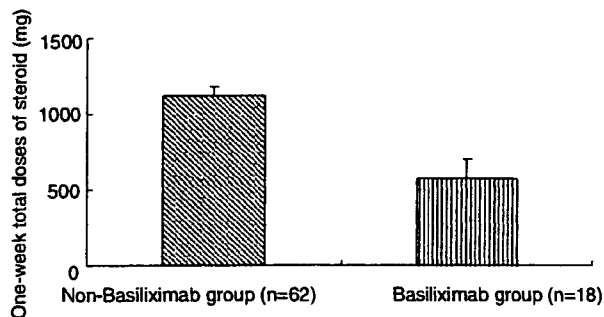


Table 4 Univariate and multivariate analysis of risk factors for biliary leakage

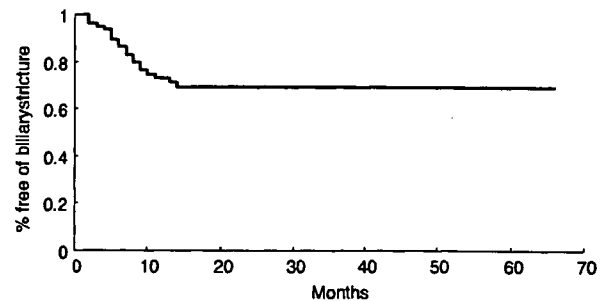
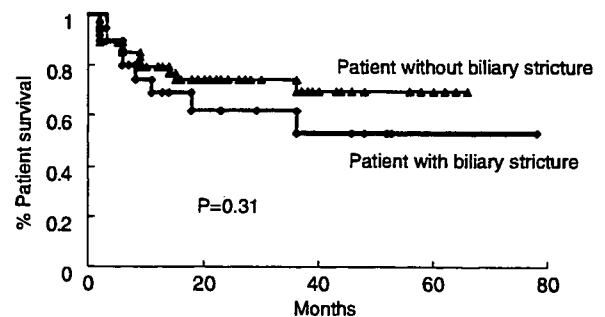
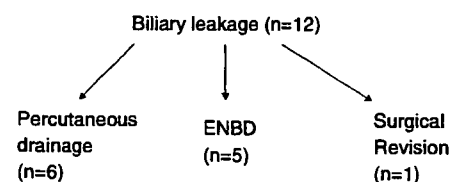
Risk factors	No. of patients with biliary leakage	<i>p</i> Value (Univariate)	<i>p</i> Value (Multivariate)
Immunosuppression		0.033	0.005
Bax (<i>n</i> =18)	0 (0%)		
Non-Bax (<i>n</i> =62)	12 (19%)		
No. of bile ducts		0.569	0.901
Single (<i>n</i> =48)	6 (12.5%)		
Non-single (<i>n</i> =32)	6 (19%)		
Ductoplasty		0.639	0.702
Yes (<i>n</i> =16)	3 (18.8%)		
No (<i>n</i> =64)	9 (14%)		
Donor age		0.066	0.065
> 50 year (<i>n</i> =28)	7 (25%)		
< 50 year (<i>n</i> =52)	5 (9.6%)		
Graft		0.636	0.512
Right (<i>n</i> =66)	9 (13.6%)		
Left (<i>n</i> =14)	3 (21%)		
MELD		0.674	0.42
> 25 (<i>n</i> =17)	2 (12.5%)		
< 25 (<i>n</i> =63)	10 (15.6%)		

**Fig. 1** Total doses of methylprednisolone that were administered for one week after living donor liver transplant (LDLT). All values are expressed as the mean \pm standard error

Management of biliary complications

For biliary leakage, ENBD was possible in five patients. Six patients with biliary leakage underwent percutaneous drainage under ultrasound guidance. One patient underwent Roux-en-Y hepaticojejunostomy. All patients were successfully treated by these modalities (Fig. 4).

Figure 5 shows the summary of the various modalities used for the treatment of biliary strictures. Initially, the patients with biliary strictures were referred for ERC. In 10 of the 20 patients, a guidewire could pass through the stricture, and these patients were treated by endoscopic internal stent placement. In the remaining 10 patients, the

**Fig. 2** Biliary stricture-free rate after LDLT**Fig. 3** Impact of biliary strictures on patient survival (Kaplan-Meier) in months**Fig. 4** Summary of the treatment modalities used for biliary leakage: endoscopic retrograde nasobiliary drainage (ENBD), percutaneous drainage, and surgical revision

guidewire could not be passed through the biliary stricture because it was too tight and the bile ducts were too kinked. No symptoms of biliary stricture were observed in two patients after ERC. Seven patients required percutaneous transhepatic biliary drainage (PTBD), and five patients underwent stenting. Consequently, six patients underwent Roux-en-Y reconstruction to repair the stricture; however, 16 months after transplantation, one patient died of sepsis secondary to chronic cholangitis.

Discussion

In the present study we observed that post-transplantation anastomotic biliary leakages and strictures occurred, respectively, in 15% and 25% of our patients who

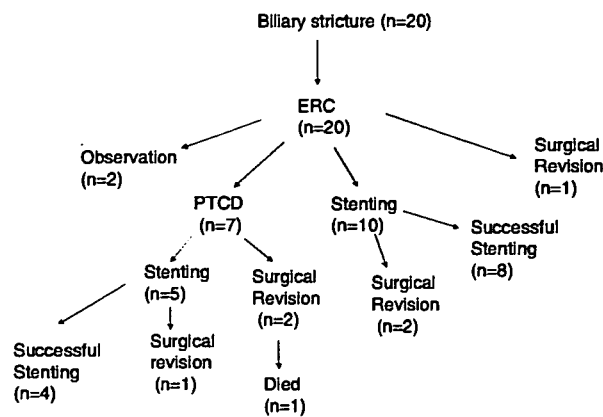


Fig. 5 Summary of the treatment modalities used for biliary stricture: endoscopic retrograde cholangiography (ERC), percutaneous transhepatic cholangiography (PTC), and surgical revision

underwent LDLT with duct-to-duct biliary reconstruction. In previous reports the incidence of biliary stricture in patients who have undergone LDLT with duct-to-duct biliary reconstruction ranges from 20% to 60% [15, 16], whereas the reported incidence of biliary stricture in patients who have undergone cadaveric-donor liver transplantation with duct-to-duct biliary reconstruction is less than 30% [17–19]. Fan et al. reported that while the incidence of biliary leakage was comparable in the two groups, the incidence of biliary stricture was significantly higher in the LDLT group than in cadaveric whole-graft liver transplantation [20].

The present study sought to identify the risk factors for biliary leakage and stricture unique to duct-to-duct biliary reconstruction. We found that patients who developed biliary leakage were vulnerable to biliary stricture. Bile leakage after liver transplantation has previously been suggested as a risk factor for the development of anastomotic strictures [4, 21, 22]. It is likely that the leakage of bile causes local inflammation, thus increasing the risk of fibrosis. Further, the leakage of bile may merely represent ischemia of the extrahepatic bile duct, and in this manner, is associated with anastomotic stricture. The immunosuppressive regimes that included basiliximab were found to be a protective factor against anastomotic leakage. Biliary leakage developed less frequently in patients treated with the basiliximab-based immunosuppressive regimes than in those treated with the non-basiliximab-based ones. The initial 1-week doses of methylprednisolone were significantly lower in the basiliximab-based immunosuppressive regimes than in those without basiliximab. In general, glucocorticosteroids impair fibroblast proliferation and collagen synthesis, and they delay wound healing [23]. Possibly, the steroid-sparing basiliximab-based immunosuppressive therapy contributed to protection against biliary leakage. Consequently, the development of biliary strictures tended to be less frequent in

the basiliximab-based immunosuppressive therapy than in the non-basiliximab-based one. However, there was no significant difference in the incidence of biliary stricture with respect to the immunosuppressive therapies by using stepwise multivariate analysis.

In our series, 40% of the liver grafts had multiple bile duct orifices. The poor outcome might be related to the complicated procedures. The Kyoto Group has reported that they experienced a higher rate of biliary complications with the use of stents for biliary reconstruction; further, no difference was observed with respect to the presence of single or multiple biliary anastomoses [4]. Salvalaggio et al. studied the impact of multiple bile duct anastomoses on the development of biliary complications after liver transplantation in children. They demonstrated that patients with multiple ducts had a higher incidence of leaks as compared to those in the single duct group, but that the incidence of strictures, both early and late, was similar in the two groups [24]. Similarly, in our study, the presence of multiple or single hepatic ducts was not a significant risk factor for biliary stricture. The extensive dissection enabled us to overcome the technical difficulty encountered with multiple and widely separated bile ducts in the liver grafts, as the corresponding orifices in the recipient hilar plate could be freely selected. The use of a bile duct with a wide orifice might result in a good outcome. Hwang et al. reported that duct-to-duct biliary reconstruction involving a small-sized duct (diameter <4 mm) was a risk factor for anastomotic stenosis [25]. However, in our study, the diameter of the graft bile duct was not related to the incidence of biliary strictures.

Ischemic changes around the anastomosis are known to be a major cause of biliary stricture. In donor operations, particularly in cases of right lobe graft, the tissues surrounding the right hepatic duct at the bifurcation must be removed in order to correctly identify the anatomy of the anterior and the posterior segmental branches. The anterior branch of the hepatic duct might be more widely exposed than the posterior branch during the dissection. Dissecting these tissues might cause the biliary stump in the right-lobe graft to become ischemic, which may then facilitate formation of a biliary stricture. The arterial blood supply of the biliary system has been described by several investigators [26–29]. A fine arterial plexus covering the surface of the biliary tract is one of the main sources of the blood supply of the biliary system. Furthermore, the vascular supply for both hepatic ducts depends on an arterial network that is bilaterally fed by the plexus that is formed by the branches of both the right and left hepatic arteries. The blood supply to the bile duct of the graft tends to be tenuous. Shortening of the segment of the donor duct results in an improved circulatory status around the biliary anastomosis, and this technique is therefore suitable for LDLT with a limited length of the graft hepatic duct [9].

Our patients with biliary strictures were treated satisfactorily by ERC, PTBD, surgery, or a combination of these. Hisatake et al. reported that 14 (73.4%) of their patients with strictures were treated endoscopically by inserting internal stents ranging from 7 to 12 Fr in size [13]. The complication of biliary stricture occurred only in one patient. Our initial treatment for duct-to-duct biliary reconstruction recipients was endoscopic stenting. Forty percent of our patients with anastomotic strictures failed to respond to endoscopic treatment because it was impossible to pass an endoscopic guidewire through the stricture because of the extreme narrowness of the duct. Those patients who failed to respond to the endoscopic treatment were treated via a percutaneous transhepatic approach. The total success rate of stenting therapy was 60%. Surgical correction had been effectively performed in patients with recurrent symptoms of cholangitis after stent removal or in those patients for whom stenting was not possible. As demonstrated in several studies, balloon dilatation and stenting via either an endoscopic or percutaneous transhepatic approach were safe and effective. Nonetheless, surgical correction should be reserved for patients who fail to respond to nonsurgical treatment.

Our patients with biliary leakages were treated satisfactorily by ENBD, percutaneous drainage, or surgery. We preferred interventional treatment rather than surgery as mentioned by Hwang et al [25]. All patients with biliary leakages were successfully treated by ENBD or percutaneous drainage, except one patient who was treated by surgery.

In summary, our experiences revealed that biliary stricture was associated with biliary leakage and that steroid-sparing basiliximab-based immunosuppressive therapy contributed to the decreased incidence of biliary leakage. The incidence of biliary stricture was not associated with the type or method of biliary reconstruction. The radiological, endoscopic, or surgical treatment for biliary complications was effective and satisfactory. However, the incidence rate of biliary stricture after LDLT is still high. Further improvements in surgical modalities and postoperative management should contribute to the decrease in the incidence of biliary complications.

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Effects of a 24-week course of interferon- α therapy after curative treatment of hepatitis C virus-associated hepatocellular carcinoma

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related HCC occurred during persistent viral infection. Eradication of HCV is essential for the prevention of HCC recurrence and improvement of survival.

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Key words: Hepatitis C virus, Hepatocellular carcinoma, Recurrence, Survival, Sustained virological response

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Abstract

AIM: To assess whether a 24-wk course of interferon (IFN) could prevent hepatocellular carcinoma (HCC) recurrence and worsening of liver function in patients with hepatitis C virus (HCV)-infected patients after receiving curative treatment for primary HCC.

METHODS: Outcomes in 42 patients with HCV infection treated with IFN- α , after curative treatment for primary HCC (IFN group), were compared with 42 matched curatively treated historical controls not given IFN (non-IFN group).

RESULTS: Although the rate of initial recurrence did not differ significantly between IFN group and non-IFN group (0%, 44%, 61%, and 67% vs 4.8%, 53%, 81%, and 87% at 1, 3, 5, and 7 years, $P = 0.153$, respectively), IFN group showed a lower rate than the non-IFN group for second recurrence (0%, 10.4%, 28%, and 35% vs 0%, 30%, 59%, and 66% at 1, 3, 5 and 7 years, $P = 0.022$, respectively). Among the IFN group, patients with sustained virologic response (SVR) were less likely to have a second HCC recurrence than IFN patients without an SVR, or non-IFN patients. Multivariate analysis identified the lack of SVR as the only independent risk factor for a second recurrence, while SVR and Child-Pugh class A independently favored overall survival.

CONCLUSION: Most intrahepatic recurrences of HCV-

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms worldwide. Chronic infection with hepatitis C virus (HCV) has been causally associated with HCC^[1-3]. Recent advances in imaging and treatment have brought about some improvement in 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 such as hepatic resection or local ablation^[4]. Reasons for this unfavorable prognosis are considered to include high intrahepatic tumor recurrence rates and sustained hepatic damage, both resulting from HCV infection^[5].

Even after curative hepatic resection for HCV-related HCC, the rate of intrahepatic tumor recurrence within 1 year is 20%-40%, rising to about 80% by 5 years^[4,6-8]. Intrahepatic recurrence of HCC may result from intrahepatic metastasis originating from the primary HCC, or from ongoing multicentric carcinogenesis related to chronic HCV infection. Underlying HCV-related hepatic damage may also compromise hepatic functional reserve, and worsen clinical outcome. Thus prevention of HCC recurrence and preservation of liver function are both high priorities for improving prognosis of patients with HCV-

related HCC.

Interferon (IFN) therapy for patients with HCV infection is effective in reducing serum alanine transaminase (ALT) activity and in eradicating HCV^[9,10], and thus IFN could have value in minimizing hepatic necrosis, inflammation and fibrosis, as well as reducing the incidence of HCC. Several recent studies have reported that IFN therapy, even after curative treatment for HCV-related HCC, could prevent HCC recurrence and improve survival^[11-17]. Unfortunately, since these studies are characterized by differing IFN regimens, definitions of IFN responses, and background characteristics of patients, results have varied and no standard IFN regimen has been established for after curative treatment of HCV-related HCC. As well, the mechanisms by which IFN suppresses HCC recurrence, including possible direct anti-tumor and anti-inflammatory effects, remain uncertain.

In the present study, recurrence and survival outcomes in matched historical controls were compared with those in patients receiving a 24-wk course of IFN- α therapy after receiving curative treatment for HCC.

MATERIALS AND METHODS

Patients

We retrospectively reviewed 495 consecutive patients treated for primary HCC associated with HCV infection at Hiroshima University Hospital from March 1992 to March 2004. Of these, 384 with HCC initially underwent therapeutic intervention with curative intent. Curative treatment was defined as complete tumor eradication, with no residual tumor visible by computed tomography, or resection of all evident tumor tissue. Medical treatment included percutaneous radiofrequency ablation (RFA), ethanol injection, and microwave coagulation therapy (MCT). Surgical treatment included hepatic resection and ablation during laparotomy.

Among these 384 patients, we administered IFN therapy to 42 who met the following eligibility criteria: age under 70 years; up to three tumors with none exceeding 30 mm in diameter, or a solitary tumor less than 50 mm in diameter; tumor-node-metastasis (TNM) stage I, II, or III; detectable serum HCV RNA; seronegativity for hepatitis B surface antigen; chronic hepatitis or compensated cirrhosis with a Child-Pugh class of A or B; platelet count above 70 000/ μ L; absence of local recurrence during the follow-up period; and absence of ectopic intrahepatic recurrence within 24 wk after treatment for primary HCC. We used the TNM classification system of the Liver Cancer Study Group of Japan as the staging system for HCC^[18]. Underlying liver conditions such as hepatitis or cirrhosis were confirmed by laboratory, pathologic and radiologic examinations. We classified liver function in chronic hepatitis as Child class A because chronic hepatitis is a known pre-cirrhotic condition. There were only a few chronic hepatitis cases: three in the IFN group and four in the non-IFN group.

As historical control subjects, we selected 42 patients with no IFN therapy after treatment for primary HCC (non-IFN group). These 42 patients, who met the eligibility

Table 1 Patient characteristics

	IFN group (n = 42)	Non-IFN group (n = 42)	P
Median age in years (range)	62 ² (45-69)	63 ¹ (40-69)	NS
Gender (male/female)	36/6	29/13	NS
Alb (g/dL)	3.9 ²	3.9 ¹	NS
PLT ($\times 10\ 000/\mu$ L)	12 ²	11.5 ¹	NS
ICG R-15 (%)	17 ¹	18 ¹	NS
CH or Child A/B	35/7	35/7	NS
Size of main tumor (mm)	20 ² (10-50)	15 ¹ (10-50)	NS
AFP (ng/mL)	26 ¹	31.4 ¹	NS
No. of HCC (single/two or three)	30/12	36/6	NS
Stage (I / II or III)	14/28	23/19	NS
Treatment of HCC (medical/surgical)	18/24	20/22	NS

IFN: interferon; Alb: albumin; PLT: platelet; ICG-R15: indocyanine green retention at 15 min; CH: chronic hepatitis; AFP: alpha-fetoprotein; HCC: hepatocellular carcinoma. ¹Median.

criteria noted above, were matched by age, gender, tumor size, TNM stage of HCC, serum albumin, platelet counts, and Child-Pugh class with patients who received IFN therapy (IFN group).

Thus, a total of 84 patients (42 in the IFN group and 42 in the non-IFN group) were enrolled. All agreed to participate in the research protocol, which was approved by the hospital research ethics board. Table 1 shows the baseline characteristics of the two groups, indicating no significant differences for age, gender, liver function, tumor characteristics, or therapeutic methods used against HCC.

IFN therapy

In the IFN group, patients received 6 MIU of natural IFN- α (human lymphoblastoid IFN, Sumiferon; Dainippon Sumitomo Pharmaceuticals, Osaka, Japan) intramuscularly every day for 2 wk, followed by three times weekly for 22 wk. IFN therapy began within 24 wk after the initial treatment for HCC. All patients were evaluated every week in an outpatient setting during IFN treatment. Qualitative detection of HCV-RNA was performed by a standardized qualitative reverse transcription-polymerase chain reaction (RT-PCR) assay at every 4 wk during and after IFN treatment.

Among the patients who received IFN therapy, 28 were of HCV genotype 1 and 14 were of HCV genotype 2. These 42 patients had various pretreatment viral loads. Twenty patients (genotype 1, $n = 11$; genotype 2, $n = 9$) had high viral loads (≥ 100 kIU/mL by PCR), and 22 (genotype 1, $n = 17$; genotype 2, $n = 5$) had low viral loads (≤ 100 kIU/mL by PCR). The 42 patients were divided into two subgroups according to virologic response, i.e. patients with *vs* without a sustained virologic response (SVR). SVR was defined as the sustained absence of serum HCV RNA for more than 24 wk after completion of IFN treatment. Absence of SVR included both persistent viremia (no response) and transient viral disappearance (transient response) during or after IFN therapy. Biochemical response was defined as ALT activity declining to a value within the normal reference range in the presence of viremia.

Follow-up

After curative treatment for primary HCC, all patients studied underwent liver function tests, serum tumor marker assays, such as those for α -fetoprotein (AFP) and protein induced by vitamin K absence or antagonist II (PIVKA-II) every month, abdominal ultrasonography every 3 mo, and dynamic computed tomography (CT) every 6 mo. If recurrence of HCC was suspected, additional examinations including CT during arteriography or tumor biopsy were performed. Recurrence of HCC was defined as any new nodules indicated by CT as hyperattenuation during hepatic arteriography or by hypoattenuation in CT performed during arteriography. Hypovascular HCC was confirmed histopathologically after fine-needle aspiration biopsy. Patients with recurrent HCC were treated medically or surgically, with curative intent if possible.

In IFN patients, including those with or without SVR, and in the non-IFN group, we compared both the rate of HCC recurrence and the survival rate. We also sought to identify significant prognostic indicators for survival and recurrence after curative treatment of primary HCC.

Statistical analysis

Chi-squared and Fisher exact tests were used for categorical variables, while Student's *t* test and the Mann-Whitney *U* test were used for continuous and ordinal variables, as appropriate. The Kaplan-Meier method was used to assess cumulative survival and recurrence rates, calculated from the date of diagnosis to the date of disease recurrence or death. Surviving patients and those who died of causes unrelated to the liver were defined as censored cases, while patients who died of causes related to the liver were defined as non-censored cases. The log-rank test was used to compare survival and recurrence curves. Univariate and multivariate predictors of survival or recurrence time were determined using the Cox proportional hazard model. Hazard ratios and their 95% confidence intervals (95% CI) were computed. $P < 0.05$ was considered to indicate statistical significance. The JMP version 5.1 statistical software package (SAS Institute, Cary, NC, USA) was used for analysis of data.

RESULTS

Virologic and biochemical responses to IFN therapy and side effects

The 42 patients receiving IFN therapy included 29 in the SVR group and 13 in the group without SVR (10 transient virological responders, 3 with no virological response). In the group without SVR, 7 biochemical responders who had a normalized ALT included 5 with transient virological responses and 2 with no virological response. Although there was no significant difference in the population of patients with HCV genotype 1 between the SVR and non-SVR group, patients in the former had significantly lower pre-IFN viral loads than patients in the latter group. In the SVR group, 24 patients received full-dose IFN therapy without dose reduction, while five patients received a reduced dose of IFN until completion

of treatment. In the group without SVR, one patient with no response discontinued IFN treatment at 16 wk because of a recurrence of HCC, while three patients with a transient response discontinued treatment because of generalized fatigue. The remainder of the group without SVR received the full course of IFN therapy. Thus, most patients were able to complete the 24-wk course.

Recurrence of HCC

In the IFN group, first recurrences of HCC developed in 20 patients after the initial treatment for HCC during a median follow-up period of 32 mo. Of these recurrences, 10 were in patients with SVR (10/29) and 10 in patients without SVR (10/13), including 7 transient virological responders and 3 with no virological response. For the 7 biochemical responders without SVR, HCC recurred in 6 patients, including 5 transient virological responders and 1 with no virological response. Of these 20 patients with recurrence, 18 were treated with local ablation therapy or surgical resection without leaving any residual tumor. The remaining 2 patients developed uncontrolled multiple HCC and were excluded from the subsequent study concerning the next recurrence. One died of HCC, while the other was treated repeatedly with hepatic arterial infusion, and has survived. Three patients in the SVR group and 7 in the group without SVR (5 transient virological responders and 2 with no virological response) had a second recurrence of HCC. Of these 10 patients with a second recurrence, 3 (2 transient virological responders and one with no virological response) developed uncontrolled HCC, while others were treated curatively with hepatic resection or local ablation therapy. In the non-IFN group, a first recurrence of HCC occurred in 30 patients during a median follow-up period of 31 mo. HCC recurred in 11 of the 17 who had a normal ALT level. Among the 30 patients with recurrent HCC, 25 were treated with local ablation therapy or surgical treatment, with no residual tumor. The remaining 5 patients who did not undergo curative therapy were treated repeatedly with transarterial chemoembolization. A second recurrence developed in 15 of the 25 patients who had curative treatment for a first recurrence. Among these 15 patients, 10 were treated curatively (9 with local ablation and 1 with hepatic resection). The remaining 5 patients had uncontrolled multiple HCC as their second recurrence.

Overall cumulative rates for first and second recurrence of HCC were compared between the groups. The 1-, 3-, 5- and 7-year rates for first recurrence in the IFN and non-IFN group were 0% *vs* 4.8%, 44% *vs* 53%, 61% *vs* 81%, and 67% *vs* 87%, respectively (Figure 1A, $P = 0.153$; no significant difference between groups). However, the 1-, 3-, 5-, and 7-year rates for second recurrence in the IFN and non-IFN group were 0% *vs* 0%, 10.4% *vs* 30%, 28% *vs* 59%, and 35% *vs* 66%, respectively (Figure 1B, $P = 0.022$). Thus, the second-recurrence rate was significantly lower in the IFN group than in the non-IFN group.

Next, the recurrence rates of HCC were compared between the SVR group, the non-SVR group and the non-IFN group. The rate of first recurrence was significantly lower in the SVR group than in the non-SVR and non-IFN group (Figure 2A). The rate of second recurrence in the

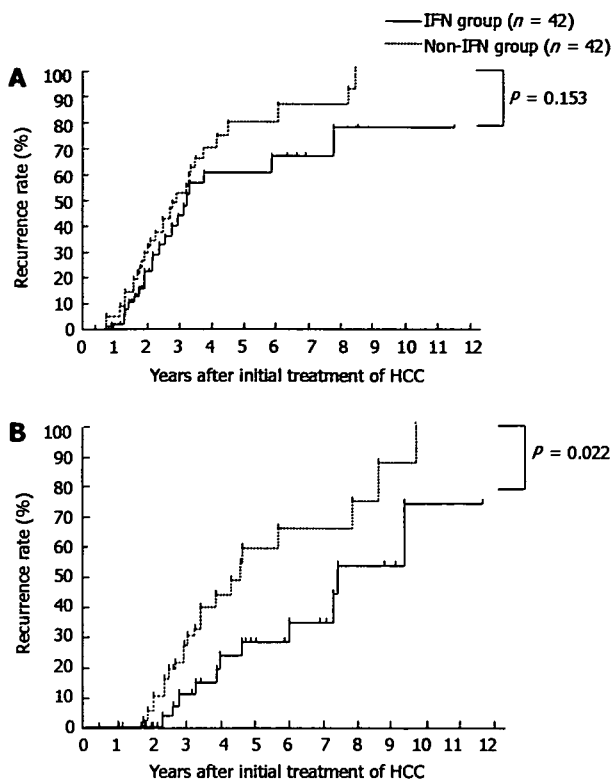


Figure 1 Cumulative recurrence rates after curative treatment of HCC. **A:** Rates of first recurrence compared between IFN and non-IFN groups, showed no significant difference ($P = 0.153$); **B:** Rates of second recurrence compared between IFN and non-IFN groups. The second recurrence rate for the IFN group was lower than that for the non-IFN group ($P = 0.022$).

SVR group was also lower than that in the non-SVR and non-IFN groups; this decrease was significantly greater than that for the rate of first recurrence (Figure 2B). No significant difference was seen in cumulative rates for first or second recurrence between the non-SVR and non-IFN groups. We also confirmed that biochemical responders in the non-SVR and non-IFN groups showed similar Kaplan-Meier curves for cumulative recurrence (data not shown). Recurrence curves were similar between the non-SVR group, including biochemical responders, and the non-IFN group, therefore, we defined these two groups as “non-SVR status” for statistical analysis. Factors found to be significantly associated with first recurrence by univariate analysis were tumor size (≥ 20 mm) and non-SVR status ($P = 0.019$, $P = 0.0067$, respectively). Multivariate analysis showed that no independent risk factor was associated with the first recurrence of HCC (data not shown), although non-SVR status tended to be associated with first recurrence ($P = 0.0657$). As shown in Table 2, univariate analysis indicated that non-SVR status, low platelet count ($< 100\,000$) and high indocyanine green retention ($\geq 20\%$) were significantly associated with second recurrence. Multivariate analysis identified only SVR status as a significant independent inhibiting factor for second recurrence of HCC.

Survival of patients

During the observation period, 13 of the total patients

studied died of liver disease. Nine died of HCC and 4 of liver failure. When we compared cumulative survival rates between the IFN and the non-IFN groups (Figure 3A), the respective rates were 100% vs 95% at 3 years, 100% vs 72% at 5 years, and 86% vs 63% at 7 years. The cumulative survival rate was significantly higher in the IFN group than in the non-IFN group ($P = 0.039$). Median survival time following the first treatment of HCC was 52.3 mo (range, 12-158) in the IFN group and 51.8 mo (range, 11-126) in the non-IFN group. In the IFN group, 2 patients died of advanced HCC, 1 with an SVR and the other without. No patients in the IFN group died of hepatic failure. In the non-IFN group, 7 patients died of HCC and four of hepatic failure.

Figure 3B shows cumulative survival curves for the SVR, non-SVR and non-IFN groups. The rate of survival in the SVR group was significantly better than that in the non-IFN group ($P = 0.029$), while no significant difference was evident between the non-SVR and non-IFN group ($P = 0.248$).

Pretreatment factors found to be significantly associated with survival by univariate analysis subsequently were evaluated by Cox regression analysis to determine independent factors. Multivariate analysis showed that SVR status and Child-Pugh class A were independent factors favorably associated with long survival (Table 3).

Liver function

Compared with the non-IFN group, patients who received IFN therapy were less likely to have worsening of hepatic dysfunction. For the SVR, non-SVR and non-IFN groups, we compared the average score for Child-Pugh classification at initial treatment of HCC with that at the time of data analysis. Median observation time was 59.8 mo in the SVR group, 45 mo in the non-SVR group, and 51.8 mo in the non-IFN group. There were no significant differences in the Child-Pugh classification score among these three groups at the time of initial treatment of HCC; however, at the time of data analysis, scores in the non-IFN group were significantly worse than in the SVR group ($P = 0.003$). No significant difference was seen between the non-SVR and non-IFN groups (Figure 4).

DISCUSSION

The present study compared historical control subjects with no IFN treatment with other subjects who were treated with IFN. Background characteristics showed no significant difference between the groups. IFN and non-IFN group did not differ significantly in their rate of first recurrence, but did differ significantly in their rate of second recurrence. According to IFN response, the recurrence rate in the SVR group was significantly lower than that in the non-SVR and non-IFN group, while recurrence rates in the non-SVR and non-IFN group did not differ significantly. Thus, SVR (i.e. HCV eradication) was the most important, and only, inhibiting factor for decreasing risk of HCC recurrence, associated with a 24-wk course of IFN- α therapy following HCC treatment.

Although several recent studies have reported the

Table 2 Factors associated with second recurrence

Variables	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
SVR	0.454	0.246-0.728	0.0005	0.457	0.243-0.757	0.0015
PLT > 100000/ μ	0.553	0.373-0.814	0.003	0.694	0.445-1.069	0.0973
ICG R-15 (< 20%)	0.667	0.450-0.965	0.032	0.685	0.447-1.035	0.0721

Cox's proportional hazards model was used.

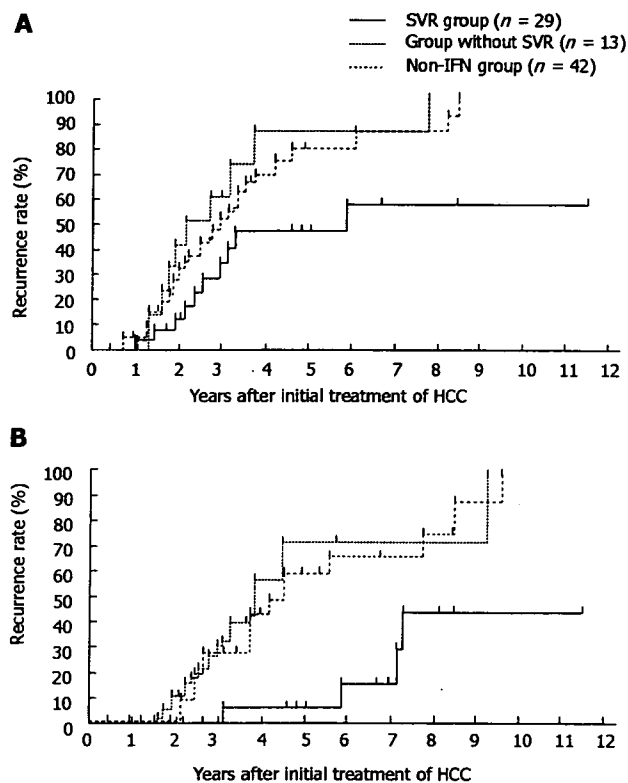


Figure 2 Cumulative recurrence rates according to SVR to IFN therapy after curative treatment of HCC. **A:** Rates of first recurrence compared among SVR, non-SVR and non-IFN groups. The rate of first recurrence of HCC in the SVR group was significantly lower than in the non-SVR and non-IFN groups ($P = 0.002$, $P = 0.016$, respectively). No significant difference in first recurrence rate was seen between the non-SVR and non-IFN groups ($P = 0.381$); **B:** Rates of second recurrence of HCC was suppressed in the SVR group compared with the non-SVR and non-IFN groups ($P = 0.0037$, $P = 0.0019$, respectively), and to a more pronounced degree than for the first recurrence rate. No significant difference in second recurrence rate was seen between the non-SVR and non-IFN groups ($P = 0.90$).

efficacy of chemoprevention with IFN after treatment of HCV-related HCC, the basis of this benefit has not been determined, since IFN has a variety of biologic effects, including antiviral, antiproliferative, immunomodulatory^[19-22] and anti-fibrogenic^[23,24] activities; growth inhibition through changes in signal transduction^[19,25,26], and activation of natural killer cells^[27] and T cells^[28,29]. Through these various effects, IFN therapy is thought to suppress tumor recurrence directly and/or indirectly.

Sakaguchi *et al.*^[15] have reported that low-dose, long-term, intermittent IFN- α therapy can, by a direct anticancer effect, inhibit intrahepatic metastasis but not

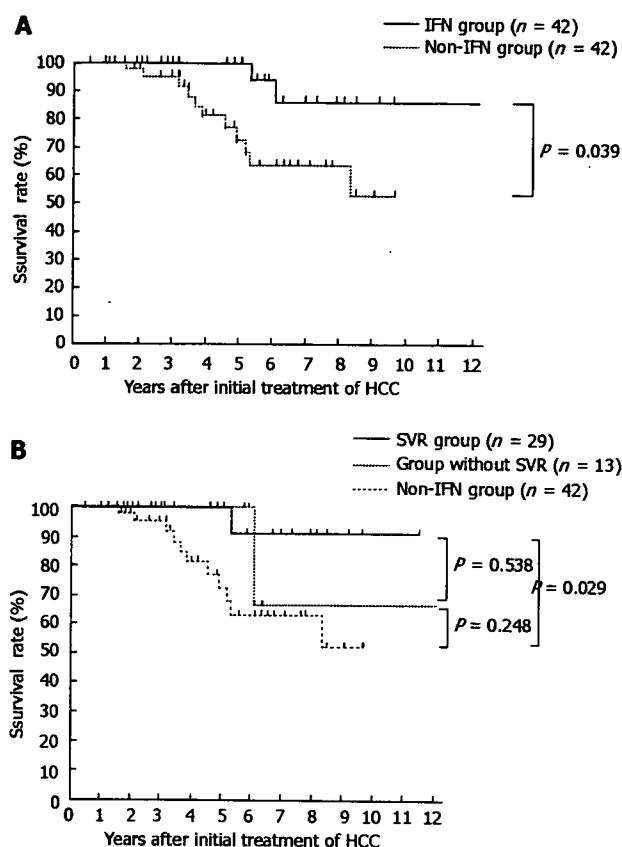


Figure 3 Cumulative survival rates after curative treatment of HCC. **A:** Comparison of cumulative survival rates in the IFN and non-IFN groups. The cumulative survival rate was significantly higher in the IFN group than in the non-IFN group ($P = 0.039$); **B:** Comparison of cumulative survival rates in the SVR, non-SVR and non-IFN groups. Although no significant overall difference was found between the SVR and non-SVR groups ($P = 0.538$), the SVR group had a particularly high survival rate compared with the non-IFN group ($P = 0.029$).

multicentric occurrences. Lai *et al.*^[20] have reported that IFN- α therapy is effective in advanced HCC. Several experimental studies have shown that IFN inhibits the growth of a human hepatoma cell line^[11,15]. In partial disagreement, however, Nishiguchi *et al.*^[12,14], Suou *et al.*^[16] and Shiratori *et al.*^[17] have reported that the rate of HCC recurrence was not different between IFN and non-IFN group during the first few years, but later became significantly lower in the IFN group. They suggested that IFN reduced HCC recurrence in the later period of observation by suppressing multicentric occurrence, as an indirect anti-tumor effect that was related to sustained hepatic inflammation. Although the present study did not have a randomized controlled design, and details of the

Table 3 Factors associated with survival

Variables	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
SVR	0.409	0.096-0.922	0.028	0.329	0.076-0.761	0.006
Child-Pugh class A	0.521	0.299-0.922	0.027	0.463	0.238-0.875	0.019
ICG R-15 (< 20%)	0.551	0.286-0.968	0.038	0.724	0.351-1.429	0.350

Cox's proportional hazards model was used.

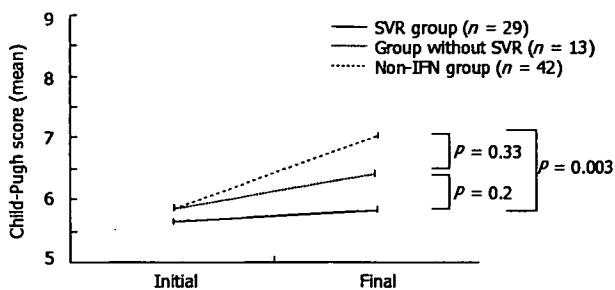


Figure 4 Influence of IFN therapy after curative treatment of HCC on Child-Pugh scores. IFN-treated patients were less likely to show deterioration of hepatic function. In particular, liver function scores in the SVR group were significantly better preserved than in the non-IFN group ($P = 0.003$). Median observation time was 59.8 mo in the SVR group, 45 mo in the non-SVR group, and 51.8 mo in the non-IFN group.

IFN protocol differed from those of others, the long-term results appear to be similar among studies. Recurrence during the first few years might involve undetectable intrahepatic metastasis, or a potential malignant tumor already existing at the time of treatment of the primary HCC; afterward, HCC might recur as multicentric new liver tumor, accompanied by sustained hepatic necrosis and inflammation. Although a direct anti-cancer effect of IFN might to some extent have directly inhibited HCC recurrence, our IFN doses were insufficient to suppress intrahepatic metastatic tumors because there was only a 24-wk treatment. Therefore, in our study, we believe that IFN therapy suppressed HCC recurrence less by a direct anti-tumor effect than by an indirect effect through inhibition of the chronic inflammation associated with HCV infection in the later period of observation.

Several studies have reported that recurrence was suppressed not only in virologic responders to IFN, but also in biochemical responders, even though HCV was not eradicated^[12-14]. However, the recurrence rates in our study did not differ significantly between biochemical responders and the non-IFN group. HCV eradication appeared to stand alone as an IFN effect capable of inhibiting recurrence, with eradication having a stronger influence against second recurrence than the first. The differences between the results of the various studies might be due to several reasons. In most previous studies, IFN therapy was given for more than 48 wk, compared with our 24 wk. Differences may also have been present in underlying hepatic inflammatory conditions such as chronic hepatitis and cirrhosis. Although such differences introduce some uncertainty to the conclusions, several recent studies suggest that HCV core protein might directly participate in hepatocarcinogenesis^[28,29], which supports the importance

of virus eradication.

Although some other recent studies have reported that IFN therapy following HCC treatment also improves liver function and survival of patients with HCV-related HCC, which of the specific IFN actions is important for these benefits remains unknown. We found that overall survival rate and preservation of liver function were significantly better in the SVR group than in the other groups, even including biochemical responders, with all subgroups without SVR resembling non-IFN patients. Favorable independent factors associated with survival by multivariate analysis were SVR and Child-Pugh class A. Thus, with a 24-wk course of IFN- α therapy, HCV eradication appears necessary for prolonging survival, suppressing HCC recurrence, and preserving liver function.

As stated above, effective management of HCV infection is needed, as well as direct treatment of the primary HCC. Although our study had limitations, such as the use of historical controls and a small number of patients, we could demonstrate a clear requirement for HCV eradication to improve survival after a short-course IFN- α therapy. Ribavirin combination or pegylated IFN therapy are considered more effective in HCV eradication than conventional IFN monotherapy^[32-34]. Several studies have indicated that pegylated IFN therapy is superior to conventional IFN when administered for 48 wk^[34-41]. Pegylated IFN therapy, with or without ribavirin, may improve prognosis in selected patients with no sustained initial response to conventional IFN. For patients who cannot undergo standard-dose IFN therapy because of limited hepatic reserve or thrombocytopenia, low-dose IFN therapy for a longer course might be effective. Nonetheless, further studies with larger controlled groups and long-term follow-up need to be performed to establish what constitutes optimal management of HCV infection after HCC treatment.

COMMENTS

Background

Risk of multicentric recurrence of hepatocellular carcinoma (HCC) and liver function deterioration remains high in hepatitis C virus (HCV)-infected patients even after receiving curative treatment for primary HCC. Most intrahepatic recurrences occurred during persistent viral infection. Although several recent studies have reported the efficacy of chemoprevention with interferon (IFN) therapy after treatment of HCV-related HCC, there was no standard IFN regimen. We investigated whether 24-week course of IFN- α therapy following curative treatment for primary HCC associated with HCV infection could suppress HCC recurrence and improve prognosis.

Research frontiers

To obtain sustained virological response (SVR) was important for suppression of HCC recurrence and for long-term survival in a 24-week course of IFN- α therapy.

Innovations and breakthroughs

Our study demonstrated that only SVR status by a 24-wk IFN- α therapy was the most important factor for decreasing risk of HCC recurrence in the later period of observation including second recurrence.

Applications

This study demonstrated that compared with non-IFN and non-SVR group, SVR group decreased the rate of recurrence, preserved liver function, and prolonged survival time in a 24-wk course of IFN- α therapy.

Peer review

This is a matched historical case controlled study concerning about the effect of 24-week short course IFN- α therapy after receiving curative treatment for primary HCC. The paper is well written and the results show that the most important factor associated with the improvement of prognosis is the SVR status.

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Low-dose intermittent interferon-alpha therapy for HCV-related liver cirrhosis after curative treatment of hepatocellular carcinoma

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Abstract

AIM: To assess the efficacy of low-dose intermittent interferon (IFN) therapy in patients with hepatitis C virus (HCV)-related compensated cirrhosis who had received curative treatment for primary hepatocellular carcinoma (HCC).

METHODS: We performed a prospective case controlled study. Sixteen patients received 3 MIU of natural IFN-alpha intramuscularly 3 times weekly for at least 48 wk (IFN group). They were compared with 16 matched historical controls (non-IFN group).

RESULTS: The cumulative rate of first recurrence of HCC was not significantly different between the IFN group and the non-IFN group (0% vs 6.7% and 68.6% vs 80% at 1- and 3-year, $P = 0.157$, respectively). The cumulative rate of second recurrence was not also significantly different between the IFN group and the non-IFN group (0% vs 6.7% and 35.9% vs 67% at 1- and 3-year, $P = 0.056$, respectively). Although the difference in the Child-Pugh classification score between the groups at initial treatment of HCC was not significant, the score was significantly worse at the time of data analysis in the non-IFN group than IFN group (7.19 ± 1.42 vs 5.81 ± 0.75 , $P = 0.0008$). The cumulative rate of deviation from objects of any treatment for recurrent

HCC was also higher in the non-IFN group than IFN group (6.7% and 27% vs 0 and 0% at 1- and 3-year, $P = 0.048$, respectively).

CONCLUSION: Low-dose intermittent IFN-alpha therapy for patients with HCV-related compensated cirrhosis after curative HCC treatment was effective by making patients tolerant to medical or surgical treatment for recurrent HCC in the later period of observation.

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Key words: Hepatitis C virus; Hepatocellular carcinoma; Interferon therapy; Liver cirrhosis; Liver function; Recurrence; Survival

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms worldwide. Approximately 80% of Japanese patients with HCC have a history of hepatitis C virus (HCV) infection, and most such patients have liver cirrhosis^[1-3]. Although recent advances in imaging techniques and treatment of HCC have improved prognosis of patients with HCV-related HCC, the outcome is still unsatisfactory; the 5-year survival rate is only 50% to 70% even after curative treatment such as hepatic resection and local ablation^[4]. The reasons for this unfavorable prognosis is considered to include high intrahepatic tumor recurrence rates and biochemical deterioration by sustained hepatic damage, both resulting from persistent HCV infection^[5]. Even after curative hepatic resection for HCV-related HCC, the rate of intrahepatic tumor recurrence within 1 year is 20% to 40%, rising to about

80% by 5 years^[4,6-8]. Intrahepatic recurrences of HCC may result from intrahepatic metastasis originating from the primary HCC or from ongoing multicentric carcinogenesis related to chronic HCV infection. In addition, sustained underlying HCV-related hepatic damage may compromise hepatic functional reserve, worsening clinical outcome. Thus, prevention of HCC recurrence and preservation of liver function are both highly important priorities in improving prognosis of patients with HCV-related HCC.

Interferon (IFN) therapy for patients with HCV infection is effective as evident by reduction of serum alanine transaminase (ALT) activity and eradication of HCV. Accordingly, IFN is valuable in minimizing hepatic necrosis, inflammation, and fibrosis, as well as reducing the likelihood of hepatocarcinogenesis^[9-16]. The primary goal of treatment of patients with HCV infection is elimination of the virus. Several studies have reported recently that IFN therapy provided after curative treatment for HCV-related HCC prevents HCC recurrences and improves survival^[17-23]. Such improvement of prognosis is more predominant when IFN therapy results in elimination of HCV RNA^[24]. However, most patients with HCV-related HCC also have liver cirrhosis. Many centers do not advocate IFN therapy of patients with compensated cirrhosis, mainly because of the disappointing sustained virological response (SVR) rates in such patients^[25]. Several studies indicated that the response of cirrhotic patients to antiviral therapy is low^[26-28]. The reasons for the low SVR rate in such patients include inability to administer IFN at recommended doses due to adverse effects and dose-limiting cytopenia. On the other hand, several investigators suggested that the use of low-dose IFN therapy for viral elimination was as effective in the treatment of cirrhotic patients with HCV as it is in non-cirrhotic patients^[29,30]. Furthermore, they indicated that the same therapy could improve the underlying liver histology. There is evidence to suggest that low-dose IFN therapy might be beneficial in HCV-related cirrhosis, not only because it prevents the progression of liver disease, but also because it reduces the risk of hepatocarcinogenesis^[31,32]. In this regard, low-dose IFN therapy seems to be tolerable without significant life-threatening adverse effects than the standard dose of IFN.

However, it is not known whether low-dose IFN after curative treatment of primary HCC could slow disease progression or reduce the rate of clinical decompensation in cirrhotic patients, in addition to prevention of HCC recurrence. Several studies used the standard dose of IFN after HCC treatment^[17,23,33], and studies using low-dose IFN therapy for HCV-related cirrhosis after HCC treatment also reported that such regimen may reduce late recurrence of HCC^[34].

In this prospective case controlled trial, we assessed the efficacy of low-dose intermittent IFN therapy on HCV-related liver cirrhosis after curative treatment of primary HCC in terms of overall survival, HCC recurrence, and liver function.

MATERIALS AND METHODS

Patients

A total of 176 consecutive patients received their initial

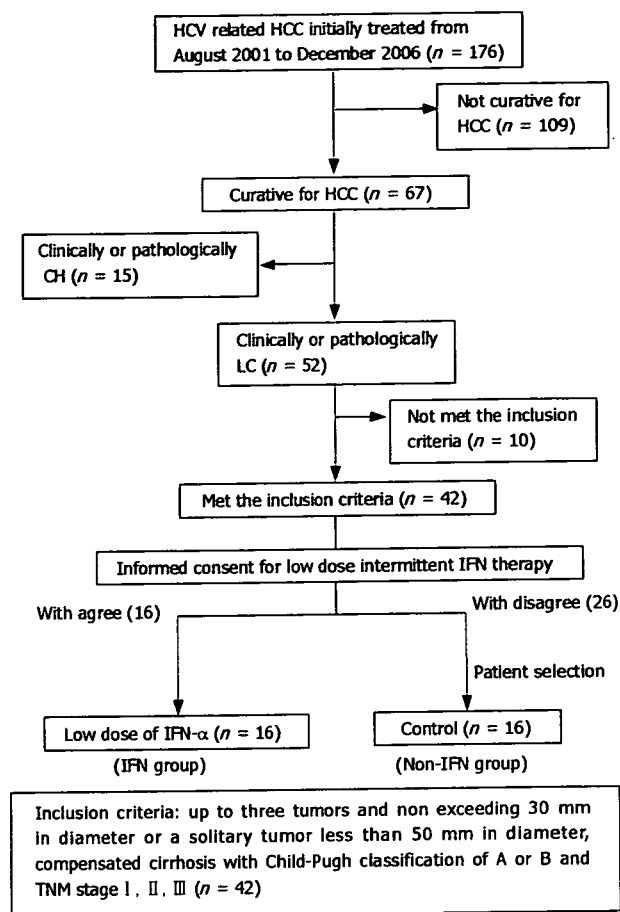


Figure 1 Schematic flow chart of enrolled patients.

treatment for HCV-related primary HCC at Hiroshima University Hospital between August 2001 and December 2006. Of these, 67 patients with HCC underwent first medical or surgical therapeutic intervention with curative intent (defined as complete tumor eradication with no visible residual tumor in computed tomographic images, or resection of all evident tumor tissue). Medical treatments included percutaneous radiofrequency (RF) ablation and ethanol injection, while surgical procedures included hepatic resection and RF ablation under laparotomy. Among these 67 patients, 52 patients with liver cirrhosis (LC), which was diagnosed clinically and pathologically, were considered for this prospective study. Figure 1 shows our study flow. Among these 52 patients with HCV-related LC, we assessed 42 patients who met the following inclusion criteria: (1) the presence of up to three tumors with none exceeding 30 mm in diameter or a solitary tumor less than 50 mm in diameter; (2) tumor-node-metastasis (TNM) stage of I, II or III; (3) detectable serum HCV RNA; (4) all seronegativity for hepatitis B marker including hepatitis B surface antigen, hepatitis B anti-core antibody and hepatitis B surface antibody; (5) compensated cirrhosis with a Child-Pugh class A or B; (6) platelet count $\geq 40000/\mu\text{L}$; and (7) absence of local recurrence during the follow-up period and of any ectopic intrahepatic recurrence within 12 wk after treatment for primary HCC. We used the TNM classification system

Table 1 Characteristics of participating patients

	Interferon group	Non-interferon group	P value
No. of patients	16	16	
Age in years (range)	68.5 ¹ (53-73)	67.5 ¹ (58-75)	NS
Gender (Male/Female)	10/6	11/5	NS
Albumin (g/dL)	3.7 ¹ (3.0-4.8)	3.7 ¹ (3.0-4.5)	NS
Platelet count ($\times 10^4$ /L)	8.0 ¹ (4.5-14.2)	8.4 ¹ (4.6-14.3)	NS
ICG R-15 (%)	17.3 ¹ (6.1-40.8)	18.2 ¹ (5-45)	NS
Alanine aminotransferase (IU/L)	59 ¹ (35-99)	58 ¹ (21-143)	NS
Alpha fetoprotein (ng/mL)	54 ¹ (5.3-293.6)	38 ¹ (5.0-1217)	NS
Child-Pugh score (A/B)	13/3	13/3	NS
Main tumor size (mm)	15 ¹ (10-50)	18 ¹ (10-40)	NS
No. of HCC tumors (single/multiple)	9/7	10/6	NS
Stage (I / II / III)	8/3/5	7/5/4	NS
Treatment (medical/surgical)	8/8	9/7	NS
HCV genotype (1/2)	12/4	14/2	NS
Viral loads (low/high)	6/10	5/11	NS

ICG-R15: Indocyanine green retention at 15 min; Low viral loads: HCV RNA < 100 KIU/mL, high viral loads: HCV RNA \geq 100 KIU/mL. ¹median.

of the Liver Cancer Study Group of Japan as a staging system for HCC^[35]. The underlying liver condition leading to LC was identified by histopathological examination of resected tissue samples. When this was not available, laboratory tests were performed including serum albumin, platelet, prothrombin time and indocyanine green retention at 15 min (ICG-R15), and radiological examination such as ultrasonography and computed tomography.

Of the 42 patients with LC who met the above eligibility criteria, 16 patients received low-dose IFN therapy after signing a written informed consent (IFN group). Of the remaining 26 patients who rejected IFN therapy, we selected 16 patients as the control (non-IFN group). These 16 patients, who met the eligibility criteria mentioned above, were matched by age, gender, tumor size, number of tumors, TNM stage of HCC, serum albumin level, platelet counts, ICG-R15 and Child-Pugh class with patients of the IFN group. Thus, a total of 32 patients (16 in the IFN group and 16 in the non-IFN group) were enrolled in this study. All agreed to participate in the research protocol, which was approved by the hospital research ethics board. Table 1 shows the baseline characteristics of patients of the two groups. The data indicates no significant differences between the groups for age, gender, liver function, tumor characteristics, and therapeutic methods used against primary HCC.

IFN therapy

In the IFN group, patients received 3 MIU of natural IFN- α (human lymphoblastoid IFN; Sumiferon, Dainippon Sumitomo Pharmaceuticals, Osaka, Japan) intramuscularly three times weekly for at least 48 wk as long as possible. IFN therapy commenced within 12 wk after initial treatment for HCC. Patients received post-treatment IFN therapy up to the detection of HCC recurrence, and then patients who could have curative treatment for recurrent HCC restarted IFN therapy when possible. However, patients who had advanced liver dysfunction or untreatable progressive HCC did not receive IFN therapy. In the control group, none of the patients received IFN therapy after curative treatment of HCC; instead, they

were on ursodeoxycholic acid (UDCA) and stronger neomycinophagen C (SNMC).

Follow-up

After curative treatment for primary HCC, all patients underwent liver function tests, serum tumor marker assays such as alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist (PIVKA)-II, every month, abdominal ultrasonography every 3 mo, and dynamic computed tomography (CT) every 6 mo. If recurrences of HCC were suspected, additional examinations including CT during arteriography or tumor biopsy were performed. Recurrence of HCC was defined as any new nodules appearing as hyperattenuation by CT during hepatic arteriography or as hypoattenuation in CT performed during arteriography. Hypovascular HCC was confirmed histopathologically by fine-needle aspiration biopsy. Patients with recurrent HCC were treated medically or surgically, with curative intent if possible. Patients without curative treatment of recurrent HCC then received transcatheter chemoembolization. After repeated transcatheter chemoembolization, patients were finally unable to receive any treatment for recurrent HCC.

End points

We analyzed the outcome of this prospective study in December 2006. We compared the rate of HCC recurrence and the survival rate between IFN group and control group. We assessed whether low-dose of IFN therapy was effective in inhibiting recurrence of HCC, preserving liver function and prolonging survival. In addition, we also assessed the cumulative rate of deviation from objective of any treatment against recurrent HCC due to progression of HCC and/or underlying liver dysfunction.

Statistical analysis

The Chi-square and Fisher exact tests were used for categorical variables, while Student's *t*-test and the Mann-Whitney *U* test were used for continuous and ordinal variables, as appropriate. The Kaplan-Meier method used to assess cumulative survival and recurrence rates calculated from the date of diagnosis to the date of

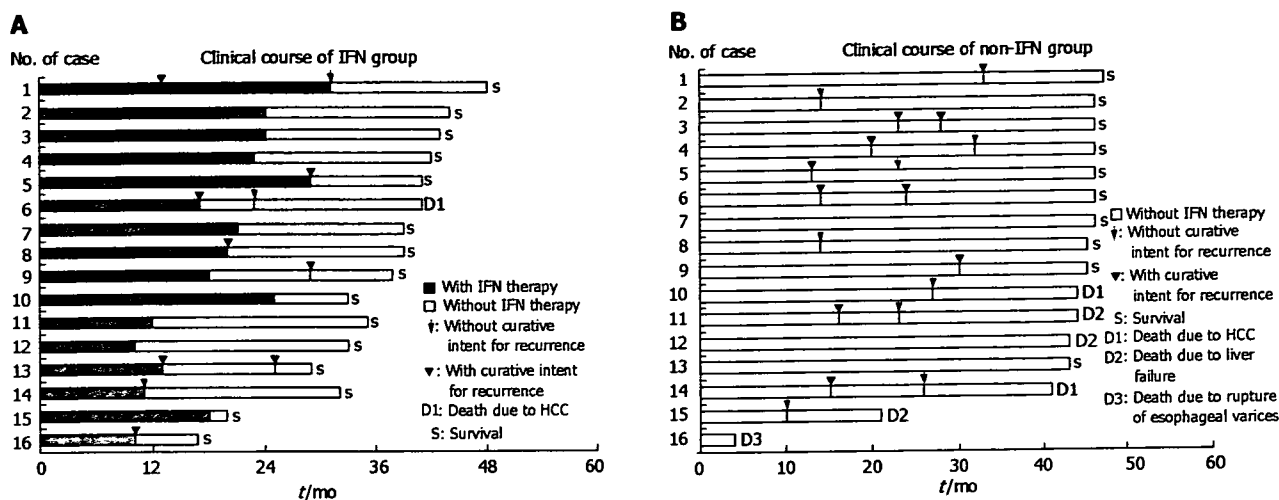


Figure 2 A: Clinical course of the interferon group. Patients who had a curative treatment for primary HCC received 3 MIU of natural interferon-alpha three times weekly for at least 48 wk as long as possible except Cases 12, 14 and 16. Recurrent HCCs were treated with or without curative treatment; B: Clinical course of the non-interferon group. Patients who had a curative treatment of primary HCC did not receive IFN therapy. Recurrent HCCs were also treated with or without curative treatment.

disease recurrence or death. Surviving patients and patients who died of causes unrelated to the liver were defined as censored cases, while patients who died of causes related to the liver were defined as noncensored cases. The log-rank test was used to compare survival and recurrence curves. *P* values below 0.05 were considered to indicate statistical significance. The JMP version 5.1 statistical software package (SAS Institute, Cary, NC) was used for analysis of data.

RESULTS

Clinical course of IFN group

Figure 2A shows the clinical course of 16 patients of the IFN group from the initial treatment of primary HCC to the date of data analysis. The duration of low-dose IFN therapy ranged from a minimum of 10 mo to a maximum of 25 mo (median 16 mo). Although 8 patients did not have HCC recurrence, HCC recurred in 8 patients after initial treatment of HCC during a median follow-up period of 37 mo. Of the recurred patients, 7 developed HCC recurrence during IFN therapy (Cases 1, 5, 6, 8, 13, 14 and 16) except 1 patient (Case 9) who had HCC recurrence after discontinuation of IFN therapy. Of the 8 patients with HCC recurrence, 4 were treated with surgical resection therapy (Cases 5, 9, 13 and 16), 3 patients with percutaneous RF ablation therapy (Cases 1, 6 and 8) and 1 patient transcatheter chemoembolization (Case 14). Of these patients, a patient with transcatheter chemoembolization (Case 14) could not have curative treatment and repeated transcatheter chemoembolization. He was excluded from the study concerning the next recurrence. Of the 7 patients with curative treatment for HCC recurrence, 2 restarted IFN therapy, one continued IFN therapy until next recurrence (Case 1), which was not curative, and the other continued until intolerant generalized fatigue (Case 8). The remaining 5 patients (Cases 5, 6, 13, 14 and 16) were followed without IFN therapy because of rejection of

IFN therapy. Although one of these 5 patients was not curative for first recurrence (Case 14), he was tolerant to repeated transcatheter chemoembolization and was still alive at the date of data analysis. Two patients without curative treatment at the second recurrence (Cases 1 and 6) were also relatively tolerant to the repeated medical treatment such as transcatheter chemoembolization. Of these patients, one died of progression of HCC in spite of repeated transcatheter chemoembolization and hepatic arterial infusion (Case 6), another was alive at the date of data analysis (Case 1). Of 3 patients without curative treatment of HCC, two survivors' status of HCC were not progressive (stage II and stage III) and underlying liver function could be tolerant to the treatment such as transcatheter chemoembolization because of relatively preserved function (Cases 1 and 14).

The 16 patients who received IFN therapy included 2 patients with virological response (Cases 2 and 3) and 14 patients who did not get SVR [3 transient responders (Cases 8, 9 and 11), and 11 non-responders (Cases 1, 4, 5, 6, 7, 10, 12, 13, 14, 15 and 16)]. Among the 14 patients who did not show SVR, 8 were biochemical responders with normalized ALT (Cases 1, 4, 5, 7, 9, 10, 13 and 16), including 4 transient responders and 4 non-responders. Two sustained virological responders who received IFN therapy for 96 wk have viral characteristics of genotype 1 and low viral load. Among the patients who did not show SVR, 7 discontinued IFN treatment because of recurrence of HCC, while 2 patients restarted IFN therapy after the curative treatment of recurrent HCC. None of the patients who received IFN therapy developed life-threatening side effects.

Clinical course of non-IFN group

Among the non-IFN group, the first recurrence of HCC occurred in 13 patients during a median follow-up period of 45 mo (Figure 2B). HCC recurred in 6 of the 7 non-IFN patients who had a sustained normalized ALT. Of the 13 patients with recurrent HCC among the non-IFN group, 4 were treated with hepatic resection (Cases 1, 4, 9 and 11),

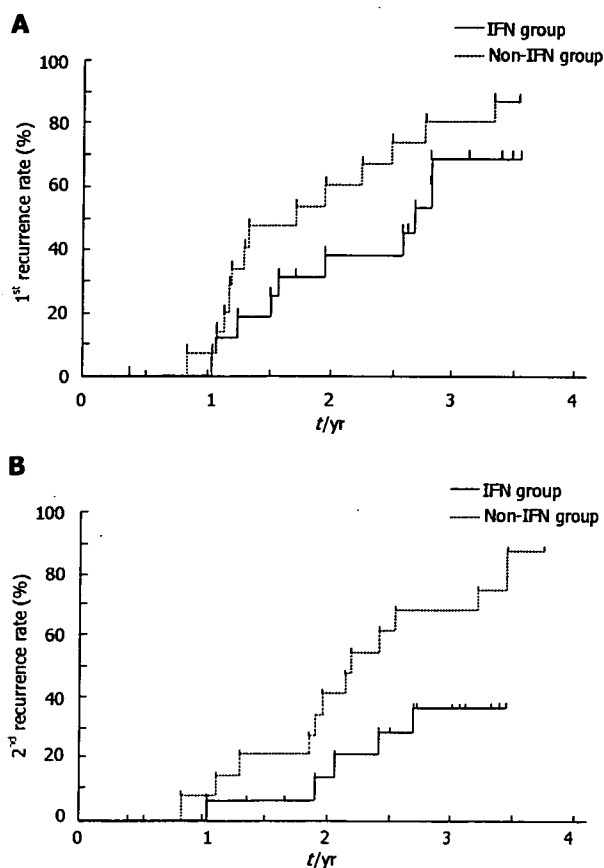


Figure 3 A: Cumulative rate of first recurrence. Rates of first recurrence for the IFN and non-IFN groups. The rate of first recurrence of HCC in the IFN group was not significantly different from that of the non-IFN group ($P = 0.157$); B: Cumulative rate of second recurrence. Rates of second recurrence for the IFN and non-IFN group. The rate of second recurrence of HCC in the IFN group was not significantly different from that of the non-IFN group ($P = 0.056$).

6 with local ablation including percutaneous RF ablation or ethanol injection (Cases 3, 5, 6, 7, 10 and 14) and 3 with transcatheter chemoembolization (Cases 2, 8 and 15). Of the 13 recurrent patients, 5 patients (2 received ethanol injection and 3 transcatheter chemoembolization) could not be treated curatively and was excluded from the study concerning the next recurrence. These 5 patients were treated repeatedly with transarterial chemoembolization after first recurrence. Among the remaining 8 patients who were treated curatively for first recurrence, 7 developed a second recurrence (Cases 3, 4, 5, 6, 9, 11 and 14). Among these 7 patients with second recurrence, 2 were treated curatively for HCC [1 with RF ablation (Case 3) and 1 with hepatic resection (Case 6)], while the remaining 5 patients were not (4 patients due to uncontrolled multiple HCC and one patient due to underlying liver dysfunction). The latter group of 5 patients received transarterial chemoembolization repeatedly after second recurrence.

Comparison of the first and second recurrence rates of HCC

We compared the overall cumulative rates for first and second recurrence between IFN and non-IFN groups (Figure 3). The 1-, 2- and 3- year rates of first recurrence

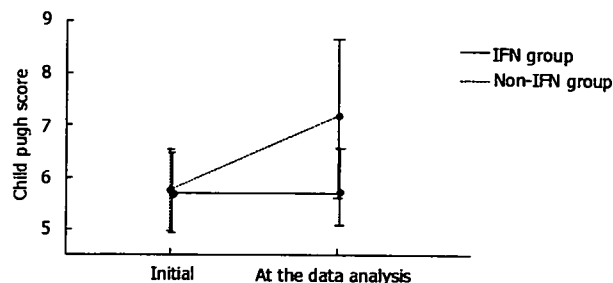


Figure 4 Effect of IFN therapy after curative treatment of HCC on Child-Pugh scores. IFN-treated patients were less likely to show deterioration of hepatic function. The average scores of Child-Pugh of the IFN group were significantly better preserved than the non-IFN group ($P = 0.0008$).

of HCC in the IFN and non-IFN group were not different (0% vs 6.7%, 38.1% vs 60% and 68.6% vs 80%, respectively, Figure 3A, $P = 0.156$). The 1-, 2- and 3-year rates of second recurrence in the IFN and non-IFN groups were 0% vs 6.7%, 13.5% vs 33.3% and 35.9% vs 67%, respectively (Figure 3B, $P = 0.056$).

Liver function

Patients of the IFN group were less likely to develop worsening of hepatic dysfunction compared with the non-IFN group. We compared the average score determined for Child-Pugh classification at initial treatment of HCC with that at the time of data analysis (Figure 4). Although the difference in the Child-Pugh classification score between the two groups at initial treatment of HCC was not significant, the score was significantly worse at the time of data analysis in the non-IFN group than IFN group ($P = 0.0008$).

Deviation from objects of any treatments for recurrent HCC

At the date of data analysis, patients who developed recurrent HCC were treated repeatedly, as possible, for the purpose of curative treatment including surgical resection and ablative therapy such as RF ablation and ethanol injection. Patients who were difficult to treat with curative intent received transcatheter chemoembolization or hepatic arterial infusion. Although patients with recurrent HCC received repeated treatments, some patients finally could not be treated because of excessive progression of HCC or liver dysfunction. Figure 5 shows that the cumulative rate of deviation from objects of any treatment for recurrent HCC between the IFN group and non-IFN group. In the IFN group, one patient could not receive treatment due to progressively advanced HCC in later period. On the other hand, 8 patients in the non-IFN group could not receive treatment because of underlying liver dysfunction ($n = 2$) and progressively advanced HCC ($n = 6$). The 1-, 2- and 3- year rates of deviation from objects of any treatment for recurrent HCC in the IFN and non-IFN group were 0% vs 6.7%, 0% vs 20% and 0% vs 27%, respectively ($P = 0.048$). Thus, the IFN group tended to be treatable for recurrent HCC compared with the non-IFN group.

Survival of patients

At the date of data analysis, 1 patient among the IFN

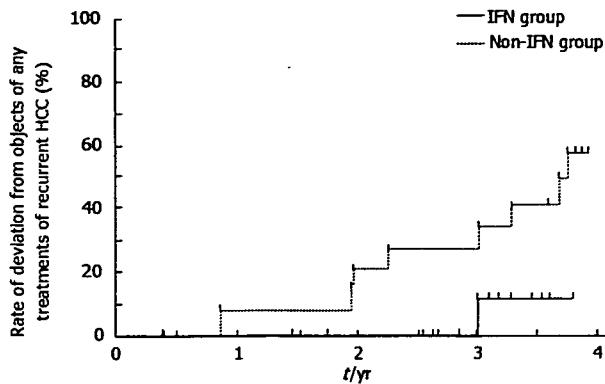


Figure 5 Cumulative rate of deviation from objects of any treatment of recurrent HCC. Recurrent HCC tended to be treatable later in the IFN group than non-IFN group ($P = 0.048$).

group and 6 patients among the non-IFN group had died of liver disease. Of the 8 recurrence patients among the IFN group, 1 died of advanced multiple HCC and none died of liver failure. On the other hand, of the 13 recurrence patients among the non-IFN group, 2 died of advanced HCC and 2 died of liver failure in spite of the relatively early stage of HCC. Among the 3 patients without recurrent HCC of the non-IFN group, 1 died of liver dysfunction and 1 died of ruptured esophageal varices.

With regard to the cumulative survival rates of the IFN and non-IFN groups (Figure 6), the respective rates of survival were 100% *vs* 93.7% at 1 year, 100% *vs* 87.5% at 2 years, 100% *vs* 87.5% at 3 years and 83.3% *vs* 61.4% at 4 years. Thus, the cumulative survival rate was not significantly different between the two groups for first 4 years after curative treatment of HCC ($P = 0.45$). The median survival time following the first treatment of HCC was 37 mo (range, 17 to 45) for the IFN group and 45 mo (range, 4 to 47) for the non-IFN group.

DISCUSSION

HCC recurrence is still a risk even if HCV-related HCC is treated with curative intent. Most of such patients with HCC have underlying liver cirrhosis, and deterioration of underlying hepatic function may be a hindrance to treatment of recurrent HCC and be associated with prognosis. The present prospective case controlled study of cirrhotic patients shows that low-dose intermittent IFN therapy after curative treatment of HCC could preserve liver function and increase the chance of treatment for recurrent tumor.

Previous studies indicated that IFN therapy after curative treatment of HCC was effective in inhibiting or delaying the development of recurrent HCC^[17-23,34,36]. Although several recent studies have reported the efficacy of chemoprevention with IFN therapy after treatment of HCV-related HCC, the basis of the benefit was not clear. Shiratori *et al.*^[23,33] and Ikeda *et al.*^[17] reported that IFN therapy in cirrhotic patients reduced recurrence of HCC and improved prognosis. Although they used standard IFN dosage per time, there are no other reports on the effect of

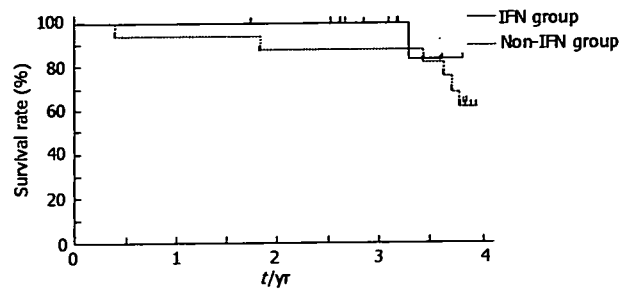


Figure 6 Cumulative survival rate. Comparison of the cumulative survival rates of the IFN and non-IFN groups. The cumulative survival rate was not significantly different between the two groups ($P = 0.45$).

low-dose IFN therapy after curative treatment of primary HCC in cirrhotic patients. Sakaguchi *et al.*^[21] reported that low-dose, long-term, intermittent IFN therapy in patients who had curative HCV-related HCC suppressed recurrence of HCC and improved survival, though it was not clear whether their patients had underlying liver cirrhosis or not. On the other hand, Mazzaferro *et al.*^[34] indicated that low-dose intermittent IFN therapy seemed to reduce late recurrence in patients with HCV-related cirrhosis after resection of HCC. Considered together, these results suggest that low-dose IFN therapy is potentially useful for cirrhotic patients when used as long as possible. However, our results of low-dose intermittent IFN therapy showed no significant difference in recurrence between those who received IFN therapy and those who did not. Unfortunately, since the difference in treatment outcome between the above three studies might be due to the use of different IFN regimens (e.g. dosage and frequency), and background characteristics of cirrhotic patients (e.g. performance status), the results varied and no standard IFN regimen to pursue after curative treatment of HCV-related HCC could be advocated.

The design of the present study was not randomized controlled type, and differed in details of the IFN protocol and characters of patients from the other studies. Although there was no significant difference in the recurrence rate between the IFN and non-IFN groups, the recurrence rate in the later period of observation including second recurrence appeared to be lower in patients with IFN therapy. Furthermore, the recurrent HCC in patients on IFN therapy did not seem to be aggressive compared with that in patients without IFN therapy, probably because they could be treated with curative intent during the observation period. Thus, low-dose intermittent IFN therapy seemed to have delayed or reduced the chance of development of recurrent HCC in the later period of observation, although IFN did not completely inhibit HCC recurrence in our cirrhotic patients.

Most cirrhotic patients cannot receive a standard full-dose IFN regimen due to underlying liver dysfunction and unfavorable complication such as cytopenia. Hence, it could be difficult to achieve SVR in most cirrhotic patients on low-dose intermittent IFN therapy. Valla *et al.*^[37] performed a randomized, controlled trial of IFN- α 2b but the results showed a lack of any benefits in terms of sustained biochemical response, liver function test

results, histology, occurrence of decompensation or HCC, or prolongation of survival. On the other hand, Everson and coworkers^[29,30] suggested that the use of low-dose IFN therapy for viral elimination was as effective in the treatment of cirrhotic patients with HCV as it is in non-cirrhotic patients. Several recent studies have reported that IFN therapy following HCC treatment improved liver function of patients with HCV-related HCC, although it is not clear which specific IFN action is important for these benefits. We also demonstrated that preservation of liver function was significantly better in the IFN group than in the non-IFN group even when HCV was not completely eradicated. Thus, hepatic functional preservation increases the chance of treatment for recurrent. Therefore, the cumulative rate of deviation from objects of any treatment for recurrent HCC might be lower in patients with IFN therapy than in patients without IFN therapy as we showed that low-dose IFN resulted in less advanced recurrence and hepatic functional preservation. Although the survival rates were not significantly different between the two groups in our observation period, we need a longer observation to determine differences in survival rates. Although we also assessed the correlation between the observed beneficial effects of the low-dose intermittent IFN therapy and HCV genotype, we could not reach the clear conclusion due to small sample size. In the future, the study with large sample size may be needed to conclude.

In our study, only about 12.5% (2/16) of patients who received IFN therapy had sustained viral elimination. And there were no significant difference in population of patients with normalized ALT between the IFN and non-IFN group ($n = 10$, $n = 7$, respectively). In spite of these results, patients treated with low-dose intermittent IFN therapy have a hepatic functional preservation greater than IFN untreated patients who received continuous medication with UDCA or SNMC after curative treatment of HCC. Although the mechanism of this reason is not well known, we suggested that the anti-inflammatory activity by low-dose intermittent IFN therapy may be stronger than medication with UDCA or SNMC and induce regression or retardation of underlying hepatic fibrosis, and finally, inhibits the progression of hepatic dysfunction.

Adverse effects such as reduction in blood counts by low-dose of IFN- α were not observed in our study, although neutropenia and/or thrombocytopenia were identified before IFN therapy. Furthermore, none of the patients required dose reduction in our study. Although 4 patients discontinued IFN therapy because of generalized fatigue, 2 of these patients restarted IFN therapy after that. Therefore, low-dose intermittent IFN- α therapy can be used relatively safely for cirrhotic patients with thrombocytopenia. However, patients who can not receive even low-doses of IFN also exist due to severe cytopenia or advanced liver cirrhosis. Medication with UDCA or SNMC or phlebotomy may be useful in decreasing ALT level for those patients.

Most cirrhotic patients who had received curative treatment for primary HCC have a limited hepatic reserve or thrombocytopenia. Therefore, low-dose intermittent IFN therapy might be effective for better prognosis. However, further studies of larger samples followed-up for

longer periods should be conducted to establish a definite conclusion about the effect of low-dose IFN therapy for the prevention of progressive liver disease and effect of treatment for recurrent HCC.

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