REFERENCES

1. **Llovet JM**: Updated treatment approach to hepatocellular carcinoma. J Gastroenterol 2005; 40(3): 225-235.
2. **Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M and Rodes J**: Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001; 35(3): 421-430.
3. **Makuuchi M and Kokudo N**: Clinical practice guidelines for hepatocellular carcinoma: the first evidence based guidelines from Japan. World J Gastroenterol 2006; 12(5): 828-829.
4. **Mazzaglia PJ, Berber E, Milas M and Siperstein AE**: Laparoscopic radiofrequency ablation of neuroendocrine liver metastases: a 10-year experience evaluating predictors of survival. Surgery 2007; 142(1): 10-19.
5. **Ribeiro MA, Jr., Rodrigues JJ, Habr-Gama A, Chaib E, D'Ipolitto G, Fonseca AZ, Saad WA, Jr. and Saad WA**: Radiofrequency ablation of primary and metastatic liver tumors--4 years experience. Hepatogastroenterology 2007; 54(76): 1170-1175.
6. **Leen E and Horgan PG**: Radiofrequency ablation of colorectal liver metastases. Surg Oncol 2007; 16(1): 47-51.
7. **White RR, Avital I, Sofocleous CT, Brown KT, Brody LA, Covey A, Getrajdman GI, Jarnagin WR, Dematteo RP, Fong Y, Blumgart LH and D'Angelica M**: Rates and patterns of recurrence for percutaneous radiofrequency ablation and open wedge resection for solitary colorectal liver metastasis. J Gastrointest Surg 2007; 11(3): 256-263.
8. **Sorensen SM, Mortensen FV and Nielsen DT**: Radiofrequency ablation of colorectal liver metastases: long-term survival. Acta Radiol 2007; 48(3): 253-258.
9. **Abitabile P, Hartl U, Lange J and Maurer CA**: Radiofrequency ablation permits an effective treatment for colorectal liver metastasis. Eur J Surg Oncol 2007; 33(1): 67-71.
10. **Chow DH, Sinn LH, Ng KK, Lam CM, Yuen J, Fan ST and Poon RT**: Radiofrequency ablation for hepatocellular carcinoma and metastatic liver tumors: a comparative study. J Surg Oncol 2006; 94(7): 565-571.
11. **Machi J, Oishi AJ, Sumida K, Sakamoto K, Furumoto NL, Oishi RH and Kylstra JW**: Long-term outcome of radiofrequency ablation for unresectable liver metastases from colorectal cancer: evaluation of prognostic factors and effectiveness in first- and second-line management. Cancer J 2006; 12(4): 318-326.
12. **Jakobs TF, Hoffmann RT, Trumm C, Reiser MF and Helmberger TK**: Radiofrequency ablation of colorectal liver metastases: mid-term results in 68 patients. Anticancer Res 2006; 26(1B): 671-680.
13. **Siperstein AE, Berber E, Ballem N and Parikh RT**: Survival after radiofrequency ablation of colorectal liver metastases: 10-year experience. Ann Surg 2007; 246(4): 559-565; discussion 565-557.
14. **Van Duijnhoven FH, Jansen MC, Junggeburt JM, van Hillegersberg R, Rijken AM, van Coevorden F, van der Sijp JR, van Gulik TM, Slooter GD, Klaase JM, Putter H and Tollenaar RA**: Factors influencing the local failure rate of radiofrequency ablation of colorectal liver metastases. Ann Surg Oncol 2006; 13(5): 651-658.
15. **Bremers AJ and Ruers TJ**: Prudent application of radiofrequency ablation in resectable colorectal liver metastasis. Eur J Surg Oncol 2007; 33(6): 752-756.
16. **Yamamoto J, Saiura A, Koga R, Seki M, Ueno M, Oya M, Azekura K, Seto Y, Ohyama S, Fukunaga S, Yamaguchi T, Kokudo N, Makuuchi M and Muto T**: Surgical treatment for metastatic malignancies. Nonanatomical resection of liver metastasis: indications and outcomes. Int J Clin Oncol 2005; 10(2): 97-102.
17. **Nouso K, Shiraga K, Uematsu S, Okamoto R, Harada R, Takayama S, Kawai W, Kimura S, Ueki T, Okano N, Nakagawa M, Mizuno M, Araki Y and Shiratori Y**: Prediction of the ablated area by the spread of microbubbles during radiofrequency ablation of hepatocellular carcinoma. Liver Int 2005; 25(5): 967-972.
18. **Lee JJ and Chu E**: An update on treatment advances for the first-line therapy of metastatic colorectal cancer. Cancer J 2007; 13(5): 276-281.
19. **Grothey A**: Is there a third-line therapy for metastatic colorectal cancer? Semin Oncol 2006; 33(6 Suppl 11): S36-38.

Alimentary Pharmacology & Therapeutics

Evolution of prognostic factors in hepatocellular carcinoma in Japan

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SUMMARY

Background
The surveillance of hepatocellular carcinoma (HCC) has become prevalent, and the modalities for its treatment have improved.

Aim
To understand the changes that occur in the characteristics and prognostic factors of HCC with time.

Methods
Newly diagnosed HCC patients were divided into two groups; patients treated before 31 December 2000 (n = 504), and after 1 January 2001 (n = 746), and their clinical backgrounds and prognostic factors were analysed.

Results
The number of patients negative for both Hepatitis B surface antigen (HBsAg) and Hepatitis C virus antibody (HCVAb) increased with time (NBNC-HCC). The size of HCC decreased in patients who were positive for HBsAg (B-HCC) or HCVAb (C-HCC), whereas no difference was observed in NBNC-HCC. The patient survival of C-HCC improved; however, no difference was detected for NBNC-HCC. In multivariate analysis, low albumin, high aspartate aminotransferase (AST), ascites, large tumour size, multiple tumour number and high alpha-fetoprotein were risk factors for survival before 2000, whereas the presence of HBsAg was additionally selected as a good prognostic factor and AST was excluded after 2001.

Conclusions
The prognostic factors as well as clinical background of HCC changed with time, and the presence of HBsAg was found to be an additional good prognostic factor after 2001.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer death in the world.[1] Globally, more than 80% of HCC cases develop in patients suffering from long-lasting viral hepatitis. Among these patients, imaging studies such as ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) are regularly performed to detect HCC at an early stage.[2-4] As a result, the proportion of HCC that can be treated by local ablation therapies or surgical resection has increased.

The effectiveness of the treatment has also increased. The mortality rates resulting from surgery have decreased,[5] and the outcomes of these patients have improved during the last few decades. Percutaneous ethanol injection therapy (PEIT), microwave coagulation therapy (MCT) and radiofrequency ablation therapy (RFA) have also been used for the treatment of small HCC, and have become more popular because they are safe and the damage they cause to the liver is minimal. Moreover, evidence-based treatment algorithms are presented by several groups and so the selection of treatment has been conducted more appropriately.[6-8]

Interferon and nucleotide analogues are drugs used to eradicate hepatitis virus infection. Recent studies have demonstrated that interferon can reduce the incidence of HCC in patients with hepatitis C virus infection and even improve the prognosis of HCC.[9, 10] Nucleotide analogues are now frequently used in patients with hepatitis B virus infection. They decrease the inflammation caused by hepatitis B virus, normalize transaminase in about 90% of the patients treated with the drugs and prolong the survival of these patients.[11] This effect was observed even in patients with HCC.[12, 13]

Although the circumstances of patients with HCC have dramatically changed as demonstrated above, few studies have been conducted to analyse the changes in the prognostic factors of HCC. In this study, we analysed the trends in HCC patients and tried to elucidate the changes that have occurred in the prognostic factors with time.

PATIENTS AND METHODS

A total of 1267 consecutive, newly diagnosed HCC patients who were admitted to Okayama University Hospital for treatment between January 1991 and February 2009 were followed up. Among these patients, 17 were excluded because they had received a liver transplant during the follow-up, so the remaining 1250 patients were enrolled in this study. The patients were divided into two groups; patients treated before 31 December 2000 (n = 504), and those treated after 1 January 2001 (n = 746), and analysed. Informed consent was obtained from all patients for use of their clinical data. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki, and was approved by the Ethical Committee of our institute.

Diagnosis

All patients were diagnosed as having HCC by using imaging modalities such as angiography, computed tomography and magnetic resonance imaging, or by tumour biopsy. The diagnostic criteria for HCC via imaging was based on previous reports of hyperattenuation at the arterial phase, hypoattenuation at the portal phase in dynamic CT or MRI, and tumour staining on angiography.[14]

Treatments and follow-up

The selection of the therapies was performed according to the evidence-based clinical practice guidelines for HCC in Japan.[8] The rate of observance of the guidelines was 74.3% and 78.0% before 2000 and after 2001 respectively. Biochemical liver function tests and US, dynamic CT or MRI were performed at least every 3 months after the initial treatment. Diagnosis of recurrence was made with the same diagnostic criteria used for the initial diagnosis. Re-treatment was performed depending on the condition of the recurrence and background liver function.

Statistical analysis

The Wilcoxon test was used to compare continuous data, and the chi-squared test was used to compare categorical data. Survival was compared using the Kaplan–Meier method, and the difference was evaluated using the log-rank test. For the analysis of prognostic factors, 15 parameters were collected: age, gender, tumour size, tumour number, alpha-fetoprotein (AFP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet count, prothrombin time (PT),

total bilirubin (T. Bil), serum albumin, hepatitis B virus surface antigen (HBsAg), hepatitis C virus antibody (HCVAb), the presence of ascites and alcohol consumption. Continuous scales and ordinal scales were categorized into two groups using the cut-off levels indicated in Tables 2 and 3. In cases before 2000, the patients who survived at the end of 2000 were no longer followed for the study from 1 January 2001 (censored at the end of 2000). The Cox proportional hazard model was used to calculate risk ratios for survival. We did not include treatment factors (e.g. nucleotide analogues, interferon and treatment modalities of HCC) because they are confounding factors in the analysis. All statistical analyses were performed using JMP software (Ver. 8.0 SAS institute, Cary, NC, USA).

RESULTS

Changes in patients' background

The clinical backgrounds of the HCC patients changed with time (Table 1). The median age at diagnosis after 2001 was greater than that before 2001 (63 vs. 67 years old, $P < 0.01$). From 2000 to 2001, the percentage of viral hepatitis decreased, and the ratio of

Table 2. The changes in patients' profiles with time in different hepatitis virus statuses

	~Dec 2000	Jan 2001~	*P*-value
Total bilirubin (mg/dL)			
B-HCC	0.90 (0.64–1.31)	0.87 (0.66–1.24)	N.S.
C-HCC	0.99 (0.75–1.37)	0.84 (0.64–1.14)	$P < 0.01$
NBNC-HCC	1.08 (0.65–1.46)	0.87 (0.61–1.23)	N.S.
Albumin (g/dL)			
B-HCC	3.69 (3.33–3.96)	3.87 (3.40–4.25)	N.S.
C-HCC	3.55 (3.22–3.90)	3.60 (3.30–3.90)	N.S.
NBNC-HCC	3.82 (3.31–4.20)	3.77 (3.42–4.10)	N.S.
Tumour size (cm)			
B-HCC	3.2 (2.1–4.9)	2.5 (1.7–3.8)	$P = 0.04$
C-HCC	2.7 (1.8–4.2)	2.1 (1.5–3.2)	$P < 0.01$
NBNC-HCC	3.2 (2.2–5.5)	3.0 (1.7–5.5)	N.S.
Tumour number (single, %)			
B-HCC	42.7	51.0	N.S.
C-HCC	54.9	56.4	N.S.
NBNC-HCC	57.6	51.6	N.S.

All numbers are medians (inter-quartile range) unless otherwise noted. B-HCC, hepatocellular carcinoma positive for hepatitis B virus surface antigen; C-HCC, hepatocellular carcinoma positive for hepatitis C virus antibody; NBNC-HCC, hepatocellular carcinoma negative for both hepatitis B virus surface antigen and hepatitis C virus antibody; N.S., not significant.

Table 1. Clinical background of 1250 patients

	~Dec 2000	Jan 2001~	
Patient number	504	746	*P*-value
Age (years)	63 (58–68)	67 (60–73)	<0.001
Gender (male)	366 (72.6%)	530 (71.1%)	0.544
HCVAb (positive)	391 (77.6%)	546 (73.2%)	<0.001*
HBsAg (positive)	93(18.5%)	108(14.5%)	
HCVAb and HBsAg negative	37(7.3%)	106(14.2%)	
Total bilirubin (mg/dL)	0.97 (0.73–1.38)	0.85 (0.64–1.17)	<0.001
Albumin (g/dL)	3.6 (3.2–3.9)	3.7 (3.3–4.0)	0.100
AST (IU/L)	63 (46–89)	54 (39–77)	<0.001
ALT (IU/L)	57(38–79)	46(31–69)	<0.001
Platelet ($\times 10^4/mm^3$)	10.1(6.8–13.8)	11.7(7.8–16.4)	<0.001
Prothrombin time (%)	82(66–97)	92(82–102)	<0.001
Ascites (present)	75(14.9%)	123(16.5%)	0.444
Alcohol (>90 g/day)	62(12.4%)	80 (10.9%)	0.438
Tumour size (mm)	28 (19–45)	22 (15–35)	<0.001
Tumour number (single)	258(53.4%)	393(55.1%)	0.561
AFP (ng/mL)	38.2 (12.4–240.9)	18.9 (6.8–86.9)	<0.001

All numbers are medians (inter-quartile range) unless otherwise noted. *P-value among three viral statuses.
HCVAb, hepatitis C virus antibody; HBsAg, hepatitis B virus surface antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein.

Table 3. Univariate analysis for the prognostic factors of HCC

	~Dec 2000 (n = 504)			Jan 2001~ (n = 746)		
	RR	95%CI	P-value	RR	95%CI	P-value
Age (>65 years)	1.25	0.97–1.61	0.08	1.11	0.84–1.49	0.44
Gender (male)	1.08	0.81–1.44	0.59	1.18	0.86–1.65	0.28
HCVAb (positive)	1.28	0.93–1.18	0.12	0.91	0.66–1.26	0.56
HBsAg (positive)	0.95	0.66–1.32	0.77	0.86	0.56–1.27	0.47
Total bilirubin (>2 mg/dL)	1.92	1.19–2.94	<0.01	2.72	1.59–4.37	<0.01
Albumin (<3.5 g/dL)	2.01	1.56–2.60	<0.01	2.65	1.95–3.60	<0.01
AST (>40 IU/L)	2.29	1.57–3.48	<0.01	1.74	1.20–2.57	<0.01
ALT (>40 IU/L)	1.17	0.88–1.57	0.25	1.09	0.80–1.51	0.56
Platelet (<10 × 10^4/mm^3)	1.29	1.00–1.66	0.04	1.12	0.82–1.52	0.44
Prothrombin time (<80%)	1.40	1.08–1.81	0.01	1.84	1.33–2.51	<0.01
Ascites (present)	1.93	1.38–2.64	<0.01	3.00	2.17–4.10	<0.01
Alcohol (>90 g/day)	0.95	0.64–1.37	0.81	0.92	0.56–1.41	0.72
Tumour size (>3 cm)	2.64	2.05–3.41	<0.01	4.00	2.99–5.37	<0.01
Tumour (multiple)	2.81	2.17–3.65	<0. 01	2.03	1.52–2.72	<0.01
AFP (>200 ng/mL)	2.20	1.67–2.87	<0.01	2.51	1.77–3.49	<0.01

RR, risk ratio; 95% CI, 95% confidence interval. Other abbreviations are the same as listed in Table 1.

hepatitis virus negative patients increased from 7.3% to 14.2% (P < 0. 01). In addition, tumour size at diagnosis became smaller, and liver functions such as bilirubin and prothrombin time were improved. Table 2 demonstrates the clinical backgrounds of the patients with different viral infection statuses. Total bilirubin of the patients who were positive for HCVAb (C-HCC) declined; however, no difference in albumin was observed in any group. The detected HCCs were smaller after 2001 in patients who were positive for HBsAg (B-HCC) or C-HCC, whereas no difference was observed in the patients without these viral markers (NBNC-HCC). The percentages of tumours over 5 cm in diameter were 23.6% and 17.8% in B-HCC (P = 0.52), 17.3% and 8.7% in C-HCC (P < 0.01) and 27.2% and 28.8% in NBNC-HCC (P = 0.86), before 2000 and after 2001 respectively.

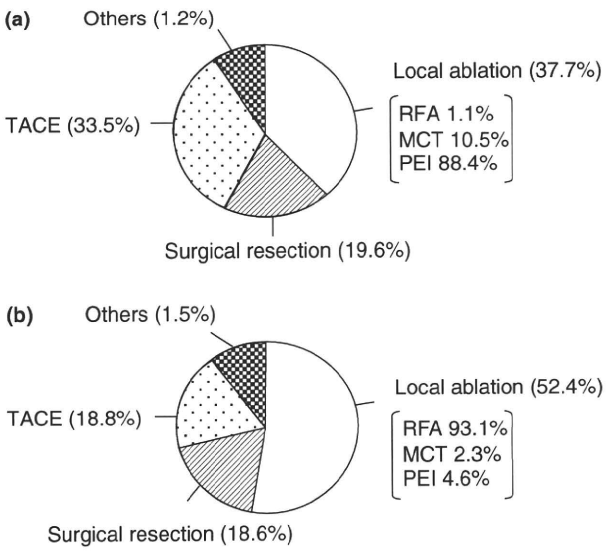

Figure 1. Changes in treatment modalities with time. The percentage of local ablation was 37.7% before December 2000 (a) and increased to 52.4% after January 2001 (b). PEI was popular before 2000; however, RFA was chosen as the standard therapy after 2001. Abbreviations: RFA, radiofrequency ablation; MCT, microwave coagulation therapy; PEI, percutaneous ethanol injection; TACE, transcatheter arterial chemoembolization.

Nucleotide analogues were used in 1.1% and 64.8% of B-HCC before 2000 and after 2001 respectively. Interferon treatment was performed in 15.5% and 19.8% of the patients who were treated before 2000 and after 2001 respectively. In all of the patients, except 22 (7 peg-interferon, 15 peg-interferon + ribavirin), treated after 2001, the treatment was carried out using conventional interferon.

Changes in treatment modalities

The treatment methods changed with time (Figure 1). The percentage of patients who received local ablation therapy increased from 37.7% (n = 190) to 52.4%

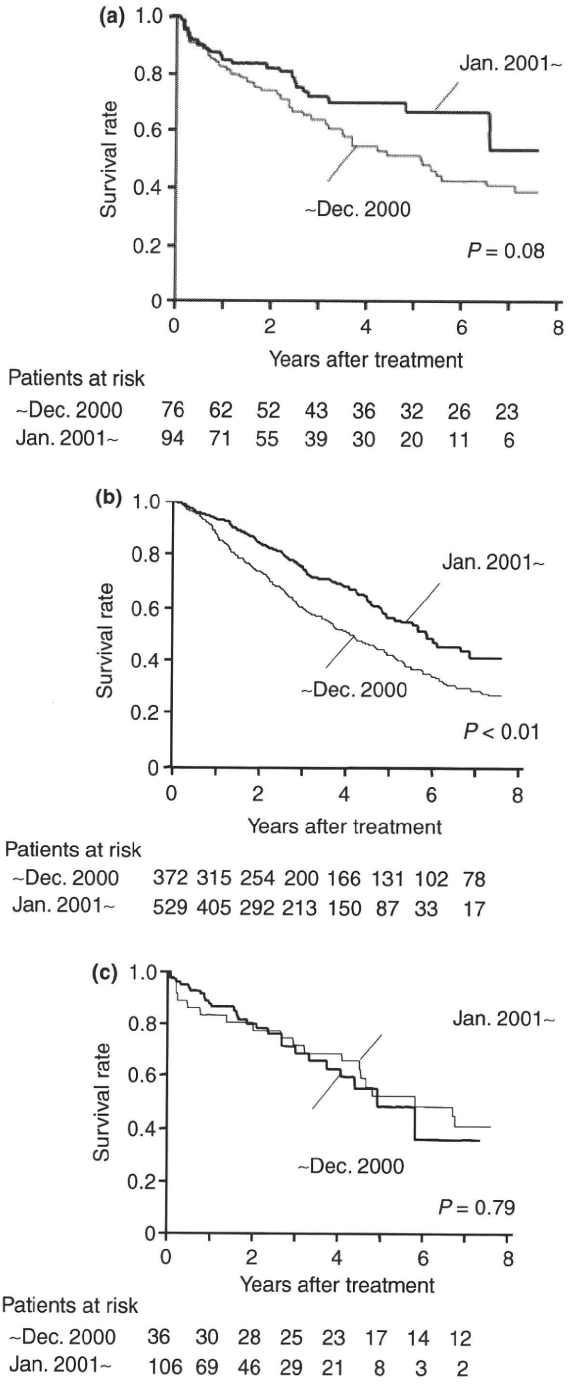

Figure 2. Survival curves of B-HCC (a), C-HCC (b) and NBNC-HCC (c). Note that the survival of C-HCC improved ($P < 0.01$) and a tendency towards improvement was observed in B-HCC ($P = 0.08$); however, no difference was observed for NBNC-HCC ($P = 0.79$). Thin line, HCC patients treated before December 2000: Thick line, HCC patients treated after January 2001.

($n = 391$). Among the patients who received local ablation therapy, PEIT was popular (168/190, 88.4%) before 2000, but RFA was chosen as the standard therapy after 2001 (364/391, 93.1%).

Changes in survival

Overall, survival of the HCC patients was prolonged after 2001. The 3- and 5-year survival rates were 63.0% and 44.2% before 2000 and 74.7% and 57.7% after 2001 respectively ($P < 0.01$). The survival of C-HCC improved ($P < 0.01$) and a tendency towards improvement was observed in B-HCC ($P = 0.08$). However, no difference was observed for NBNC-HCC ($P = 0.79$, Figure 2).

Changes of risk factors for survival

Among the 15 parameters, high T. Bil (>2 mg/dL), low albumin (<3.5 g/dL), high AST (>40 IU/mL), low platelet count ($<10 \times 10^4$), low PT (<80%), the presence of ascites, large tumour size (>3 cm), multiple tumour number and high AFP (>200 ng/mL) were the risk factors for survival before 2000 according to univariate analysis (Table 3). These risk factors were the same as the factors for survival after 2001, except that low platelet count was not selected. In multivariate analysis, low albumin, high AST, the presence of ascites, large tumour size, multiple tumour number and high AFP were the risk factors for survival before 2000, whereas positive HBsAg in addition to low albumin, the presence of ascites, large tumour size, multiple tumour number and high AFP were selected as risk factors for survival after 2001 (Table 4).

DISCUSSION

Many studies have been conducted to elucidate the factors that define the prognosis of HCC.[15–17] The factors can be classified generally into two categories. One is background liver factors such as bilirubin, and albumin, and the other is tumour factors such as the size and number of tumours. The results of this study are comparable with those of previous reports in terms of containing factors belonging to both categories; however, several new insights have emerged by examining the changes in prognostic factors with time.

When we analysed HCC altogether or limited to viral hepatitis-related HCC (B-HCC and C-HCC), we found that they were detected earlier and that the prognosis

Table 4. Multivariate analysis for the prognostic factors of HCC

	~Dec 2000 (n = 504)			Jan 2001~ (n = 746)		
	RR	95% CI	P-value	RR	95% CI	P-value
Age (>65 years old)	1.06	0.80–1.39	0.66	1.22	0.85–1.78	0.27
Gender (male)	1.05	0.78–1.44	0.71	1.36	0.94–2.02	0.10
HCVAb (positive)	1.34	0.82–2.24	0.23	0.74	0.48–1.16	0.18
HBsAg (positive)	1.15	0.68–1.90	0.58	0.39	0.21–0.71	<0.01
Total bilirubin (>2 mg/dL)	1.19	0.68–1.98	0.52	1.46	0.79–2.57	0.21
Albumin (<3.5 g/dL)	1.41	1.03–1.93	0.02	1.94	1.30–2.89	<0.01
AST (>40 IU/L)	1.86	1.13–3.12	0.01	1.59	0.96–2.65	0.06
ALT (>40 IU/L)	0.75	0.53–1.09	0.13	0.73	0.49–1.10	0.13
Platelet (<10 × 10^4/mm^3)	1.10	0.81–1.50	0.51	1.10	0.74–1.62	0.62
Prothrombin time (<80%)	1.29	0.96–1.74	0.08	1.13	0.75–1.68	0.54
Ascites (present)	1.50	1.04–2.13	0.02	1.93	1.28–2.86	<0.01
Alcohol (>90 g/day)	0.89	0.58–1.33	0.58	0.69	0.39–1.15	0.16
Tumour size (>3 cm)	2.27	1.69–3.04	<0.01	3.92	2.79–5.53	<0.01
Tumour (multiple)	2.09	1.58–2.79	<0.01	1.66	1.20–2.32	<0.01
AFP (>200 ng/mL)	1.89	1.33–2.50	<0.01	2.05	1.38–3.01	<0.01

Abbreviations are the same as listed in Table 3.

improved after 2001; however, neither early detection nor the improvement of prognosis was achieved in patients with NBNC-HCC. Hepatitis B or C infections are well-known risk factors for the occurrence of HCC; therefore, these patients were regularly surveyed for HCC.[18] Moreover, nationwide surveillance of hepatitis virus infection was started in 2002 in Japan, and many high-risk patients were identified. It is well known that screening for HCC has a survival benefit.[19, 20] Therefore, HCC was detected at an early stage after 2001 and thus the survival of such patients was prolonged. Nevertheless, surveillance has not been established for patients with NBNC-HCC because the risk factors are not well understood, except for excessive alcoholic drinking and nonalcoholic steatohepatitis.[18] As a result, the prognosis of patients with NBNC-HCC remains poor. The recent increase in metabolic syndrome may increase the likelihood of patients developing nonalcoholic steatohepatitis; therefore, careful follow-up of these patients is necessary to improve patient survival of NBNC-HCC.

In this study, hepatitis B virus infection was a good prognostic factor after 2001, according to multivariate analysis. For patients with HCC, prognosis (including risk of death, metastasis and recurrence after surgery) is reported to be worse in patients with higher serum HBV DNA levels.[21] Lamivudine treatment was started in September 2000 in Japan. In fact, 64.8% of patients with B-HCC were treated with nucleotide analogues after 2001, whereas only 1 patient (1.1%) was treated with Lamivudine before 2000. Nucleotide analogues are known to improve inflammation of the liver caused by hepatitis B virus infection and to prolong survival of patients with B-HCC.[12, 13] The use of nucleotide analogues in addition to the prevalence of surveillance of patients with hepatitis B infection may result in the selection of hepatitis B virus infection as a good prognostic factor after 2001.

Interferon (IFN) has been shown by randomized controlled trials to decrease the late recurrence after curative therapies and has also been proven to improve the survival of patients with C-HCC.[10, 22] However, hepatitis C virus infection was not a good prognostic factor before 2000 or after 2001. In contrast to the nucleotide analogues used for the therapy of hepatitis B virus, IFN has been used for the treatment of hepatitis C virus from the early 90s. The sustained virus response (SVR) rate was quite low for IFN monotherapy, especially for cases in genotype 1b with a high virus titre (2~10%), which is the dominant status of the patients in Japan.[23, 24] Even after combination therapy with peg-interferon and ribavirin for 48 weeks, the SVR rate was about 50%,[25, 26] which is lower than the response rate of lamivudine (90%). Although IFN therapy for HCV infection is similar to the nucleotide analogues used for HBV infection in

terms of being a therapy against the causative virus of HCC, the response rate of IFN therapy may be too low for HCVAb to be a good prognostic factor. In addition, the percentage of candidates for IFN treatment was much lower than that for nucleotide analogues. Many patients with C-HCC are of advanced age and cannot tolerate IFN therapy. In this study population, only 15.5% and 19.8% of C-HCC were treated with IFN before 2000 and after 2001 respectively. With the development of new drugs such as protease inhibitors, the response rate might be improved and the presence of HCVAb might be a good prognostic factor in the next decade.

Although we did not analyse the rate of recurrence or the content of repeat therapies in this study, we nevertheless clearly indicated the changes in prognostic factors of HCC with time. The prognosis of the patients with HCC improved with time. Early detection of B-HCC and C-HCC has been achieved and the presence of HBsAg was found to be a good prognostic factor after 2001. On the contrary, the number of patients with NBNC-HCC has increased with time, and the prognosis of these patients has not changed. Further examination of the risk factors of NBNC-HCC and subsequent establishment of an effective surveillance system for these patients will be necessary to improve the future prognosis of HCC patients.

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REFERENCES

1 Parkin DM. Global cancer statistics in the year 2000. *Lancet Oncol* 2001; **2**: 533–43.
2 Bolondi L. Screening for hepatocellular carcinoma in cirrhosis. *J Hepatol* 2003; **39**: 1076–84.
3 Adams PC, Arthur MJ, Boyer TD, *et al.* Screening in liver disease: report of an AASLD clinical workshop. *Hepatology* 2004; **39**: 1204–12.
4 Trevisani F, Cantarini MC, Labate AM, *et al.* Surveillance for hepatocellular carcinoma in elderly Italian patients with cirrhosis: effects on cancer staging and patient survival. *Am J Gastroenterol* 2004; **99**: 1470–6.
5 Ikai I, Arii S, Kojiro M, *et al.* Reevaluation of prognostic factors for survival after liver resection in patients with hepatocellular carcinoma in a Japanese nationwide survey. *Cancer* 2004; **101**: 796–802.
6 Bruix J, Sherman M, Llovet JM, *et al.* Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421–30.
7 Llovet JM. Updated treatment approach to hepatocellular carcinoma. *J Gastroenterol* 2005; **40**: 225–35.
8 Makuuchi M, Kokudo N. Clinical practice guidelines for hepatocellular carcinoma: the first evidence based guidelines from Japan. *World J Gastroenterol* 2006; **12**: 828–9.
9 Yoshida H, Shiratori Y, Moriyama M, *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 1999; **131**: 174–81.
10 Kudo M. Impact of interferon therapy after curative treatment of hepatocellular carcinoma. *Oncology* 2008; **75**(Suppl 1): 30–41.
11 Liaw YF. Prevention and surveillance of hepatitis B virus-related hepatocellular carcinoma. *Semin Liver Dis* 2005; **25**(Suppl 1): 40–7.
12 Matsumoto A, Tanaka E, Rokuhara A, *et al.* Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: A multicenter retrospective study of 2795 patients. *Hepatol Res* 2005; **32**: 173–84.
13 Piao CY, Fujioka S, Iwasaki Y, *et al.* Lamivudine treatment in patients with HBV-related hepatocellular carcinoma-using an untreated, matched control cohort. *Acta Med Okayama* 2005; **59**: 217–24.
14 Honda H, Ochiai K, Adachi E, *et al.* Hepatocellular carcinoma: correlation of CT, angiographic, and histopathologic findings. *Radiology* 1993; **189**: 857–62.
15 Mann CD, Neal CP, Garcea G, Manson MM, Dennison AR, Berry DP. Prognostic molecular markers in hepatocellular carcinoma: a systematic review. *Eur J Cancer* 2007; **43**: 979–92.
16 Sala M, Forner A, Varela M, Bruix J. Prognostic prediction in patients with hepatocellular carcinoma. *Semin Liver Dis* 2005; **25**: 171–80.
17 Varela M, Sala M, Llovet JM, Bruix J. Review article: natural history and prognostic prediction of patients with hepatocellular carcinoma. *Aliment Pharmacol Ther* 2003; **17**(Suppl 2): 98–102.
18 El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557–76.
19 Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004; **130**: 417–22.
20 Tanaka H, Nouso K, Kobashi H, *et al.* Surveillance of hepatocellular carcinoma in patients with hepatitis C virus infection may improve patient survival. *Liver Int* 2006; **26**: 543–51.
21 Chen CJ, Yang HI, Iloeje UH, REVEAL-HBV Study Group. Hepatitis B virus DNA levels and outcomes in chronic hepatitis B. *Hepatology* 2009; **49**: S72–84.
22 Shiratori Y, Shiina S, Teratani T, *et al.* Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. *Ann Intern Med* 2003; **138**: 299–306.
23 Shiratori Y, Kato N, Yokosuka O, *et al.* Predictors of the efficacy of interferon

therapy in chronic hepatitis C virus infection. Tokyo-Chiba Hepatitis Research Group. *Gastroenterology* 1997; 113: 558–66.

24 Poynard T, Marcellin P, Lee SS, *et al.* Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; **352**: 1426–32.

25 Manns MP, McHutchison JG, Gordon SC, *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958–65.

26 Fried MW, Shiffman ML, Reddy KR, *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975–82.

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ORIGINAL ARTICLE

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

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

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

Introduction

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

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

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

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

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

Patients and methods

Patients

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

Diagnosis

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

IFN therapy

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

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

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

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

Follow-up of the patients

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

Statistical analysis

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

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

Results

Characteristics of the patients

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

Table 1 Profiles and laboratory tests of the patients

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

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

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

* P values less than 0.05 were considered statistically significant

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

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

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

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

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

Adherence and side effects of IFN therapy

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

Cumulative survival rates of hepatocellular carcinoma

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

Recurrence of hepatocellular carcinoma

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

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

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

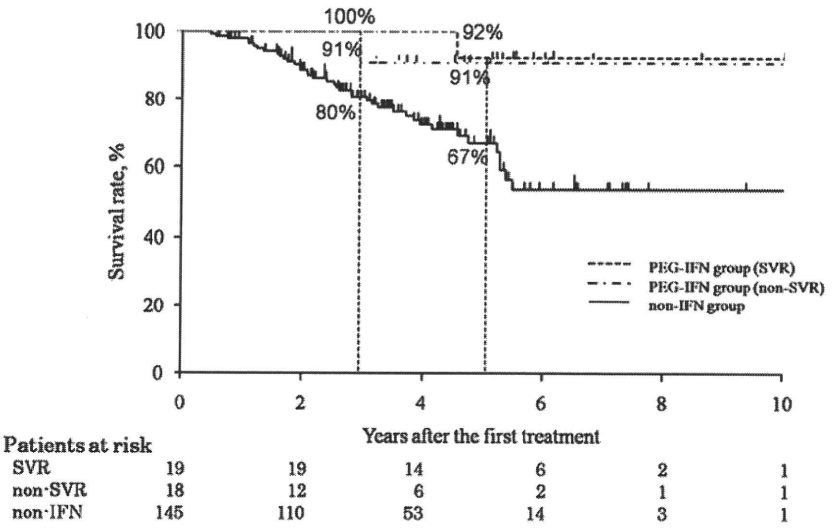

Fig. 1 Cumulative survival rates of pegylated interferon (PEG-IFN) group and non-interferon (non-IFN) group. Two patients in the PEG-IFN group died during the observation period. The survival rate was significantly different between the three groups ($P = 0.01$). *SVR* sustained virological response

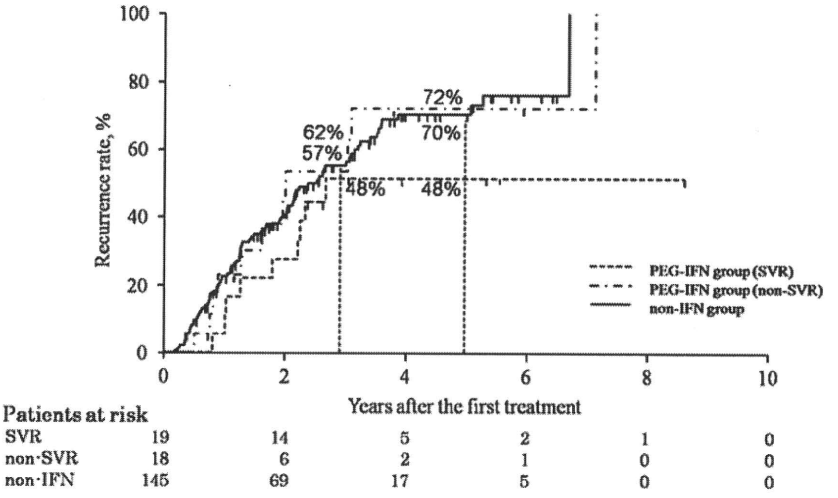

Fig. 2 The rates of first hepatocellular carcinoma (HCC) recurrence. The recurrence rate in SVR patients tended to be lower than that in non-IFN patients (48 vs. 70% at 5 years, respectively; $P = 0.05$); however, there was no significant difference between non-SVR patients and non-IFN patients (72 vs.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

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

Propensity score matched analysis

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

Prognostic factors and risk factors of HCC recurrence

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

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

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

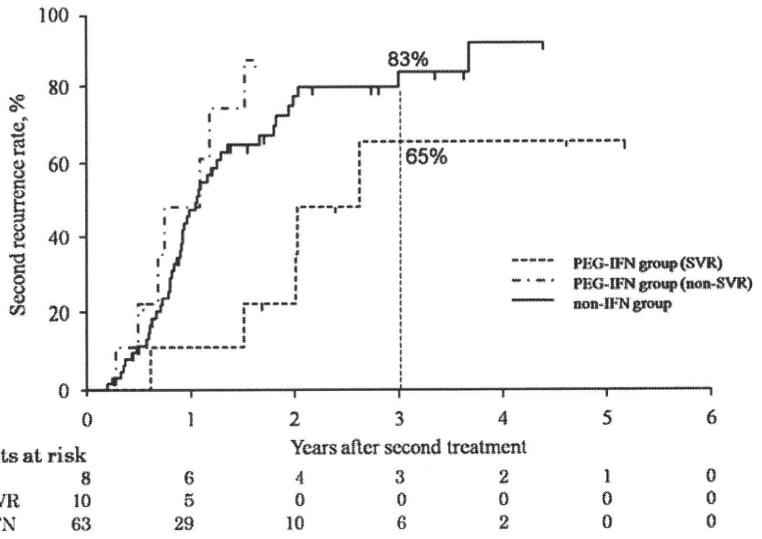

Fig. 3 Rates of second HCC recurrence. The second recurrence rate in the SVR group was significantly lower than that in the non-IFN group (65 vs. 83% at 3 years, respectively; $P = 0.03$. *SVR* sustained virological response

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

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

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

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

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

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

Discussion

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

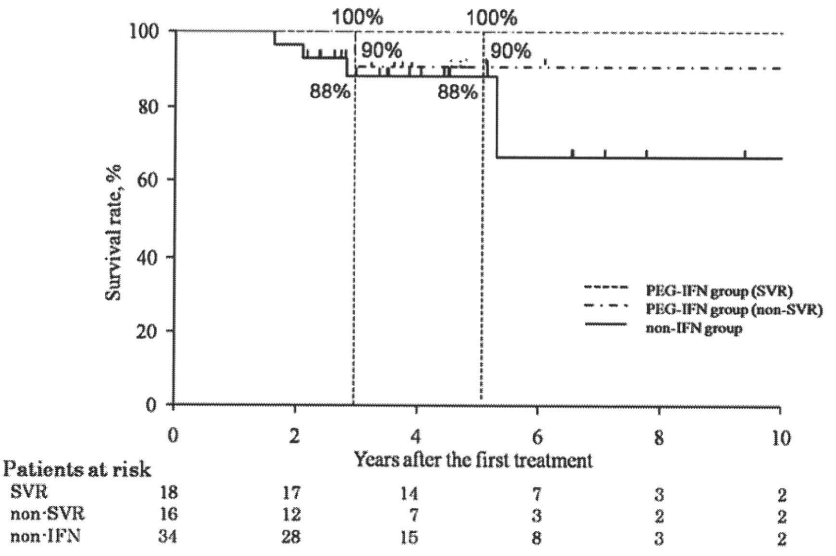

Fig. 4 Cumulative survival rates of PEG-IFN group and non-IFN group after propensity score (PS) matching. Overall-survival rate of the PEG-IFN group was higher than that of non-IFN group ($P = 0.04$). *SVR* sustained virological response

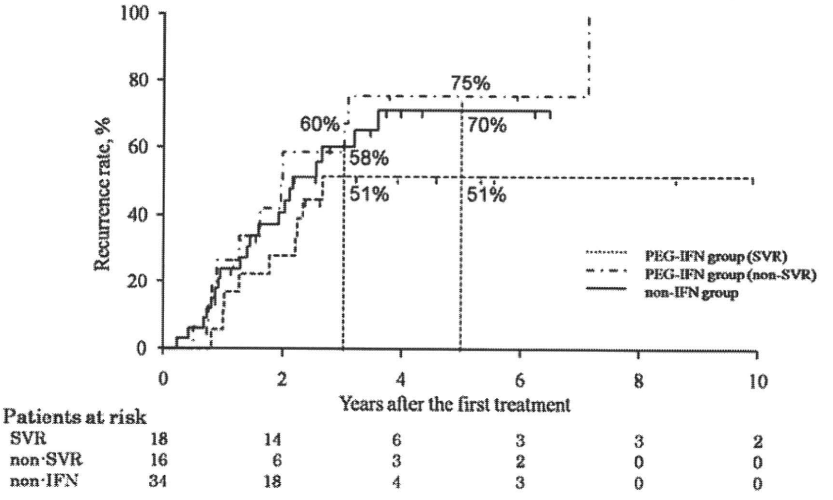

Fig. 5 Rates of first HCC recurrence after PS matching. We found no significant differences between the two groups with respect to first HCC recurrence ($P = 0.55$). *SVR* sustained virological response

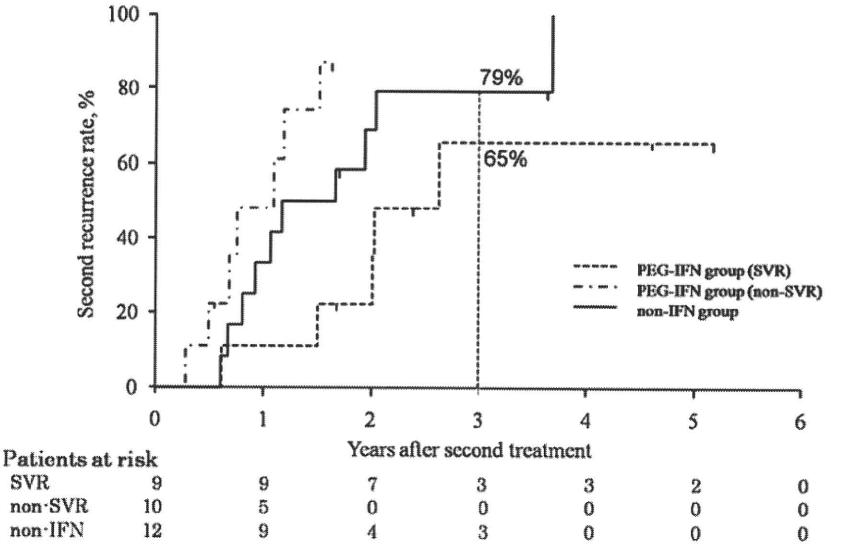

Fig. 6 Rates of second HCC recurrence after PS matching. The second recurrence rate in the SVR group was significantly lower than that in the non-IFN group (65 vs. 79% at 3 years, respectively; $P = 0.01$), although no statistical difference was observed between the IFN group and non-IFN group ($P =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

Table 4 Factors contributing to survival after HCC development

	Univariate analysis		Multivariate analysis	
	RR (95% CI)	*P* value	RR (95% CI)	*P* value
Interferon-related variables				
Application of interferon therapy	3.24 (1.52–11.0)	<0.01*	2.72 (1.29–9.04)	<0.01*
Response to interferon therapy (SVR vs. non-SVR + non-IFN)	10.5 (2.33–121)	<0.01*	–	
Variables at the first treatment of HCC				
Age (<60 years)	0.59 (0.29–1.32)	0.19		
T.Bil (<1.0 mg/dl)	2.68 (1.45–5.02)	<0.01*	1.69 (0.87–3.31)	0.11
ALB (≧3.5 g/dl)	3.45 (1.86–6.55)	<0.01*	2.51 (1.29–4.98)	<0.01*
ALT (<80 IU/l)	0.74 (0.35–1.45)	0.40		
PT (≧70%)	1.48 (0.63–3.06)	0.33		
PLT (≧10 × 10^4/mm^3)	1.63 (0.88–3.07)	0.11		
AFP (<100 ng/ml)	1.42 (0.66–2.81)	0.34		
DCP (<40 mAU/ml)	1.06 (0.56–1.99)	0.84		
Maximum tumor size (<30 mm)	1.48 (0.70–2.87)	0.28		
Number of tumors (single)	0.98 (0.45–1.94)	0.97		

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

* *P* values less than 0.05 were considered statistically significant

Table 5 Risk factors contributing to first recurrence of hepatocellular carcinoma (HCC)

	Univariate analysis		Multivariate analysis	
	RR (95% CI)	*P* value	RR (95% CI)	*P* value
Interferon-related variables				
Application of interferon therapy	1.31 (0.97–1.84)	0.07		
Response to interferon therapy (non-SVR + non-IFN vs. SVR)	1.92 (1.01–4.15)	0.04*	1.60 (0.83–3.48)	0.16
Variables at the first treatment of HCC				
Age (≧60 years)	1.29 (0.76–2.37)	0.35		
T.Bil (≧1.0 mg/dl)	1.15 (0.75–1.72)	0.50		
ALB (<3.5 g/dl)	1.55 (1.03–2.29)	0.03*	1.70 (1.11–2.56)	0.01*
ALT (≧80 IU/l)	0.97 (0.63–1.46)	0.91		
PT (<70%)	0.74 (0.41–1.27)	0.30		
PLT (<10 × 10^4/mm^3)	1.26 (0.85–1.85)	0.23		
AFP (≧100 ng/ml)	1.50 (0.91–2.36)	0.11		
DCP (≧40 mAU/ml)	1.45 (0.97–2.17)	0.06		
Maximum tumor size (≧30 mm)	1.71 (1.07–2.65)	0.02*	1.65 (1.02–2.59)	0.04*
Number of tumors (multiple)	1.60 (1.02–2.43)	0.03*	1.66 (1.05–2.56)	0.02*

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

* *P* values less than 0.05 were considered statistically significant

However, there have been few trials involving PEG-IFN therapy.

In this study, the overall survival rate of PEG-IFN-treated patients was higher than that of non-IFN patients, and the HCC recurrence rate after curative therapy for HCC in SVR patients was significantly lower than that in non-IFN patients. The survival rates are not different, although the rates of first and second recurrence of the PEG-IFN group (SVR) and PEG-IFN group (non-SVR) were different. The main reason for this discrepancy is that

Table 6 Risk factors contributing to second recurrence of HCC

	Univariate analysis		Multivariate analysis	
	RR (95% CI)	*P* value	RR (95% CI)	*P* value
Interferon-related variables				
Application of interferon therapy	1.97 (0.97–2.15)	0.06		
Response to interferon therapy (non-SVR + non-IFN vs. SVR)	2.77 (1.20–8.05)	0.01*	2.51 (1.06–7.40)	0.03*
Variables at the time of first recurrence of HCC				
Age (≧60 years)	0.81 (0.41–1.77)	0.57		
T.Bil (≧1.0 mg/dl)	1.70 (0.89–3.12)	0.10		
ALB (<3.5 g/dl)	2.81 (1.55–5.09)	<0.01*	2.65 (1.46–4.83)	<0.01*
ALT (≧80 IU/l)	1.36 (0.72–2.69)	0.34		
PT (<70%)	2.47 (0.98–5.46)	0.05		
PLT ($<10 \times 10^4/mm^3$)	0.94 (0.52–1.70)	0.86		
AFP (≧100 ng/ml)	2.13 (0.86–4.54)	0.09		
DCP (≧40 mAU/ml)	1.46 (0.78–2.76)	0.23		
Maximum tumor size (≧30 mm)	1.26 (0.64–2.31)	0.47		
Number of tumors (multiple)	1.21 (0.67–2.13)	0.51		

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

* *P* values less than 0.05 were considered statistically significant

few patients died during follow up in both groups. In addition, we observed a significant effect of PEG-IFN (SVR) in the prevention of recurrence by two different analyses (PS score matched analysis and multivariate analysis), although the effect was limited to the prevention of second recurrence, and the term of surveillance was relatively short because PEG-IFN was only available in Japan after 2004. The results were quite similar to those of reports on conventional non-PEG-IFN therapy [17].

We conducted propensity score (PS) matched analysis to adjust the clinical background of the patients in each group. PS in this analysis is a probability of choosing PEG-IFN treatment among the patients that was calculated using seven covariates. By matching the score of the patients in the PEG-IFN group and non-IFN group, we could reconstruct a situation similar to randomization.

PEG-IFN is considered to be more beneficial than non-PEG because it results in the SVR rate being higher and the IFN concentration being maintained at a high level for a longer period [40, 41], which is favorable for its action as a direct anticancer agent. However, there was no difference between conventional IFN and PEG-IFN with regard to the prevention of only late (second) recurrence. We did not compare the effect of PEG-IFN with that of non-PEG-IFN directly, but our results that non-SVR was an independent risk factor for second recurrence but not for first recurrence suggested that IFN treatment after curative treatment of HCC is more beneficial for the suppression of de novo HCC than for preventing the progression of preexisting very small HCC or intrahepatic metastasis, regardless of the type of interferon used.

In the PEG-IFN group, tumor size at HCC recurrence was smaller (13 vs. 16 mm, respectively; $P = 0.03$) and liver function tended to be better (T.Bil, ALB, PLT, PT) than in the non-IFN group. These results suggested that PEG-IFN might inhibit the growth of recurrent tumors as well as preserve liver function, although the inhibitory effect does not appear to be sufficient for complete prevention of recurrence.

PEG-IFN therapy after curative treatment of HCC was generally well tolerated in our study. Among the 37 patients, the PEG-IFN dose had to be reduced for 8 patients (21%); however, only 3 (8%) discontinued treatment with the drug because of adverse events. This rate was similar to that of the non-PEG-IFN group after HCC treatment (8–15%) [17–24]. However, PEG-IFN therapy has fewer side effects than non-PEG-IFN therapy, such as high-grade fever and general fatigue. The good adherence of patients to treatment should be noted, with a low rate of withdrawal as a consequence of adverse events [32]. The number of elderly patients with HCC will increase in the future. Because of fewer side effects and a higher rate of SVR, HCV-related HCC treatment with PEG-IFN should be considered for these elderly patients.

The weak point of this study is that it is a retrospective study and it is difficult to eliminate biases completely even with PS analysis, although no statistical difference was observed between the PEG-IFN group and non-IFN group.

In conclusion, the present study suggests that PEG-IFN therapy after curative treatment of HCC can improve the prognosis and inhibit the recurrence of HCV-related HCC. This work involved a nonrandomized study, so further prospective studies with a larger number of cases are required to reach firm conclusions.

Conflict of interest No author has any conflict of interest.

References

1. Tsukuma H, Hiyama T, Tanaka S et al (1993) Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 328:1797–1801
2. Takano S, Yokosuka O, Imazeki F et al (1995) Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. Hepatology 21:650–655
3. Shiratori Y, Shiina S, Imamura M et al (1995) Characteristic difference of hepatocellular carcinoma between hepatitis B- and C-viral infection in Japan. Hepatology 22:1027–1033
4. Ikeda K, Saitoh S, Tsubota A et al (1993) Risk factors for tumor recurrence and prognosis after curative resection of hepatocellular carcinoma. Cancer (Phila) 71:19–25
5. Tateishi R, Shiina S, Teratani T et al (2005) Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. Cancer (Phila) 103:1201–1209
6. Mazziotti A, Grazi GL, Cavallari A (1998) Surgical treatment of hepatocellular carcinoma on cirrhosis: a Western experience. Hepatogastroenterology 45(suppl 3):1281–1287
7. Livraghi T, Goldberg SN, Lazzaroni S et al (1999) Small hepatocellular carcinoma: treatment with radio-frequency ablation versus ethanol injection. Radiology 210:655–661
8. Kubo S, Nishiguchi S, Shuto T et al (1999) Effects of continuous hepatitis with persistent hepatitis C viremia on outcome after resection of hepatocellular carcinoma. Jpn J Cancer Res 90:162–170
9. Kumada T, Nakano S, Takeda I et al (1997) Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. Hepatology 25:87–92
10. Shimada M, Takenaka K, Gion T et al (1996) Prognosis of recurrent hepatocellular carcinoma: a 10-year surgical experience in Japan. Gastroenterology 111:720–726
11. Nagasue N, Uchida M, Makino Y et al (1993) Incidence and factors associated with intrahepatic recurrence following resection of hepatocellular carcinoma. Gastroenterology 105:488–494
12. Davis GL, Balart LA, Schiff ER et al (1989) Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. Hepatitis Interventional Therapy Group. N Engl J Med 321:1501–1506
13. Di Bisceglie AM, Martin P, Kassianides C et al (1989) Recombinant interferon alfa therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. N Engl J Med 321:1506–1510
14. Nishiguchi S, Kuroki T, Nakatani S et al (1995) Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 346:1051–1055
15. Yu ML, Lin SM, Chuang WL et al (2006) A sustained virological response to interferon or interferon/ribavirin reduces hepatocellular carcinoma and improves survival in chronic hepatitis C: a nationwide, multicentre study in Taiwan. Antivir Ther 11:985–994
16. Tanaka H, Tsukuma H, Kasahara A et al (2000) Effect of interferon therapy on the incidence of hepatocellular carcinoma and mortality of patients with chronic hepatitis C: a retrospective cohort study of 738 patients. Int J Cancer 87:741–749
17. Shiratori Y, Shiina S, Teratani T et al (2003) Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. Ann Intern Med 138:299–306
18. Kubo S, Nishiguchi S, Hirohashi K et al (2001) Effects of long-term postoperative interferon alpha therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. A randomized, controlled trial. Ann Intern Med 134:963–967
19. Kubo S, Nishiguchi S, Hirohashi K et al (2002) Randomized clinical trial of long-term outcome after resection of hepatitis C virus-related hepatocellular carcinoma by postoperative interferon therapy. Br J Surg 89:418–422
20. Mazzaferro V, Romito R, Schiavo M et al (2006) Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. Hepatology 44:1543–1554
21. Lin SM, Lin CJ, Hsu CW et al (2003) Prospective randomized controlled study of interferon-alpha in preventing hepatocellular carcinoma recurrence after medical ablation therapy for primary tumors. Cancer (Phila) 100:376–382
22. Lo CM, Liu CL, Chan SC et al (2007) A randomized, controlled trial of postoperative adjuvant interferon therapy after resection of hepatocellular carcinoma. Ann Surg 245:831–842
23. Ikeda K, Arase Y, Saitoh S et al (2000) Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor: a prospective randomized study of hepatitis C virus-related liver cancer. Hepatology 32:228–232
24. Sun HC, Tang ZY, Wang L et al (2006) Postoperative interferon alpha treatment postponed recurrence and improved overall survival in patients after curative resection of HBV-related hepatocellular carcinoma: a randomized clinical trial. J Cancer Res Clin Oncol 132:458–465
25. Sakaguchi Y, Kudo M, Fukunaga T et al (2005) Low-dose, long-term, intermittent interferon-alpha-2b therapy after radical treatment by radiofrequency ablation delays clinical recurrence in patients with hepatitis C virus-related hepatocellular carcinoma. Intervirology 48:64–70
26. Suou T, Mitsuda A, Koda M et al (2001) Interferon alpha inhibits intrahepatic recurrence in hepatocellular carcinoma with chronic hepatitis C: a pilot study. Hepatol Res 20:301–311
27. Hung CH, Lee CM, Wang JH et al (2005) Antiviral therapy after non-surgical tumor ablation in patients with hepatocellular carcinoma associated with hepatitis C virus. J Gastroenterol Hepatol 20:1553–1559
28. Jeong S, Aikata H, Katamura Y et al (2007) Low-dose intermittent interferon-alpha therapy for HCV-related liver cirrhosis after curative treatment of hepatocellular carcinoma. World J Gastroenterol 13:5188–5195
29. Kudo M, Sakaguchi Y, Chung H et al (2007) Long-term interferon maintenance therapy improves survival in patients with HCV-related hepatocellular carcinoma after curative radiofrequency ablation. A matched case-control study. Oncology 72(suppl 1):132–138
30. Someya T, Ikeda K, Saitoh S et al (2006) Interferon lowers tumor recurrence rate after surgical resection or ablation of hepatocellular carcinoma: a pilot study of patients with hepatitis B virus-related cirrhosis. J Gastroenterol 41:1206–1213
31. Cammà C, Di Bona D, Schepis F et al (2004) Effect of peg-interferon alfa-2a on liver histology in chronic hepatitis C: a meta-analysis of individual patient data. Hepatology 39:333–342
32. Lindsay KL, Trepo C, Heintges T et al (2001) A randomized, double-blind trial comparing pegylated interferon alfa-2b to

interferon alfa-2b as initial treatment for chronic hepatitis C. Hepatology 34:395–403
33. Kumada H, Okanoue T, Onji M et al (2010) The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, Ministry of Health, Labour, Welfare of Japan. Guidelines for the treatment of chronic hepatitis, cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. Hepatol Res 40:8–13
34. Parsons LS, Ovation Research Group (2001) Reducing bias in a propensity score matched-pair sample using greedy matching techniques. In: Proceedings of the Twenty-Sixth Annual SAS Users Group International Conference. SAS Institute Inc., Cary, pp 214–226
35. Hisaka T, Yano H, Ogasawara S et al (2004) Interferon-alpha-Con1 suppresses proliferation of liver cancer cell lines in vitro and in vivo. J Hepatol 41:782–789
36. Ogasawara S, Yano H, Momosaki S et al (2007) Growth inhibitory effects of IFN-beta on human liver cancer cells in vitro and in vivo. J Interferon Cytokine Res 27:507–516
37. Nakaji M, Yano Y, Ninomiya T et al (2004) IFN-alpha prevents the growth of pre-neoplastic lesions and inhibits the development of hepatocellular carcinoma in the rat. Carcinogenesis (Oxf) 25:389–397
38. Yano H, Ogasawara S, Momosaki S et al (2006) Growth inhibitory effects of pegylated IFN alpha-2b on human liver cancer cells in vitro and in vivo. Liver Int 26:964–975
39. Miki A, Yano Y, Kato H et al (2008) Anti-tumor effect of pegylated interferon in the rat hepatocarcinogenesis model. Int J Oncol 32:603–608
40. Chung RT, Andersen J, Volberding P et al (2004) Peginterferon Alfa-2a plus ribavirin versus interferon alfa-2a plus ribavirin for chronic hepatitis C in HIV-coinfected persons. N Engl J Med 351:451–459
41. Carrat F, Bani-Sadr F, Pol S et al (2004) Pegylated interferon alfa-2b vs standard interferon alfa-2b, plus ribavirin, for chronic hepatitis C in HIV-infected patients: a randomized controlled trial. JAMA 292:2839–2848