low AFP (≤ 20 ng/mL), meaning that a small amount of AFP-L3 production could have easily raised the percentage of AFP-L3. Small constitutive expression of AFP-L3 by FUT8 activation in cirrhotic liver as well as the possibility of occult HCC in some patients might have led to the low specificity in cases with low AFP.

Despite the low sensitivity and specificity of AFP-L3, its increase was closely related to the prognosis of HCC patients; the risk ratio was 8.36 and was the highest among the factors examined in this study. The sensitivities of AFP at 20 ng/mL cut-off were reported to be approximately 25–55% and 53–70% when the tumor size was less than or equal to 2 cm and 5 cm, respectively.[14,15,20–23] The sensitivity of AFP in our department is 62%,[14] meaning that approximately 40% of our patients have previously undergone unreliable AFP-L3 measurements. Measuring AFP-L3 with the new method provides a good prediction of prognosis in large patient populations with normal AFP (≤ 20 ng/mL).

In the present study, we demonstrated the prognostic value of AFP-L3 in patients with low AFP (≤ 20 ng/mL). The outcome of LC patients with high AFP-L3 is a future issue that must be solved.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research of Japan Society for the Promotion of Science (KAKENHI 21890146). We thank Miss Asuka Maeda for assistance in carrying out experiments.

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Hepatology Research

Hepatology Research 2011; 41: 405–416 doi: 10.1111/j.1872-034X.2011.00785.x

Original Article

Long-term outcome and hepatocellular carcinoma development in chronic hepatitis B or cirrhosis patients after nucleoside analog treatment with entecavir or lamivudine

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Aim: We conducted this prospective study to elucidate the long-term outcome and incidence of hepatocellular carcinoma (HCC) development after nucleos(t)ide analog (NA) treatment in patients with chronic hepatitis B (CHB) or cirrhosis.

Methods: CHB or cirrhosis patients without past NA treatment or HCC were started on entecavir (ETV) or lamivudine (LVD), and prospectively followed up with monthly blood tests, and with abdominal imaging every 6 months in CHB and every 3 months in cirrhosis patients.

Results: A total of 256 subjects with CHB ($n = 194$) or cirrhosis ($n = 62$) received ETV ($n = 129$) or LVD ($n = 127$) for 4.25 years (range: 0.41–10.0). After NA treatment, serum HBV DNA, alanine aminotransferase and α-fetoprotein (AFP) dropped significantly, along with significant increases in serum albumin and prothrombin time. Drug-resistance developed in 60 cases in the LVD group and in only one case in the ETV group. HCC developed in 35 patients, and the incidence at years 1, 3, 5, 7 and 10 was significantly higher in patients with cirrhosis (8.1%, 17.5%, 43.2%, 46.7% and 53.4%, respectively) than chronic hepatitis (1.6%, 3.5%, 3.5%, 7.1% and 29.6%, respectively), with no difference between ETV and LVD. After NA treatment, the sensitivity/specificity for HCC of AFP and des-γ-carboxy prothrombin (DCP) was 45.7%/97.3% and 33.3%/96.2%, respectively, with the specificity of AFP being higher than at baseline (64.4%), at the cut-off of 10 ng/mL.

Conclusion: NA exerted a long-term efficacy and improved hepatic reservation in CHB and cirrhosis. After NA treatment, AFP dropped to lower than 10 ng/mL with marked elevation of specificity, leading to an earlier detection of HCC.

Key words: α-fetoprotein, chronic hepatitis B, entecavir, hepatitis B virus, hepatocellular carcinoma, lamivudine

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a serious health problem because of its potential to induce a variety of liver diseases, namely, acute hepatitis, chronic hepatitis (CH), liver cirrhosis, hepatocellular carcinoma (HCC) and fulminant hepatic failure.

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Conflicts of interest: None.

Received 5 December 2010; revision 8 January 2011; accepted 16 January 2011.

The prevalence of HBV carriage is reported to be 350–400 million people worldwide, and the prevalence rate of HBV infection in Japan is estimated to be 0.8%.[1,2] It has been reported that 15–20% of chronic hepatitis B (CHB) patients progress to cirrhosis within 5 years and that the annual incidence of HCC is 2.8%.[3] Nucleos(t)ide analogs (NA) suppress HBV DNA replication by inhibiting HBV DNA polymerase activity in the reverse transcription process from pregenomic RNA derived from a HBV closely covalent circular (ccc)DNA template.[4] Three NA anti-HBV agents, namely lamivudine (LVD), adefovir-dipivoxil (ADV) and entecavir (ETV), have been approved for coverage by the health

insurance program in Japan. They have been shown to be effective in decreasing serum HBV DNA and alanine aminotransferase (ALT) levels, improving hepatic reservation and liver histology.[5–11] It has also been reported that LVD decreases the risk of developing liver failure and hepatocarcinogenesis.[12,13]

A high serum HBV DNA load has been shown to be the most critical risk factor for HCC in HBV carriers.[14] Therefore, current treatment guidelines for patients with CHB or cirrhosis stress the importance of suppression of the serum HBV DNA load by antiviral treatment including NA administration in order to minimize the risk of liver disease progression and hepatocarcinogenesis.[15–18] However, the long-term effect of NA treatment on prognosis, especially on development of HCC in patients with CHB or cirrhosis, has not been fully elucidated.

Therefore, we designed this prospective cohort study to elucidate the virological and biochemical treatment effects and the long-term prognosis – particularly with respect to development of HCC – of patients with CHB or cirrhosis, who were started on LVD treatment, with or without co-administration with ADV, and ETV treatment.

METHODS

Study design

THIS STUDY WAS a prospective, non-randomized open-label cohort study on NA treatment for patients with CHB or cirrhosis without complications or past history of HCC. It was started as a study on LVD treatment for CHB or cirrhosis in November 2000, and then later modified to include ETV treatment when ETV was approved for coverage by the health insurance program and became commercially available in Japan in September 2006. This study was designed and performed in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines. The study was approved by the Institutional Committee for Human Rights. This study was also registered for the University Hospital Medical Information Network CTR system (ID: UMIN 000000594).

Subject population

The eligible patients were men and women aged 20 years or older, diagnosed with CHB or cirrhosis, without previous NA administration, and without complications or past history of HCC. The diagnosis of CHB and cirrhosis was made by positive hepatitis Bs antigen and positive serum HBV DNA with elevated serum ALT. The indication criteria for NA included serum HBV DNA greater than 5 log copies/mL with an elevated ALT level over twice the upper normal limit (ULN) or with complications of hepatic insufficiency, such as jaundice, ascites or encephalopathy.

The diagnosis of cirrhosis was confirmed by liver histology, clinical signs (encephalopathy or ascites), evidence of esophagogastric varices by endoscopy, or imaging modalities such as ultrasonography (US), computed tomography (CT) or magnetic resonance imaging (MRI).

All patients were deemed to be without complications of HCC by US, CT or MRI performed within 3 months before enrollment. Patients with acute hepatitis, fulminant hepatitis, alcoholic liver injury, co-infection with hepatitis C virus, autoimmune hepatitis, primary biliary cirrhosis, non-alcoholic steatohepatitis or hereditary liver diseases were excluded. All patients were informed of the aim and methodology of the study, received a written synopsis and gave their written consent to participate. The patients were started on LVD 100 mg/day or ETV 0.5 mg/day p.o. Some patients who had been started on ETV as participants of ETV phase II clinical study in Japan were also enrolled in this study.[9] In this paper, the study population consisted of the subjects enrolled between November 2000 and December 2009, and the patients' data between enrollment and November 2010 were used.

Patient follow up

The patients were followed up every month with a medical consultation and the following blood examinations: serum albumin, total bilirubin, aspartate AST, ALT, prothrombin time (PT%), platelet count (Plt), hepatitis B e-antigen (HBeAg), anti-HBe antibody by chemiluminescence immunoassay (Chemi-luminescent Immunoassay; Abbott Japan, Tokyo), and serum HBV DNA by polymerase chain reaction (PCR) assay (Roche Amplicor PCR assay; Roche Diagnostics, Tokyo, Japan), real-time PCR assay (Cobas TaqMan HBV Auto; Roche Diagnostics), or transcription-mediated amplification (TMA) assay (Chugai Diagnostics, Tokyo, USA). The detection ranges for the HBV DNA assay of PCR, real-time PCR and TMA were from 2.6–7.6 log copies/mL, from 1.8–8.8 log copies/mL and from 3.7–8.7 log genome equivalent/mL, respectively, permitting close determination with nearly equal quantitative accuracies. Levels of HBV DNA under the lower limit of normal (LLN) or over the ULN were assigned the value of the LLN or the ULN, respectively, in statistical analysis.

Drug resistance was confirmed by virological breakthrough and defined as an increase in serum HBV DNA by more than 1 log copy/mL greater than nadir. If virological breakthrough developed in patients receiving LVD, co-administration of ADV 10 mg/day or change to ETV 1.0 mg/day was selected. If virological breakthrough developed in patients receiving ETV, ETV was stopped and changed to co-administration of LVD 100 mg/day and ADV 10 mg/day.

Surveillance and diagnosis of HCC

As surveillance for HCC, α-fetoprotein (AFP) by electrochemiluminescent immunoassay (Roche Diagnostics) and des-g-carboxy prothrombin (DCP) by electrochemiluminescent immunoassay (Sanko, Tokyo, Japan) were measured alternately every month. Image diagnosis by US, dynamic enhanced CT or dynamic enhanced MRI was performed every 6 months in CH patients and every 3 months in cirrhosis patients. If elevation of AFP or DCP was observed, imaging diagnosis was performed within a month. Diagnosis of HCC was confirmed by the finding of enhanced arterial contrast uptake followed by washout in the portal venous phase and equivalent phase by dynamic enhanced CT or dynamic enhanced MRI, or histologically by fine-needle tumor biopsy. The methods used for the confirmation of diagnosis and staging of HCC conformed to the standards by Liver Cancer Study Group of Japan.[19]

End-points

The primary end-point was the development of HCC. Secondary end-points included changes in serum HBV DNA, HBeAg, AST, ALT, serum albumin, PT%, AFP, DCP, Plt and Child–Turcotte–Pugh class between baseline and the latest visit. Seroconversion was defined as loss of HBeAg and development of anti-HBe. In the case of patients who developed HCC during the study period, the last data gathered before the first detection of HCC by imaging were adopted as the latest data. In the patients who developed HCC, the size, number and stage of tumors were also estimated. The sensitivity and specificity of AFP and DCP were estimated using the values at diagnosis of HCC for the patients who developed HCC, and those at the latest visit for patients without HCC. Only the specificity of AFP and DCP, without sensitivity, was estimated at baseline using the values of AFP and DCP at baseline, when no patients had yet developed HCC.

Statistical analysis

Parameters represented by continuous variables were expressed as the median and range (minimum and maximum). Parameters at baseline were compared between ETV patients and LVD patients by Mann–Whitney *U*-test for continuous variables and by χ^2-test for categorical data. Each parameter at the latest visit was compared with the corresponding one at baseline by Mann–Whitney *U*-test for continuous variables and by χ^2-test for categorical data. The incidence of HCC development was estimated by Kaplan–Meier analysis, and compared between the patients with CH and cirrhosis, and between the patients started on ETV and those administrated LVD, using a log–rank test. Receiver–operator curves (ROC) for serum AFP and DCP were estimated to search for the optimal cut-off value to distinguish between the patients with and without HCC. A *P*-value of less than 0.05 was considered statistically significant. Statistical analysis was performed with JMP software ver. 5.01J.

RESULTS

Study populations and baseline characteristics (Table 1)

A TOTAL OF 256 patients were enrolled in this study. ETV and LVD were administrated to 129 and 127 patients, respectively, as the first-line NA. The baseline characteristics and demographics of all patients, the ETV group and the LVD group, are shown in Table 1. All patients were Japanese, and consisted of 179 men and 77 women, with a median age of 50 years. The median follow-up period was 4.25 years in all patients, 2.96 years in ETV patients and 5.97 years in LVD patients.

As for viral markers, the median serum HBV DNA level was 7.0 log copies/mL and HBeAg was positive in 132 patients of the total patient group, with no significant difference between the ETV and LVD groups.

The clinical diagnosis in all patients was CH in 194 and cirrhosis in 62 patients and there was no significant difference in distribution between the ETV and LVD groups. In the 62 patients with cirrhosis, the Child–Turcotte–Pugh class was A, B and C in 43, 16 and three patients, respectively. The serum albumin concentration, PT% and Plt were significantly lower in the LVD group than the ETV group, indicating that hepatic reservation was somewhat lower in the LVD group.

Liver biopsy was performed and assessed using the New Inuyama Classification system in 166 patients in

Table 1 Baseline characteristics of the subjects

	All ($n = 256$)	ETV ($n = 129$)	LVD ($n = 127$)	*P*-value
Sex (male/female)†	179/77	86/43	93/34	0.2964
Age (years)‡	50 (22–88)	51 (26–88)	50 (22–81)	0.4369
Follow-up period (years)‡	4.25 (0.41–10.0)	2.86 (0.41–7.47)	5.97 (0.51–10.0)	<0.0001*
Fibrosis stage (1/2/3/4)†	42/57/50/17	23/31/21/6	19/26/29/11	0.2665
Activity grade (1/2/3)†	49/80/37	29/34/18	20/46/19	0.2276
Clinical diagnosis (CH/LC)†	194/62	101/28	93/34	0.2934
CTP class A/B/C (in LC patients)†	43/16/3	23/4/1	20/12/2	0.1253
HBV DNA (log copies/mL)‡	7.0 (2.6–8.8)	6.8 (2.6–8.8)	7.3 (3.0–8.7)	0.1763
HBeAg (positive/negative)†	132/124	67/62	65/62	0.952
Total bilirubin (mg/dL)‡	0.87 (0.2–22.67)	0.86 (0.2–5.19)	0.875 (0.38–22.67)	0.0617
Albumin (g/dL)‡	4.0 (2.07–5.0)	4.1 (2.3–5.0)	3.96 (2.0–5.0)	0.0090*
AST (IU/L)‡	66 (12–1216)	61 (12–811)	73 (19–1216)	0.0427*
ALT (IU/L)‡	87 (13–1660)	82 (13–1250)	96 (14–1660)	0.0569
Platelet count (×104/μL)‡	14.0 (3.9–47.2)	17.5 (3.5–32.8)	15.7 (3.6–52.2)	<0.001*
Prothrombin time (%)‡	88.0 (27.8–132.0)	93.0 (58.0–132.0)	83.0 (27.8–122.6)	<0.001*
AFP (ng/mL)‡	5.8 (1.4–1057.1)	5.7 (1.4–820.6)	5.8 (1.4–1057.1)	0.839
DCP (mAU/mL)‡	20.0 (6.0–145.0)	19.0 (6.0–145.0)	20.0 (10.0–89.0)	0.2282

*Different between ETV and LVD groups with statistical significance.
†Values are numerical and analyzed by χ^2-test.
‡Values are median (range) and analyzed by Mann–Whitney *U*-test.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CH, chronic hepatitis; CTP, Child–Turcotte–Pugh; DCP, des-γ-carboxy prothrombin; ETV, entecavir; HBeAg, hepatitis B e-antigen; LC, liver cirrhosis; LVD, lamivudine.

total. Fibrosis stages of 1, 2, 3 and 4 were observed in 42, 57, 50 and 17 patients, and activity grades of 1, 2 and 3 were observed in 49, 80 and 37 patients in total, both with no significant difference between the ETV and LVD groups.

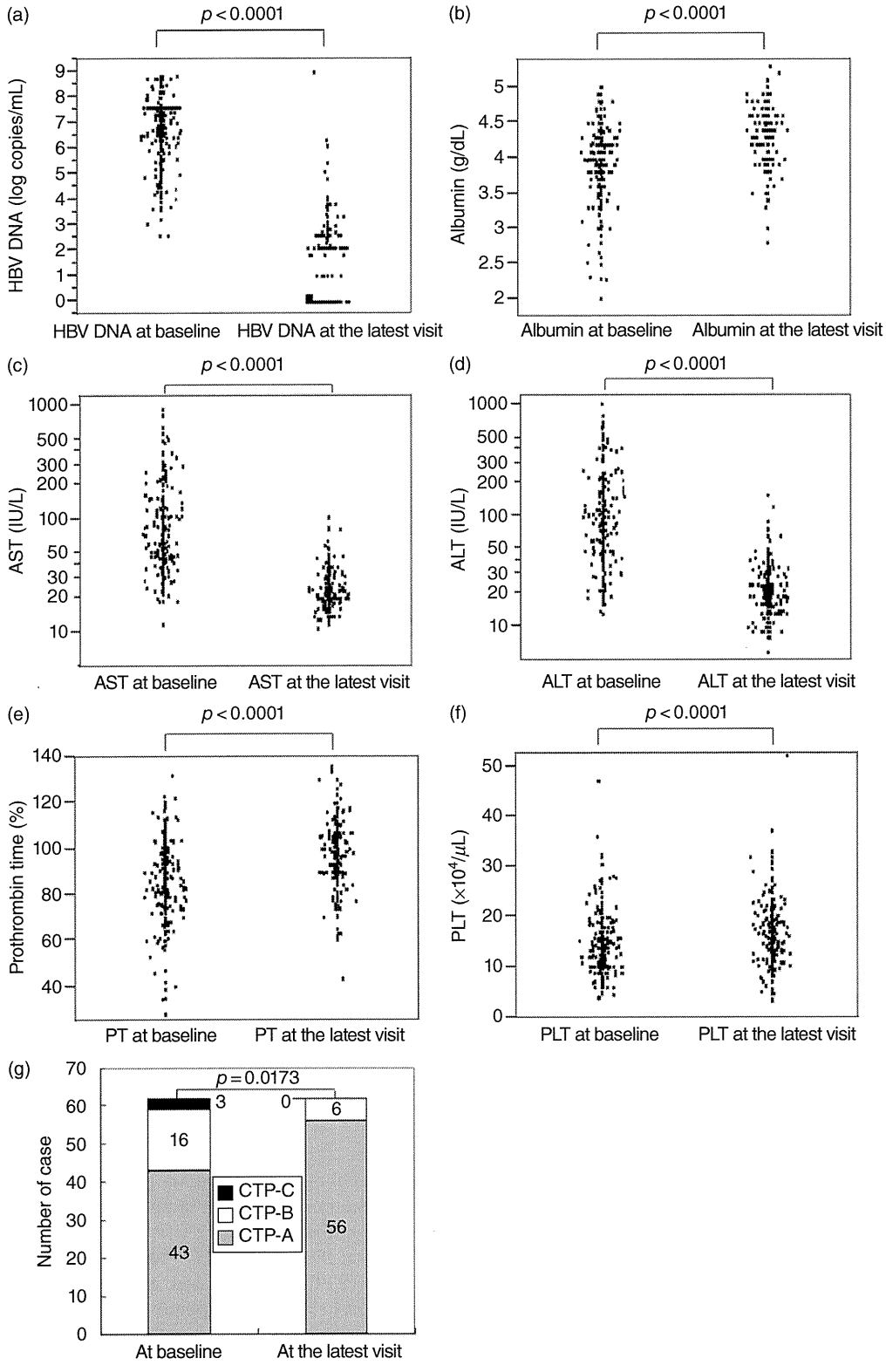
As for tumor markers, the median AFP was 5.8 ng/mL and the median DCP was 20.0 mAU/mL, and neither marker was significantly different between the ETV and LVD groups. The complication of HCC was neglected in all patients by the inclusion criteria.

Changes in virological and biochemical markers (Fig. 1)

Median serum HBV DNA dropped significantly from 7.0 log copies/mL at baseline to 2.1 log copies/mL at the latest visit ($P < 0.0001$) (Fig. 1a). Serum HBV DNA was under the LLN in 220 out of 256 cases at the latest visit. There was no difference in the latest serum HBV DNA or the rate of decline between the ETV and LVD groups. Among the 132 patients with positive HBeAg at baseline, HBeAg seroconversion was observed in 48 patients (36.4%) after a median period of 4.25 years.

Median serum albumin was significantly elevated from 4.0 g/dL at baseline to 4.4 g/dL at the latest visit ($P < 0.0001$) (Fig. 1b). The median AST dropped from 66 to 22 IU/L ($P < 0.0001$), and the median ALT dropped from 87 to 19 IU/L ($P < 0.0001$) (Fig. 1c,d). The median PT% was elevated from 88% to 100% ($P < 0.0001$), and the median Plt increased from 14.0 to $16.7 \times 10^4/\mu L$ ($P < 0.0001$) (Fig. 1e,f). By subgroup

Figure 1 Comparison of the measured parameters between baseline and the latest visit (a–f) by Mann–Whitney *U*-test for continuous valuables and (g) by χ^2-test for Child–Turcotte–Pugh class distribution. (a) Median serum hepatitis B virus (HBV) DNA dropped significantly from 7.0 log copies/mL at baseline to 2.1 log copies/mL at the latest visit ($P < 0.0001$). (b) Median serum albumin was significantly elevated from 4.0 g/dL at baseline to 4.4 g/dL at the latest visit ($P < 0.0001$). (c) Median aspartate aminotransferase (AST) dropped significantly from 66 IU/L at baseline to 22 IU/L at the latest visit ($P < 0.0001$). (d) Median alanine aminotransferase (ALT) dropped significantly from 87 IU/L at baseline to 19 IU/L at the latest visit ($P < 0.0001$). (e) Median prothrombin time (PT) was significantly elevated from 88% at baseline to 100% at the latest visit ($P < 0.0001$). (f) Median platelet count (PLT) increased significantly from 14.0 at baseline to $16.7 \times 10^4/\mu L$ at the latest visit ($P < 0.0001$). (g) In the 62 patients with cirrhosis, the distribution of Child–Turcotte–Pugh class A, B and C changed from 43, 16 and three at baseline to 56, six and zero at the latest visit, with significant difference by χ^2-test ($P = 0.0173$).



analysis, these parameters changed similarly and significantly in both the ETV and LVD groups. In the 62 patients with cirrhosis, the distribution of Child–Turcotte–Pugh class A, B and C changed from 43, 16 and three at baseline to 56, six and zero at the latest visit, and the change in each class was significantly different by χ^2-test ($P = 0.0173$) (Fig. 1g).

Drug resistance

Among the 127 patients of the LVD group, virological resistance cumulatively developed in 60 patients during the follow-up period; co-administration of ADV was selected in 53 of these patients, and change to ETV was selected in the other seven patients. In the 129 patients of the ETV group, resistance to ETV developed in one patient, and the protocol was changed to co-administration of LVD and ADV for this patient.

Tumor markers (Fig. 2)

Median AFP dropped significantly from 5.8 ng/mL at baseline to 2.9 ng/mL at the latest visit without or before development of HCC ($P < 0.0001$) in both the ETV and LVD groups (Fig. 2a). There was no significant difference in median DCP between baseline (20.0 mAU/mL) and the latest visit (19.0 mAU/mL) (Fig. 2b). Median serum AFP levels changed from 5.8 ng/mL at baseline to 5.1, 3.9, 3.1 and 2.8 ng/mL at months 2, 4, 6 and 8 after NA treatment (Fig. 2c). In patients who developed HCC in the follow-up period, median AFP levels were 4.9, 5.3 and 5.4 ng/mL at months 6, 4 and 2 before diagnosis of HCC, and 6.6 ng/mL at diagnosis of HCC (Fig. 2d).

Development and characteristics of HCC (Table 2, Fig. 3)

During the follow-up period, HCC developed in 35 out of the total 256 patients, or 11 patients from the ETV

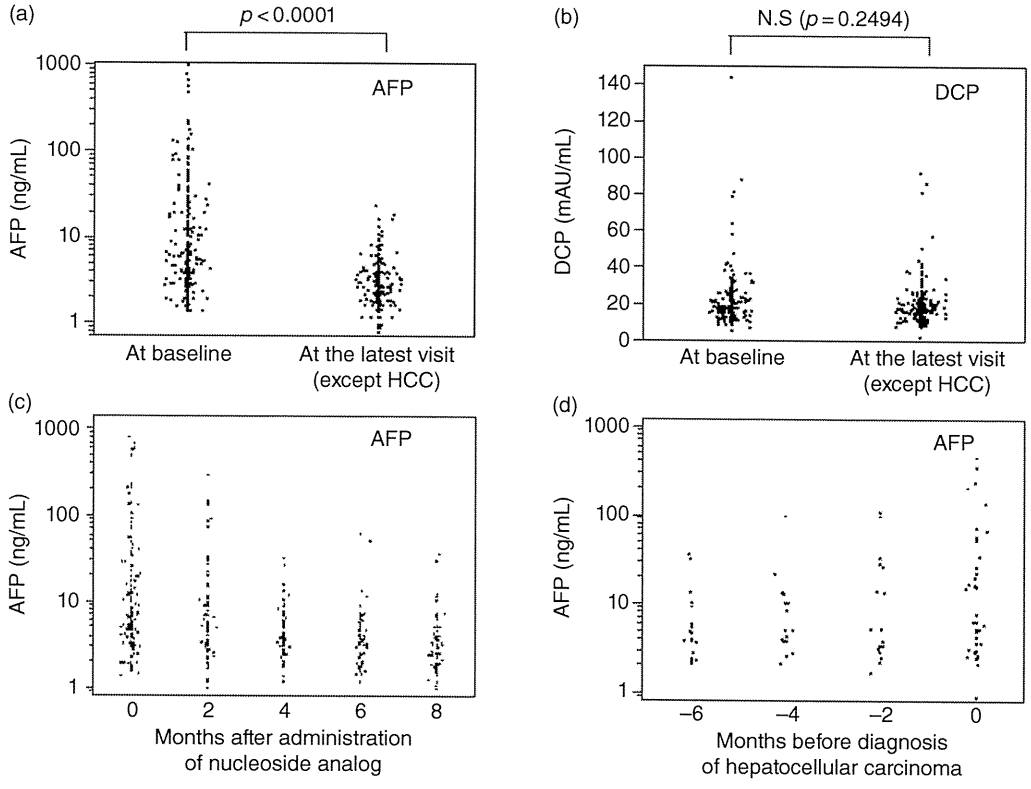


Figure 2 Changes in serum α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP). (a) Median AFP dropped significantly from 5.8 ng/mL at baseline to 2.9 ng/mL at the latest visit ($P < 0.0001$ by Mann–Whitney *U*-test). (b) Median DCP at the latest visit, 19.0 mAU/mL, was not changed significantly from that at baseline, 20.0 mAU/mL ($P = 0.2494$ by Mann–Whitney *U*-test). (c) Median AFP levels changed from 5.8 ng/mL at baseline, to 5.1, 3.9, 3.1 and 2.8 ng/mL at months 2, 4, 6 and 8 after NA treatment. (d) In the patients who developed hepatocellular carcinoma (HCC), median AFP levels were 4.9, 5.3 and 5.4 ng/mL at months 6, 4 and 2 before diagnosis of HCC, and 6.6 ng/mL at diagnosis of HCC.

Table 2 Data at development of hepatocellular carcinoma

	$n = 35$
Background (CH/LC)†	14/21
NA (ETV/LVD)†	11/24
Duration of NA administration (years)‡	4.25 (0.4–10.0)
Size (cm)‡	1.9 (0.9–3.2)
Number (1/2/3)†	21/10/4
Stage 1/2/3/4A†	15/14/5/1
Treatment (Hr/RFA/TACE/PMCT)†	18/14/2/1
AFP (ng/mL)‡	6.6 (0.9–459)
DCP (mAU/mL)‡	27.0 (10–456)

†Values are numerical.
‡Values are median (range).
AFP, α-fetoprotein; CH, chronic hepatitis; DCP, des-γ-carboxy prothrombin; ETV, entecavir; Hr, hepatic resection; LC, liver cirrhosis; LVD, lamivudine; NA, nucleoside analog; PMCT, percutaneous microwave coagulation therapy; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization.

group and 24 patients from the LVD group. The background liver disease in these 35 patients at baseline was CH in 14 patients and cirrhosis in 21 patients. The mean duration from start of NA administration to HCC development was 4.25 years in total (Table 2). The cumulative incidence of HCC development at years 1, 3, 5, 7 and 10 in the all patients estimated by Kaplan–Meier analysis was 3.2%, 6.9%, 12.4%, 16.8% and 34.0%, respectively (Fig. 3a). It was 1.6%, 3.5%, 3.5%, 7.1% and 29.6% in CH, and 8.1%, 17.5%, 43.2%, 46.7% and 53.4% in cirrhosis patients, respectively, and the incidence in patients with cirrhosis was significantly higher than that in the patients with CH ($P < 0.0001$) (Fig. 3b). There was no difference in HCC development between the ETV group and LVD group ($P = 0.680$) (Fig. 3c). The cumulative incidence of HCC development was significantly ($P = 0.0352$) higher in patients who developed LVD resistance in the follow-up period, as compared with patients without LVD resistance (Fig. 3d).

The characteristics of HCC at diagnosis were as follows: the median diameter was 1.9 cm (ranging 0.9–3.2 cm), the number of tumors was 1, 2 and 3 in 21, 10 and four cases, and the tumor stage was 1, 2, 3 and 4A in 15, 14, five and one case, respectively. Treatment of HCC consisted of hepatic resection in 18 patients, radiofrequency ablation (RFA) in 14 patients, transcatheter arterial chemoembolization in two patients and percutaneous microwave coagulation therapy in one patient. The median AFP value at diagnosis of HCC was 6.6 ng/mL (as described previously), ranging from 0.9–459. The median DCP value at diagnosis of HCC was 27.0 mAU/mL, ranging from 10–456 (Table 2). There was no significant difference in these characteristics between the ETV and LVD groups.

Sensitivity and specificity of AFP and DCP (Table 3, Fig. 4)

Receiver–operator curve analysis indicated that the area under the curve of AFP was 0.797 and that of DCP was 0.736 (Fig. 4). ROC analysis suggested that an AFP value of approximately 10 ng/mL and DCP value of approximately 40 mAU/mL provided an optimal balance between sensitivity and specificity. The sensitivity and specificity of AFP by the cut-off value of 10 ng/mL was 45.7% and 97.3%, respectively, at diagnosis of HCC or at the latest visit. At baseline, the specificity of AFP by the cut-off value of 10 ng/mL was 64.4%. The sensitivity and specificity of DCP by the cut-off value of 40 mAU/mL was 33.3% and 96.2%, respectively, at diagnosis of HCC or at the latest visit. At baseline, the specificity of DCP was 95.1% by the cut-off value of 40 mAU/mL. (The sensitivity of AFP and DCP could not be estimated at baseline when all patients were without complication and/or past history of HCC.)

Using the combination of AFP and DCP, the sensitivity (AFP >10 ng/mL and/or DCP >40 mAU/mL) was 64.7%, and the specificity (AFP ≤10 ng/mL and DCP ≤40 mAU/mL) was 93.4%. At baseline, the specificity was 74.5%.

DISCUSSION

ALTHOUGH ANTI-HBV NA have a potent antiviral activity,[5–11] they cannot delete the HBV cccDNA template. Therefore, the aim of NA treatment is to realize a long-term improvement in the outcome of patients by sustained suppression of HBV proliferation and disease progression. The present study showed that NA treatment continuously exerted long-lasting effects on viral suppression, control of disease activity and improvement of hepatic reservation. Namely, median serum HBV DNA dropped to 2.1 log copies/mL, and HBV DNA was under the LLN in 220 out of 256 cases at the latest visit. The activity of liver disease was also suppressed, as shown by the reduction and normalization of AST and ALT. Hepatic reservation was significantly improved, as shown by the elevation in serum albumin concentration and PT%, and the Child–Turcotte–Pugh class was improved in the majority of patients with cirrhosis. It therefore seems reasonable to

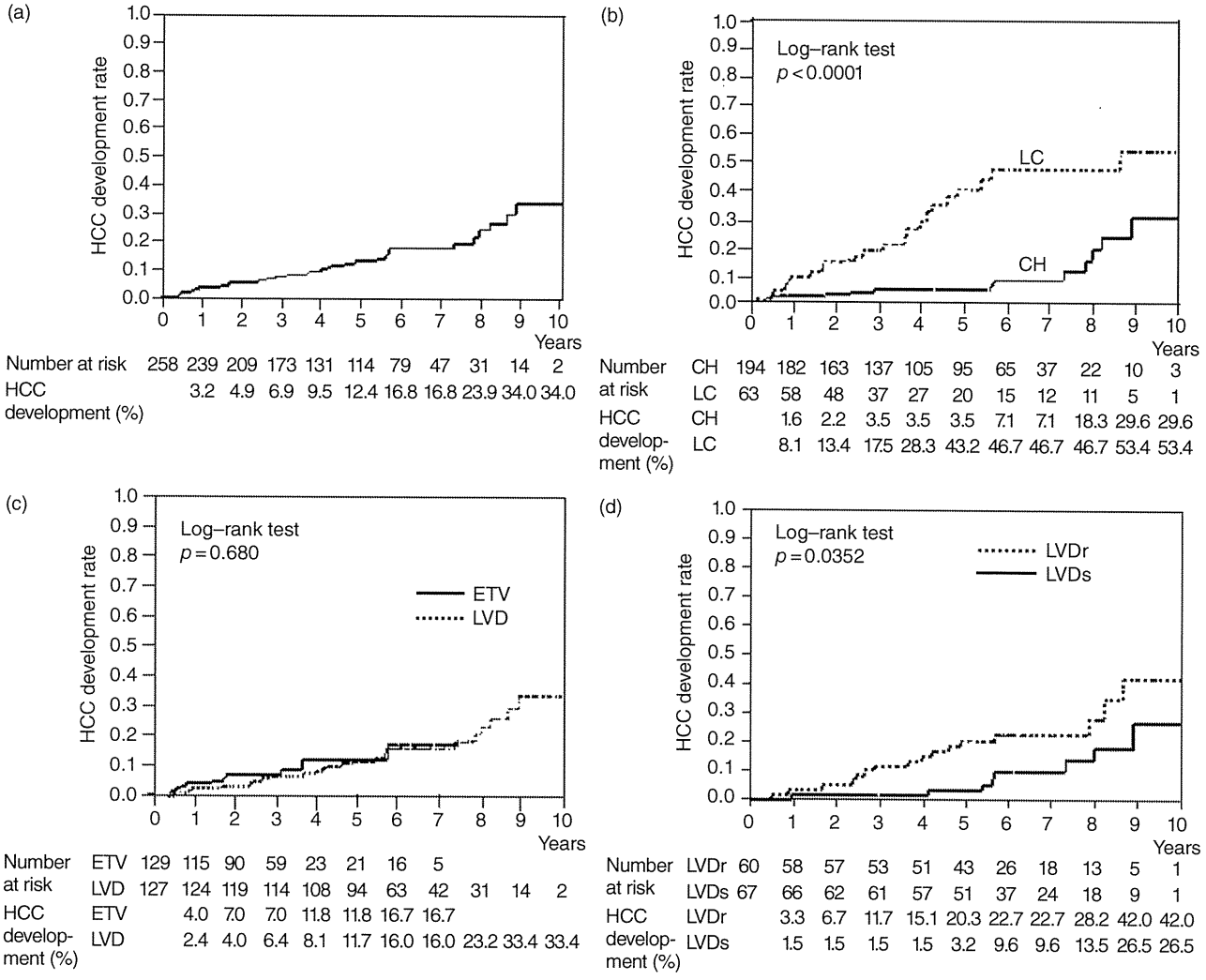


Figure 3 Kaplan–Meier analysis of the incidence of hepatocellular carcinoma (HCC) development with comparison by log-rank test. (a) The cumulative incidence of HCC development at years 1, 3, 5, 7 and 10 in the all patients was 3.2%, 6.9%, 12.4%, 16.8% and 34.0%, respectively. (b) The cumulative incidence of HCC development at years 1, 3, 5, 7 and 10 was 1.6%, 3.5%, 3.5%, 7.1% and 29.6% in chronic hepatitis (CH) patients, and was 8.1%, 17.5%, 43.2%, 46.7% and 53.4% in liver cirrhosis (LC) patients, respectively, with significantly higher incidence in LC than CH patients by log-rank test ($P < 0.0001$). (c) There was no significant difference between the cumulative incidence of HCC between the entecavir (ETV) and lamivudine (LVD) groups by log-rank test ($P = 0.680$). (d) The cumulative incidence of HCC development at years 1, 3, 5, 7 and 10 was 3.3%, 11.7%, 20.3%, 22.7% and 42.0%, respectively, in the patients who developed LVD resistance. It was significantly ($P = 0.0352$) higher as compared with that in patients without LVD resistance, namely, 1.5%, 1.5%, 3.2%, 9.6% and 27.3%, respectively. LVDr, lamivudine resistant; LVDs, lamivudine sensitive.

conclude that NA induced an improvement in the long-term outcome of CHB and cirrhosis patients.

As for drug resistance, LVD resistance cumulatively developed in 60 of 127 patients after a median follow up of 4.25 years, while ETV resistance developed in only one of 129. It was clearly shown that ETV should be selected as the first-line NA for NA-naive patients, as recommended by recent treatment guidelines.[15–18] In patients with LVD resistance, however, co-administration with ADV rescued the antiviral and biochemical effects, and improved hepatic reservation to the same levels as in the ETV group.

The most serious complication of HBV infection is HCC. The annual incidence of HCC from HBV carriers

Table 3 Sensitivity and specificity of AFP and DCP for HCC

Cut-off value	At diagnosis of HCC and at the latest visit						At baseline
	Sensitivity (%)					Specificity (%)	Specificity (%)
	All ($n = 35$)	Stage 1 ($n = 15$)	Stage 2 ($n = 14$)	Stage 3 ($n = 5$)	Stage 4A ($n = 1$)		
AFP							
10 ng/mL	45.7	47.0	57.1	20.0	0	97.3	64.4
40 ng/mL	25.7	40.0	28.6	0	0	100	84.6
100 ng/mL	14.3	13.3	21.4	0	0	100	92.0
DCP							
28 mAU/mL	51.5	60.0	50.0	0	100	90.6	82.7
40 mAU/mL	33.3	33.3	35.7	0	100	96.2	95.1
AFP >10 ng/mL and/or DCP >40 mAU/mL	64.7	66.0	71.4	20.0	100	93.4	74.5

AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

has been reported to be 1% and 3% in patients with CH and cirrhosis, respectively.[20] There are three ways to prevent death from HBV-related HCC: decreasing the risk factors of HCC development, early detection of HCC and a curative treatment. The risk factors include high serum HBV DNA, positive HBeAg, genotype C, precore and core promoter mutations, high ALT levels, cirrhosis, male sex, aging, alcohol, aflatoxin and possibly some single nucleotide polymorphisms.[4,21–25] In these factors, it has been shown that the most important risk is a high serum HBV DNA level.[14] From this point of view, NA treatment, which suppresses HBV replication potently and durably, is naturally expected to suppress hepatocarcinogenesis.[26]

Although interferon has been reported to reduce the incidence of HCC development,[27–29] there have been only a few prospective studies on the effect of NA treatment for suppression of HBV-related HCC development, so far.[12]

In the present prospective study, the cumulative incidence of HCC development at years 1, 3, 5, 7 and 10 after NA treatment was 1.6%, 3.5%, 3.5%, 7.1% and

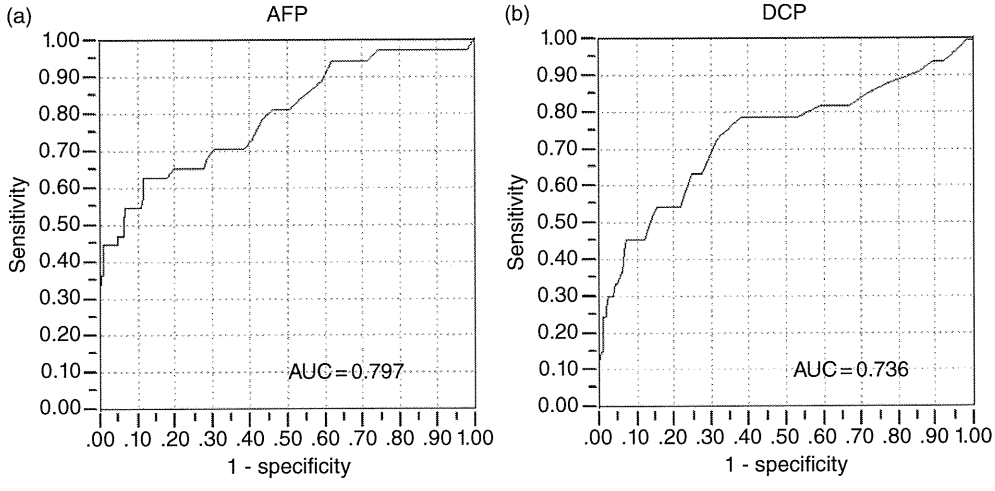


Figure 4 Receiver–operator curve, a plot of sensitivity versus (1 – specificity) over the entire range of the test results was analyzed in search for the optimal cut-off value to distinguish hepatocellular carcinoma (HCC) and non-HCC. (a) The area under the curve (AUC) of α-fetoprotein (AFP) was 0.797. The optimal cut-off value of AFP was approximately 10 ng/mL. (b) The AUC of des-γ-carboxy prothrombin (DCP) was 0.736. The optimal cut-off value of DCP was approximately 40 mAU/mL.

29.6% in CH, and 8.1%, 17.5%, 43.2%, 46.7% and 53.4% in cirrhosis patients, respectively, and there was no difference between ETV and LVD groups. The cumulative incidence of HCC development was almost equivalent to, or somewhat higher than, the conventional reports. In this study, the patient eligibility for enrollment included serum HBV DNA greater than 5 log copies/mL, an elevated ALT level over twice the ULN, or complications of hepatic insufficiency. In other words, we estimated that CHB and cirrhosis patients would have somewhat higher activities or be at more advanced stages. This might be a reason for a higher incidence of HCC development in this study. A direct comparison between patients with and without NA treatment might be necessary for an accurate estimation of the impact of NA on hepatocarcinogenesis. However, such a controlled trial could not be performed due to ethical reasons. Thus, it was a limit of this study that the impact of NA on hepatocarcinogenesis could not be directly estimated.

The majority of the patients who developed HCC, 29 out of 35 cases, were detected at stage 1 or 2. It seemed that scheduled surveillance enabled early detection of HCC, as previously reported,[30] and radical and curative treatments could be performed in 33 out of 35 patients owing to improved hepatic reservation by NA treatment.

Most recent guidelines for HCC screening recommend surveillance every 6 months using US and AFP.[31] The guideline of Japan recommended US screening every 6 months for CH, and every 3 months for cirrhosis, with AFP, AFP-L3 and DCP measurement.[32] Although US is superior to AFP in both sensitivity and specificity for detecting HCC,[31] detection of small HCC by US is frequently difficult because of a very rough parenchymal US-appearance called "mesh-like" in HBV-cirrhosis. In such cases, an elevated AFP level, a continuously elevating AFP in particular, may suggest the development of HCC. Thus, AFP should be employed in combination with imaging.[15]

The sensitivity and specificity of AFP for diagnosis of HCC is insufficient, as previously reported.[33] The sensitivity of AFP for diagnosis of HCC is reported to be approximately 60% at a cut-off of 20 ng/mL,[34,35] and 22–29% at a cut-off of 200 ng/mL.[35,36] The specificity of AFP is reported to be approximately 81–91%[34,35] at a cut-off of 20 ng/mL. AFP may be frequently pseudopositive in CH and cirrhosis. In some guidelines, AFP levels of 100, 200 or 400 ng/mL are offered as cut-off values to elevate specificity.[37]

The present study, however, showed a comparatively high (45.7%) sensitivity of AFP, considering the high ratio of HCC at stage 1 and 2, after NA treatment, at a cut-off value of 10 ng/mL. It is noteworthy that the specificity of AFP, at the cut-off value of 10 ng/mL, became remarkably elevated to 97.3% after NA treatment, although it was merely 64.4% at baseline.

Therefore, the cut-off value of AFP should be lowered to 10 ng/mL in patients receiving NA. The significance of lowering the cut-off level, while maintaining a high specificity, is that it leads to early detection of HCC. A minimal elevation of AFP over 10 ng/mL can result in a high rate of prediction of the development of HCC in NA-treated patients. A similar result has been reported elsewhere.[38]

The sensitivity and specificity of DCP for diagnosis of small HCC, at a cut-off value of 40 mAU/mL, have been reported to be 37–41% and 87–97%, respectively.[35,39–41] In the present study, the sensitivity and specificity of DCP at a cut-off value of 40 mAU/mL was 33.3% and 96.2%, respectively. There was no significant difference in the specificity of DCP, unlike AFP, between baseline and after NA treatment. The combination of AFP and DCP provided a sensitivity of 64.7% and specificity of 93.4%. A recent consensus by the Japan Society of Hepatology recommended a combination of AFP and DCP in high-risk patients.[42]

Recently, it has been speculated that elevated AFP itself is an independent risk factor for HCC. Some reports have shown that AFP can promote the growth of HCC cells.[43–45] In addition, a high AFP level has been correlated with more aggressive behavior and poor prognosis of HCC patients.[46,47] Therefore, a high level of serum AFP can be considered to contribute to the development of HCC rather than merely serving as a tumor marker. Although it remains controversial whether a decrease of AFP could directly reduce the risk and incidence of HCC or not, suppression of AFP production by NA treatment may possibly lead to a reduction in the cellular potential for hepatocarcinogenesis.

In conclusion, the present study demonstrated that long-term NA treatment for CHB and cirrhosis leads to a sustained virological and biochemical effect and improvement of hepatic reservation. Although the impact of NA on hepatocarcinogenesis remained undetermined, it was shown that early detection of HCC was possible by a scheduled surveillance program, and curative treatment could be performed owing to early detection and restored hepatic reservation. The serum AFP level was significantly reduced to lower than 10 ng/mL by NA treatment, resulting in marked elevation of specificity. In NA-treated patients with CHB and cirrhosis, the cut-off value of AFP for HCC surveillance should be

lowered to 10 ng/mL. It is important to note that a minimal elevation of AFP to over 10 ng/mL results in highly accurate prediction of HCC development, leading to earlier detection.

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Int J Clin Oncol (2011) 16:210–220
DOI 10.1007/s10147-010-0150-x

ORIGINAL ARTICLE

Effect of pegylated interferon therapy on intrahepatic recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma

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Received: 7 July 2010 / Accepted: 2 November 2010 / Published online: 9 December 2010

Abstract
Background We wished to determine whether pegylated interferon (PEG-IFN) therapy after curative treatment of hepatocellular carcinoma (HCC) prevents a recurrence of HCC.
Methods Thirty-seven HCC patients with hepatitis C virus (HCV) infection who were treated with PEG-IFN after curative treatment (PEG-IFN group) and 145 controls without IFN therapy (non-IFN group) were enrolled. The overall survival and recurrence-free survival rates were compared between the groups, and the predisposing factors for recurrence and survival were analyzed. The rates were also examined by propensity score (PS) matched analysis that could minimize selection biases.
Results The median follow-up period was 3.7 years. The 5-year survival rate in the PEG-IFN group (91%) was significantly higher than that in the non-IFN group (56%; $P < 0.01$). The rate of the second recurrence but not that of the first recurrence of HCC in the sustained virological responder (SVR) group was lower than that in the non-IFN group ($P = 0.03$). Improvement of survival by PEG-IFN and low rate of second recurrence in the SVR group were also observed in PS matched analysis. Multivariate analysis revealed that PEG-IFN therapy and high serum albumin were good prognostic factors for survival. Although low serum albumin and large and multiple tumors were risk factors for the first recurrence, non-SVR and low serum albumin were risk factors for the second recurrence.
Conclusion PEG-IFN-therapy after curative treatment of HCC improved the rate of survival, and SVR was found to be closely correlated with the prevention of recurrence.

Keywords Hepatitis C virus · Hepatocellular carcinoma · Recurrence · Survival · PEG-IFN

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. Chronic infection with hepatitis C virus (HCV) is one of the major causes of HCC [1–3], and the percentage of HCC patients with HCV infection is about 70% in Japan. Recent advances in imaging and treatment modalities have improved the prognosis of patients with HCV-related HCC, but outcomes are still unsatisfactory. The 5-year survival rate is only 50–70%, even after curative treatment [4, 5], such as surgical resection and percutaneous ablation [percutaneous ethanol injection therapy (PEIT), microwave coagulation therapy (MCT), and radiofrequency thermal ablation (RFA)] [6, 7]. This unfavorable prognosis is caused by high intrahepatic tumor recurrence rates and sustained hepatic damage, both correlated with sustained viral infection [8]. The rate of intrahepatic tumor recurrence within 1 year is

20–40%, rising to about 80% by 5 years [9–11]. Thus, alleviation of the effect of HCV is a high priority for improving the prognosis of patients with HCV-related HCC.

Interferon (IFN) therapy is effective in reducing serum alanine transaminase (ALT) activity and in eradicating HCV [12, 13]. Thus, IFN could have value in minimizing hepatic necrosis, inflammation, and fibrosis, as well as reducing the incidence of HCC. In 1995, a small randomized controlled trial (RCT) showed a reduction in the incidence of HCC in cirrhotic patients with HCV infection by IFN treatment [14]. Yu et al. [15] reported that the cumulative incidences of HCC were 12.2% and 35.2% in IFN-treated and untreated chronic hepatitis C patients, respectively ($P = 0.001$). Tanaka et al. [16] also reported that interferon therapy decreased the risk of developing HCC by 48% compared with that in a control group ($P = 0.064$). In addition, several recent studies have shown that IFN therapy, even after curative treatment of HCV-related HCC, could prevent recurrence and improve the rate of survival [17–30]. Because these studies used different IFN regimens and the background characteristics of patients were diverse, the results varied, and no standard IFN regimen has been established for patients after curative treatment of HCV-related HCC.

Recently, the administration of pegylated interferon (PEG-IFN) has become the standard treatment for patients with chronic HCV infection. Treatment with PEG-IFN and oral ribavirin produces a virological response in more than 50% of patients, which is better than that in conventional α-IFN therapy [31, 32]. However, there are few reports that demonstrate the effect of PEG-IFN therapy after curative treatment of HCV-related HCC.

The present study involves analysis of the efficacy of PEG-IFN after the curative treatment of HCC for the prevention of HCC recurrence and for improving the rate of survival.

Patients and methods

Patients

From January 1997 until March 2009, 358 consecutive patients with HCV-related HCC underwent curative treatment as an initial treatment at Okayama University Hospital. Here, curative treatment is defined as surgical operation (resection; $n = 86$), RFA ($n = 228$), PEIT ($n = 30$), or MCT ($n = 14$). Among the patients, 176 patients were excluded because 163 patients had previously received IFN therapy and, for 13 patients, information was lacking on whether they had previously received IFN treatment. The remaining 182 patients were enrolled in the study. Informed consent was obtained from all patients for use of their clinical data. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki, and was approved by the ethical committees of the institute. This study is a retrospective cohort study.

Diagnosis

HCC was diagnosed on the basis of typical findings by ultrasonography, computed tomography (CT) scans, and magnetic resonance imaging (MRI) scans (hyperattenuation in the arterial phase and hypoattenuation in the portal-venous phase). The imaging diagnoses were confirmed by at least two imaging modalities. The diagnosis of HCC was confirmed histopathologically with ultrasound-guided biopsy in nine patients because no typical findings were identified in imaging modalities.

IFN therapy

After curative treatment of primary HCC and confirmed that no residual tumor was existed by imaging modalities, 37 of the 182 patients were assigned to PEG-IFN therapy (PEG-IFN group). The remaining 145 patients did not receive any IFN treatment (non-IFN group). IFN treatment was performed on patients who agreed to use IFN after receiving a full explanation regarding the benefits and side effects of the treatment and who met the following inclusion criteria: (1) tumor–node–metastasis (TNM) stage of I, II, or III; (2) detectable serum HCV-RNA; (3) seronegative for hepatitis B virus surface antigen; (4) Child-Pugh class A or B; (5) platelet count above 80,000/mm^3; and (6) age less than 75 years. In the PEG-IFN group, 15 patients received 90–180 μg pegylated interferon alpha-2a (Pegasys; F-Hoffmann-La Roche, Basel, Switzerland) subcutaneously once per week for 24–48 weeks, and 22 patients received 60–100 μg pegylated interferon alpha-2b (PegIntron; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (Rebetol; Schering-Plough) at 600–800 mg/body for 24–48 weeks, according to the guideline on medical care for chronic hepatitis C prepared by the Ministry of Health, Labor and Welfare of Japan [33]. The median period between the day of curative treatment and PEG-IFN therapy was 242 days.

Patients stopped posttreatment PEG-IFN therapy when HCC recurrence was detected or if the hemoglobin level was <8.5 g/dl, the leukocyte count was <1,000/mm^3, the neutrophil count was <500/mm^3, or the platelet count was <50,000/mm^3, and then restarted the therapy after the treatment of HCC whenever possible.

In the control group (non-IFN group), the patients were prescribed ursodeoxycholic acid (UDCA) and the stronger neo-minophagen C (SNMC).

A sustained virological response (SVR) was defined as HCV-RNA negativity, determined by reverse transcription-polymerase chain reaction, more than 6 months after the termination of IFN therapy. The rest of the patients were considered to have exhibited a nonsustained virological response (non-SVR).

Follow-up of the patients

After curative treatment of primary HCC, all patients underwent liver function tests every 1–2 months, and ultrasonography or three-phase dynamic CT scanning every 3 months. The serum levels of alpha-fetoprotein (AFP), AFP-L3, and des-γ-carboxy prothrombin (DCP) were also determined every 2–3 months. The recurrence of HCC was diagnosed using the same criteria as for the initial development of HCC.

Statistical analysis

Statistical analysis was performed using SAS version 9.1 package and JMP software, version 8.0 (SAS Institute, Cary, NC, USA). Differences between two groups were evaluated using the unpaired Student's t test. The χ^2 test or Fisher's exact probability test was used to compare categorical data. Cumulative incidence curves were determined with the Kaplan–Meier method, and the differences between groups were assessed using the log-rank test. Possible risk factors for survival and HCC recurrence were examined by the Cox proportional hazards regression model with the following 12 variables: interferon-related variables (application of interferon therapy, response to interferon therapy, and HCV genotype), background, liver function, and tumor factors at the first treatment and at recurrence of HCC [age, alanine aminotransferase (ALT), albumin (ALB), total bilirubin (T.Bil), platelet counts (PLT), prothrombin time (PT), AFP, DCP, maximum tumor size, and tumor number]. Parameters that proved to be significant in the univariate analysis were tested by the multivariate Cox proportional hazards regression model.

We also conducted propensity score (PS) matched analysis that can adjust the clinical background of the patients in each group. To calculate PS, we used seven covariates: sex of patients, and variables at the time of development of HCC (age at the time of development of HCC, ALT, ALB, T.Bil, PLT, maximum tumor size, and tumor numbers). The propensity score of choosing the IFN treatment was calculated, followed by matching IFN group and non-IFN group according to a greedy matching technique [34]. The survival and recurrence rates of matched patients were compared by the Kaplan–Meier method and the differences were evaluated by the log-rank test. A P value less than 0.05 was considered statistically significant.

Results

Characteristics of the patients

Table 1 shows the clinical features of the patients in the PEG-IFN and non-IFN (control) groups at the first treatment of HCC, and Table 2 shows their data at the first recurrence of HCC. Clinical and laboratory characteristics were similar in both groups, but those in the PEG-IFN group were slightly younger (63 vs. 67 years old), and

Table 1 Profiles and laboratory tests of the patients

Variables	PEG-IFN	Non-IFN	P value
Number of patients	37	145	
Age (years)	63 (48–77)	67 (43–85)	<0.01*
Sex (male)	29 (78%)	95 (65%)	0.10
HCV genotype (1b high/others/unknown)	23/14/0	55/30/60	0.83
Response to IFN therapy (SVR/non-SVR)	19/18		
Observation period (years)	4.5 (0.8–12.7)	3.3 (0.3–10.8)	0.01*
T.Bil (mg/dl)	0.7 (0.3–2.7)	0.9 (0.2–2.9)	0.04*
ALB (g/dl)	3.9 (2.5–4.7)	3.7 (2.2–4.6)	<0.01*
ALT (IU/l)	75 (17–168)	54 (14–183)	<0.01*
PLT ($\times$1,000/mm^3)	141 (31–307)	96 (34–281)	<0.01*
PT (%)	94 (62–118)	85 (48–145)	0.01*
AFP (ng/ml)	12 (1.6–1,729)	16.9 (0.6–54,535)	0.49
DCP (mAU/ml)	26 (0–5,230)	34 (0–66,700)	0.52
Number of tumors (solitary)	27 (72%)	105 (72%)	0.34
Size of main tumor (mm)	18 (7–55)	20 (9–74)	0.11
Disease stage (I/II/III/IVA)	16/15/6/0	47/48/44/6	0.88

All variables are shown as the median (range in parentheses) unless otherwise noted

IFN interferon, *PEG-IFN* pegylated interferon, *HCV* hepatitis C virus, *SVR* sustained virological response, *ALB* albumin, *T.Bil* total bilirubin, *ALT* alanine aminotransferase, *PLT* platelet, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

* P values less than 0.05 were considered statistically significant

Table 2 Profiles and laboratory tests of the patients at first recurrence

Variables	PEG-IFN	Non-IFN	*P* value
Number of patients	18	63	
Sex (male)	14 (78%)	40 (63%)	0.24
HCV genotype (1b high/others/unknown)	12/6/0	26/13/24	0.89
Response to IFN therapy (SVR/non-SVR)	8/10		
Treatment method (RFA/ope/PEIT/MCT/other)	15/0/0/1/2	50/4/5/2/2	0.20
T.Bil (mg/dl)	0.7 (0.4–1.4)	0.9 (0.3–2.6)	0.18
ALB (g/dl)	3.7 (2.9–5.0)	3.2 (2.8–4.6)	0.20
ALT (IU/l)	38 (9–295)	50 (16–137)	0.70
PLT ($\times$1,000/mm^3)	105 (39–250)	97 (43–31.2)	0.48
PT (%)	89 (65–117)	83 (35–124)	0.16
AFP (ng/ml)	12 (2.6–144)	11 (1.1–835)	0.40
DCP (mAU/ml)	23 (10–661)	41 (10–28,132)	0.51
Number of tumors (solitary)	11 (61%)	40 (63%)	0.71
Size of main tumor (mm)	13 (6–20)	15 (9–29)	0.16
Disease stage (I/II/III/IVA)	11/5/2/0	36/22/4/1	0.54

All variables are shown as the median (range) unless otherwise noted

IFN interferon, *PEG-IFN* pegylated interferon, *HCV* hepatitis C virus, *RFA* radiofrequency thermal ablation, *ope* operation, *PEIT* percutaneous ethanol injection therapy, *MCT* microwave coagulation therapy, *SVR* sustained virological response, *ALB* albumin, *T.Bil* total bilirubin, *ALT* alanine aminotransferase, *PLT* platelet, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

exhibited higher levels of ALB (3.9 vs. 3.7 g/dl), ALT (78 vs. 54 IU/l), and PLT (141 vs. 96 $\times$ 1,000/mm^3) than those in the non-IFN group. The median follow-up was 4.6 years for patients receiving PEG-IFN and 3.6 years for the controls. In the PEG-IFN group, 19 patients exhibited an SVR (12 monotherapy and 7 combination therapy), 2 were biochemical responders, and the other 17 patients were nonresponders.

Adherence and side effects of IFN therapy

Life-threatening adverse events were not observed in this study. In 11 cases of mild to moderate toxicity (5 thrombocytopenia, 3 anemia, and 3 neutropenia), IFN dose was reduced by 50%. Three patients eventually discontinued treatment with the drug because of adverse events: depression and severe malaise ($n = 1$), hemolytic anemia ($n = 1$), and IFN retinopathy ($n = 1$). In 8 cases with moderate toxicity, IFN treatment could be continued.

Cumulative survival rates of hepatocellular carcinoma

In this study, 2 patients in the PEG-IFN group and 39 patients in the non-IFN group died. All the patients who died had recurrence of HCC. The overall survival rate of PEG-IFN patients was higher than that of non-IFN patients (Fig. 1). Five-year survival rates of the PEG-IFN and non-IFN groups were 91% and 65%, respectively ($P < 0.01$).

Recurrence of hepatocellular carcinoma

At the end of the study, recurrence of HCC had occurred in 8 patients (42%) in the SVR group, 10 (55%) in the non-SVR group, and 63 (43%) in the non-IFN group.

The rate of first HCC recurrence after curative therapy of HCC in SVR patients tended to be lower than that in non-IFN patients (48 vs. 70% at 5 years, respectively, $P = 0.05$; Fig. 2); however, there was no significant difference between non-SVR patients and non-IFN patients (72 vs. 70% at 5 years, respectively; $P = 0.73$). In addition, there was no significant difference between the PEG-IFN group and the non-IFN group (58 vs. 70% at 5 years, respectively; $P = 0.17$). At first HCC recurrence, there was no significant difference in tumor number or liver function between the PEG-IFN and non-IFN groups; however, maximum tumor size in the PEG-IFN group was smaller than that in the non-IFN group (13 vs. 16 mm, respectively; $P = 0.03$). Fifteen of the 17 patients in the PEG-IFN group underwent curative treatment at the first recurrence of HCC.

The rate of second recurrence was not significantly different between the PEG-IFN and non-IFN groups (78 vs. 83% at 3 years, respectively; $P = 0.26$). However, the rate in the SVR group was significantly lower than that in the non-IFN group (65 vs. 83% at 3 years, respectively, $P = 0.03$; Fig. 3). At second HCC recurrence, in the PEG-IFN group, maximum tumor size was smaller (12 vs.

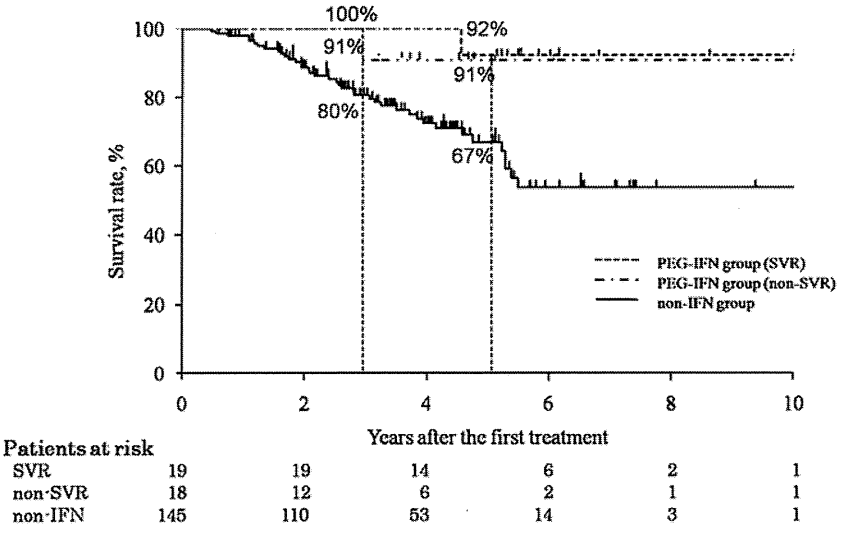

Fig. 1 Cumulative survival rates of pegylated interferon (PEG-IFN) group and non-interferon (non-IFN) group. Two patients in the PEG-IFN group died during the observation period. The survival rate was significantly different between the three groups ($P = 0.01$). *SVR* sustained virological response

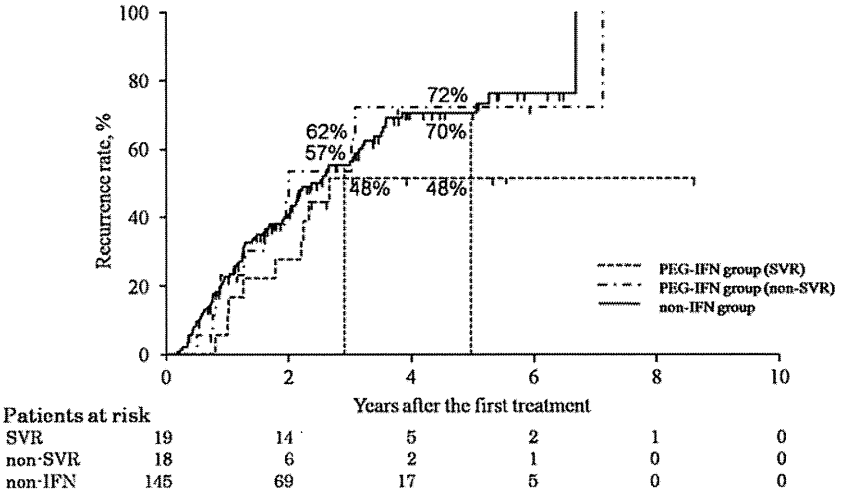

Fig. 2 The rates of first hepatocellular carcinoma (HCC) recurrence. The recurrence rate in SVR patients tended to be lower than that in non-IFN patients (48 vs. 70% at 5 years, respectively; $P = 0.05$); however, there was no significant difference between non-SVR patients and non-IFN patients (72 vs. 70% at 5 years, respectively; $P = 0.73$). *SVR* sustained virological response

15 mm, respectively; $P = 0.02$) and serum ALB was higher (3.3 vs. 3.1 g/dl, respectively; $P = 0.04$) than that in the non-IFN group.

Propensity score matched analysis

To minimize the biases of the PEG-IFN group and non-IFN group, we conducted a propensity score (PS) matched analysis. Thirty-four matched pairs were selected from the PEG-IFN group and non-IFN group by PS. No significant difference in clinical characteristics was observed between the groups (Table 3). Eighteen patients exhibited an SVR [11 monotherapy and 7 combination therapy, 9 (43%) genotype 1b high and 9 (69%) others]. Overall survival rate of the PEG-IFN group was higher than that of the non-IFN group ($P = 0.04$; Fig. 4). Although no significant difference in the first and second HCC recurrence ($P = 0.55$ and 0.62, respectively) was observed between the IFN group and non-IFN group, the rate of second recurrence in the SVR group was significantly lower than that in the non-IFN group (65 vs. 79% at 3 years, respectively, $P = 0.01$; Figs. 5, 6).

Prognostic factors and risk factors of HCC recurrence

To identify the factors that contributed to survival and the recurrence of HCC, a Cox proportional hazard analysis was performed.

Univariate analysis showed that PEG-IFN therapy, low T.Bil, and high serum ALB were independent factors favorably associated with long survival. Among the factors that were significant in the analysis, PEG-IFN therapy [risk ratio = 2.72; 95% confidence interval (CI), 1.29–9.04] and a serum ALB level >3.5 g/dl (risk ratio = 2.51; 95% CI, 1.29–4.98) were shown to be significantly associated with better survival in the multivariate analysis (Table 4).

On the other hand, non-SVR, low ALB, and large and multiple tumors at the initial treatment were significantly

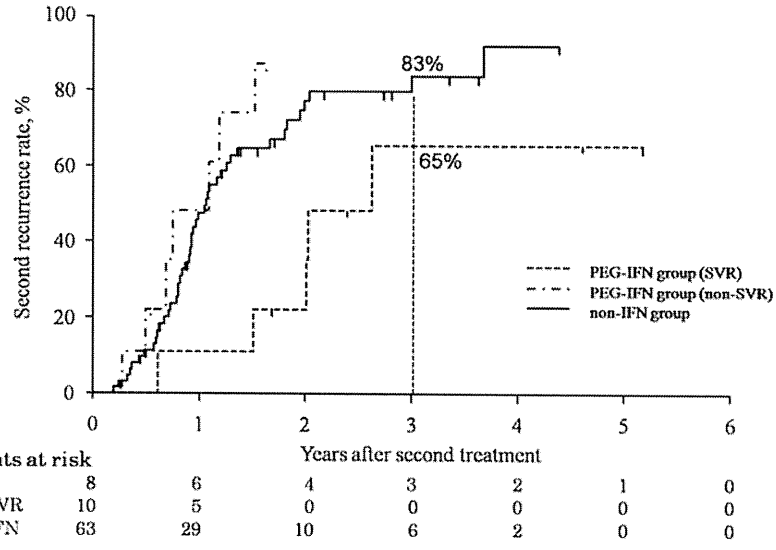

Fig. 3 Rates of second HCC recurrence. The second recurrence rate in the SVR group was significantly lower than that in the non-IFN group (65 vs. 83% at 3 years, respectively; $P = 0.03$. *SVR* sustained virological response

Table 3 Profiles and laboratory tests of the patients (propensity score matched cases)

Variables	PEG-IFN	Non-IFN	*P* value
Number of patients	34	34	
Age (years)	64 (48–77)	64 (43–85)	0.97
Sex (male)	26 (76%)	29 (85%)	0.48
HCV genotype (1b high/others/unknown)	21/13/0	17/8/9	0.62
Response to IFN therapy (SVR/non-SVR)	18/16		
Observation period (years)	4.6 (0.8–12.7)	3.4 (0.8–10.8)	0.22
T.Bil (mg/dl)	0.7 (0.3–2.7)	0.7 (0.43–1.8)	0.77
ALB (g/dl)	3.9 (2.5–4.7)	3.6 (3.1–4.7)	0.83
ALT (IU/l)	69 (17–168)	61 (17–183)	0.43
PLT ($\times$1,000/mm^3)	147 (31–307)	137 (42–216)	0.49
PT (%)	95 (62–118)	85 (52–110)	0.07
AFP (ng/ml)	11 (1.6–1,729)	10.8 (1.3–11,006)	0.38
DCP (mAU/ml)	29 (0–5,230)	27 (0–66,700)	0.34
Number of tumors (solitary)	25 (74%)	27 (79%)	0.81
Size of main tumor (mm)	19 (7–55)	21 (9–50)	0.06
Disease stage (I/II/III/IVA)	14/14/6/0	12/11/9/2	0.27

All variables are shown as the median (range) unless otherwise noted

IFN interferon, *PEG-IFN* pegylated interferon, *HCV* hepatitis C virus, *SVR* sustained virological response, *ALB* albumin, *T.Bil* total bilirubin, *ALT* alanine aminotransferase, *PLT* platelet, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

associated with first recurrence of HCC in univariate analysis. Multivariate analysis showed that low ALB (risk ratio = 1.70; 95% CI, 1.11–2.56) and large (risk ratio = 1.65; 95% CI, 1.02–2.59) and multiple (risk ratio = 1.66; 95% CI, 1.05–2.56) tumors were independent risk factors; however, response to PEG-IFN therapy was not determined to be a significant factor for the first recurrence of HCC (risk ratio = 1.60; 95% CI, 0.83–3.48; Table 5).

Regarding the second recurrence of HCC, non-SVR (risk ratio = 2.51; 95% CI, 1.06–7.40) and low ALB at the first recurrence of HCC (risk ratio = 2.56; 95% CI, 1.46–4.83) were found to be independent risk factors in multivariate analysis as well as univariate analysis (Table 6).

Discussion

Persistent active hepatitis is common in the advanced stage of chronic HCV infection and is a risk factor for the development of HCC. Several reports have shown the inhibitory effects of IFN therapy on the development of HCC. In these reports, the inhibitory effect was considered to be the result of the remission of inflammation, necrosis, and fibrosis in addition to the direct action of IFN on tumor cells [35–39]. Recently, several studies were conducted to show the effect of IFN therapy after curative treatment of HCC, which reduced the risk for recurrence and improved the rate of survival. To date, reports on eight randomized control trials (RCTs) [17–24] and six non-RCTs [25–30] on this effect have been published.

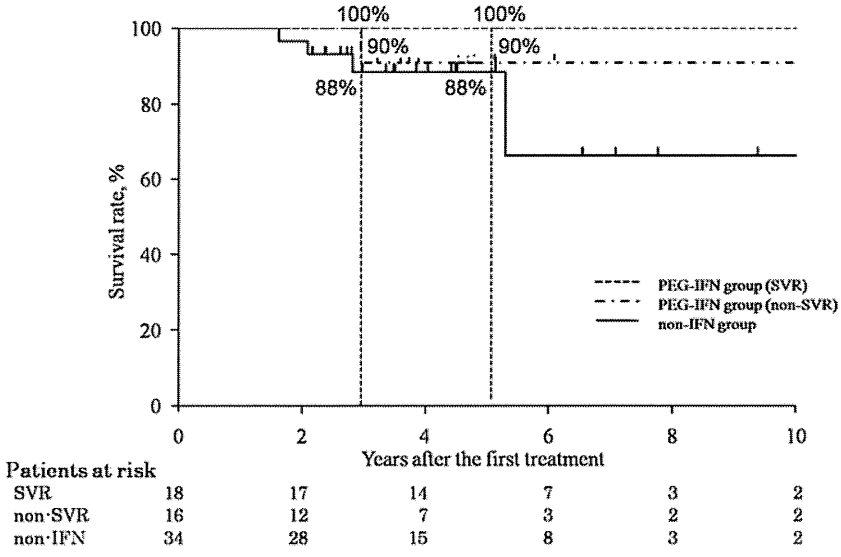

Fig. 4 Cumulative survival rates of PEG-IFN group and non-IFN group after propensity score (PS) matching. Overall-survival rate of the PEG-IFN group was higher than that of non-IFN group ($P = 0.04$). *SVR* sustained virological response

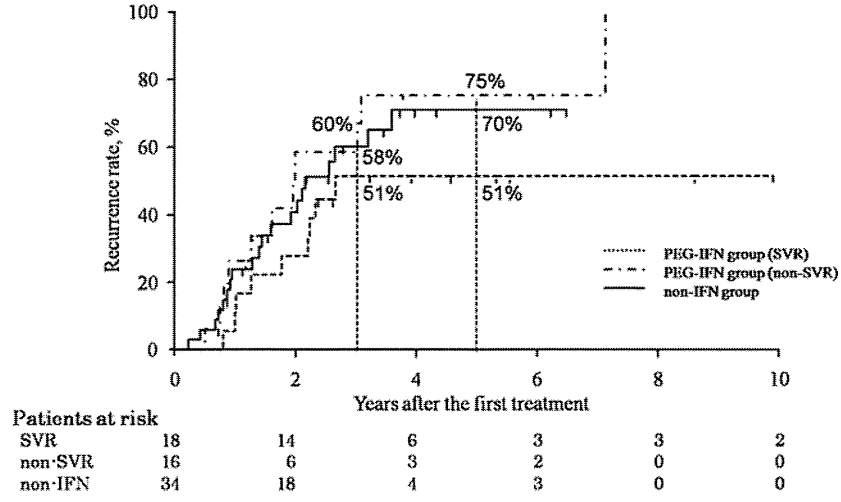

Fig. 5 Rates of first HCC recurrence after PS matching. We found no significant differences between the two groups with respect to first HCC recurrence ($P = 0.55$). *SVR* sustained virological response

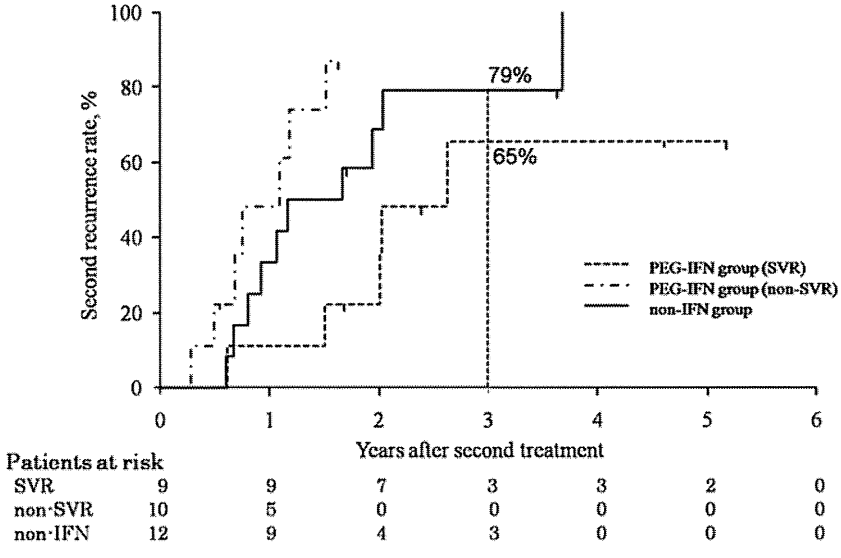

Fig. 6 Rates of second HCC recurrence after PS matching. The second recurrence rate in the SVR group was significantly lower than that in the non-IFN group (65 vs. 79% at 3 years, respectively; $P = 0.01$), although no statistical difference was observed between the IFN group and non-IFN group ($P = 0.62$). *SVR* sustained virological response