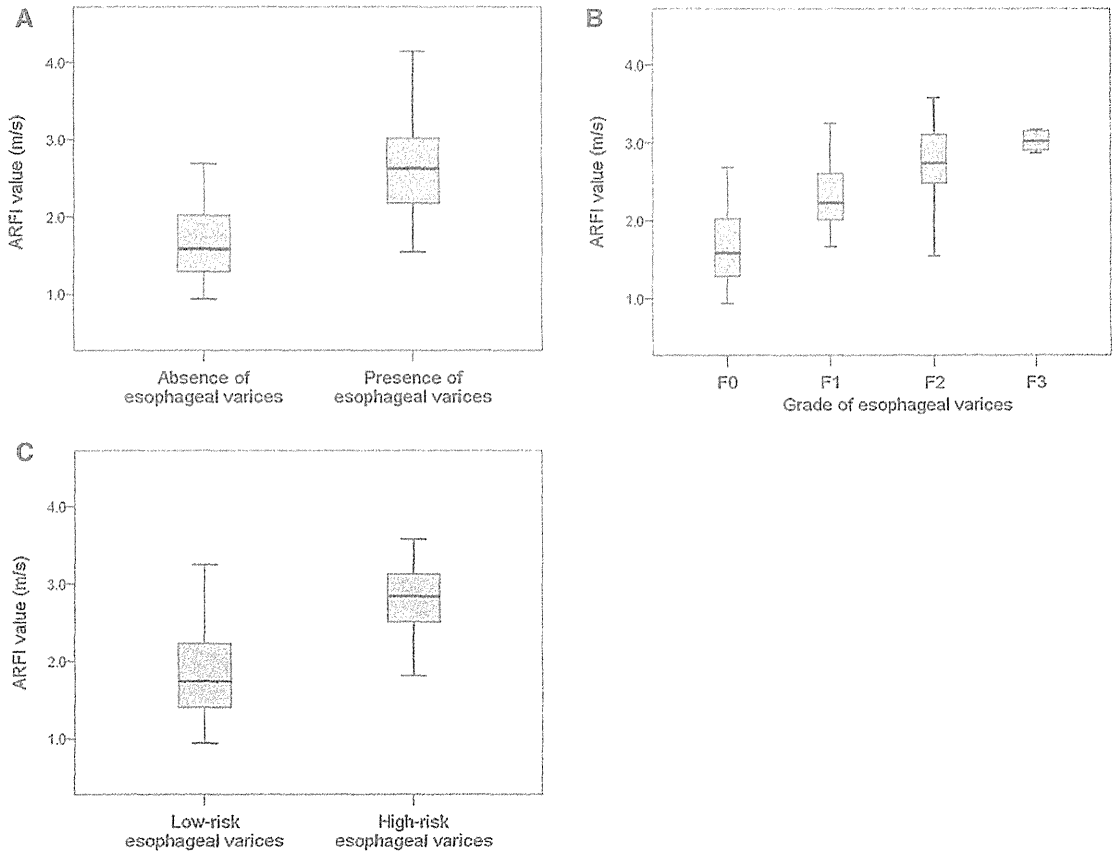


**Fig. 1** Correlation between ARFI values and esophageal varices. The *top* and *bottom* of each *box* represent the first and third quartiles, respectively. The *middle line* represents the median. Correlation between ARFI values and **a** the presence or absence of esophageal varices, **b** the grade of esophageal varices, **c** low-risk esophageal varices or high-risk esophageal varices

ARFI value with high-risk EVs was significantly higher than that with low-risk EVs ($p < 0.001$) (Fig. 1c).

## Diagnostic performances and optimal cutoff values for the presence of EVs

The ROC curves for diagnosis of the presence of EVs by ARFI, platelet count, FIB-4, and APRI are shown in Fig. 2. AUROCs (95 % CIs) were as follows: ARFI, 0.890 (0.825–0.955); platelet count, 0.735 (0.632–0.838); FIB-4, 0.745 (0.642–0.848); APRI, 0.684 (0.573–0.795). ARFI had the best diagnostic performance for predicting EVs compared with all other parameters (ARFI vs. platelet counts, $p = 0.0047$; ARFI vs. FIB-4, $p = 0.0041$; ARFI vs. APRI, $p = 0.0001$).

Table 2 shows the Se, Sp, NPV, and PPV of the optimal cutoff values for diagnosis of the presence of EVs. The optimal cutoff value of ARFI was 2.05 m/s with the best performance of Se (83 %), Sp (76 %), PPV (78 %), and NPV (81 %) compared with those of platelet count, FIB-4, and APRI.

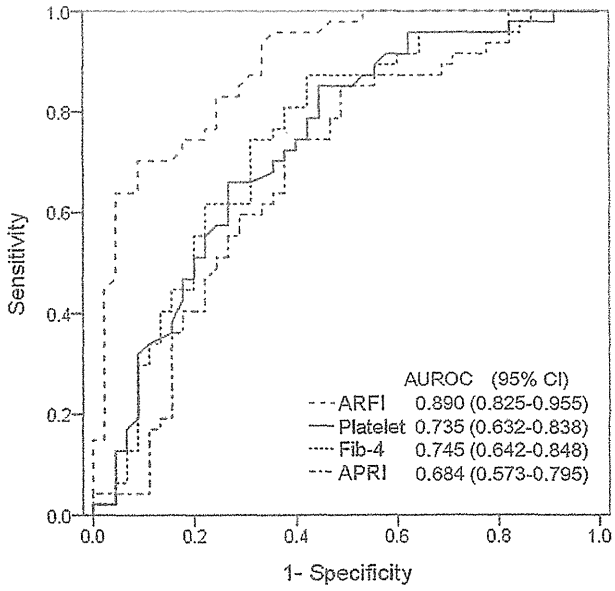


**Fig. 2** Receiver operating characteristics curves of ARFI, platelet count, FIB-4, and APRI for detecting the presence of esophageal varices

Table 2 Diagnostic performance of ARFI, platelet count, FIB-4, and APRI for detecting the presence of esophageal varices

| | Cutoff | Se (%) | Sp (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|
| ARFI | 2.05 | 83 | 76 | 78 | 81 |
| Platelet | 8.25 | 67 | 67 | 71 | 67 |
| FIB-4 | 6.21 | 71 | 69 | 73 | 69 |
| APRI | 1.50 | 59 | 64 | 67 | 64 |

*Se* sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value

### Diagnostic performances and optimal cutoff values for high-risk EVs

The ROC curves for diagnosis of high-risk EV by ARFI, platelet count, FIB-4, and APRI are shown in Fig. 3. AUROCs (95 % CIs) were as follows: ARFI, 0.868 (0.792–0.943); platelet count, 0.659 (0.547–0.771); FIB-4, 0.741 (0.635–0.847); APRI, 0.669 (0.555–0.784). ARFI had the best diagnostic performance for predicting EV compared with all other parameters (ARFI vs. platelet count, $p = 0.0004$; ARFI vs. FIB-4, $p = 0.0109$; ARFI vs. APRI, $p = 0.0002$).

Table 3 shows the Se, Sp, PPV, and NPV of the optimal cutoff values for diagnosis of high-risk EVs. The optimal cutoff value of ARFI was 2.39 m/s with the best performance of Se (81 %), Sp (82 %), PPV (69 %), and NPV (89 %) compared with those of platelet count, FIB-4, and APRI.

### Diagnostic performance of ARFI cutoff value in validation set

In the validation set, the optimal cutoff values of ARFI analyzed by the training set for diagnosis of the presence of EVs (2.05 m/s) and high-risk EVs (2.39 m/s) were prospectively analyzed. The ARFI cutoff value for the presence of EVs showed good performance with Se (85 %), Sp (81 %), PPV (89 %), and NPV (77 %), and that for high-risk EVs with Se (83 %), Sp (77 %), and NPV (92 %), but not PPV (59 %) (Table 4). Thus, the diagnostic performance of ARFI for the presence of EVs and high-risk EVs in the validation set were almost equal to those in the training set.

## Discussion

Screening EGD for EVs is clinically important in the management of patients with cirrhosis. However, EGD is an examination that is not readily accepted by patients [24]. Therefore, there has been increasing interest in developing non-invasive methods for prediction of EVs. Recently,

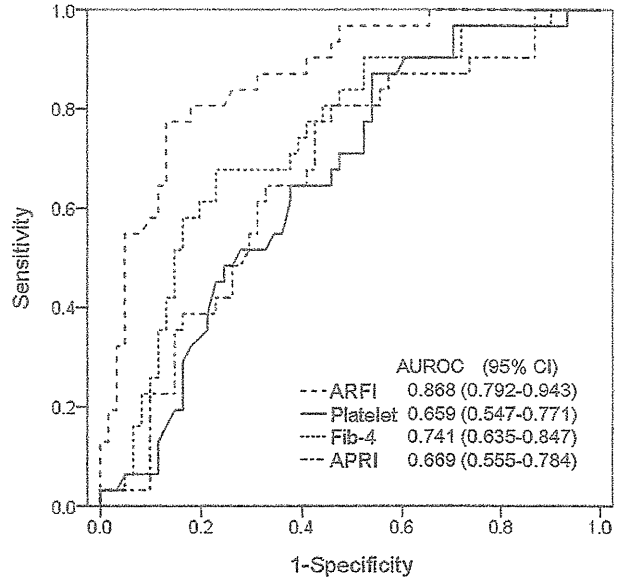


Fig. 3 Receiver operating characteristics curves of ARFI, platelet count, FIB-4, and APRI for diagnosis of high-risk esophageal varices

Table 3 Diagnostic performance of ARFI, platelet count, FIB-4, and APRI for detecting high-risk esophageal varices

| | Cutoff | Se (%) | Sp (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|
| ARFI | 2.39 | 81 | 82 | 69 | 89 |
| Platelet | 7.95 | 64 | 63 | 49 | 63 |
| FIB-4 | 7.70 | 67 | 78 | 63 | 78 |
| APRI | 1.62 | 64 | 68 | 53 | 68 |

*Se* sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value

Table 4 Diagnostic performance of ARFI for detecting the presence of esophageal varices and high-risk esophageal varices in training and validation sets

| | Cutoff | Set | Se (%) | Sp (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|
| Presence | 2.05 | Training | 83 | 76 | 78 | 81 |
| | | Validation | 85 | 81 | 89 | 77 |
| High-risk | 2.39 | Training | 81 | 82 | 69 | 89 |
| | | Validation | 83 | 77 | 59 | 92 |

*Se* sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value

several serum markers and imaging methods have been shown to correlate well with liver fibrosis and can replace liver biopsy in the diagnosis of liver fibrosis [18]. Several of these methods have been tried for non-invasive assessment of EV prediction. Another simple test, the platelet count, has not been a good predictor of the presence of EVs in patients with cirrhosis (AUROC 0.63) [8]. In a large cirrhotic cohort, FIB-4 showed an AUROC of 0.64 for the

presence of EVs and 0.63 for large EVs, and APRI showed an AUROC of 0.57 for the presence of EVs and 0.60 for large EVs [9]. In the present study, the diagnostic performances for the presence of EVs and high-risk EVs by platelet count (AUROC 0.735 and 0.659, respectively), FIB-4 (AUROC 0.745 and 0.741, respectively), and APRI (AUROC 0.684 and 0.669, respectively) were superior to those by previously reported findings [8, 9]. Nevertheless, ARFI showed better diagnostic performances (AUROC 0.890 and 0.868, respectively) than these serum fibrosis markers in this study.

In this study, we examined patients with HCV infection. A large study evaluating the performance of TE for diagnosis of cirrhosis showed variation in optimal liver stiffness cutoff values depending on the underlying cause of HCV infection, HBV infection, and alcoholic liver disease or NASH [25]. The factors which contribute to increasing liver stiffness other than fibrosis were reported as follows: severe inflammation which was characterized by ALT elevation often displayed in patients with chronic HBV infection [26–29], perisinusoidal fibrosis which was common in patients with alcoholic liver disease and NASH [25], and alcohol consumption characterized by AST elevation [30]. In addition, pooled meta-analysis suggested lower diagnostic performance for liver fibrosis in patients with chronic HBV infection. That is, the AUROC of diagnosis of the fibrosis (METAVIR fibrosis score $\geq$2) by ARFI in patients with chronic HBV infection was lower than that in those with chronic HCV infection (AUROC 0.79 vs. 0.88) [19]. This result could be related to architectural abnormalities, characterized by inhomogeneous liver fibrosis and macronodular cirrhosis, which is common in patients with HBV-related cirrhosis [25]. Thus, we suggest that liver stiffness measurement (LSM) for diagnosis of liver fibrosis should be evaluated according to the underlying disease, and the patients who were limited to HCV-related cirrhosis were examined in this study.

Recently, prediction of the presence of EVs and large or high-risk EVs by TE or ARFI has been reported in several studies [28, 31–36]. However, the diagnostic performances differed greatly among these reports; AUROC for the presence of EVs was from 0.58 to 0.84 and that for large or high-risk EVs was from 0.58 to 0.83 [28, 31–36]. The reason for these differences of AUROCs is considered to arise as a result of the underlying liver disease. In fact, Pritchett et al. [31] reported that the AUROC for predicting large varices by TE in patients with only HCV-related cirrhosis was higher than those with various cirrhosis etiologies except HCV-related (AUROC 0.78 vs. 0.72). Thus, as well as the evaluation of the LSM for diagnosis of liver fibrosis, the correlation between EVs and the liver stiffness by ARFI should be evaluated according to the specific etiology of liver diseases.

Our study is the first assessment of the prediction of EVs by ARFI for a patient group with homogeneous cirrhotic disease of HCV etiology. This led to the very good diagnostic performance for predicting the presence of EVs or high-risk EVs (AUROC 0.890 or 0.868, respectively) in the training cohort, and these results were also confirmed to prospectively validate another cohort of HCV-related cirrhosis patients whose characteristics differed from the training cohort. The results of our study showed better diagnostic performance than those of the past studies described above. Only one report showed high diagnostic performance of TE for the presence of EVs and high-risk EVs among patients with only HCV (AUROC 0.87 and 0.84, respectively) [37]. TE can show diagnostic performance for EVs comparable to ARFI. However, ARFI may have some advantages over TE for LSM. For one, the rate of unsuccessful TE was reported to be as high as 18.9 %, mostly because of obesity, ascites, and patients with narrow intercostal spaces [16]. On the other hand, ARFI is not limited by these conditions and the rate of unsuccessful results was reported to be 2.9 % overall [19]. In the present study, ARFI could be successfully performed in all patients. Moreover, ARFI is superior in terms of its convenience because it is integrated into a conventional ultrasonography (US) system using conventional probes and can be performed during standard US examinations. In addition, the ROI, which is 10 mm long and 6 mm wide, is smaller than that of TE and can be chosen while performing real-time B-mode imaging. Therefore, we can see the ROI while avoiding nearby interfering structures such as blood vessels and minimize the measurement error. Further study is needed to clarify whether ARFI surpasses TE in LSMs.

In recent studies, the relationship between spleen stiffness and the presence of EVs and high-risk or large EVs has been assessed. Takuma et al. [36] reported the high diagnostic performance of spleen stiffness for the presence of EVs and high-risk EVs (AUROC 0.933 and 0.930, respectively). On the other hand, Vermehren et al. [34] showed that the diagnostic performance of spleen stiffness for predicting large EVs was low (AUROC 0.58). Thus, the diagnostic values of EVs or portal hypertension by spleen stiffness remain controversial. The mechanism of spleen stiffness arising as portal hypertension develops should be investigated.

The limitation of our study is that a serial prospective study by ARFI for the development of EVs for a specific individual was not done. Therefore, the ideal interval for ARFI measurement to follow-up on EVs remains unclear. Further study is needed.

In conclusion, for patients with HCV-related cirrhosis, we showed that LSM by ARFI can non-invasively predict the presence of EVs or high-risk EVs. This indicates that the non-invasive ARFI can be useful in selecting patients with HCV-related cirrhosis who need screening EGD or EGD at short intervals for the diagnosis of the progression to high-risk EVs.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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# Risk factors for hepatocellular carcinoma in hepatitis C patients with normal alanine aminotransferase treated with pegylated interferon and ribavirin

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SUMMARY. Pegylated interferon (Peg-IFN) plus ribavirin combination therapy is effective in patients with hepatitis C virus (HCV) infection and normal alanine aminotransferase levels (NALT). However, it remains unclear whether the risk of hepatocellular carcinoma (HCC) incidence is actually reduced in virological responders. In this study, HCC incidence was examined for 809 patients with NALT (ALT ≤ 40 IU/mL) treated with Peg-IFN alpha-2b and ribavirin for a mean observation period of 36.2 ± 16.5 months. The risk factors for HCC incidence were analysed by Kaplan–Meier method and Cox proportional hazards model. On multivariate analysis among NALT patients, the risk of HCC incidence was significantly reduced in patients with sustained virological response (SVR) or relapse compared with those showing nonresponse (NR) (SVR *vs* NR, hazard ratio (HR): 0.16, $P = 0.009$, relapse *vs* NR, HR: 0.11, $P = 0.037$). Other risk factors were older age (≥65 years *vs* <60 years, HR: 6.0, $P = 0.032$, 60–64 *vs* <60 years, HR: 3.2, $P = 0.212$) and male gender (HR: 3.9, $P = 0.031$). Among 176 patients with PNALT (ALT ≤ 30 IU/mL), only one patient developed HCC and no significant risk factors associated with HCC development were found. In conclusion, antiviral therapy for NALT patients with HCV infection can lower the HCC incidence in responders, particularly for aged and male patients. The indication of antiviral therapy for PNALT (ALT ≤ 30 IU/mL) patients should be carefully determined.

*Keywords:* hepatitis C, hepatocellular carcinoma, normal alanine aminotransferase levels, Peg-IFN plus ribavirin combination therapy, persistent normal alanine aminotransferase levels.

## INTRODUCTION

The goal of treatment for patients with hepatitis C virus (HCV) infection is not only elimination of the virus, but also prevention of hepatocellular carcinoma (HCC) and thus of death due to liver disease. Therefore, the indication of antiviral therapy should be based on whether or not antiviral therapy can suppress the incidence of HCC.

In the era of interferon (IFN) monotherapy, patients with chronic HCV infection and normal alanine transaminase (NALT) were not considered as candidates for antiviral therapy because SVR rates were very low (6–15%) and ALT flare-up occurred in some cases (47–62%) on IFN therapy [1–3]. Recently, however, the antiviral efficacy of pegylated interferon (Peg-IFN) plus ribavirin combination therapy for patients with chronic HCV infection has led to

Abbreviations: ALT, alanine aminotransferase; CT, computed tomography; EALT, elevated alanine aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; NALT, normal alanine aminotransferase; NR, nonresponse; Peg-IFN, Pegylated interferon; PNALT, persistent normal alanine aminotransferase; SVR, sustained virological response.

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equivalent antiviral effects for patients with NALT levels, compared with those with elevated ALT levels (EALT) [4,5]. Moreover, ALT flare-up after Peg-IFN plus ribavirin combination therapy for patients with NALT has been reported to be rare [4]. Thus, patients with chronic HCV infection and NALT have come to be considered as candidates for Peg-IFN plus ribavirin combination therapy. What remains unknown is whether or not HCC incidence can be reduced in patients with HCV infection and NALT responding to antiviral therapy. In this study, this is what we tried to elucidate.

Another issue is the risk factors for HCC incidence in patients with HCV infection and NALT. For example, among chronic hepatitis patients with NALT, it is still controversial whether or not the viral load of HCV is related to HCC incidence, although high levels of HBV DNA have been revealed to be a risk factor for HCC incidence in patients with hepatitis B virus (HBV) infection [6]. We therefore also examined patients with chronic HCV infection and NALT, who are at high risk of HCC.

## PATIENTS AND METHODS

This retrospective, multicentre study was conducted by Osaka University Hospital and institutions participating in the Osaka Liver Forum. Of 4640 patients with chronic HCV infection who had been treated by Peg-IFN plus ribavirin combination therapy between December 2004 and December 2009, this study enrolled 809 patients with NALT (ALT ≤ 40 IU/mL) at the start of the therapy who had not suffered from HCC. Among them, 431 patients showed persistent normal alanine aminotransferase (PNALT), defined as an ALT value of ≤40 IU/mL (PNALT40) or ≤30 IU/mL (PNALT30), on two to three occasions separated by at least a month over a period of 6 months [7,8]. PNALT30–40 was defined as the observation of ALT values of 31–40 IU/mL on at least one occasion among PNALT40 patients. This study excluded patients who developed HCC within 12 months from the start of Peg-IFN plus ribavirin combination therapy, patients with co-infection with hepatitis B or human immunodeficiency virus, patients with drug-induced or alcoholic liver disorder and patients with autoimmune hepatitis. The protocol was performed after obtaining informed consent from each patient before treatment in accordance with the ethical guidelines of the 1975 Declaration of Helsinki amended in 2002.

### *Treatment protocol*

All patients received Peg-IFN alpha-2b (PEGINTRON; Merck & Co. Inc. Whitehouse Station, NJ, USA) plus ribavirin (REBETOL; Merck & Co). The serum HCV RNA levels were qualitatively analysed using the COBAS AMPLICOR HCV test, version 2.0 (lower limit of detection 50 IU/mL; Roche Diagnostics, Branchburg, NJ, USA), and the COBAS AMPLICOR HCV MONITOR test, version 2.0 (detection range 6–5000 K IU/mL; Roche Diagnostics). Among the patients with HCV genotype 1, as a rule, the treatment duration was 48 weeks. However, patients with detectable HCV RNA (≥50 IU/mL) at week 12 and undetectable HCV RNA (<50 IU/mL) at week 24 were treated for 72 weeks. Patients with HCV genotype 2 were treated for 24 weeks.

### *Definition of virological response*

A sustained virological response (SVR) was defined as HCV RNA being undetectable at the end of treatment and at 24 weeks after completion of treatment. A relapse was defined as undetectable HCV RNA at the end of treatment, but detectable HCV RNA at 24 weeks after completion of treatment. A nonresponse (NR) was defined as detectable HCV RNA at the end of treatment.

### *Histological evaluation*

Liver biopsy was performed immediately before initiating the Peg-IFN plus ribavirin combination therapy. Liver biopsy specimens were scored using the METAVIR system, and the grade of activity and stage of fibrosis were evaluated [9].

### *Surveillance of HCC*

Ultrasonography or computed tomography (CT) was carried out before Peg-IFN plus ribavirin combination therapy and every 3–6 months during the follow-up period. New space-occupying lesions detected or suspected at the time of ultrasonography were further examined by CT or hepatic angiography. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the CT findings. If no typical image of HCC was observed, fine-needle aspiration biopsy was carried out with the patient's consent, or the patient was carefully followed until a diagnosis was possible with a definite observation by CT or angiography.

### *End point*

The observation period started from the date of the start of Peg-IFN plus ribavirin combination therapy. Patients who developed HCC and patients who were retreated after completion of Peg-IFN plus ribavirin combination therapy were defined as censored cases at that point in time.

### *Statistical analysis*

Baseline data for various demographic, biochemical and virological characteristics of the patients were expressed as mean ± SD. The variables of age, sex, body mass index, HCV genotype, grading and staging of liver histology, platelet count, serum ALT level and virological response to the