Table 1 Baseline characteristics of 40 enrolled patients with recurrent hepatitis C after LDLT before interferon therapy

		Non-SVR		
	SVR (n = 11)	IFN (n = 17)	Withdrawal (n = 12)	P
Age (years)	55 (17–68)	57 (39–66)	58 (15–70)	0.724*
Males/Females	7/4	12/5	5/7	0.281†
Time since LDLT (months)	11.5 (4.2–39.1)	10.6 (1.1–51.2)	5.9 (1.8–85.3)	0.316*
HCV genotype 1/non-1	8/3	15/2	12/0	0.141†
HCV RNA (kIU/mL)	1120 (289–5000)	2810 (74–5000)	2320 (498–5000)	0.850*
White cell count (/μL)	4000 (2200–9000)	4600 (1300–6900)	4400 (1700–6900)	0.991*
Neutrophil count (/μL)	2220 (1235–4140)	2040 (793–4816)	2642 (836–4623)	0.884*
Haemoglobin (g/dL)	12.4 (11.6–17)	11.6 (9.2–15.5)	11.65 (8.9–15.2)	0.096*
Platelet count (10^4/μL)	11.7 (5.9–58.1)	11.3 (4.8–32.4)	14.9 (7.6–40)	0.529*
PT (INR)	1.00 (0.92–1.19)	1.04 (0.93–1.67)	1.07 (0.87–1.34)	0.561*
AST (IU/L)	106 (27–352)	78 (30–258)	107 (44–464)	0.539*
ALT (IU/L)	106 (38–395)	82 (37–275)	157.5 (40–354)	0.619*
ALP (IU/L)	492 (233–1954)	479 (234–828)	636 (306–2977)	0.221*
γ-GTP (IU/L)	293 (41–1447)	107 (29–457)	122.5 (23–1417)	0.147*
Bilirubin (mg/dL)	0.9 (0.4–1.8)	0.9 (0.4–2.6)	1.25 (0.3–10.4)	0.530*
Albumin(g/dL)	3.7 (3.3–4.7)	3.8 (2.7–4.5)	3.5 (2.9–4.4)	0.329*
METAVIR score				
A 0/1/2/3	0/8/3/0	0/8/8/1	0/7/5/0	0.594†
F 0/1/2/3/4	1/8/2/0/0	1/9/7/0/0	5/5/2/0/0	0.066†
Immunosuppression				
Tacrolimus	8	16	7	0.257†
Tacrolimus + MMF	2	0	3	
Tacrolimus + prednisolone	1	0	2	
Cyclosporine	0	1	0	
Cyclosporine + MMF	0	1	0	
Trough level for tacrolimus (ng/mL)	5.9 (3.4–8.7)	5.95 (3.3–10.9)	6.4 (3.8–9.1)	0.752*

PT, prothrombin time; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transpeptidase; MMF, mycophenolate mofetil; LDLT, living donor liver transplantations; SVR, sustained virological response. Qualitative variables are shown in number; and quantitative variables expressed as median (range). *Kruskal–Wallis test, †chi-square test.

that, steroid administration was terminated. Mycophenolate mofetil (MMF) was administered to patients who experienced refractory rejection or required reduction in tacrolimus or cyclosporine doses because of adverse events.

Virological assays

Hepatitis C virus genotype was determined using a genotyping system based on polymerase chain reaction (PCR) of the core region using genotype-specific PCR primers [17]. Serum HCV RNA load was evaluated once a month during treatment and 24 weeks after treatment, using PCR and an Amplicor HCV assay (Cobas Amplicor HCV Monitor; Roche Molecular Systems, Pleasanton, CA, USA).

Statistical analysis

Wilcoxon and Kruskal–Wallis tests, chi-square tests and t-tests were used to analyse the continuous variables, categorical variables and histological changes, respectively. The Kaplan–Meier method was used to estimate the rates of patients who showed a progression of fibrosis to stage F3 or F4 after the initiation of the interferon therapy; log-rank tests were used to compare these rates among groups. Significance was defined as $P < 0.05$.

RESULTS

Characteristics of patients

Hepatitis C virus RNA concentrations and histological evidence were used to diagnose 80 patients with recurrent hepatitis C after LDLT. These patients were given one of two combination therapies: interferon and ribavirin (n = 40) or peginterferon and ribavirin (n = 40) at Kyoto University between January 2001 and April 2007. Thirty-one of the 80 patients who received the combination therapy achieved SVR (Fig. 1). Among the remaining 49 non-SVR patients,

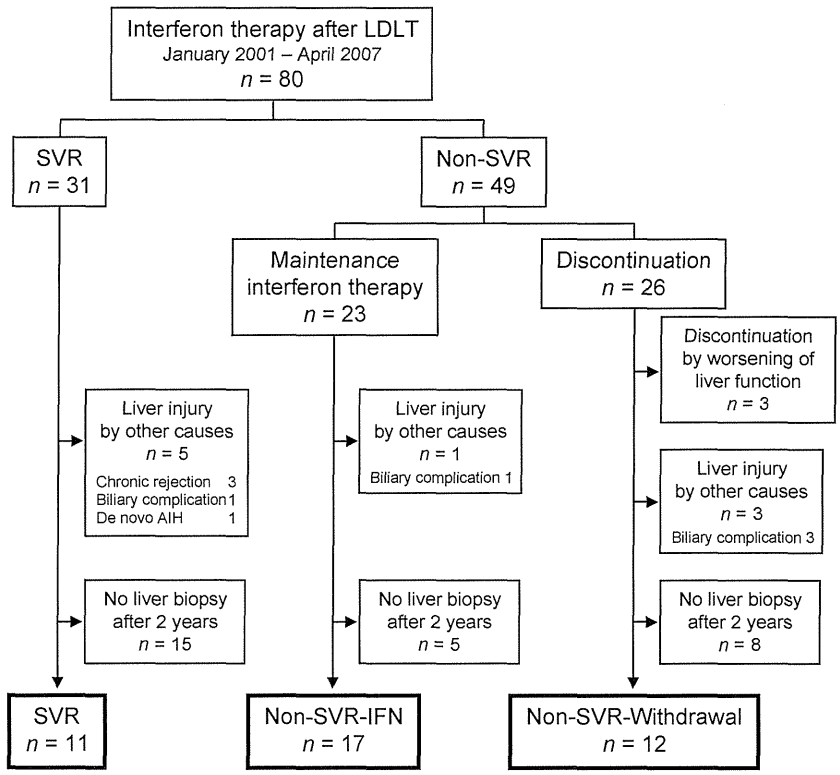

Fig. 1 Flow diagram showing the outcome of interferon therapy for patients with recurrent hepatitis C after living donor liver transplantation and indicating the classification of patients in this study.

23 (47%) received the low-dose peginterferon maintenance therapy, while 26 (53%) discontinued treatment within 12 months and did not receive low-dose peginterferon maintenance therapy as this was the patients' wish (n = 4), because of general fatigue (n = 4), recurrent hepatocellular carcinoma (n = 4), worsening of liver function (n = 3), biliary complications (n = 3), heart failure (n = 2), brain haemorrhage (n = 1), dementia (n = 1), sinusitis (n = 1), anaemia (n = 1), neutropenia (n = 1), and haemosputum (n = 1).

Of the 31 SVR patients, five were excluded because of chronic rejection (n = 3), biliary complications (n = 1) and *de novo* AIH (n = 1). Fifteen patients did not have liver biopsies more than 2 years after the initiation of the interferon therapy, mainly because liver function tests were normal. The remaining 11 patients were classified as the SVR group for analysis in this study. Among the 23 patients who received maintenance therapy, one patient with biliary complications and five patients who did not have liver biopsy more than 2 years after the initiation of therapy were excluded from the study. The remaining 17 patients were classified into the non-SVR-IFN group. Among the 26 patients who discontinued treatment within 12 months, three patients who initially experienced worsening of liver function were excluded because of the rapid progression of HCV; an additional three patients were excluded because of biliary complications. Eight patients were excluded because they had no liver biopsies taken more than 2 years after the initiation of the treatment. The remaining 12 patients were classified into the non-SVR-Withdrawal group. Cumulatively, we analysed the long-term histological changes of 40 patients: 11 in the SVR group (27.5% of the total), 17 in the non-SVR-IFN group (42.5% of the total) and 12 in the non-SVR-Withdrawal group (30% of the total).

There were no significant differences in the baseline characteristics among patients in the SVR, non-SVR-IFN, and non-SVR-Withdrawal groups (Table 1). The median age of patients at the beginning of therapy was 56.5 years (range, 15–70 years). The treatment started at a median of 9.5 months (range, 1.1–85.3 months) after LDLT. Thirty-five patients (88%) were infected with HCV genotype 1b. HCV genotypes of the remaining patients were 2a (n = 3), 2b (n = 1) and undetermined (n = 1). Median serum HCV RNA load was 2290 kIU/mL (range, 73.7–5000 kIU/mL); i.e. most patients had an extremely high viral load. Before the treatment, the necroinflammatory activity of all patients was A1 or greater, and 33 patients (83%) had a fibrosis score of F1 or greater. Among patients receiving tacrolimus for immunosuppression, the median serum trough level was 5.95 ng/mL (range, 3.3–10.9).

Effect of maintenance interferon therapy on liver histology

To evaluate the efficacy of long-term peginterferon therapy on histological changes, we compared scores between final biopsy samples (median, 44.0 months; range, 24.0–81.3 months) and those taken prior to treatment. Five patients in the non-SVR-IFN group discontinued maintenance

therapy between 26.5 and 53.1 months after the initiation of the treatment because of the adverse events. For these patients, the biopsies taken just before or within 3 months after discontinuation of the treatment were analysed as final biopsies. Despite the variation in time between pretreatment and final biopsy sample collection, there were no significant differences in the duration among the three groups ($P = 0.547$). Median duration from initiation of interferon therapy to final liver biopsy was 41.9 months (range, 24.0–81.3 months) in the SVR group, 41.7 months (range, 26.5–68.4 months) in the non-SVR-IFN group and 46.5 months (range, 30.4–79.6 months) in the non-SVR-Withdrawal group.

There were no significant differences in baseline activity grades or fibrosis stages of patients in the three treatment groups when they were first diagnosed with recurrent hepatitis C (Table 1). However, there were noticeable differences among the three groups by the end of treatment (Fig. 2a). The activity grade of all patients in the SVR and non-SVR-IFN groups improved or remained stable, whereas it deteriorated in 6 (50%) of 12 patients in the non-SVR-Withdrawal group. The fibrosis stage deteriorated in all patients in the non-SVR-Withdrawal group; nine of these patients (75%) deteriorated by more than one stage. In contrast, only four patients (24%) in the non-SVR-IFN group deteriorated, all by only a single stage. Furthermore, three patients actually improved. In the SVR group, fibrosis stage decreased or remained stable in 10 of 11 patients (91%).

In patients in the SVR and non-SVR-IFN groups, the mean activity grade was markedly reduced in the final biopsy, compared to the pretreatment biopsy (Fig. 2b). In contrast, patients in the non-SVR-Withdrawal group experienced an increase in activity grade. The differences between the non-SVR-Withdrawal group and both the SVR and the non-SVR-IFN groups were statistically significant ($P < 0.001$). The mean changes in fibrosis stage in the SVR and non-SVR-IFN groups were −0.18 and +0.06, respectively, suggesting that fibrosis did not change during the follow-up period. However, there was an obvious increase (+2.2) among patients in the non-SVR-Withdrawal group, indicating marked progression of fibrosis.

The Kaplan–Meier analysis allowed us to investigate whether patients in the three treatment groups experienced different progression rates to late-stage fibrosis (Fig. 2c). No patient in the SVR group and only 1 patient (6%) in the non-SVR-IFN group developed fibrosis stage F3 or F4, whereas nine patients (75%) in the non-SVR-Withdrawal group progressed to these stages. The rates of fibrosis progression were significantly higher in the non-SVR-Withdrawal group than in the non-SVR-IFN and SVR groups ($P = 0.0049$ and $P = 0.0086$, respectively). There was no significant difference between the SVR group and the non-SVR-IFN group ($P = 0.3980$). Five-year progression rates to F3 or F4 were 0% in the SVR group, 14% in the non-SVR-IFN group and 54% in the non-SVR-Withdrawal group.

Safety and tolerability of maintenance interferon therapy

Five of 17 patients (29%) who received low-dose maintenance peginterferon treatment discontinued interferon therapy because of biliary complications ($n = 2$), neutropenia ($n = 1$), anaemia ($n = 1$) and *de novo* AIH ($n = 1$), between 26.5 and 53.1 months after its initiation. The biliary complications were not related to interferon therapy. Patients with neutropenia and anaemia recovered after discontinuing interferon therapy and were able to resume therapy within months (3 and 10, respectively). Steroid therapy alleviated the *de novo* AIH, but the patients did not resume interferon therapy.

DISCUSSION

Studies have repeatedly shown the benefits of achieving SVR via interferon therapy after liver transplantation. For instance, the durability of the SVR is associated with improvements in hepatic inflammation and histological regression of fibrosis over the long-term [18–23]. In contrast, efficacy of interferon therapy for non-SVR patients after liver transplantation had not previously been investigated. Here, we have demonstrated that long-term peginterferon maintenance therapy suppresses histological progression of recurrent hepatitis C after LDLT.

Maintenance interferon therapy was recently shown to have no influence on either histological or clinical outcomes in patients with nontransplant hepatitis C [24]. This conclusion was drawn after observing that the rate of fibrosis progression was similar between treatment and control groups following a 3.5-year randomized controlled trial of low-dose peginterferon. As a large number of patients with advanced fibrosis were enrolled in the randomized controlled trial, it is difficult to compare with our study in which the number of patients studied is much smaller and patients with advanced fibrosis were not enrolled. In the current study after liver transplantation, however, we demonstrated that low-dose maintenance interferon therapy reduced necroinflammatory activity and fibrosis scores in non-SVR patients to levels similar to those in SVR patients. Furthermore, we found that non-SVR patients who discontinued treatment had significantly worse scores once no longer receiving therapy.

Although these results clearly suggest that low-dose peginterferon maintenance therapy is beneficial for non-SVR patients with recurrent hepatitis C after liver transplantation, the mechanism behind this positive response is unknown. Progression of hepatitis C and development of fibrosis after discontinuation of interferon treatment has been shown to proceed more rapidly in patients who have undergone liver transplantation [20,21]. Our results, indicating that activity grade and fibrosis stage markedly deteriorated in non-SVR patients who discontinued maintenance treatment, support these previous findings. Thus, such a

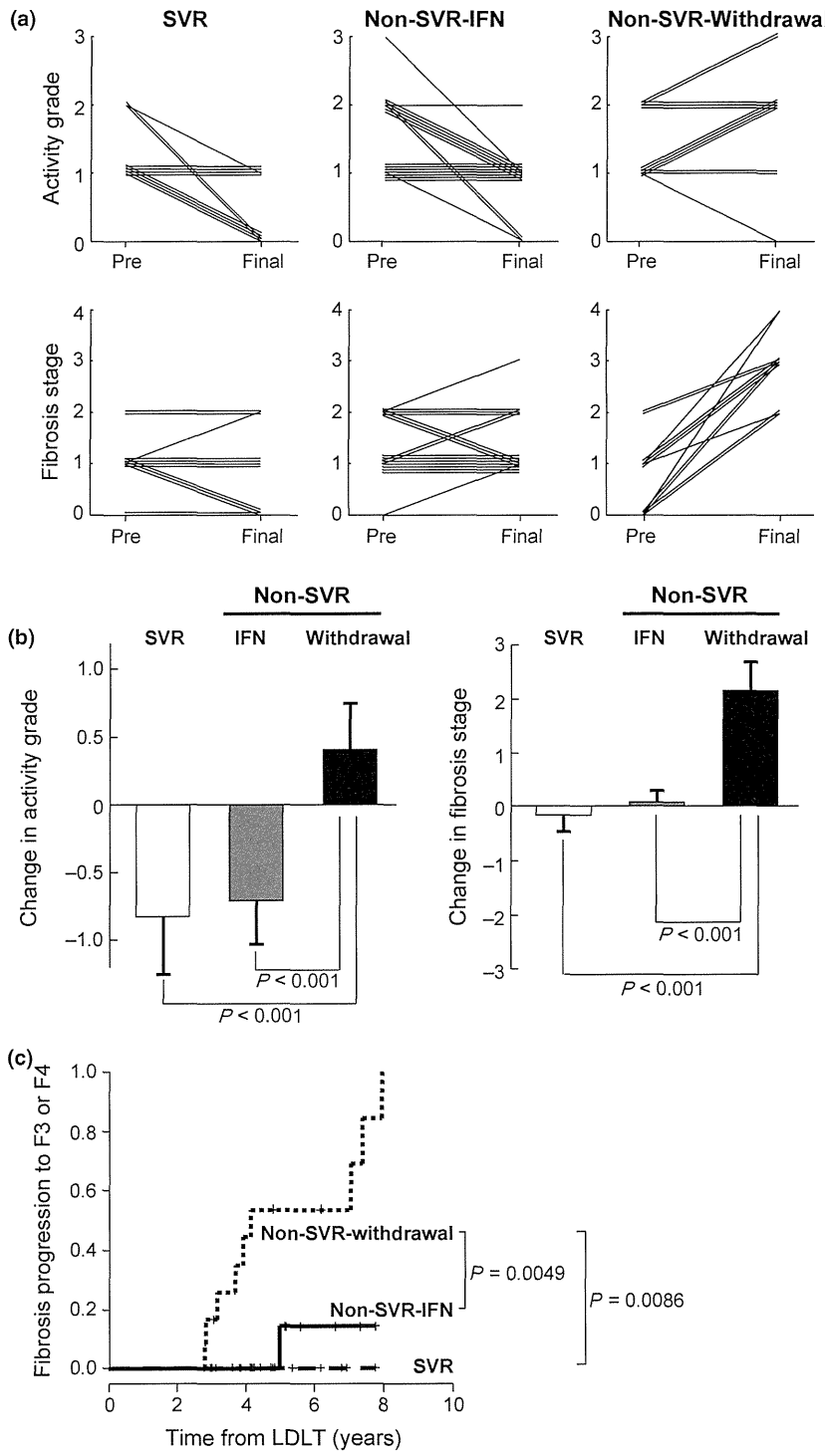

Fig. 2 Effect of maintenance interferon therapy on liver histology: (a) Changes in activity grade (upper) and fibrosis score (lower) of individual patients before interferon therapy (Pre) and at final biopsy (final). (b) Mean changes of liver activity grade (left) and fibrosis stage (right) between pretreatment liver biopsy and the final liver biopsy in each of the three treatment groups. The error bars represent 2 SEs. (c) Kaplan–Meier estimates of the progression rates among patients whose fibrosis advanced to F3 or F4. The dashed line indicates the sustained virological response (SVR) group, the solid line indicates the non-SVR-IFN group and the dotted line indicates the non-SVR-Withdrawal group.

rapid progression of recurrent hepatitis C in patients who discontinued interferon therapy may have highlighted the beneficial effect of the low-dose peginterferon maintenance therapy.

Another issue is the tolerability and safety of long-term peginterferon maintenance treatment. In this study, five patients (29%) discontinued the treatment during the peginterferon maintenance treatment, but only three did so for reasons directly related to the treatment. While two of these patients recovered simply by discontinuing the treatment, the third did require steroid pulse therapy to treat *de novo* AIH. Overall, however, the maintenance therapy did not result in the incidence of major adverse events, suggesting that it is both a tolerable and a safe treatment method.

Our work shows that long-term, low-dose peginterferon administration is an effective method for inhibiting the

progression of liver damage for recurrent hepatitis C after liver transplantation. Unfortunately, this was not a randomized control study, and only a small number of patients were eligible for research. Therefore, we recommend further work to more fully explore the effects of this treatment and to improve the outcomes for patients who do not achieve SVR.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Decrease in alpha-fetoprotein levels predicts reduced incidence of hepatocellular carcinoma in patients with hepatitis C virus infection receiving interferon therapy: a single center study

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Abstract
Background Increasing evidence suggests the efficacy of interferon therapy for hepatitis C in reducing the risk of hepatocellular carcinoma (HCC). The aim of this study was to identify predictive markers for the risk of HCC incidence in chronic hepatitis C patients receiving interferon therapy.
Methods A total of 382 patients were treated with standard interferon or pegylated interferon in combination with ribavirin for chronic hepatitis C in a single center and evaluated for variables predictive of HCC incidence.
Results Incidence rates of HCC after interferon therapy were 6.6% at 5 years and 13.4% at 8 years. Non-sustained virological response (non-SVR) to antiviral therapy was an independent predictor for incidence of HCC in the total study population. Among 197 non-SVR patients, independent predictive factors were an average alpha-fetoprotein (AFP) integration value ≥10 ng/mL and male gender. Even in patients whose AFP levels before interferon therapy were ≥10 ng/mL, reduction of average AFP integration value to <10 ng/mL by treatment was strongly associated with a reduced incidence of HCC. This was significant compared to patients with average AFP integration values of ≥10 ng/mL ($P = 0.009$).
Conclusions Achieving sustained virological response (SVR) by interferon therapy reduces the incidence of HCC in hepatitis C patients treated with interferon. Among non-SVR patients, a decrease in the AFP integration value by interferon therapy closely correlates with reduced risk of HCC incidence after treatment.

Keywords Alpha-fetoprotein · Hepatocellular carcinoma · Hepatitis C · Interferon

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Introduction

Hepatitis C virus (HCV) infection is a predominant cause of liver cirrhosis and hepatocellular carcinoma (HCC) in many countries, including Japan, the United States, and countries of Western Europe [1–5]. The annual incidence of HCC in patients with HCV-related cirrhosis ranged from 1 to 8% [6–9]. Even in the absence of liver cirrhosis, patients with chronic hepatitis caused by HCV infection are at a high risk of developing HCC. Indeed, a large-scale Japanese cohort study showed that the annual incidence of HCC is 0.5% among patients with stage F0 or F1 fibrosis and 2.0, 5.3, and 7.9% among those with F2, F3, and F4 fibrosis, respectively [9]. Periodic surveillance is recommended to detect HCC as early as possible in patients with HCV-related chronic liver disease; however, this may not be cost-effective. For patients with chronic hepatitis C, more effective detection and prevention of HCC is being sought by two important routes: (1) the attempt to discover noninvasive predictive markers and (2) development of treatment strategies to reduce the risk of HCC. There have been several attempts to discover non-invasive markers capable of predicting the risk of HCC incidence in patients with chronic hepatitis C [6, 10]. For example, a cohort

derived from the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) Trial identified older age, African American race, lower platelet count, higher alkaline phosphatase, and esophageal varices as risk factors for HCC [11].

There have also been a number of studies to evaluate the effect of anti-viral treatment of chronic hepatitis C on the incidence of HCC [12–19]. The results were summarized in a meta-analysis, which concluded that the effect of interferon on risk of HCC is mainly apparent in patients achieving a sustained virological response (SVR) to interferon therapy [13]. In addition, a number of studies have suggested the incidence of HCC is reduced in treated patients compared to historical controls [12, 15, 16, 19]. However, the recent HALT-C randomized control trial revealed that long-term pegylated interferon therapy does not reduce the incidence of HCC among patients with advanced hepatitis C who do not achieve SVRs. Reduction in the risk of HCC by maintenance therapy was shown only in patients with cirrhosis [14, 17]. These controversial results suggest that interferon therapy reduces the risk of HCC only in a group of patients with HCV-related chronic liver disease. Thus, it is important to evaluate the risk of HCC development in hepatitis C patients receiving interferon therapy and it will be clinically useful to discover markers distinguishing high- and low-risk groups.

Serum alpha-fetoprotein (AFP) has been widely used as a diagnostic marker of HCC [20–22]. However, elevation of serum AFP levels is often found in non-neoplastic liver diseases without evidence of HCC, including acute liver injury and chronic viral hepatitis [23–27], especially among patients with advanced chronic hepatitis C [28]. An increase of AFP after liver damage is interpreted as a sign of dedifferentiated hepatic regeneration [27]. There have been some reports that AFP is a significant predictor of HCC in patients with chronic hepatitis C [4, 5, 29]. In addition, it has recently been shown that AFP levels decrease in response to interferon administration in patients with chronic hepatitis C [30, 31], and that long-term interferon therapy for aged patients with chronic HCV infection is effective in decreasing serum AFP levels and preventing hepatocarcinogenesis [32, 33]. However, little is known about the relationship between changes in serum AFP level over time during interferon therapy and the development of HCC.

The aim of this large single center study was to identify predictive markers for the risk of HCC development in patients receiving interferon therapy for chronic hepatitis C. For this purpose, patients treated with standard or pegylated interferon, in combination with ribavirin, for chronic hepatitis C were enrolled and subjected to scheduled periodic surveillance for HCC and a number of potential predictive markers, including AFP and alanine aminotransferase (ALT) integration values, at a single center.

Materials and methods

Patients

Between January 2002 and April 2010, 528 patients with chronic hepatitis C received combination therapy with standard interferon and ribavirin ($n = 84$) or pegylated interferon and ribavirin ($n = 444$) at Osaka Red Cross Hospital. Eligibility criteria for treatment were positivity for serum HCV RNA and histological evidence of chronic hepatitis C ($n = 427/444$; 80.9%), or positivity for serum HCV RNA, liver enzyme levels greater than the normal upper limit, and an ultrasound image demonstrating chronic liver damage ($n = 101/444$; 19.1%). Exclusion criteria for treatment were as follows: neutrophil count <750 cells/μL, platelet count <50,000 cells/μL, hemoglobin level ≤9.0 g/dL, and renal insufficiency (serum creatinine levels >2 mg/dL).

Of 528 patients who received interferon therapy for chronic hepatitis C, 146 were excluded from this study for the following reasons: follow-up <24 weeks after the termination of the interferon therapy ($n = 122$), previously treated for HCC ($n = 22$), or occurrence of HCC during or within 24 weeks after treatment ($n = 2$). Therefore, 382 patients were enrolled for the study and were retrospectively analyzed.

To detect early-stage HCC, ultrasonography, dynamic contrast enhanced computed tomography (CT), dynamic contrast enhanced magnetic resonance imaging (MRI), and/or measurement of tumor markers (including AFP) were performed for all patients at least every 6 months. HCC was diagnosed radiologically as liver tumors displaying arterial hypervascularity and venous or delayed phase washout by dynamic contrast enhanced CT or MRI.

The study protocol was approved by the Ethics Committee at Osaka Red Cross Hospital and performed in compliance with the Helsinki Declaration.

Treatment protocol and definition of responses to treatment

The basic treatment protocol for patients with chronic hepatitis C consisted of 6 mega units of interferon-α-2b 3 times a week or 1.5 μg/kg of pegylated interferon α-2b once a week, combined with ribavirin at an oral dosage of 600–1000 mg/day. Duration of the treatment was 48–72 weeks for those with HCV genotype 1 and serum HCV RNA titer of >5 log IU/mL, and 24 weeks for all other patients.

Patients who were negative for serum HCV RNA for >6 months after completion of interferon therapy were defined as showing an SVR. Patients whose serum ALT levels decreased to the normal range and remained normal for >6 months after the termination of interferon therapy were defined as showing a sustained biochemical response (SBR).

Patients who did not achieve SVR received ursodeoxycholic acid and/or glycyrrhizin containing preparation (Stronger Neo-Minophagen C), when serum ALT levels were higher than the upper limit of normal.

Virological assays

HCV genotype was determined by polymerase chain reaction (PCR) amplification of the core region of the HCV genome using genotype-specific PCR primers [34]. Serum HCV RNA load was evaluated once a month during and 24 weeks after treatment using a PCR assay (Cobas Amplicor HCV Monitor, Roche Molecular Systems, Pleasanton, CA, USA).

Measurement of AFP and calculation of average integration value

AFP was measured in serum samples obtained from each patient at intervals of 1–3 months. The median number of examinations was 15 (range 1–70) in each patient. Serum AFP levels were determined by enzyme-linked immunosorbent assay, which was performed using a commercially available kit (ELISA-AFP, International Reagents, Kobe, Japan). Integration values of AFP and ALT were calculated as described in previous reports [35]. For example, the integration value of AFP was calculated as follows, $(y_0 + y_1) \times x_1/2 + (y_1 + y_2) \times x_2/2 + (y_2 + y_3) \times x_3/2 + (y_3 + y_4) \times x_4/2 + (y_4 + y_5) \times x_5/2 + (y_5 + y_6) \times x_6/2$, i.e., the area of each trapezoid representing an AFP value was measured the sum of the resulting values used to calculate the integration value (Fig. 1). The average integration value was obtained by dividing the integration value by the observation period from initiation of the treatment.

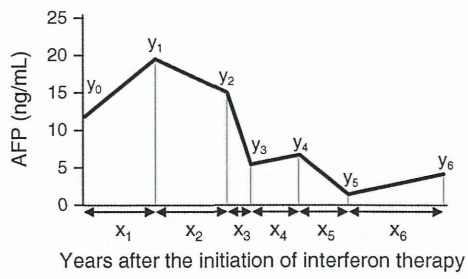

Fig. 1 Example plot of data used for calculation of average integration value of alpha-fetoprotein (AFP)

Statistical analysis

The Kaplan–Meier method was used to estimate the rates of development of HCC in patients after interferon therapy. Log-rank tests were used to evaluate the effects of predictive factors on incidence of HCC. Significance was defined as $P < 0.05$. Multivariate Cox regression analysis using the stepwise method was used to evaluate the association between HCC incidence and patient characteristics, and to estimate hazard ratio (HR) with a 95% confidence interval (CI). A P value of 0.1 was used for variable selection and was regarded as statistically significant. SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Results

Characteristics of patients and incidence of HCC

This study included 382 patients treated for chronic hepatitis C with standard interferon or pegylated interferon in combination with ribavirin. Baseline clinical and virological characteristics of patients included in the study are summarized in Table 1. The median age of the patients at the outset of therapy was 59.0 years (range 18–81 years) and the median follow-up period was 4.1 years (range 0.1–8.4 years). The majority of patients were infected with HCV genotype 1b ($n = 229$; 60%), and median serum HCV RNA load was 6.1 log IU/mL (range 2.3–7.3 log IU/mL). Baseline (before interferon therapy) median serum AFP level was 6.9 ng/mL (range 1.6–478.3 ng/mL).

During follow-up, 23 patients (4.9%) developed HCC. The cumulative incidences of HCC, which was estimated using the Kaplan–Meier method, were 3.1, 6.6, and 13.4% at 3, 5, and 8 years, respectively (Fig. 2).

Predictive factors for incidence of HCC in all patients

Predictive factors for incidence of HCC in all 382 patients were analyzed using log-rank tests (Table 2). Univariate analysis showed that age ≥70 years ($P = 0.040$), non-SVR ($P < 0.0001$), non-SBR ($P = 0.027$), average ALT integration value ≥40 IU/L ($P = 0.001$), baseline AFP ≥10 ng/mL ($P = 0.005$), average AFP integration value ≥10 ng/mL ($P < 0.0001$), and baseline platelet count <150,000 platelets/μL ($P = 0.001$) were all significantly associated with the incidence of HCC. After multivariate analysis, the only variable remaining in the model was non-SVR (HR 8.413, 95% CI 1.068–66.300, $P = 0.043$).

Table 1 Characteristics of 382 patients with hepatitis C treated with interferon therapy in this study

Age (years)	59.0 (18–81)
[a]Males/females	192/190
Observation period (years)	4.1 (0.1–8.4)
[a]IFN + RBV/PEG-IFN + RBV	69/313
HCV genotype 1/2/unclassified	229/57/96
HCV RNA (log IU/mL)	6.1 (2.3–7.3)
White blood cell count (/μL)	4950 (2050–9970)
Hemoglobin (g/dL)	14.0 (10.3–18.8)
Platelet (10^4/μL)	15.0 (5.3–36.4)
AST (IU/L)	56 (17–244)
ALT (IU/L)	67 (16–416)
Bilirubin (mg/dL)	0.8 (0.3–2.4)
AFP (ng/mL)	6.9 (1.6–478.3)

Qualitative variables ([a]) are shown in number, and quantitative variables expressed as median (range)

IFN interferon, *RBV* ribavirin, *PEG-IFN* pegylated interferon, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *AFP* alpha-fetoprotein

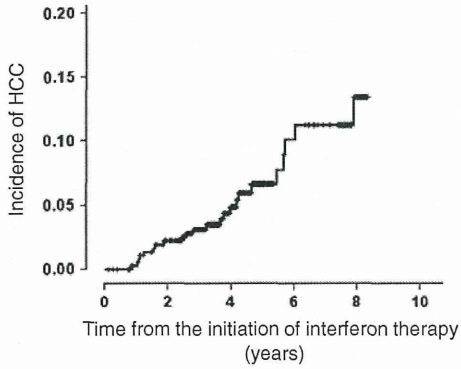

Fig. 2 Incidence of hepatocellular carcinoma (HCC) in 382 patients with hepatitis C who received interferon therapy, estimated using the Kaplan–Meier method

Further, although patients with average AFP integration values ≥10 ng/mL also appeared to have an increased risk of HCC, the difference did not reach statistical significance in the multivariate analysis ($P = 0.050$) (Table 3).

Predictive factors for incidence of HCC in non-SVR patients

Because non-SVR was the only predictive factor across the entire study cohort, to clarify predictive factors for incidence of HCC within this group, the same variables were further analyzed in non-SVR patients alone. By univariate analysis, average AFP integration value ≥10 ng/mL ($P = 0.019$) and baseline platelet count <150,000 ($P = 0.0022$) (Table 2) were again identified as significant predictive factors for incidence of HCC. In addition, male gender was significantly associated with incidence of HCC in non-SVR patients ($P = 0.022$). Multivariate analysis, however, indicated that only two variables were independently associated with incidence of HCC in non-SVR patients: average AFP integration value ≥10 ng/mL (HR 4.039, 95% CI 1.570–10.392, $P = 0.004$), and male gender

Table 2 Univariate analysis of predictive factors for incidence of hepatocellular carcinoma in all 382 and 197 non-SVR patients

Factors	All ($n = 382$)			Non-SVR ($n = 197$)		
	No.	Incidence of HCC ($n = 23$)		No.	Incidence of HCC ($n = 22$)	
		No. (%)	*P* value[a]		No. (%)	*P* value[a]
Age (years)						
<70	359	19 (5)	0.040	182	18 (10)	0.089
≥70	23	4 (17)		15	4 (27)	
Sex						
Female	190	8 (4)	0.125	111	8 (7)	0.022
Male	192	15 (8)		86	14 (16)	
HCV genotype						
1	229	12 (5)	0.452	137	12 (9)	0.796
Non-1	57	1 (2)		10	1 (10)	
Virological response						
SVR	185	1 (1)	<0.0001			
Non-SVR	197	22 (11)				
Biochemical response						
SBR	282	12 (4)	0.027	102	11 (11)	0.857
Non-SBR	86	11 (13)		81	11 (14)	
ALT before IFN therapy						
<40	79	2 (3)	0.274	39	2 (5)	0.319
≥40	301	21 (7)		158	20 (13)	
ALT integration value						
<40	238	6 (3)	0.001	79	5 (6)	0.153
≥40	142	17 (12)		118	17 (14)	
AFP before IFN therapy						
<10	230	7 (3)	0.005	102	7 (7)	0.124
≥10	116	14 (12)		75	13 (17)	
AFP integration value						
<10	258	8 (3)	<0.0001	115	8 (6)	0.019
≥10	63	12 (19)		53	11 (21)	
Platelet before IFN therapy						
<150,000	187	20 (11)	0.001	121	19 (16)	0.022
≥150,000	194	3 (2)		76	3 (4)	

[a] Log-rank test

SVR sustained virological response, *SBR* sustained biochemical response, *ALT* alanine aminotransferase, *IFN* interferon, *AFP* alpha-fetoprotein

Table 3 Multivariate analysis of the predictive factors for incidence of hepatocellular carcinoma in all 382 patients

Factors	Hazard ratio	95% CI	*P* value
Virological response			
SVR	1		
Non-SVR	8.413	1.068–66.300	0.043
AFP integration value			
<10	1		
≥10	2.580	0.999–6.659	0.050

SVR sustained virological response, *IFN* interferon, *AFP* alpha-fetoprotein

Table 4 Multivariate analysis of predictive factors for incidence of hepatocellular carcinoma in 197 non-SVR patients

Factors	Hazard ratio	95% CI	*P* value
AFP integration value			
<10	1		
≥10	4.039	1.570–10.392	0.004
Sex			
Female	1		
Male	3.636	1.383–9.563	0.009

AFP alpha-fetoprotein

(HR 3.636, 95% CI 1.383–9.563, $P = 0.009$) (Table 4). There was no significant difference in other variables including those identified as predictive factors in the entire study population (i.e., age, non-SBR, ALT integration value, AFP before interferon therapy) (Table 2).

AFP integration value as a predictive factor for HCC

Further analysis focused on the AFP integration value as this was the strongest predictive factor for incidence of HCC in non-SVR patients. Of the 382 patients, both baseline and AFP integration values were available for 321. These were divided into four groups: (1) AFP "low–low," (2) AFP "low–high," (3) AFP "high–low," and (4) AFP "high–high," for baseline AFP-average AFP integration values, respectively, where "high" is ≥10 ng/mL and "low" is <10 ng/mL. As shown in Fig. 3a, of the 321 patients, 211 (65.7%) showed baseline AFP levels <10 ng/mL. Of these 211, 207 (98%), were in the AFP low–low group, and only four in the AFP low–high groups. Baseline characteristics, including age, gender, serum HCV-RNA, aspartate aminotransferase (AST), ALT, bilirubin, white blood cell, hemoglobin, platelet, observation periods, and number of times of AFP measurement, were not different between AFP high–low group and high–high group. However, AFP-low group, which is a combination of the low–high and low–low groups, showed significantly lower AST level ($P < 0.00001$), lower ALT level ($P < 0.00001$), higher platelet count ($P < 0.00001$), shorter observation period ($P = 0.01448$), and fewer number of times of AFP examination ($P = 0.00035$), compared to both AFP high–high and AFP high–low group. Six patients (2.8%) with baseline AFP levels <10 ng/mL developed HCC in the follow-up period and none of these patients were among the four low–high group patients. Even in patients with high baseline AFP levels, incidence of HCC was only 3.9% among the AFP high–low group (2 of 51 patients). In contrast, 20.3% of patients in the AFP high–high group developed HCC during the follow-up period.

The incidence rate of HCC in three patient groups, "AFP-low" (a combination of the "low–high" and "low–low" groups),"high–low," and "high–high", was estimated using the Kaplan–Meier method and compared using log-rank tests (Fig. 3b). The rate of HCC incidence was significantly higher in the AFP high–high group compared to both the AFP high–low group and patients with low baseline AFP levels ($P = 0.009$ and 0.001, respectively). There was no significant difference between patients with low baseline AFP levels and the AFP high–low group. The 7-year incidence rate of HCC was 32.3% in the AFP high–high group, compared to only 6.6% in the AFP high–low group, and 8.1% in all patients with low pre-treatment levels.

Discussion

It is well recognized that the most effective strategy for the prevention of HCC development in patients with chronic hepatitis C is likely to be the complete elimination of the HCV infection accompanied by the resultant normalization of liver function [7, 12, 13, 15, 16, 19]. Indeed, we confirmed here that non-SVR is the most significant predictive factor for incidence of HCC in patients receiving interferon therapy for chronic hepatitis C. However, it should be noted that the risk of HCC, even in non-SVR patients, differs between individuals. In the current study, we identified AFP integration value and male gender as independent risk factors for incidence of HCC in non-SVR patients. The incidence of HCC was significantly reduced in individuals with average AFP integration values < 10 ng/mL after interferon therapy, which suggests that the decrease of AFP by interferon therapy lowers the risk of developing HCC. Indeed, even where patients had high baseline AFP levels, incidence of HCC was reduced when the AFP integration value decreased after interferon therapy. Thus, our current findings identify AFP integration value as a useful predictive marker of HCC development in non-SVR patients.