Dig Dis Sci
DOI 10.1007/s10620-011-1934-1

ORIGINAL ARTICLE

Effect of Previous Interferon Treatment on Outcome After Curative Treatment for Hepatitis C Virus-Related Hepatocellular Carcinoma

Hirokazu Miyatake · Yoshiyuki Kobayashi · Yoshiaki Iwasaki · Shin-ichiro Nakamura · Hideki Ohnishi · Kenji Kuwaki · Junichi Toshimori · Hiroaki Hagihara · Kazuhiro Nouso · Kazuhide Yamamoto

Received: 26 July 2011 / Accepted: 21 September 2011

Abstract
Background and Aims Treatment of chronic hepatitis C virus (HCV) infection with interferon (IFN) prevents the development of hepatocellular carcinoma (HCC). The purpose of this study was to clarify the effect of previous IFN treatment before the development of HCC on recurrence and survival in HCV-related HCC patients.
Methods Three hundred ninety-five patients who underwent curative treatment for HCV-related HCC were enrolled. Of these, 124 had received IFN treatment before the development of HCC (17 achieved sustained virological response [SVR group] and 107 did not [non-SVR group]), whereas 271 patients had never received IFN treatment (IFN-untreated group). The first and second recurrence and survival rates in these patient groups were statistically analyzed.
Results The first HCC recurrence rate was similar among patient groups. In contrast, the second HCC recurrence rate was significantly lower in the SVR group than in the non-SVR group ($p = 0.003$) and the IFN-untreated group ($p = 0.006$). In multivariate analysis, platelet count ($p = 0.033$) and number of tumors ($p = 0.001$) were associated with the first HCC recurrence, while SVR ($p = 0.002$) was the only factor associated with the second HCC recurrence. The survival rate was higher in the SVR group than in non-SVR and IFN-untreated groups, and SVR to previous IFN treatment was an independent factor associated with better survival ($p < 0.001$).
Conclusions SVR to previous IFN treatment before the development of HCV-related HCC was associated with lower risk of the second recurrence of HCC and better survival.

Keywords Hepatocellular carcinoma · Hepatitis C virus · Previous interferon therapy · Recurrence · Survival

Introduction

Chronic hepatitis and cirrhosis following hepatitis C virus (HCV) infection are major risk factors for hepatocellular carcinoma (HCC) [1–3]. Particular risk factors for developing HCV-related HCC in patients are advanced stage fibrosis, male gender, older age, heavy drinking, and high serum alanine aminotransferase (ALT) levels [4, 5]. Interferon (IFN) therapy improves hepatic inflammation and inhibits the progression of hepatic fibrosis [6]. Furthermore, treating patients with IFN with chronic HCV infection can prevent the development of HCC, particularly in patients with sustained virological response (SVR) to IFN therapy [7–13]. In contrast, HCC is liable to frequently recur even after curative therapy primarily because of its multicentric occurrence, leading to a poor prognosis [14–19]. The recurrence rate after resection of HCV-related HCC is higher in patients with HCV viremia than in those without it [20]. It has been reported that IFN therapy after resection or ablation of HCC reduces recurrence and improves prognosis in patients with HCV-related HCC [21–28]. However, no complete investigation has been performed of the possible effect of IFN therapy before HCC development on the outcome of curative treatment for

H. Miyatake · Y. Kobayashi · Y. Iwasaki (✉) · S. Nakamura · H. Ohnishi · K. Kuwaki · J. Toshimori · H. Hagihara · K. Nouso · 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: yiwasaki@cc.okayama-u.ac.jp

Published online: 12 October 2011

HCV-related HCC particularly in relation to the response to IFN treatment. Only a few relevant studies involving limited number of patients with previous IFN therapy are available [29–32].

The purpose of this study was to clarify the effect of previous IFN treatment before the development of HCV-related HCC on recurrence and prognosis after curative treatment of HCC in a large cohort of patients.

Patients and Methods

Patients

Between 1995 and 2006, 733 consecutive patients with HCC positive for HCV antibody and HCV RNA were diagnosed at Okayama University Hospital. Three hundred thirty-eight patients who did not receive curative treatment for HCC or undergo IFN therapy after the development of HCC were excluded from the study (Fig. 1). Inclusion criteria were as follows: (1) no evidence of HCC before consulting the Okayama University Hospital, (2) absence of hepatitis B surface antigen, (3) absence of co-existing liver diseases such as autoimmune hepatitis or primary biliary cirrhosis, and (4) absence of a history of alcohol abuse.

HCV infection was diagnosed on the basis of identification of anti-HCV antibodies using the first, second, or third enzyme-linked immunosorbent assays (Ortho Diagnostics, Tokyo, Japan). HCV RNA was identified by reverse transcription-polymerase chain reaction (RT-PCR) [33].

HCC was suspected on the basis of several imaging methods, including abdominal ultrasonography (US), dynamic computed tomography (CT), magnetic resonance imaging, and angiography. Diagnosis of HCC was confirmed by needle biopsy, by surgically resected tumor specimens, or by typical radiological findings on hepatic angiography or dynamic CT.

The study was conducted in accordance with the Helsinki Declaration and approved by the Ethical Committee of the institution.

Treatment

Of the 395 patients receiving curative treatment of HCC, 103 were treated with surgical resection and 292 with percutaneous tumor ablation (PTA) [34–37], that is, percutaneous ethanol injection therapy (PEIT) ($n = 116$), percutaneous microwave coagulation therapy (PMCT) ($n = 11$), or radiofrequency ablation (RFA) ($n = 165$). There were no patients who underwent liver transplantation or other modes of HCC treatment. The choice between surgical resection and PTA were determined according to the extent of tumor and hepatic functional reserve as assessed by Child's classification [38]. If the liver tumor consisted of fewer than three nodules that were less than 3 cm in diameter, patients were indicated

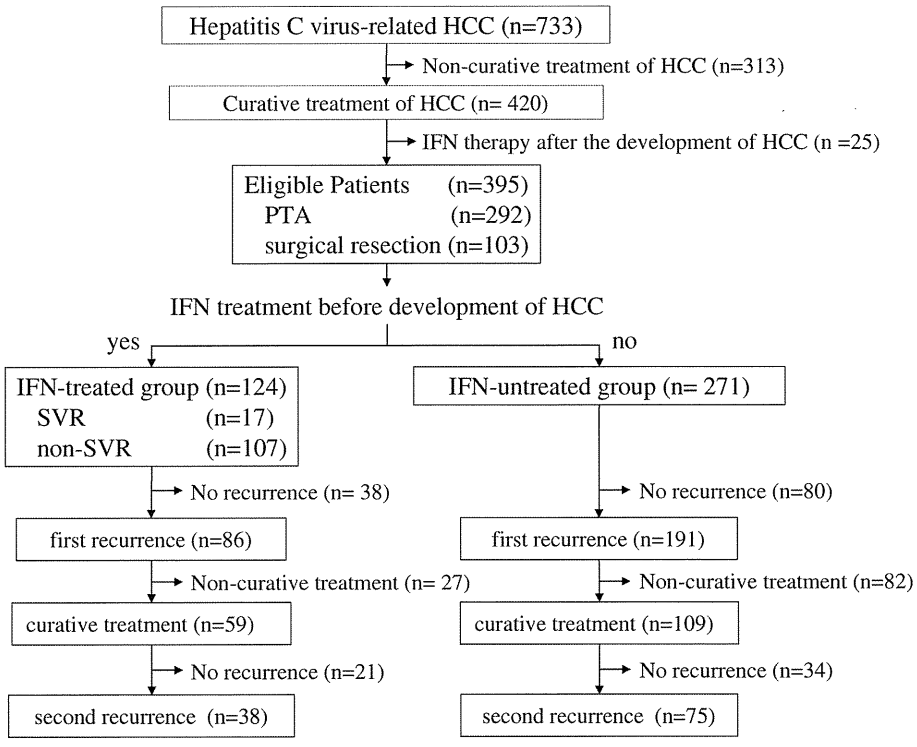

Fig. 1 Schematic presentation of patients with HCV-related hepatocellular carcinoma (HCC). Patients with HCV-related HCC who were diagnosed at Okayama University Hospital were classified into three groups according to their previous IFN treatment and response to that treatment. One hundred twenty-four patients had received IFN treatment before the development of HCC (IFN-treated group) and the remaining 271 had not (IFN-untreated group). Patients who had undergone IFN treatment before the development of HCC were further classified according to their response into a sustained virological response (SVR) group or a non-SVR group. Patients were regularly screened for HCC

for PTA. When a patient was indicated for both surgery and PTA, the modality of treatment was determined by patient choice after obtaining fully informed consent. PEIT was carried out under US guidance using a 15- or 20-cm-long needle (21 gauge) (Hakko, Chikuma, Japan) [35], PMCT was performed under US guidance using a 15-cm-long guide needle (14 gauge) according to the procedure described previously [37], and RFA was executed under US guidance using a 15- or 20-cm-long guide needle (16 gauge) (Tyco Healthcare Japan, Tokyo, Japan) [36]. PTA was repeated until complete necrosis of all HCC lesions was confirmed by dynamic CT. Treatment of HCC was considered curative, when no viable HCC lesions were detected on dynamic CT 3 months after completion of the treatment.

Of the 395 patients receiving curative treatment for HCC, 124 had received either human lymphoblastoid IFN, recombinant IFN-alpha 2a, or recombinant IFN-alpha 2b monotherapy for chronic HCV infection before the development of HCC (IFN-treated group), whereas 271 had not (IFN-untreated group) (Fig. 1). Patients received 6 million units of IFN by intramuscular injection three times weekly for 24 weeks as outpatients. If patients could not tolerate this dose, the IFN dose was reduced to 3 million units. SVR was defined as HCV RNA (as determined by RT-PCR; detection limit, 10^2 copies/ml) negativity for over 6 months after the termination of IFN therapy. SVR was achieved in 17 of the 124 patients (SVR group) and the remaining 107 were regarded as non-SVR (non-SVR group) (Fig. 1).

Follow-up of Patients

Patients attended a monthly medical consultation at the Okayama University Hospital outpatient clinic. Blood biochemical markers, including α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), were measured every 1–2 months; US was performed every 2–3 months, and dynamic CT was performed every 6 months. If HCC recurrence was suspected, further imaging examinations including dynamic CT, magnetic resonance imaging, abdominal angiography, or US-guided tumor biopsy were performed to confirm the diagnosis.

New HCC foci as well as local recurrent nodules at tumor, node, metastasis (TNM) stage I, II, and III, were mainly treated by a second course of PTA; local recurrent nodules at TNM stage IV were treated with transarterial chemoembolization or chemotherapy. Further development of HCC and survival of patients (tumor recurrence rate and survival rate) were analyzed in relation to the time interval after treatment of HCC.

Table 1 Demographic and clinical characteristics of patients with HCV-related HCC

Groups	IFN-treated		IFN-untreated (n = 271)	p^a	p^b
	SVR (n = 17)	Non-SVR (n = 107)			
Characteristics					
Sex (men/women), n	13/4	60/47	187/84	0.049	0.112
Age (years)	63 (52–71)	65 (46–82)	67 (33–85)	0.018	0.061
Laboratory data					
Total bilirubin (mg/dl)	0.74 (0.40–1.29)	0.85 (0.36–3.28)	0.91 (0.16–4.13)	0.194	0.171
Albumin (g/dl)	4.4 (3.7–4.8)	3.7 (2.5–4.8)	3.6 (2.2–4.7)	<0.001	<0.001
Prothrombin time (%)	93 (70–121)	85 (47–142)	85 (40–145)	0.355	0.023
ALT (IU/l)	22 (10–54)	55 (12–198)	60 (14–201)	0.058	<0.001
Platelet count ($\times 10^4$/μl)	16.6 (8.4–30.3)	9.2 (2.8–37.2)	10.1 (3.2–31.9)	0.980	<0.001
Child–Pugh stage (A/B/C), n	17/0/0	87/20/0	213/54/4	0.236	0.049
Tumor-related variables					
Number of tumors (single/multiple), n	15/2	76/31	192/79	0.603	0.136
Size of largest tumor (mm)	20 (8–40)	18 (10–53)	20 (9–74)	0.033	0.942
AFP (ng/ml)	13 (1.9–25,716)	24 (1.7–3,480)	20 (0.6–54,535)	0.956	0.297
DCP (mAU/ml)	34 (1–35,000)	46 (10–56,000)	46 (1–66,700)	0.294	0.195
Initial treatment of HCC					
PTA/surgical resection, n	6/11	79/28	207/64	0.100	0.002

Laboratory data and tumor-related variables are at the development of HCC. Continuous variables are given as medians with ranges

HCV hepatitis C virus, *HCC* hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation

[a] IFN-treated versus IFN-untreated

[b] SVR versus non-SVR

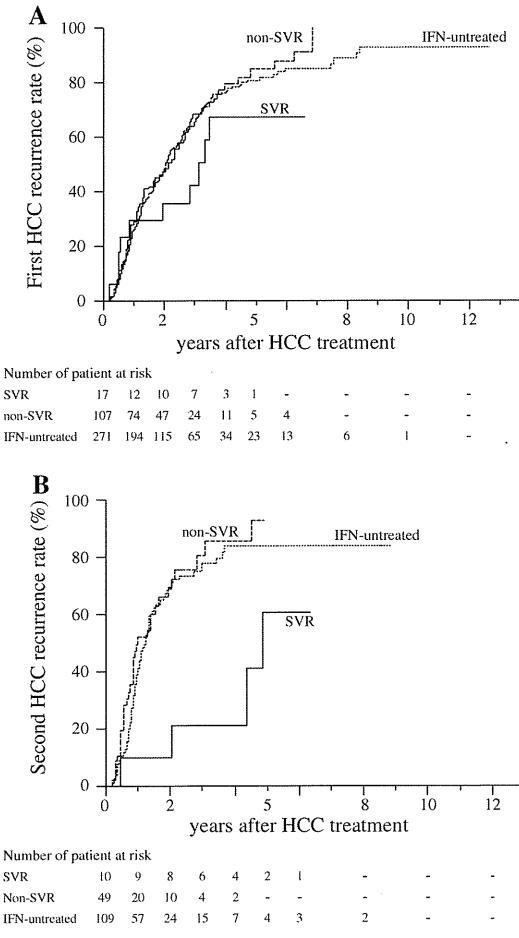

Fig. 2 Cumulative first (**a**, $n = 395$) and second (**b**, $n = 168$) HCC recurrence rates in patients with curative treatment of HCC according to the previous IFN treatment and response to the treatment. The first HCC recurrence rates were similar among SVR, non-SVR, and IFN-untreated groups (**a**). However, the second HCC recurrence rate in the SVR group at 2 years after HCC treatment was significantly lower than that in the non-SVR group (10 vs. 69%, $p = 0.003$) and the IFN-untreated group (10 vs. 70%, $p = 0.006$) (**b**)

Statistical Analysis

Statistical analysis was performed using JMP statistical discovery software, version 8.0 (SAS Institute Inc., Cary, NC). Differences between two groups were evaluated using the unpaired Student's t test or the Mann–Whitney U test. The Chi-square test or the 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 patient groups were assessed using the logrank test. Possible risk factors for recurrence of HCC and survival included both IFN-related variables and variables at the development and recurrence of HCC (age, total bilirubin level, albumin level, prothrombin time, ALT level, platelet count, number of tumors, largest tumor size, AFP level, and DCP level). Tumor associated variables, number of tumors and size of largest tumor, were transformed into categorical data consisting of two ordinal numbers by the median value. Variables exhibiting p values less than 0.10 in univariate analysis were subjected to a stepwise Cox proportional hazards regression analysis. A risk ratio with a 95% confidence interval was denoted for each analysis. p values less than 0.05 were considered statistically significant.

Results

Demographic and clinical characteristics of patients at the development of HCC are shown in Table 1. The patient group comprised 260 men and 135 women (73 men and 51 women in the IFN-treated group), and median age was 58 years (65 years in the IFN-treated group). Of the 395 patients (80%), 317 were classified as Child–Pugh stage A. Significant differences were observed between IFN-treated and untreated patients in sex, age, albumin level, and size of largest tumor. On the other hand, significant differences were observed between IFN-treated patients with SVR and non-SVR in albumin level, prothrombin time, ALT level, platelet count, Child–Pugh stage, and initial treatment of HCC. This indicated better hepatic functional reserve in SVR patients than in non-SVR patients.

The median follow-up period after curative treatment of HCC for patients with and without IFN treatment was 3.8 years and 3.5 years, respectively. In the IFN-treated group, patients underwent IFN therapy 7.2 (0.8–17.4) (median and range) years before development of HCC. Of the 395 patients, 277 (70%) had recurrence of HCC during a median follow-up period of 2.1 (1.8–2.4) years [including 86 of 124 IFN-treated patients (69%)]. Of the 168 patients receiving curative treatment for the first recurrence of HCC, 113 (67%) had a second HCC recurrence during a median follow-up period of 1.3 (1.0–1.4) years [including 38 of 59 IFN-treated patients (64%)] (Fig. 1).

HCC Recurrence Rates

The rates of the first and second HCC recurrence after curative treatment of primary HCC in each treatment group are shown in Fig. 2. In the IFN-treated group, 86 patients (10 with SVR and 76 with non-SVR) had the first HCC recurrence and 38 (four with SVR and 34 with non-SVR) had the second HCC recurrence during the follow-up period. The average times to the first and second HCC recurrence were 632 and 1,069 days, 661 and 401 days, and 666 and 428 days in SVR, non-SVR, and IFN-untreated groups, respectively. The rates of the first recurrence at 2 years in SVR, non-SVR, and IFN-untreated groups were 36, 47, and 48%, respectively. The differences between these rates were not statistically significant

($p = 0.410$) (Fig. 2a). However, the rates of the second HCC recurrence at 2 years were significantly lower in the SVR group than in the non-SVR group (10 vs. 69%, $p = 0.003$) and in the IFN-untreated group (10 vs. 70%, $p = 0.006$) (Fig. 2b). There was no significant difference in the second HCC recurrence rates between non-SVR and IFN-untreated groups ($p = 0.441$). In multivariate analysis, platelet count ($p = 0.033$) and number of tumors ($p = 0.001$) were independent factors associated with the first recurrence of HCC (Table 2), whereas SVR to previous IFN therapy ($p = 0.002$) was the only factor associated with lower risk for the second recurrence of HCC (Table 3).

Overall Survival

Survival rates after curative treatment of primary HCC in each group are shown in Fig. 3. A tendency was observed toward a higher survival rate in the IFN-treated group than in the IFN-untreated group but it was not significant ($p = 0.053$) (Fig. 3a). In contrast, survival rates at 5 years were higher in the SVR group (100%) than in non-SVR (73%) and IFN-untreated groups (62%) ($p = 0.004$) (Fig. 3b). No significant difference was observed in the survival rates between non-SVR and IFN-untreated groups ($p = 0.450$). In multivariate analysis, SVR to previous IFN therapy ($p < 0.001$), albumin level ($p = 0.006$), number of tumors ($p = 0.007$), and AFP level ($p = 0.046$) were independent factors associated with overall death after curative treatment of primary HCC (Table 4).

Discussion

In the present study, we have demonstrated that patients with SVR to previous IFN treatment before development of HCC showed lower risk for the second recurrence of HCC and better survival compared to patients with non-SVR to previous IFN treatment or IFN-untreated patients. Several studies have demonstrated that IFN therapy reduces the risk of HCC development among chronic hepatitis C patients. On the other hand, a few reports are available on the influence of previous IFN therapy before the development of HCC on patient outcomes after curative treatment of HCV-related HCC. It was initially reported that HCV-related HCC patients who received IFN therapy before development of HCC showed lower recurrence rates and better survival rates, independent of response to IFN therapy, compared to those without previous IFN therapy [29, 30]. It has recently been reported that patients showing

Table 2 Risk factors for the first recurrence of HCC ($n = 395$)

Variable	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Sex (male)	1.17 (0.91–1.51)	0.229	–	
IFN-related variables				
IFN-untreated	1			
Non-SVR	1.07 (0.82–1.39)	0.623	–	
SVR	0.68 (0.34–1.22)	0.209	–	
Variables at the development of HCC				
Age (≥60 years)	1.13 (0.84–1.56)	0.434	–	
Total bilirubin (≥1.0 mg/dl)	1.07 (0.83–1.37)	0.579	–	
Albumin (<3.5 g/dl)	1.34 (1.04–1.71)	0.022	1.24 (0.95–1.61)	0.108
Prothrombin time (<70%)	1.07 (0.79–1.43)	0.664	–	
ALT (≥40 IU/l)	1.09 (0.83–1.43)	0.542	–	
Platelet count(<10 × 10^4/μl)	1.37 (1.08–1.75)	0.009	1.34 (1.04–1.75)	0.026
Tumor-related variables				
Number of tumors (multiple vs. single)	1.66 (1.27–2.15)	<0.001	1.63 (1.24–2.14)	0.001
Size of largest tumor (≥20 mm)	1.24 (0.98–1.57)	0.074	1.22 (0.94–1.59)	0.140
AFP (≥100 ng/ml)	1.45 (1.07–1.92)	0.016	1.30 (0.96–1.74)	0.093
DCP (≥40 mAU/ml)	1.33 (1.02–1.75)	0.034	1.11 (0.85–1.44)	0.448
Initial treatment of HCC				
PTA/surgical resection	1.09 (0.84–1.43)	0.530	–	

HCC hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation, *CI* confidence interval

Table 3 Risk factors for the second recurrence of HCC ($n = 168$)

Variable	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Sex (male)	1.07 (0.73–1.61)	0.719	–	
IFN-related variables				
IFN-untreated	1		1	
Non-SVR	1.17 (0.77–1.74)	0.447	1.09 (0.68–1.72)	0.718
SVR	0.27 (0.08–0.65)	0.002	0.10 (0.01–0.50)	0.002
Variables at the development of HCC				
Age (≥60 years)	1.50 (0.91–2.61)	0.115	–	
Total bilirubin (≥1.0 mg/dl)	1.08 (0.72–1.60)	0.701	–	
Albumin (<3.5 g/dl)	1.04 (0.68–1.57)	0.847	–	
Prothrombin time (<70%)	1.18 (0.70–1.89)	0.529	–	
ALT (≥40 IU/l)	1.30 (0.86–2.01)	0.220	–	
Platelet count ($<10 \times 10^4/\mu l$)	1.00 (0.69–1.47)	0.984	–	
Number of tumors (multiple vs. single)	1.57 (1.04–2.32)	0.033	1.51 (0.93–2.42)	0.098
Size of largest tumor (≥20 mm)	0.91 (0.63–1.32)	0.613	–	
AFP (≥100 ng/ml)	0.65 (0.38–1.06)	0.084	0.77 (0.39–1.39)	0.391
DCP (≥40 mAU/ml)	0.81 (0.54–1.23)	0.331	–	
Initial treatment of HCC				
PTA/surgical resection	1.12 (0.75–1.69)	0.595	–	
Variables at the first recurrence of HCC				
Age (≥60 years)	0.97 (0.46–2.39)	0.950		
Total bilirubin (≥1.0 mg/dl)	0.94 (0.59–1.46)	0.785	–	
Albumin (<3.5 g/dl)	1.67 (1.06–2.61)	0.029	1.47 (0.90–2.36)	0.125
Prothrombin time (<70%)	1.24 (0.60–2.30)	0.531	–	
ALT (≥40 IU/l)	1.49 (0.95–2.40)	0.083	1.21 (0.75–2.01)	0.452
Platelet count ($<10 \times 10^4/\mu l$)	1.13 (0.74–1.73)	0.573	–	
Number of tumors (multiple vs. single)	2.09 (1.37–3.13)	<0.001	1.47 (0.91–2.34)	0.112
Size of largest tumor (≥20 mm)	0.96 (0.62–1.45)	0.840	–	
AFP (≥100 ng/ml)	0.72 (0.32–1.41)	0.355	–	
DCP (≥40 mAU/ml)	1.05 (0.67–1.63)	0.842	–	

HCC hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation, *CI* confidence interval

biochemical response, with or without SVR to previous IFN therapy, showed higher tumor-free survival rates after surgery than those without such a response to IFN or those without previous IFN therapy [31, 32]. In these previous reports, a biochemical response as well as SVR to previous IFN therapy was associated with favorable outcome, demonstrating the importance of response to previous IFN therapy for the outcome after surgery of HCV-related HCC.

However, in the present study, patients with non-SVR showed similar recurrence and survival rates as IFN-untreated patients. Furthermore, no difference was observed in the recurrence and survival rates among non-SVR patients with and without biochemical response to previous IFN therapy (data not shown). In fact, only patients with SVR to previous IFN therapy showed better outcome than those with non-SVR or IFN-untreated patients. Therefore, the present data indicate that SVR but not biochemical response without SVR to previous IFN treatment is a predictor of favorable outcome in patients who have developed HCC.

The reason for the difference between the present and previous studies in the outcome of non-SVR patients with biochemical response to previous IFN therapy is currently unknown. In patients with HCV-related chronic hepatitis and cirrhosis, who received IFN therapy and showed normalization of ALT levels, suppression of primary HCC development and better survival rates have been independently demonstrated of eradication of HCV infection by the IFN therapy [10, 11, 13, 39]. However, this

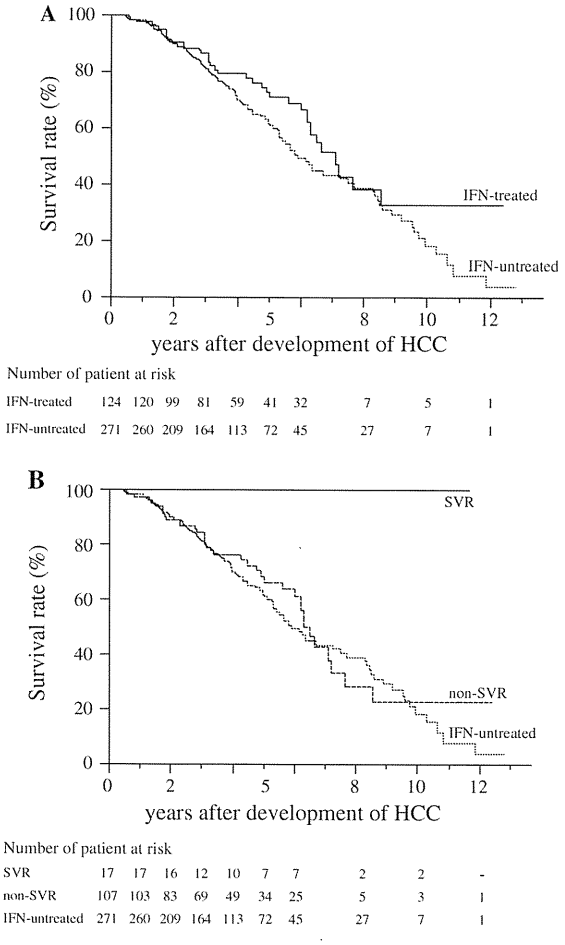

Fig. 3 Overall survival rates of HCV-related HCC patients ($n = 395$) according to their previous IFN treatment before development of HCC (**a**) and their response to the treatment (**b**). A tendency was observed toward a higher survival rate in the IFN-treated group than in the IFN-untreated group but it was not significant ($p = 0.053$) (**a**). On the other hand, the survival rate of the SVR group was significantly higher than those of non-SVR and IFN-untreated groups ($p = 0.004$) (**b**)

suppression observed for primary carcinogenesis in non-SVR patients with biochemical response to IFN therapy does not appear to be the case for secondary carcinogenesis in the present study. The period after IFN therapy was much longer in the present study than in the previous reports on primary carcinogenesis. The IFN therapy had preceded to the HCC development, that should have required long incubation after the termination of IFN treatment, and in the present study the observation of HCC recurrence and survival started with the curative treatment of the primary HCC. In patients who had sustained biochemical response but had not eradicated HCV infection, we and others demonstrated that platelet count transiently increases following IFN therapy but decrease over the following 3 years after the termination of IFN therapy. On the other hand, in patients with SVR an increase followed by persistence in platelet counts was observed [40, 41]. These observations suggest the progression of fibrosis during a longer incubation period after IFN therapy, even in the non-SVR patients with biochemical response to the therapy. Therefore, the suppressive effect of IFN therapy on development of HCC may not persist beyond the development of primary HCC particularly in these patients.

It has also been demonstrated that HCV core transgenic mice can develop HCC without apparent hepatitis [42]. Therefore, besides active hepatitis, which involves persistent hepatocyte death and regeneration, and should result in both genetic and epigenetic disorders as well as increased oxidative stress, the presence and persistence of HCV infection and viral products such as core protein may themselves play an important role in the development of HCC in non-SVR patients with biochemical response. Thus, patients with SVR who had eradicated HCV infection should have a lower incidence of HCC recurrence and higher survival rates than non-SVR patients with biochemical response.

In the present study, patients with SVR showed a better overall survival rate than other groups. However, although patients with SVR showed lower rates of the second HCC recurrence, this was not the case for the first HCC recurrence. Although both SVR and non-SVR groups have a carcinogenic background during the development of primary HCC, the carcinogenic potential in SVR patients may be gradually attenuated because of the eradication of HCV infection, whereas it may increase in those with non-SVR because of persistence of HCV infection and relapse of hepatitis, finally leading to progression of fibrosis over a longer period. However, a substantial time may be required before differences between patients with and without SVR become apparent, and these differences eventually become significant in the second recurrence of HCC.

It should also be noted that IFN-treated patients enrolled in the present and previous studies are a selected cohort, since the incidence rates of HCC development in patients treated with IFN should be lower than in those untreated with IFN [13]. This is particularly the case for patients with SVR to previous IFN treatment, whose risk for development of HCC is less than one fifth of that for IFN-untreated patients [13]. Reported risk factors for HCC development in patients who received IFN therapy include advanced fibrosis, lower platelet count, advanced age, male gender, and regular drinking [8, 9, 12, 13, 43]. Therefore, in the present study, HCC patients who received IFN therapy before the development of HCC may have demonstrated many of these characteristics, making them more prone to develop HCC than those not developing HCC after IFN therapy and not included in this study. Furthermore, it has been suggested that cirrhotic patients who develop primary

Table 4 Analysis of factors associated with overall death after curative treatment for primary HCC ($n = 395$)

Variable	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Sex (male)	1.02 (0.73–1.44)	0.911	–	
IFN-related variables				
IFN-untreated	1		1	
Non-SVR	0.86 (0.59–1.24)	0.445	1.05 (0.71–1.54)	0.794
SVR	<0.01 (0–0.17)	<0.001	<0.01 (0–0.26)	<0.001
Variables at the development of HCC				
Age (≥60 years)	1.06 (0.72–1.63)	0.773	–	
Total bilirubin (≥1.0 mg/dl)	1.45 (1.04–2.01)	0.028	1.21 (0.82–1.76)	0.332
Albumin (<3.5 g/dl)	2.07 (1.49–2.89)	<0.001	1.70 (1.16–2.49)	0.007
Prothrombin time (<70%)	1.44 (0.99–2.06)	0.059	0.97 (0.65–1.43)	0.874
ALT (≥40 IU/L)	1.12 (0.78–1.67)	0.531	–	
Platelet count (<10 × 10^4 μl)	1.72 (1.23–2.41)	0.001	1.35 (0.93–1.96)	0.118
Tumor-related variables				
Number of tumors (multiple vs. single)	1.59 (1.10–2.26)	0.014	1.71 (1.16–2.46)	0.007
Size of largest tumor (≥20 mm)	1.15 (0.83–1.60)	0.395	–	
AFP (≥100 ng/ml)	1.71 (1.17–2.45)	0.006	1.50 (1.00–2.18)	0.047
DCP (≥40 mAU/ml)	1.33 (0.91–1.98)	0.145	–	
Initial treatment of HCC				
PTA/surgical resection	1.69 (1.16–2.53)	0.006	1.03 (0.68–1.60)	0.882

HCC hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation, *CI* confidence interval

HCC may already be at a "carcinogenic stage" and have a higher potential to develop intrahepatic multicentric carcinogenesis than those without HCC [15]. Patients who have already developed HCC may have background features such as greater age and impaired liver function because of more advanced fibrosis. Therefore, the observed recurrence and survival rates in the present study are those of selected patients who were already at the carcinogenic stage, and are thus biased in comparison to previous observations on primary prevention of HCC development in patients who had received IFN therapy. Recently, Imai et al. reported that an inhibitory effect of IFN therapy on development of HCC in older patients was limited to patients with SVR [44]. This also supports the notion that patients already at a carcinogenic stage or with risk factors associated with HCC development, such as greater age or advanced fibrosis, require eradication of HCV infection in order to achieve a significantly better prognosis.

The present observation highlights the importance of eradication of HCV in order to prevent HCC recurrence and to achieve better survival in this patient group. Plenty of reports are available that demonstrated the favorable effect of IFN therapy on the recurrence of HCC and survival particularly in patients who achieved SVR [21–28]. Therefore, re-treatment with more potent IFN therapies, such as combination therapy of PEGylated IFN plus ribavirin [45], should be recommended for patients who previously underwent IFN treatment without achieving SVR.

The present study has limitations as it is retrospective in nature, and thus, patients enrolled were biased in favor of experience of IFN treatment, and also HCC patients with previous IFN treatment were a selected population from a large cohort of patients who had undergone IFN treatment. Also, information on the histological data that may have influence on the outcome of HCC patients was not available in the present study. Further prospective studies are required to address these issues.

In conclusion, the present study demonstrated that patients with SVR to IFN treatment before the development of HCV-related HCC showed lower second HCC recurrence rates and higher survival rates than those with non-SVR to previous IFN treatment or IFN-untreated patients. Therefore, treatment with potent antiviral therapy is recommended for patients in the latter groups in order to suppress recurrence and improve survival by eradicating HCV infection.

Acknowledgments The authors thank Dr. Kenji Miyoshi and Dr. Shota Iwadou for contribution to this study.

References

1. Di Bisceglie AM. Hepatitis C and hepatocellular carcinoma. *Semin Liver Dis.* 1995;15:64–69. doi:10.1055/s-2007-1007263.
2. Shiratori Y, Shiina S, Imamura M, et al. Characteristic difference of hepatocellular carcinoma between hepatitis B- and C- viral infection in Japan. *Hepatology.* 1995;22:1027–1033.
3. Tanaka K, Hirohata T, Koga S, et al. Hepatitis C and hepatitis B in the etiology of hepatocellular carcinoma in the Japanese population. *Cancer Res.* 1991;51:2842–2847.
4. Colombo M, de Franchis R, Del Ninno E, et al. Hepatocellular carcinoma in Italian patients with cirrhosis. *N Engl J Med.* 1991;325:675–680. doi:10.1056/NEJM199109053251002.
5. Tsukuma H, Hiyama T, Tanaka S, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med.* 1993;328:1797–1801. doi:10.1056/NEJM199306243282501.
6. Shiratori Y, Imazeki F, Moriyama M, et al. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med.* 2000;132:517–524.
7. International Interferon-alpha Hepatocellular Carcinoma Study Group. Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. *Lancet.* 1998;351:1535–1539.
8. Imai Y, Kawata S, Tamura S, et al. Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. *Ann Intern Med.* 1998;129:94–99.
9. Kasahara A, Hayashi N, Mochizuki K, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology.* 1998;27:1394–1402. doi:10.1002/hep.510270529.
10. Nishiguchi S, Kuroki T, Nakatani S, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet.* 1995;346:1051–1055.
11. Nishiguchi S, Shiomi S, Nakatani S, et al. Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. *Lancet.* 2001;357:196–197. doi:10.1016/S0140-6736(00)03595-9.
12. Okanoue T, Itoh Y, Minami M, et al. Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. Viral Hepatitis Therapy Study Group. *J Hepatol.* 1999;30:653–659.
13. 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;131:174–181.
14. Belghiti J, Panis Y, Farges O, Benhamou JP, Fekete F. Intrahepatic recurrence after resection of hepatocellular carcinoma complicating cirrhosis. *Ann Surg.* 1991;214:114–117.
15. Ikeda K, Arase Y, Kobayashi M, et al. Significance of multicentric cancer recurrence after potentially curative ablation of hepatocellular carcinoma: a long-term cohort study of 892 patients with viral cirrhosis. *J Gastroenterol.* 2003;38:865–876. doi:10.1007/s00535-003-1163-2.
16. Lam VWT, Ng KKC, Chok KSH, et al. Risk factors and prognostic factors of local recurrence after radiofrequency ablation of hepatocellular carcinoma. *J Am Coll Surg.* 2008;207:20–29. doi:10.1016/j.jamcollsurg.2008.01.020.
17. N'Kontchou G, Mahamoudi A, Aout M, et al. Radiofrequency ablation of hepatocellular carcinoma: long-term results and prognostic factors in 235 Western patients with cirrhosis. *Hepatology.* 2009;50:1475–1483. doi:10.1002/hep.23181.
18. Okada S, Shimada K, Yamamoto J, et al. Predictive factors for postoperative recurrence of hepatocellular carcinoma. *Gastroenterology.* 1994;106:1618–1624.
19. Shiina S, Tagawa K, Niwa Y, et al. Percutaneous ethanol injection therapy for hepatocellular carcinoma: results in 146 patients. *AJR Am J Roentgenol.* 1993;160:1023–1028.
20. Kubo S, Nishiguchi S, Shuto T, et al. Effects of continuous hepatitis with persistent hepatitis C viremia on outcome after resection of hepatocellular carcinoma. *Jpn J Cancer Res.* 1999;90:162–170.
21. Breitenstein S, Dimitroulis D, Petrowsky H, Puhan MA, Mullhaupt B, Clavien PA. Systematic review and meta-analysis of interferon after curative treatment of hepatocellular carcinoma in patients with viral hepatitis. *Br J Surg.* 2009;96:975–981. doi:10.1002/bjs.6731.
22. Ikeda K, Arase Y, Saitoh S, et al. 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.* 2000;32:228–232. doi:10.1053/jhep.2000.9409.
23. Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Kinoshita H. Randomized clinical trial of long-term outcome after resection of hepatitis C virus-related hepatocellular carcinoma by postoperative interferon therapy. *Br J Surg.* 2002;89:418–422. doi:10.1046/j.0007-1323.2001.02054.x.
24. Kubo S, Nishiguchi S, Hirohashi K, et al. 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.* 2001;134:963–967.
25. Mazzaferro V, Romito R, Schiavo M, et al. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology.* 2006;44:1543–1554. doi:10.1002/hep.21415.
26. Shen YC, Hsu C, Chen LT, Cheng CC, Hu FC, Cheng AL. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): a meta-regression approach. *J Hepatol.* 2010;52:889–894. doi:10.1016/j.jhep.2009.12.041.
27. 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.
28. Singal AK, Freeman DH Jr, Anand BS. Meta-analysis: interferon improves outcomes following ablation or resection of hepatocellular carcinoma. *Aliment Pharmacol Ther.* 2010;32:851–858. doi:10.1111/j.1365-2036.2010.04414.x.
29. Komorizono Y, Sako K, Yamasaki N, et al. Outcome of patients with hepatitis C virus-related hepatocellular carcinoma occurring after interferon therapy. *Anticancer Res.* 2002;22:3573–3578.
30. Kubo S, Nishiguchi S, Hirohashi K, et al. Influence of previous interferon therapy on recurrence after resection of hepatitis c virus-related hepatocellular carcinoma. *Jpn J Cancer Res.* 2001;92:59–66.
31. Uenishi T, Kubo S, Hirohashi K, et al. Relationship between response to previous interferon therapy and postoperative recurrence of hepatitis C virus-related hepatocellular carcinoma. *Hepatol Res.* 2002;24:404–412.
32. Uenishi T, Nishiguchi S, Tamori A, et al. Influence of interferon therapy on outcome after surgery for hepatitis C virus-related hepatocellular carcinoma. *Hepatol Res.* 2006;36:195–200. doi:10.1016/j.hepres.2006.07.011.

33. Kato N, Yokosuka O, Omata M, Hosoda K, Ohto M. Detection of hepatitis C virus ribonucleic acid in the serum by amplification with polymerase chain reaction. *J Clin Invest.* 1990;86: 1764–1767. doi:10.1172/JCI114903.
34. Farmer DG, Rosove MH, Shaked A, Busuttil RW. Current treatment modalities for hepatocellular carcinoma. *Ann Surg.* 1994;219:236–247.
35. Lee MJ, Mueller PR, Dawson SL, et al. Percutaneous ethanol injection for the treatment of hepatic tumors: indications, mechanism of action, technique, and efficacy. *AJR Am J Roentgenol.* 1995;164:215–220.
36. Livraghi T, Goldberg SN, Lazzaroni S, Meloni F, Solbiati L, Gazelle GS. Small hepatocellular carcinoma: treatment with radio-frequency ablation versus ethanol injection. *Radiology.* 1999;210:655–661.
37. Seki T, Wakabayashi M, Nakagawa T, et al. Ultrasonically guided percutaneous microwave coagulation therapy for small hepatocellular carcinoma. *Cancer.* 1994;74:817–825.
38. Arii S, Yamaoka Y, Futagawa S, et al. Results of surgical and nonsurgical treatment for small-sized hepatocellular carcinomas: a retrospective and nationwide survey in Japan. The Liver Cancer Study Group of Japan. *Hepatology.* 2000;32:1224–1229. doi: 10.1053/jhep.2000.20456.
39. Yoshida H, Arakawa Y, Sata M, et al. Interferon therapy prolonged life expectancy among chronic hepatitis C patients. *Gastroenterology.* 2002;123:483–491.
40. Moriyama M, Matsumura H, Aoki H, et al. Long-term outcome, with monitoring of platelet counts, in patients with chronic hepatitis C and liver cirrhosis after interferon therapy. *Intervirology.* 2003;46:296–307. doi:10.1159/000073209.
41. Taniguchi H, Iwasaki Y, Fujiwara A, et al. Long-term monitoring of platelet count, as a non-invasive marker of hepatic fibrosis progression and/or regression in patients with chronic hepatitis C after interferon therapy. *J Gastroenterol Hepatol.* 2006;21: 281–287. doi:10.1111/j.1440-1746.2006.04201.x.
42. Moriya K, Fujie H, Shintani Y, et al. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. *Nat Med.* 1998;4:1065–1067. doi:10.1038/2053.
43. Iwasaki Y, Takaguchi K, Ikeda H, et al. Risk factors for hepatocellular carcinoma in Hepatitis C patients with sustained virologic response to interferon therapy. *Liver Int.* 2004;24:603–610. doi:10.1111/j.1478-3231.2004.0956.x.
44. Imai Y, Tamura S, Tanaka H, et al. Reduced risk of hepatocellular carcinoma after interferon therapy in aged patients with chronic hepatitis C is limited to sustained virological responders. *J Viral Hepat.* 2010;17:185–191. doi:10.1111/j.1365-2893.2009.01163.x.
45. Huang JF, Yu ML, Huang CF, et al. The efficacy and safety of pegylated interferon plus ribavirin combination therapy in chronic hepatitis c patients with hepatocellular carcinoma post curative therapies—a multicenter prospective trial. *J Hepatol.* 2011;54:219–226. doi:10.1016/j.jhep.2010.07.011.

Alimentary Pharmacology & Therapeutics

Evolution of prognostic factors in hepatocellular carcinoma in Japan

K. NOUSO*,†, Y. KOBAYASHI†, S. NAKAMURA†, S. KOBAYASHI†, J. TOSHIMORI†, K. KUWAKI†, H. HAGIHARA†, H. ONISHI†, Y. MIYAKE*,†, F. IKEDA*,†, H. SHIRAHA†, A. TAKAKI†, Y. IWASAKI†, H. KOBASHI† & K. YAMAMOTO†

*Department of Molecular Hepatology; †Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

Correspondence to:
Dr K. Nouso, Department of Molecular Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama-city, Okayama, 700-8558 Japan.
E-mail: nouso@cc.okayama-u.ac.jp

Publication data
Submitted 26 August 2009
First decision 15 September 2009
Resubmitted 16 October 2009
Accepted 19 October 2009
Epub Accepted Article 22 October 2009

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.

Aliment Pharmacol Ther **31**, 407–414

doi:10.1111/j.1365-2036.2009.04179.x

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.

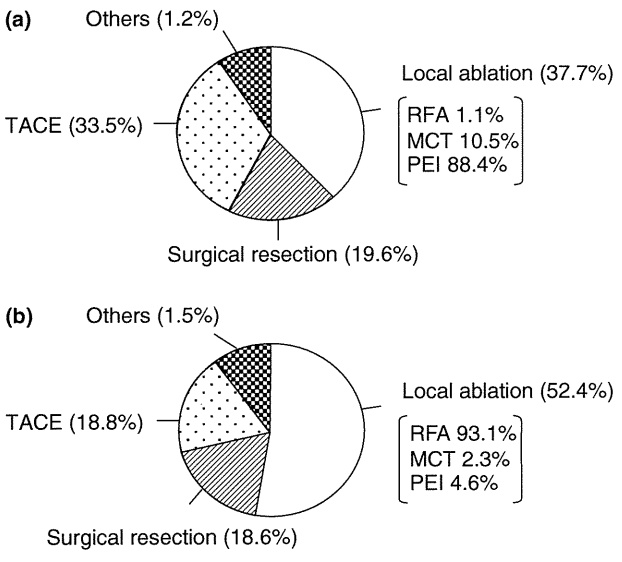

Figure 1. Changes in treatment modalities with time. The percentage of local ablation was 37.7% before December 2000 (a) and increased to 52.4% after January 2001 (b). PEI was popular before 2000; however, RFA was chosen as the standard therapy after 2001. Abbreviations: RFA, radiofrequency ablation;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

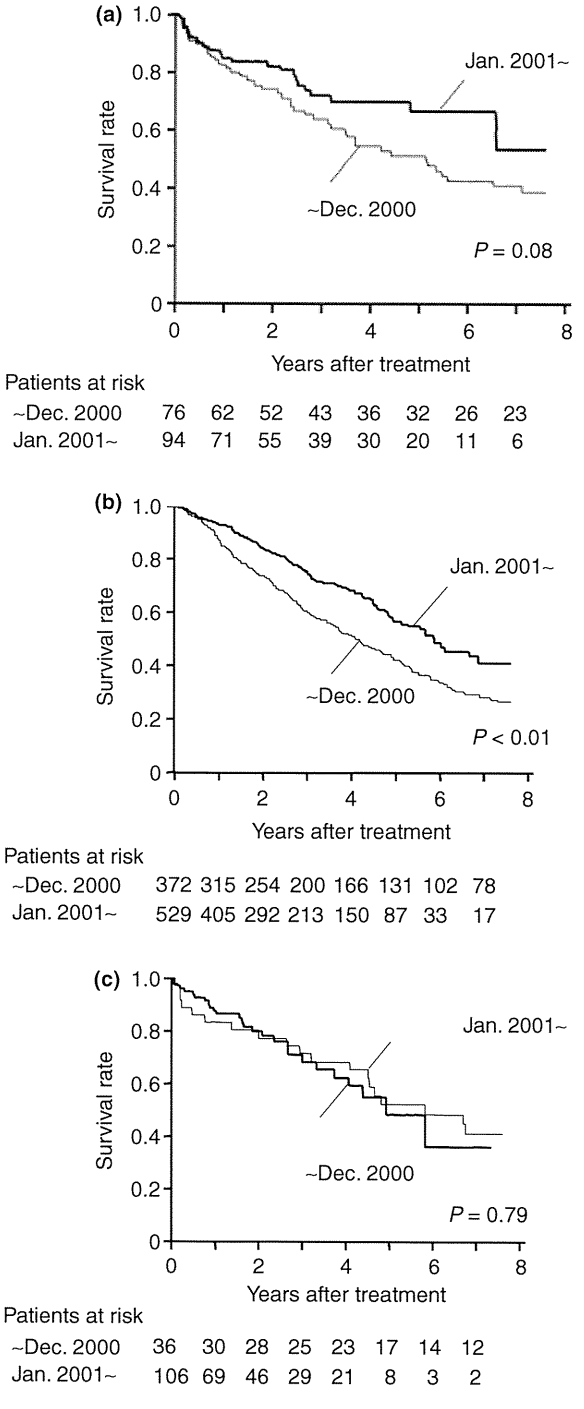

Figure 2. Survival curves of B-HCC (a), C-HCC (b) and NBNC-HCC (c). Note that the survival of C-HCC improved ($P < 0.01$) and a tendency towards improvement was observed in B-HCC ($P = 0.08$); however, no difference was observed for NBNC-HCC ($P = 0.79$). Thin line, HCC patients treated before December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

ACKNOWLEDGEMENT

Declaration of personal and funding interests: None.

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.

Liver INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL ASSOCIATION FOR THE STUDY OF THE LIVER

Liver International ISSN 1478-3223

CLINICAL STUDIES

Time-dependent analysis of predisposing factors for the recurrence of hepatocellular carcinoma

Shouta Iwadou[1], Kazuhiro Nouso[2,3], Kenji Kuwaki[3], Yoshiyuki Kobayashi[3], Shinichiro Nakamura[3], Hironori Tanaka[4], Kenji Miyoshi[3], Hideki Ohnishi[3], Yasuhiro Miyake[2,3], Hidenori Shiraha[3], Yoshiaki Iwasaki[3], Yasushi Shiratori[5] and Kazuhide Yamamoto[3]

1 Department of Internal Medicine, Hiroshima City Hospital, Hiroshima, Japan
2 Department of Molecular Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan
3 Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan
4 Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan
5 Department of Internal Medicine, Miyamoto Hospital, Ibaraki, Japan

Keywords
hepatocellular carcinoma – recurrence – time-dependent analysis

Correspondence
Shouta Iwadou, Department of Internal Medicine, Hiroshima City Hospital, 7-33 Motomachi, Naka-ku, Hiroshima-city, Hiroshima 730-8518, Japan
Tel: +082 221 2291
Fax: +082 223 1447
e-mail: shorter@abox.so-net.ne.jp

Received 26 September 2009
Accepted 11 March 2010

DOI:10.1111/j.1478-3231.2010.02252.x

Abstract

Background/aim: There are many reports dealing with the risk factors for hepatocellular carcinoma (HCC) recurrence. However, in most of these reported studies, factors were analysed only at the initial treatment stage, and the predisposing factors for the recurrence during follow-up have not been well studied. The aim of this study is to evaluate the predisposing factors after treatments. *Methods:* Two hundred and seventy-one consecutive HCC patients curatively treated between January 1994 and March 2004 were followed up and analysed. The recurrence rate was estimated by the Kaplan–Meier method and the predisposing factors were evaluated by time-fixed Cox regression analysis and by time-dependent covariate analysis using multiple parameters. *Results:* The mean follow-up period was 4.86 years and recurrence was observed in 169 patients (62.4%). The recurrence rates were 27.9, 65.1 and 84.3% at 1, 3 and 5 years respectively. Among the variables determined before treatment, predisposing factors for recurrence were low serum albumin [$\leq$3.5 g/dl, hazard ratio (HR) = 1.47, 95% confidence interval (CI) = 1.07–2.01] and multiple tumour number (HR = 2.04, 95% CI = 1.46–2.84) by time-fixed multivariate analysis. In the time-dependent analysis, six variables with 12 013 plots were examined. The multivariate analysis revealed that high des- γ-carboxy prothrombin (DCP $\geq$ 40 mAU/ml, HR = 2.33, 95% CI = 1.61–3.39), high α-fetoprotein (AFP $\geq$ 100 ng/ml, HR = 2.01, 95% CI = 1.3–3.35) and high alanine aminotransferase (ALT $\geq$ 40 IU/L, HR = 1.52, 95% CI = 1.1–2.1) were significant predisposing factors for recurrence. *Conclusion:* Predisposing factors for the recurrence of HCC after treatment are different from those before treatments and special cautions are required when AFP, DCP or ALT is high during follow-up.

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer-related deaths in the world (1). HCC is known to occur in patients who suffer from hepatitis and cirrhosis, especially those with hepatitis B virus (HBV) or hepatitis C virus (HCV) infection. The annual incidence of HCC was found to be 3% in a retrospective series of Caucasian patients with HCV-related compensated cirrhosis (2), and it was 5–7% in Japan (3).

Despite the advancement of surveillance systems and the progress in the curative treatment of HCC, few patients can avoid HCC recurrence. The recurrence rate after tumour ablation therapies, such as percutaneous ethanol injection therapy (PEI) and radio-frequency ablation (RFA), was 64–91% at 5 years, and was also high after surgical resection of HCC (4–7). The annual recurrence rates given in these reports were 20–40% after curative treatments.

There are many reports regarding the predisposing factors for HCC recurrence, e.g., size of tumour, tumour number, safety margin, presence of capsule formation and tumour markers such as α-fetoprotein (AFP) and

des- γ-carboxy prothrombin (DCP) (4–9). Although there are some differences in the hazard ratios of factors among the studies, which may be caused by different treatment modalities and patients' profiles, the factors can be classified into two categories: so-called tumour factors and background liver factors. Most of the factors presented in the above studies were based on time-fixed parameters that were obtained before the initial treatment of HCC.

In a clinical setting, a periodical screening of HCC with imaging modalities including ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI), which are gold standard for screening, and repeated blood tests including those for tumour markers such as AFP have been recommended after the initial treatment of HCC (10). Because the values of many factors change over time, it is rational to determine the predisposing factors for the recurrence of HCC in a time-dependent manner.

The usefulness of time-dependent analysis, which involves the analysis of the change in certain variables after the initial treatment in order to predict recurrence, was reported for colon cancer, prostate cancer, breast cancer and metastatic bone cancer (11–14). However, there are few studies dealing with the predisposing factors for the recurrence of HCC with multiple time-dependent covariates.

The aim of this study is to determine the factors that are important to measured repeatedly during follow-up after the curative treatment of HCC.

Material and methods

Patients

Among the 485 consecutive newly diagnosed HCC patients who were treated and participated in our follow-up programme at Okayama University Hospital between January 1994 and March 2004, 271 HCC patients were curatively treated by surgical resection or tumour ablation, and were enrolled in this study (Fig. 1). All the patients were followed up for at least 6 months, and four patients were excluded because of the ingestion of warfarin or vitamin K, which may affect DCP concentration.

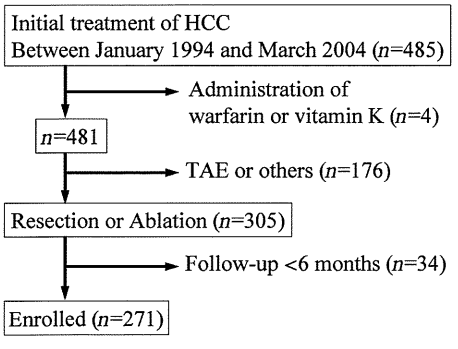

Fig. 1. Flow chart of consecutive 271 patients who received curative treatments. HCC, hepatocellular carcinoma; TAE, transcatheter arterial embolization.

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 the institute.

Diagnosis

The diagnosis of HCC was carried out by at least two imaging modalities including CT, MRI and angiography, as described previously (15). Briefly, diagnostic findings included enhancement at the arterial phase, washout at the portal phase in dynamic CT (section thickness = 5–8 mm) or MRI and tumour staining in angiography. In cases that did not meet the diagnostic criteria, HCC was confirmed by histological findings of tumour-directed biopsies ($n = 45$).

Treatments

Surgical resection, PEI, RFA and microwave coagulation therapy (MCT) were performed on 96, 86, 76 and 13 patients respectively. The selection of the therapies was performed according to the evidence-based clinical practice guidelines for HCC in Japan (16). Segmental transcatheter arterial injection or transcatheter arterial chemoembolization (TACE) was carried out before these treatments [34 patients (35.4%), 39 patients (45.3%), 52 patients (68.4%) and 10 patients (76.9%) respectively]. We performed TACE before the therapies in cases when HCC was extruded from the surface of the liver and was likely to rupture or when the tumour was too big to evaluate the ablated margin without the information of lipiodol retention at HCC visualized on CT after the ablation. The procedures of PEI, MCT and RFA are described elsewhere (15).

The extent of ablation was evaluated by CT or MRI after each session and the treatments were considered as curative when the ablated area completely engulfed the pretreatment lesions, as determined by a dynamic CT scan at days 2–7 after the therapies.

Follow-up of patients

A follow-up was conducted every 1–2 months at outpatient clinics by blood tests, including those for tumour markers (AFP and DCP), total bilirubin (T.Bil), albumin, alanine aminotransferase (ALT) and platelet counts. These factors were reported to correlate with the tumour recurrence or the prognosis of the patients (17–27). The screening of HCC recurrence was performed by US every 3 months and dynamic CT or MRI was performed every 6 months. HCC recurrence was defined by the same criteria used for the initial diagnosis. When recurrence was detected, the lesions were treated by local ablation therapies, TACE or surgical resection, depending on the state of the tumour and liver function. The follow-up