factors and background factors at the initial treatment of HCC.

Recently, time-dependent analyses using repetitively measured prognostic variables have been reported and their usefulness has been suggested [8-11]. Boberg *et al.* have reported that the prediction of prognosis improves when the change in a covariate over time is included in the prognostic index of primary sclerosing cholangitis [8]. Murtaugh *et al.* have developed an updated model for primary biliary cirrhosis that can be used to predict short-term survival at any time in the course of the disease [11].

HCC frequently recurs even after curative treatments such as hepatectomy or radiofrequency ablation (RFA). Stage and the liver residual function at recurrence of tumor may often vary in patients whose prognoses have been predicted to be the same by conventional methods. Therefore, re-evaluation of prognosis after initial treatment is a rational way of achieving a better prediction of survival.

The purpose of this study was to build a prognostic model of HCC using time-dependent covariates, which we can then use to re-evaluate the prognosis at any stage of the disease.

Materials and Methods

Patients. We examined 563 consecutive patients who were newly diagnosed as having HCC and received initial treatment of HCC at Okayama University Hospital from January, 1995 to June, 2007. We divided the patients into a training group (n = 336) and a testing group (n = 227). Patients for whom the last digit of their identification data (ID) number was 0-5 were assigned to the training group, regardless of condition, and those for whom the last digit was 6-9 was assigned to the testing group. We built prognostic models with the training group and validated them with the testing group. The patients who were alive at the end of June, 2007 were no longer followed in the study and were assumed considered to be "censored". The average observation period was 3.0 years. Informed consent was obtained from patients for the 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 the institute.

Diagnosis. HCC was diagnosed by abdominal ultrasonography, abdominal computerized tomography (CT), magnetic resonance imaging (MRI), abdominal angiography or tumor biopsy. The diagnostic criteria for HCC via imaging were based on previous reports of hyperattenuation at the arterial phase, hypoattenuation at the portal phase in dynamic CT or MRI, and tumor staining on angiography [12]. The patients with hepatic masses who did not satisfy the above criteria underwent ultrasound-guided fine-needle biopsy with histologically confirmed HCC. We classified HCC morphologically according to the criteria outlined by the Liver Cancer Study Group of Japan [13].

Treatments. The selection of therapies was performed in accordance with the evidence-based clinical practice guidelines for HCC in Japan [14].

For the initial treatment of HCC, 150 (27%), 129 (23%), and 73 patients (13%) received RFA, hepatectomy, and percutaneous ethanol injection therapy (PEIT), respectively (Table 1). One hundred and thirty-one people (23%) underwent transcatheter arterial chemoembolization (TACE)/transcatheter arterial infusion (TAI) without local ablation therapies. TACE/TAI was performed before PEIT and RFA in 36% (26/73) and 66% (99/150) of the patients, respectively.

Follow-up. We performed blood tests at every outpatient visit (at least once every 3 months). The examined factors were as follows: bilirubin, serum albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum creatinine, platelet count, prothrombin time and tumor markers (AFP and des-gamma-carboxy prothrombin, DCP). Ultrasound, dynamic CT, or MRI were also performed every 3-4 months. When HCC recurred, re-treatment was performed depending on patient conditions, tumor stage and background liver function, according to the same clinical indications as for the first intervention.

Time-fixed model construction. Survival duration was calculated from the date of initial treatment to the date of liver-related death. Examined covariates were as follows: nine background factors (presence of ascites, age, bilirubin, albumin, AST, ALT, creatinine, platelet count, and prothrombin time) and six tumor-related factors (main tumor size, tumor number, presence of portal vein invasion or distant metastasis, AFP, and DCP). We conducted univariate survival analysis using a Cox proportional

Table 1 Baseline characteristics of 563 patients with HCC

	Total	Training Group (n = 336)	Testing Group (n = 227)	*p*-value
Male (%)	407 (72%)	241 (72%)	166 (73%)	0.72
Etiology (B/C/B+C/other)	82/413/14/54	49/245/9/33	33/168/5/21	1.00
Child-Pugh grade (A/B/C)	395/142/26	240/82/14	155/60/12	0.68
Age at treatment (years)†	65 (23-85)	65 (23-83)	65 (28-85)	0.73
Tumor number (single)	306 (54%)	183 (54%)	123 (54%)	0.95
Main tumor size (mm)†	25 (8-160)	25 (9-160)	25 (8-160)	0.91
Tumor stage (Ⅰ/Ⅱ/Ⅲ/Ⅳ)	145/185/167/65	91/107/98/40	54/78/69/25	0.80
AFP (ng/ml)†	23 (0.5-455,560)	22 (0.5-455,560)	25 (1.4-116,870)	0.76
DCP (mAU/ml)†	23 (0-455,560)	44 (0-410,500)	56 (0-317,000)	0.46
Number of hospitalization†	1 (1-12)	2 (1-12)	1 (1-8)	0.44
Number of liver-related death	218 (38.8%)	123 (36.6%)	95 (41.9%)	0.15
Histologic differentiation				
Well differentiated	95 (17%)	55 (16%)	40 (18%)	0.97
Moderately differentiated	97 (17%)	57 (17%)	40 (18%)	
Poorly differentiated	15 (3%)	9 (3%)	6 (3%)	
Not examined	356 (63%)	215 (64%)	141 (61%)	
Treatment of HCC				
RFA	150 (27%)	94 (28%)	56 (25%)	0.80
MCT	13 (2%)	8 (2%)	5 (2%)	
PEIT	73 (13%)	49 (15%)	24 (11%)	
Liver resection	129 (23%)	72 (21%)	57 (25%)	
TACE/TAI	131 (23%)	75 (22%)	56 (25%)	
Chemotherapy	49 (8%)	28 (8%)	21 (9%)	
Liver transplantation	8 (1%)	4 (1%)	4 (2%)	
Others	10 (2%)	6 (2%)	4 (2%)	

†Data are shown as median (range). AFP, alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin; RFA, radiofrequency ablation; MCT, microwave coagulation therapy; PEIT, percutaneous ethanol injection therapy; TACE, transcatheter arterial chemoembolization; TAI, transcatheter arterial infusion.

hazards model [15] with these covariates at the initial treatment. For continuous variables or category variables with 3 values or more, we prepared multiple cut-off values for each factor and adopted the one with the highest likelihood.

Factors exhibiting significant values in univariate analysis were selected to build the model and were used for time-fixed multivariate Cox regression analysis. We built the model consisting of 8 factors where the goodness of fit of the model was optimized with the best option of the PHREG procedure of SAS 9.1.3. We assumed the value of the logarithm hazard corresponding to each factor rounded to 0.5 units as the "weighted score (WS)" and summed the score. The integer part of the total score was defined as the prognostic index (PI). When the score was more than 5, we considered it to be PI 5.

Time-dependent model construction. We adopted the method of Murtaugh *et al.* to incorporate the change in the covariate over time in the model. In this way, 721 survival data points were generated for the training group and 465 for the testing group. Missing values were estimated from the previously recorded value of the variable [16]. The same 9 background factors and 6 tumor-related factors that we adopted in the time-fixed model construction were examined.

The cut-off values of each covariate were determined and used to build a model by the same method as that for the time-fixed model construction.

Validation. To evaluate the validity of the time-fixed and time-dependent models that we built, we applied these models to the testing group. We examined conformity between observed prognosis and PI of all patients in the testing group and calculated the c-index [17]. The c-index is defined as the proportion of all usable patient pairs in which the predictions and outcome are concordant.

The c-indexes and the 95% confidence intervals of CLIP scores, JIS scores, and Okuda classification

[18] were calculated in the testing group, and the precision of the prognostic values was compared with that obtained with our models. SAS 9.1.3 and JMP 7.0.2 (SAS Institute) were used for all analyses.

Results

Patient background. The background factors of 563 patients are shown in Table 1. The average age was 64.4 years old. Four hundred and thirteen patients (73%) were positive for hepatitis C virus antibody, and 82 patients (15%) were positive for hepatitis B virus antigen. No difference in values was observed between the training and testing groups. The 1-year, 3-year, and 5-year survival rates were 88%, 67%, and 52%, respectively.

Time-fixed model. Based on the univariate analysis, the presence of ascites, bilirubin, albumin, AST, ALT, prothrombin time, main tumor size, tumor number, portal vein invasion, distant metastasis, AFP, and DCP were closely related to survival. The 8 selected factors and WS values were as follows: main tumor size (WS = 1), portal vein invasion (WS = 1.5), tumor number (WS = 1), distant metastasis (WS = 2), AFP (WS = 0.5), bilirubin (WS = 0.5), albumin (WS = 0.5), and prothrombin time (WS = 1) (Table 2). The 1-year survival rates in PI 0, 1, 2, 3, 4, and 5 of the training group were 100%, 97%, 96%, 75%, 20%, and 10%, and the 5-year survival rates were 69%, 70%, 43%, 19%, 0%, and 0%, respectively. The survival curves for each PI are shown in Fig. 1. With the exception of that between PI0 and PI1, statistically significant differences were found between each survival period.

Time-dependent model. Based on the univariate analysis, the factors related to survival were the presence of ascites, bilirubin, albumin, AST, prothrombin time, main tumor size, tumor number, portal vein invasion, distant metastasis, AFP, and DCP. From the results of the multivariable analysis, a prognosis model consisting of main tumor size (WS = 0.5), portal vein invasion (WS = 0.5), tumor number (WS = 1.5), distant metastasis (WS = 1.5), AFP (WS = 0.5), DCP (WS = 1), bilirubin (WS = 0.5), and albumin (WS = 0.5) was made (Table 3). The 1-year survival rates in PI 0, 1, 2, 3, 4, and 5 of the training group were 99%, 92%, 76%, 39%, 15%, and 0%, and the 5-year survival rates were 84%, 75%, 31%, 0%, 10%, and 0%, respectively. The survival curves for each PI are shown in Fig. 2. Between all pairs of 2 adjacent prognostic indices, statistically significant differences of survival were observed. Median survival times (MST) and 95% confidence intervals of survival duration of PI 1, 2, 3, 4, and 5 were 7.9 years (5.0–18.0 years), 2.5 years (1.6–4.9 years), 0.7 years (0.4–1.4 years), 0.4 years (0.1–0.6 year), and 0.2 years (0.1–0.4 year), respectively.

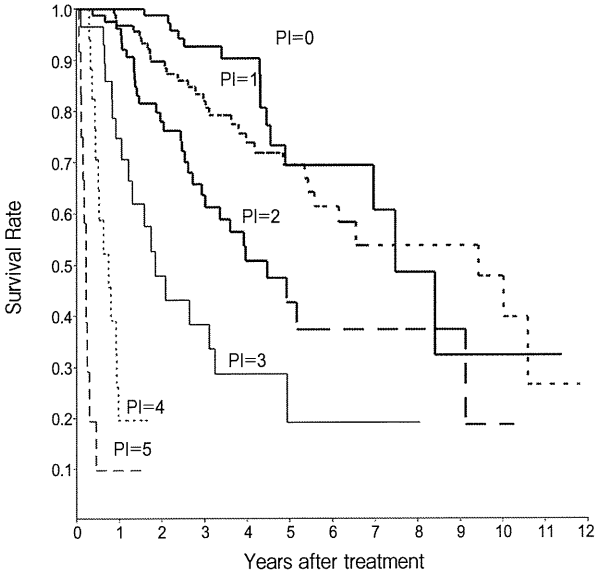
Model fitness. We applied conventional CLIP scores, JIS scores, Okuda classifications, and our prognostic models to the testing group, and compared the goodness of fit of the models in terms of the c-index. Regarding our time-fixed and time-dependent model, CLIP scores, JIS scores, the Okuda classification, and survival curves in the testing group are shown in Fig. 3(A)–(E). The c-indexes and the 95% confidence intervals of our time-fixed and time-dependent model, CLIP scores, JIS scores, and Okuda

Table 2 Time-fixed model

Factor	β	SE	RR	95%CI	*p*-value	Weighted score
Main tumor size (30mm <)	0.79	0.24	2.20	1.39–3.50	<0.001	1
Portal vein invasion (vp2<)	1.65	0.33	5.19	2.75–9.80	<0.001	1.5
Tumor number (3<)	1.24	0.23	3.45	2.18–5.46	<0.001	1
Distant metastasis (present)	1.78	0.43	5.92	2.55–13.71	<0.001	2
AFP (400ng/ml<)	0.53	0.27	1.70	1.01–2.86	0.045	0.5
Serum bilirubin (1.0mg/dl<)	0.12	0.22	1.13	0.73–1.74	0.587	0.5†
Serum albumin (<3.5g/dl)	0.61	0.22	1.84	1.19–2.83	0.006	0.5
Prothrombin time (<80%)	0.86	0.23	2.34	1.51–3.71	<0.001	1

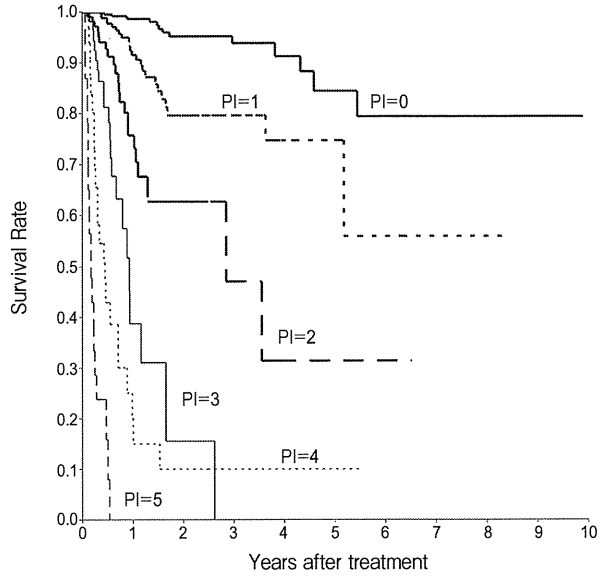
†Because the logarithm hazard of the serum bilirubin was less than 0.25, we defined 0.5, which was the minimum of the score, as the weighted score of serum bilirubin.

β, parameter of each factor; SE, standard error of β; RR, risk ratio; 95%CI, 95% confidence interval of RR.



PI	Patients at risk					
	0	2	4	6	8	10
0	92	70	30	10	3	2
1	98	73	40	20	9	6
2	81	43	18	5	4	1
3	29	10	4	1	1	0
4	18	0				
5	12	0				

Fig. 1 Survival rate in each PI of the time-fixed model for the training group. The 1-year survival rates in PI 0, 1, 2, 3, 4, and 5 of the training group were 100%, 97%, 96%, 75%, 20%, and 10%, and the 5-year survival rates were 69%, 70%, 43%, 19%, 0%, and 0%, respectively. With the exception of that between PI0 and PI1, statistically significant differences were found between each survival period.



PI	Patients at risk					
	0	2	4	6	8	10
0	270	108	34	12	1	0
1	207	42	10	2	1	0
2	113	6	1	1	0	
3	57	1	0			
4	33	2	1	0		
5	23	0				

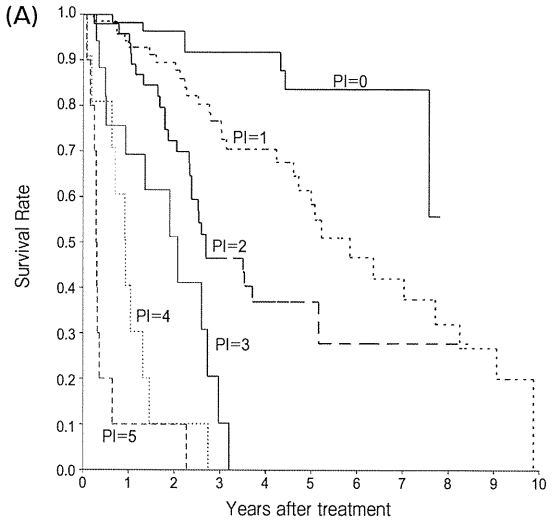
Fig. 2 Survival rates in each PI of the time-dependent model for the training group. The 1-year survival rates in PI 0, 1, 2, 3, 4, and 5 of the training group were 99%, 92%, 76%, 39%, 15%, and 0%, and the 5-year survival rates were 84%, 75%, 31%, 0%, 10%, and 0%, respectively. Between all pairs of two adjacent prognostic indices, statistically significant differences of survival were observed.

Table 3 Time-dependent model

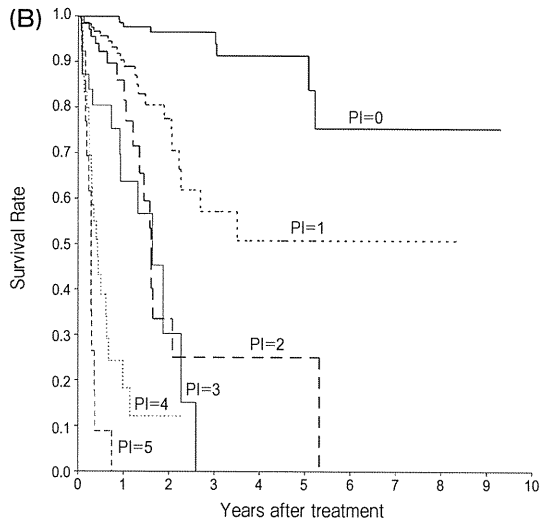
Factor	β	SE	RR	95%CI	p-value	Weighted score
Main tumor size (30mm<)	0.48	0.24	1.62	1.01-2.59	0.045	0.5
Portal vein invasion (vp1<)	0.46	0.26	1.58	0.95-2.65	0.080	0.5
Tumor number (3<)	1.61	0.23	5.00	3.21-7.78	<0.001	1.5
Distant metastasis (present)	1.39	0.28	4.02	2.35-6.89	<0.001	1.5
AFP (1,000ng/ml<)	0.63	0.23	1.88	1.20-2.95	0.006	0.5
DCP (1,000ng/ml<)	0.94	0.25	2.56	1.57-4.16	<0.001	1
Serum bilirubin (1.0mg/dl<)	0.70	0.22	2.00	1.31-3.06	0.001	0.5
Serum albumin (<3.5g/dl)	0.67	0.24	1.96	1.24-3.11	0.004	0.5

β, parameter of each factor; SE, standard error of β; RR, risk ratio; 95%CI, 95% confidence interval of RR.

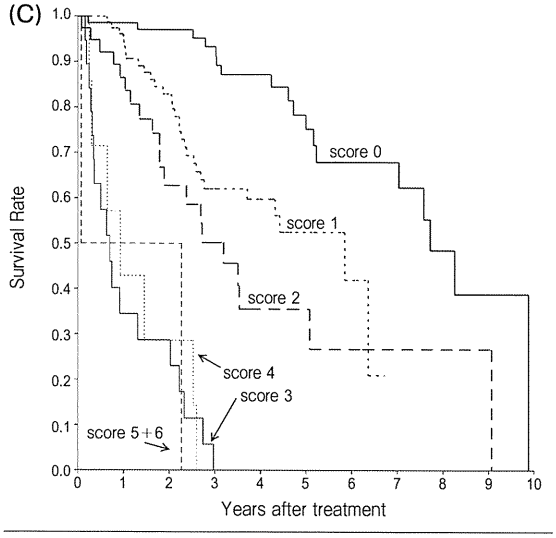
classification are shown in Table 4. The c-index of our time-dependent model was higher than that of all of the time-fixed prognostic models, indicating that the prognostic estimation of the time-dependent model was the best.



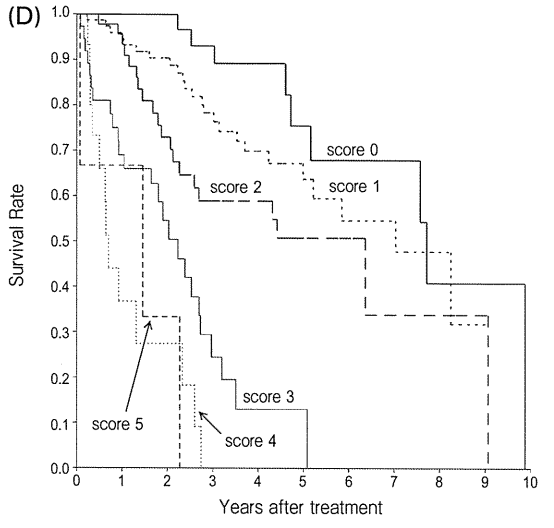
PI	Patients at risk					
	0	2	4	6	8	10
0	60	44	26	10	0	
1	72	51	27	11	6	0
2	40	29	8	2	1	0
3	19	5	0			
4	11	1	0			
5	10	1	0			



PI	Patients at risk					
	0	2	4	6	8	10
0	184	71	22	6	3	0
1	127	23	7	1	1	0
2	76	5	2	0		
3	31	2	0			
4	30	1	0			
5	13	0				

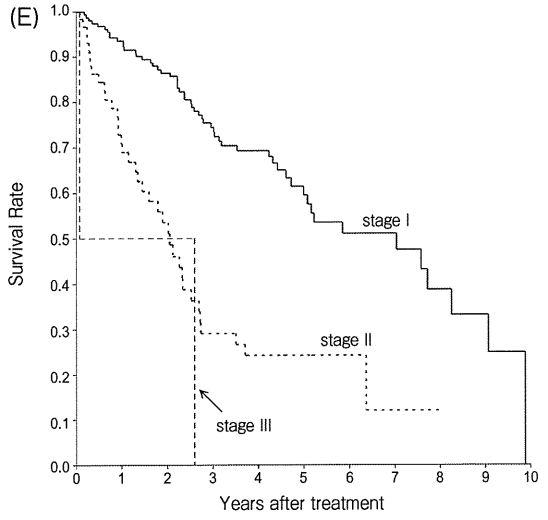


PI	Patients at risk					
	0	2	4	6	8	10
0	71	57	34	16	5	0
1	82	51	21	11	6	0
2	40	15	6	3	2	0
3	20	5	0			
4	8	2	0			
5+6	2	0				



PI	Patients at risk					
	0	2	4	6	8	10
0	38	31	16	9	2	0
1	79	57	28	10	3	0
2	49	27	17	5	2	0
3	38	13	1	0		
4	17	3	0			
5	3	1	0			

Fig. 3 Survival rate in each prognostic score of the time-fixed model and our time-dependent model for the testing group. Our time-fixed model (A), our time-dependent model (B), CLIP scores (C), JIS scores (D), and Okuda classification (E) are shown.



stage	Patients at risk					
	0	2	4	6	8	10
I	160	109	54	21	7	0
II	62	22	8	3	0	
III	2	1	0			

Fig. 3 Survival rate in each prognostic score of the time-fixed model and our time-dependent model for the testing group. Okuda classification (E) are shown.

Table 4 C-index of models

	c-index (95%CI)	
	Time-fixed	Time-dependent
Our model	0.775 (0.477–0.990)	0.870 (0.603–1.000)
CLIP	0.741 (0.432–0.975)	—
JIS	0.727 (0.418–0.966)	—
Okuda	0.609 (0.226–0.946)	—

CLIP, Cancer of the Liver Italian Program; JIS, Japan Integrated Staging Score; Okuda, Okuda staging system; 95%CI, 95% confidence interval.

Discussion

The utilization of time-dependent covariates has been shown to help in predicting the prognosis of several diseases. We used time-dependent covariates for constructing a prognostic model of HCC and demonstrated its superiority in this study. The c-indexes of our time-dependent model and the time-fixed model were higher than those of pre-existing time-fixed scores such as CLIP scores, JIS scores, and Okuda stage.

One of the characteristics of our time-dependent model is that the weight of tumor-related factors is higher than that in other models, including our time-fixed model. Among the eight factors constituting the time-dependent model, 6 are tumor-related. Of the 6.5 total points for the total weighted score, 5.5 are for tumor-related factors.

The weights of tumor-related factors in 3 conventional prognostic models were different. The tumor-related factors in the CLIP scoring system are tumor morphology, AFP and portal vein thrombus, so the weight is 4 of 6. There is only one tumor-related factor, tumor size, in the Okuda staging system out of four covariates. The JIS scoring system consists of 2 equally weighted factors, which are the TNM classification and Child-Pugh grade, meaning that half of the score is from tumor-related factors. Among the 3 conventional prognostic models, the CLIP score was superior to the 2 other models in terms of its ability to estimate prognosis. Our time-fixed model consists of 5 tumor-related factors out of 8 total factors, and the c-index is very similar to that of the CLIP score.

These results indicate that a higher weight of tumor-related factors in prognostic models may increase the ability to predict prognosis in this study population and might be one of the reasons for the striking superiority of our time-dependent model.

Liao *et al.* [10] investigated 108 patients with HCC smaller than 5 cm and built a time-dependent prognostic model with a time-dependent Cox regression model. The method used was very similar to that used in the present study and also suggested the superiority of the adoption of time-dependent factors for predicting prognosis. The prognostic model in this previous study consists of 6 factors: AFP, serum albumin, AST, serum bilirubin, alkaline phosphatase (ALP), and prothrombin time. They selected only AFP as a tumor-related factor. They considered other tumor-related factors such as tumor number and diameter only as baseline parameters and did not examine change over time. One of the possible explanations for the difference in covariates between their study and our model is the difference in the study population. They dealt with only HCC treated with PEIT, meaning that they seemed to treat HCC curatively and that the effects of tumor factors such as tumor size decreased and were excluded from the

model. In contrast, the patients in our study were not limited to those with small HCC.

The superiority of the time-dependent model for prediction of the prognosis of HCC was clearly demonstrated; however, there were some limitations to this study. There are several other factors associated with prognosis of HCC such as lens culinaris agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3). The adoption of these factors may result in the construction of a better model.

The hepatitis C virus is a maximal pathogenesis factor of HCC, and the most of patients of this study are transmitted to the virus. The contribution of mutations in the hepatitis C virus core gene has been reported to be a virus side factor associated with liver carcinogenesis [19]. Also, single nucleotide polymorphisms (SNPs) of IL28B have been reported as a host factor involved in the treatment of chronic hepatitis C [20]. The adoption of genetic information from the virus and the host may result in the construction of a more correct model in the future.

Because this was a study based at a single institution, it has not been shown that we can extrapolate our results to other institutions or countries. The skill in RFA and selective TACE differ, and patient backgrounds also differ. The index might be too precise to apply to all cases. But there is also merit in the study being limited to a single institution. Because blood tests and imaging studies were performed according to the same surveillance algorithm, the lead-time bias that influenced the model was minimized. As for all the prognostic factors, minor modifications depending on individual clinical circumstances are advisable for obtaining better predictive ability.

The original point in this study is that we used time-dependent covariates in a model based on the premise that prognosis changes drastically with the state at recurrence. We can predict prognosis even if HCC recurs and tumor stage and liver residual function change using this time-dependent model. The potential to estimate the prognosis of HCC is better than that of the traditional time-fixed models. More studies to verify its effectiveness for different populations are needed to confirm that the index can be widely used.

References

1. Zhu AX: Development of sorafenib and other molecularly targeted agents in hepatocellular carcinoma. Cancer (2008) 112: 250-259.
2. Umemura T, Ichijo T, Yoshizawa K, Tanaka E and Kiyosawa K: Epidemiology of hepatocellular carcinoma in Japan. J Gastroenterol (2009) 44 Suppl 19: 102-107.
3. Tateishi R, Yoshida H, Shiina S, Imamura H, Hasegawa K, Teratani T, Obi S, Sato S, Koike Y, Fujishima T, Makuuchi M and Omata M: Proposal of a new prognostic model for hepatocellular carcinoma: an analysis of 403 patients. Gut (2005) 54: 419-425.
4. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC and Williams R: Transection of the oesophagus for bleeding oesophageal varices. Br J Surg (1973) 60: 646-649.
5. Cancer of the Liver Italian Program (CLIP) investigators: A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology (1998) 28: 751-755.
6. Kudo M, Chung H and Osaki Y: Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging Score (JIS score). J Gastroenterol (2003) 38: 207-215.
7. Bruix J and Llovet JM: Prognostic prediction and treatment strategy in hepatocellular carcinoma. Hepatology (2002) 35: 519-524.
8. Boberg KM, Rocca G, Egeland T, Bergquist A, Broome U, Caballeria L, Chapman R, Hultcrantz R, Mitchell S, Pares A, Rosina F and Schrumpf E: Time-dependent Cox regression model is superior in prediction of prognosis in primary sclerosing cholangitis. Hepatology (2002) 35: 652-657.
9. Christensen E, Altman DG, Neuberger J, De Stavola BL, Tygstrup N and Williams R: Updating prognosis in primary biliary cirrhosis using a time-dependent Cox regression model. PBC1 and PBC2 trial groups. Gastroenterology (1993) 105: 1865-1876.
10. Liao CS, Yang KC, Yen MF and Hsiu-Hsi Chen T: Time-varying predictors for clinical surveillance of small hepatocellular carcinoma. Cancer J (2005) 11: 226-233.
11. Murtaugh PA, Dickson ER, Van Dam GM, Malinchoc M, Grambsch PM, Langworthy AL and Gips CH: Primary biliary cirrhosis: prediction of short-term survival based on repeated patient visits. Hepatology (1994) 20: 126-134.
12. Honda H, Ochiai K, Adachi E, Yasumori K, Hayashi T, Kawashima A, Fukuya T, Gibo M, Matsumata T, Tsuneyoshi M and *et al.*: Hepatocellular carcinoma: correlation of CT, angiographic, and histopathologic findings. Radiology (1993) 189: 857-862.
13. The Liver Cancer study Group of Japan: General rules for the clinical and pathological study of primary liver cancer 2nd English Ed. Tokyo (2003).
14. Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, Matsuyama Y, Okazaki M, Okita K, Omata M, Saida Y, Takayama T and Yamaoka Y: Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res (2008) 38: 37-51.
15. Cox DR: Regression models and life-tables. J R Stat Soc [B] (1972) 34: 187-202.
16. Siddiqui O and Ali MW: A comparison of the random-effects pattern mixture model with last-observation-carried-forward (LOCF) analysis in longitudinal clinical trials with dropouts. J Biopharm Stat (1998) 8: 545-563.
17. Harrell FE, Jr, Califf RM, Pryor DB, Lee KL and Rosati RA:

Evaluating the yield of medical tests. JAMA (1982) 247: 2543-2546.
18. Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, Nakajima Y and Ohnishi K: Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer (1985) 56: 918-928.
19. Fishman SL, Factor SH, Balestrieri C, Fan X, Dibisceglie AM, Desai SM, Benson G and Branch AD: Mutations in the hepatitis C virus core gene are associated with advanced liver disease and hepatocellular carcinoma. Clin Cancer Res (2009) 15: 3205-3213.
20. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB and Carrington M: Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature (2009) 461: 798-801.

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

ORIGINAL ARTICLE

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

Hiroaki Hagihara · Kazuhiro Nouso · Yoshiyuki Kobayashi · Yoshiaki Iwasaki · Shinichiro Nakamura · Kenji Kuwaki · Junichi Toshimori · Hirokazu Miyatake · Hideki Ohnishi · Hidenori Shiraha · Kazuhide Yamamoto

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

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

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

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

H. Hagihara (✉) · K. Nouso · Y. Kobayashi · Y. Iwasaki · S. Nakamura · K. Kuwaki · J. Toshimori · H. Miyatake · H. Ohnishi · H. Shiraha · K. Yamamoto
Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Kita-ku, Okayama 700-8558, Japan
e-mail: h2mof1977@gmail.com

K. Nouso
Department of Molecular Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

H. Miyatake
Department of Gastroenterology and Hepatology, Mitoyo General Hospital, Kagawa, Japan

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 (×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.

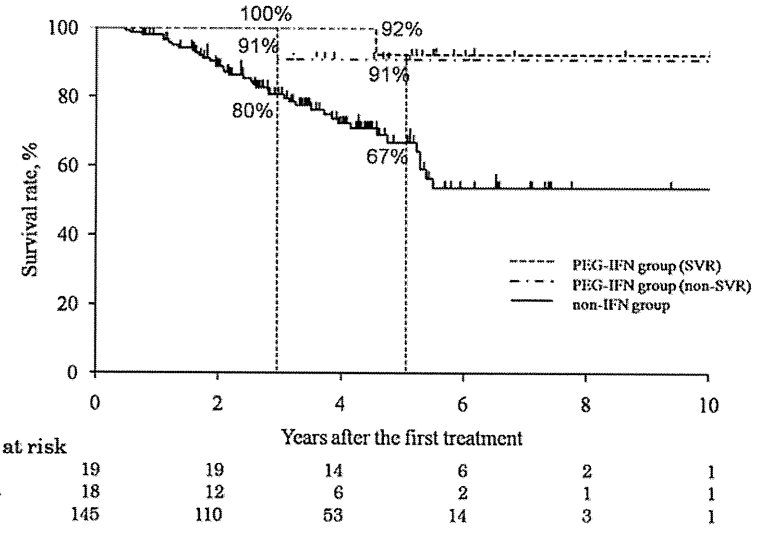


Fig. 1 Cumulative survival rates of pegylated interferon (PEG-IFN) group and non-interferon (non-IFN) group. Two patients in the PEG-IFN group died during the observation period. The survival rate was significantly different between the three groups ($P = 0.01$). *SVR* sustained virological response

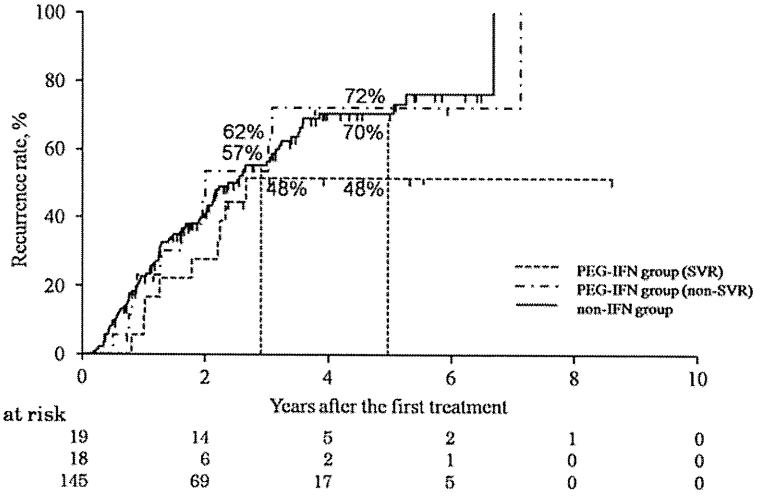


Fig. 2 The rates of first hepatocellular carcinoma (HCC) recurrence. The recurrence rate in SVR patients tended to be lower than that in non-IFN patients (48 vs. 70% at 5 years, respectively; $P = 0.05$); however, there was no significant difference between non-SVR patients and non-IFN patients (72 vs.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

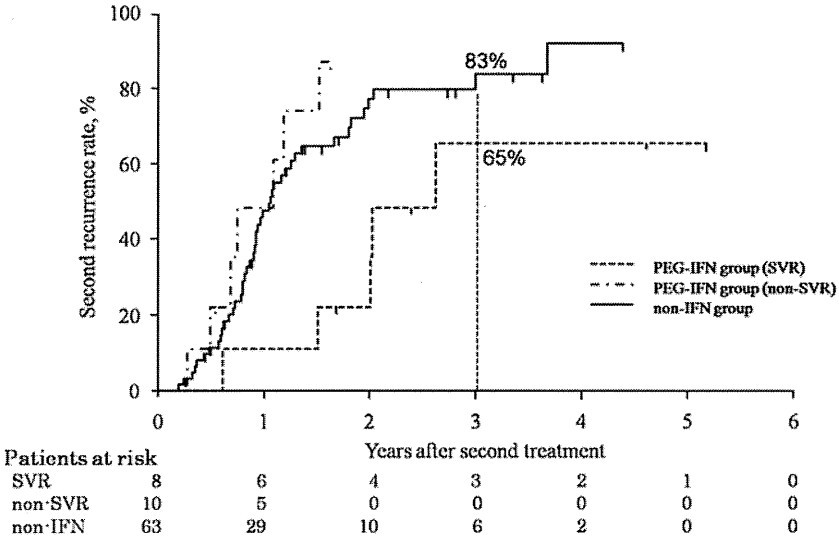


Fig. 3 Rates of second HCC recurrence. The second recurrence rate in the SVR group was significantly lower than that in the non-IFN group (65 vs. 83% at 3 years, respectively; $P = 0.03$. *SVR* sustained virological response

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.

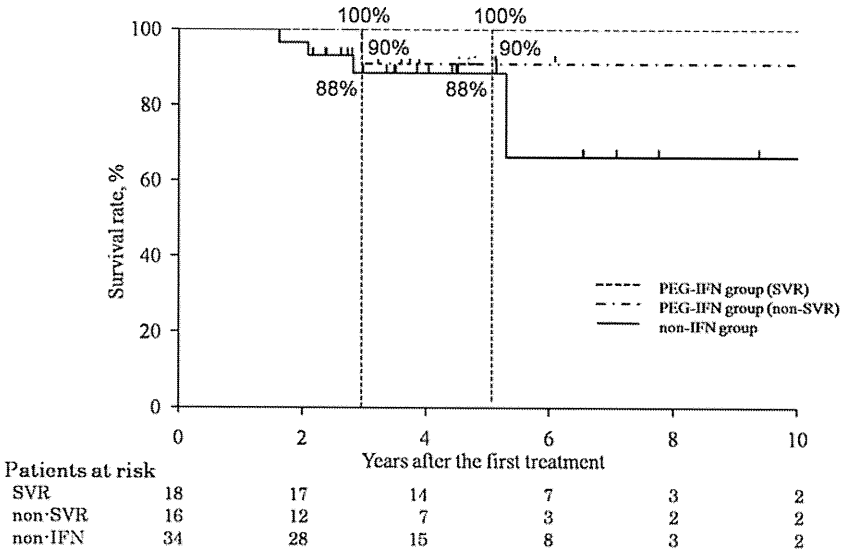


Fig. 4 Cumulative survival rates of PEG-IFN group and non-IFN group after propensity score (PS) matching. Overall-survival rate of the PEG-IFN group was higher than that of non-IFN group ($P = 0.04$). *SVR* sustained virological response

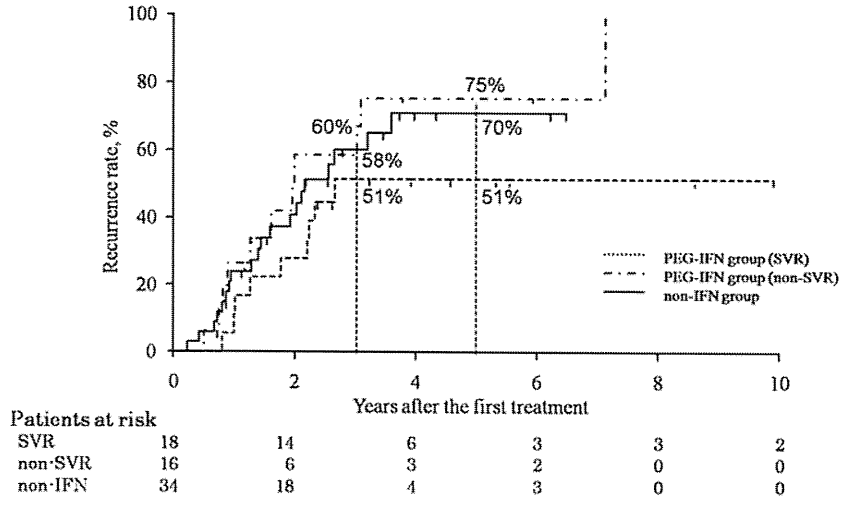


Fig. 5 Rates of first HCC recurrence after PS matching. We found no significant differences between the two groups with respect to first HCC recurrence ($P = 0.55$). *SVR* sustained virological response

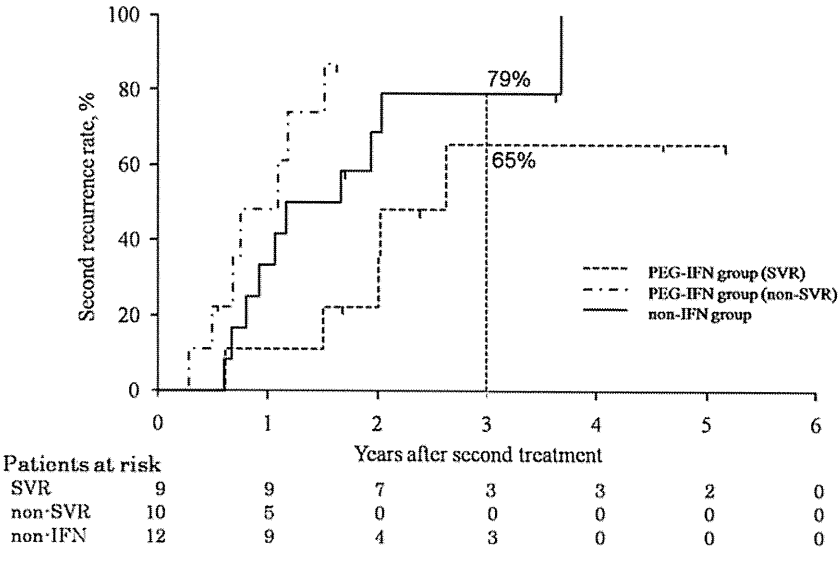


Fig. 6 Rates of second HCC recurrence after PS matching. The second recurrence rate in the SVR group was significantly lower than that in the non-IFN group (65 vs. 79% at 3 years, respectively; $P = 0.01$), although no statistical difference was observed between the IFN group and non-IFN group ($P =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

がん化学療法の進歩

B. 各論 臓器別がん治療

(12) 肝がんに対する化学療法

池田 健次*

肝がんは小型で少数であれば，外科的・内科的な根治的治療が行われるため，化学療法は行われない。腫瘍が大型・多発・脈管侵襲など，根治的除去が不可能な場合に化学療法が考慮される。背景肝の肝硬変による肝機能不良が強い場合には十分な抗がん剤の使用は困難である。以上のように，肝がんに対する化学療法は肝がん条件・背景肝条件の両者を勘案して行うことが必要で，門脈血流が十分に存在する場合には動注化学療法や肝動脈化学塞栓療法として経動脈的に行われ，門脈血流が不十分な場合には持続動注化学療法として経動脈的に，もしくは抗がん剤・分子標的薬内服として使用されることが多い。肝外転移に対しては静注・内服で抗がん剤が使用されることがある。

Key Words：ソラフェニブ：Sorafenib，ミリプラチン：Miriplatin，
分子標的薬：Molecular Targeting Drug，
動注化学療法：Intra-arterial chemotherapy

I はじめに

肝がん治療に関する先進国であるわが国では，C型肝炎の新規感染の激減を受けて，肝がん死亡数は横ばいから減少傾向を示し始めている。しかし，実際の臨床の場では，いまだ肝がん治療のニーズは衰えず，肝がん治療・肝がん予防の努力が続けられている。

各種肝がんの治療法について，肝細胞がんの治療アルゴリズムとして総論を述べたのち，外科切除，局所治療，経動脈的治療，抗がん剤治療の順にエビデンスとしての観点から最新の知見を示す。

II 肝細胞がんの治療法選択の基準

肝細胞がんの病態に応じた治療法の選択基準として，幕内班の『肝細胞癌治療アルゴリズム』(図)が推奨されている[1]。この肝がん治療に関するアルゴリズムは肝障害度・腫瘍数・腫瘍径の3因子をもとに設定されてお

Chemotherapy for hepatocellular carcinoma

*虎の門病院肝臓内科・部長 Kenji Ikeda