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.

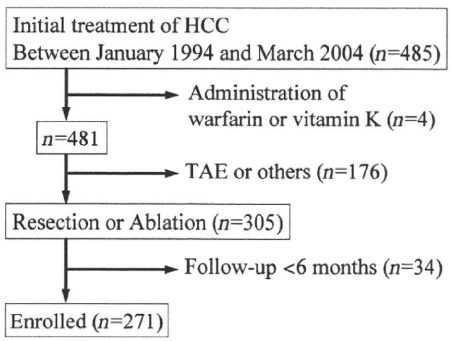

Fig. 1. Flow chart of consecutive 271 patients who received curative treatments. HCC, hepatocellular carcinoma; TAE, transcatheter arterial embolization.

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

period of this analysis was defined as the interval between the date of the initial treatment and the date of death, the date of dropping out from the follow-up programme, or the end of programme in January 2005. The average period was 4.86 years (range: 0.5–9.5 years).

Measurement of serum des- γ-carboxy prothrombin and α-fetoprotein concentrations

The serum AFP concentrations were measured using a commercially available enzyme immunoassay (EIA) kit, and serum DCP concentrations were determined using a revised EIA kit (Eitest PIVKA-II kit, Eisai, Tokyo, Japan) or an electrochemiluminescence immunoassay kit (Picolumi PIVKA-II kit, Sanko Junyaku, Tokyo, Japan).

Statistical analysis

Cumulative recurrence rates after the initial therapies were examined using the Kaplan–Meier method. Cox univariate analysis was used for the time-fixed analysis of the predisposing factors for HCC recurrence, with 13 parameters determined before the therapies. Factors exhibiting significant values in the analysis were further analysed by the Cox multivariate proportional hazard model. For the time-dependent analysis, we chronologically measured six serum parameters: T.Bil, ALT, platelet counts, albumin, AFP and DCP.

The six variables were measured every 6 months (± 2 months) from the initial treatment to the end of this study. For missing data, the actual value obtained before the miss was used. The cut-off values of these parameters were as follows: DCP, 40 mAU/ml; AFP, 100 ng/ml; ALT, 40 IU/ml; T.Bil, 2 mg/dl; albumin, 3.5 g/dl and platelet counts, 100×10^9 cells/L. The utilities of time-dependent analysis were in accordance with those given by Gail (14). The proportional hazard model of Cox, with a time-dependent covariate, was used to analyse serial data in this study. A particular advantage of this method is the ease with which missing marker data can be handled. Methods to yield estimates and confidence intervals (CIs) for model parameters are outlined both for continuous and for grouped time–response data. For grouped data, a likelihood ratio test of the proportional hazard assumption was adopted.

We used SAS version 9.1 and JMP IN for statistical analyses (SAS Institute, Cary, NC, USA).

Results

Clinical backgrounds

The clinical backgrounds of the enrolled patients are listed in Table 1. The median age of the patients was 71 years, and 210 (77.5%) of the patients suffered from HCV infection. Most of the patients showed preserved liver function, and 214 patients (79.0%) were classified as Child–Pugh grade A. The median tumour size was 21 mm, and 191 patients (70.5%) had a single tumour. DCP and AFP were above or equal to 40 mAU/ml and 100 ng/ml in 109 patients (40.2%) and 68 patients (25.1%) before the initial treatments respectively. Portal invasion was observed by imaging modalities in 17 (6.5%) patients and none of the patients had venous or bile duct invasion.

Table 1. Clinical backgrounds of enrolled patients

Variables	Values*
Host-related factors	
Age (years)	71 (35–87)
Gender (male)	191 (70.5%)
Antibody to hepatitis C virus (positive)	210 (77.5%)
Hepatitis B virus surface antigen (positive)	49 (18.1%)
Child–Pugh classification grade (A/B/C)	214/55/2
Ascites (presence)	49 (18.1%)
Serum total bilirubin (mg/dl)	0.88 (0.16–3.18)
Serum albumin (g/dl)	3.72 (2.33–4.88)
Prothrombin time (%)	83 (36–197)
AST (IU/L)	55 (14–180)
ALT (IU/L)	51 (10–189)
Platelet counts (10^9 cells/L)	103 (34–424)
Tumour-related factors	
Number of tumours	
1/2/3	191/47/33
Size of the largest tumour (mm)	21 (8–135)
Portal invasion (presence)	17 (6.3%)
DCP (mAU/ml)	27 (0–66 700)
AFP (ng/ml)	21 (0.6–137 560)

*Values are presented as median (range) unless otherwise noted.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des- γ-carboxy prothrombin.

Recurrence and survival rates

Recurrence was observed in 169 patients (62.4%). The recurrence rates were 27.9, 65.1 and 86% at 1, 3 and 5 years respectively (Fig. 2). The patients were re-treated by resection and local ablation in 14 patients and 84 patients respectively. The survival rates of the patients were 97.3, 76.4 and 55.6% at 1, 3 and 5 years respectively (Fig. 3).

Time-fixed analysis

Among the 13 variables examined, predisposing factors for recurrence were low serum albumin (≤3.5 g/dl) and multiple tumour number by time-fixed univariate analysis. Multivariate analysis revealed that low serum albumin (HR = 1.47, 95% CI = 1.07–2.01, $P = 0.02$) and multiple tumour number (HR = 2.04, 95% CI = 1.46–2.84, $P < 0.01$) were also significant predisposing factors for recurrence. Neither AFP nor DCP was a significant predisposing factor in the time-fixed analyses (Table 2).

Time-dependent analysis

Six parameters were measured repeatedly after the treatment. The total number of samples used in the time-dependent covariate analysis was 12 013, and the number

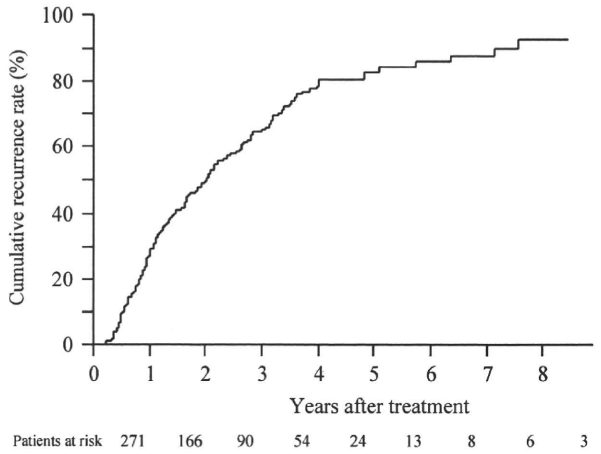

Fig. 2. Kaplan–Meier estimation of the cumulative recurrence rate of consecutive 271 patients which were 27.9, 65.1 and 86% at 1, 3 and 5 years respectively.

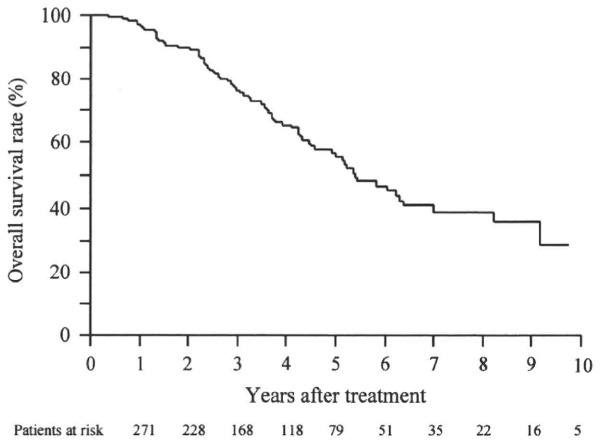

Fig. 3. Kaplan–Meier estimation of the overall survival rate of consecutive 271 patients which were 97.3, 76.4 and 55.6% at 1, 3 and 5 years respectively.

of missing data points was 763 (5.97%). The time-dependent univariate analysis revealed that high DCP (≥40 mAU/ml), high AFP (≥100 ng/ml), high total bilirubin (≥2 mg/dl), low serum albumin (≤3.5 g/dl) and high ALT (≥40 IU/L) were the predisposing factors for recurrence. Among these parameters, high DCP (HR = 2.33, 95% CI = 1.61–3.39, $P<0.01$), high AFP (HR = 2.01, 95% CI = 1.3–3.35, $P<0.01$) and high ALT (HR = 1.52, 95% CI = 1.1–2.1, $P<0.01$) were also the significant predisposing factors for recurrence in multivariate analysis (Table 3).

Discussion

There have been several studies dealing with the risk factors for HCC recurrence (5, 6, 28–32). Although the factors were not identical in these studies because of differences in the patients enrolled and the cutoff values of the factors, most of them were classified into two categories: tumour factors and background liver factors. The predisposing factors for recurrence before the initial treatment determined in our study also consisted of a tumour factor (tumour number) and a background liver factor (serum albumin), and are not inconsistent with factors identified in the previous reports (5, 6, 28–32). According to published reports, many physicians focus on these factors and follow up patients with HCC. However, in most of the reported studies, analysis of variables recorded at the time of HCC treatment (time-fixed analysis) was performed, which predicts the patients' outcome with factors before treatments or at the first HCC recurrence (33, 34).

Analysis of dynamic variables recorded during follow-up, after HCC therapy (time-dependent covariate analysis), can weigh repeatedly measured factors and elucidate the key factors that must be focused on during follow-up. Using this method, we identified AFP and DCP, two major tumour markers of HCC, as the major

Table 2. Time-fixed analysis at initial treatment for hepatocellular carcinoma recurrence

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Age (≥70 years)	1.04	0.73–1.84	0.82			
Gender (male)	1.17	0.84–1.64	0.34			
Antibody to hepatitis C virus (positive)	1.32	0.90–1.93	0.15			
HBsAg (positive)	0.83	0.55–1.25	0.36			
Ascites (present)	1.38	0.89–2.12	0.16			
Serum total bilirubin (≥2 mg/dl)	1.17	0.48–2.87	0.74			
Serum albumin (≤3.5 g/dl)	1.57	1.15–2.15	<0.01	1.47	1.07–2.01	0.02
ALT (≥40 IU/L)	1.21	0.87–1.68	0.24			
Platelet counts (≤100 × 10^9 cells/L)	1.33	0.98–1.80	0.07			
Size of tumour (>20 mm)	1.18	0.87–1.60	0.29			
Number of tumours (multiple)	2.13	1.53–2.97	<0.01	2.04	1.46–2.84	<0.01
DCP (≥40 mAU/ml)	1.14	0.83–1.55	0.43			
AFP (≥100 ng/ml)	1.32	0.93–1.87	0.12			

This analysis is based on data collected at the time of initial therapy.

AFP, α-fetoprotein; ALT, alanine aminotransferase; HR, hazard ratio; 95% CI, 95% confidence interval; DCP, des-γ-carboxy prothrombin.

Table 3. Time-dependent analysis for hepatocellular carcinoma recurrence

	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P* value
DCP (≥40 mAU/ml)	2.46	(1.72–3.51)	<0.01	2.33	(1.61–3.39)	<0.01
AFP (≥100 ng/ml)	2.4	(1.52–3.77)	<0.01	2.01	(1.3–3.35)	<0.01
Serum total bilirubin (≥2 mg/dl)	1.99	(1.2–3.31)	<0.01	1.6	(.94–2.75)	0.09
Serum albumin (≤3.5 g/dl)	1.3	(0.95–1.76)	<0.01	1.05	(0.75–1.48)	0.77
Platelet counts (≤100 × 10^9 cells/L)	1.07	(0.79–1.45)	0.67	0.87	(.62–1.21)	0.41
ALT (≥40 IU/L)	1.55	(1.13–2.12)	<0.01	1.52	(1.1–2.1)	0.01

AFP, α-fetoprotein; ALT, alanine aminotransferase; HR, hazard ratio; 95% CI, 95% confidence interval; DCP, des-γ-carboxy prothrombin.

predisposing factors for recurrence. These factors have been known to be useful for the prediction or detection of the occurrence and recurrence of HCC. Aoyagi *et al.* (30) reported that simultaneous determinations of AFP and DCP are useful for monitoring recurrence in patients with HCC after treatment because they increase independently. Oka *et al.* reported that patients who had AFP levels of 20 ng/ml or more, who exhibited transient increases in AFP or both should be treated as a super-high-risk group for HCC (29). In our study, the relative risks of elevated AFP and DCP during follow-up were calculated as 2.40 and 2.46 respectively. These ratios are higher than those of other factors such as serum bilirubin, albumin and ALT, as determined by the time-dependent analysis. They are also higher than those of any other factor in the time-fixed analysis. Therefore, we should ensure the measurement of both AFP and DCP periodically after treatments, as well as examine these super-high-risk patients with imaging modalities. The repetitive measurement might result in the increase of the patients who could receive the second curative treatment.

Alanine aminotransferase is known to be correlated with the inflammatory activity of hepatitis and was found to be a predisposing factor for the recurrence of HCC in this time-dependent analysis; however, few studies have demonstrated the importance of ALT in time-fixed analysis. The importance of the repeated measurement of ALT has been reported in a cohort study conducted by Tarao *et al.* (35). In this study, HCV-associated cirrhotic patients with a high average ALT level showed a rapid development of HCC after surgical resection of HCC. Although the report is not on a randomized study, our finding for the time-dependent analysis supported the conclusion of this study. ALT level fluctuates, and so repeated measurement is necessary to correctly evaluate the effect of elevated ALT.

Chronologically measured data is important to understand the clinical course. Chen *et al.* reported a predictive survival model of HCC with time-dependent prognostic factors and showed good predictive validity (36, 37). The factors that they used for constructing the model were AFP, AST, ALT, bilirubin, albumin, alkaline phosphatase and prothrombin time. Interestingly, these factors did not coincide with prognostic factors that were reported by time-fixed analysis as we observed in our study. From this point of view, it appears that the time-fixed analysis is not sufficient to determine the factors that should be measured during follow-up, and time-dependent analysis is indispensable.

In this study, we demonstrated the importance of chronological measurements of AFP, DCP and ALT to predict HCC recurrence by a time-dependent covariate analysis. Further examination is necessary to construct a recurrence model using these factors and to achieve the early detection of recurrence and improve patients' survival.

References

1. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907–17.
2. Colombo M, De Franchis R, Del Ninno E, *et al.* Hepatocellular carcinoma in Italian patients with cirrhosis. *N Engl J Med* 1991; **325**: 675–80.
3. 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**: 1797–801.
4. Williams R, Melia WM, Johnson PJ. Serum alpha-foetoprotein in hepatocellular carcinoma – value in diagnosis, and prognosis. *Ann Acad Med Singapore* 1980; **9**: 245–50.
5. Koike Y, Shiratori Y, Sato S, *et al.* Risk factors for recurring hepatocellular carcinoma differ according to infected hepatitis virus-an analysis of 236 consecutive patients with a single lesion. *Hepatology* 2000; **32**: 1216–23.
6. Arii S, Tanaka J, Yamazoe Y, *et al.* Predictive factors for intrahepatic recurrence of hepatocellular carcinoma after partial hepatectomy. *Cancer* 1992; **69**: 913–9.
7. Izumi R, Shimizu K, Ii T, *et al.* Prognostic factors of hepatocellular carcinoma in patients undergoing hepatic resection. *Gastroenterology* 1994; **106**: 720–7.
8. Ko S, Nakajima Y, Kanehiro H, *et al.* Influence of associated viral hepatitis status on recurrence of hepatocellular carcinoma after hepatectomy. *World J Surg* 1996; **20**: 1082–6.

9. Shirabe K, Takenaka K, Taketomi A, *et al.* Postoperative hepatitis status as a significant risk factor for recurrence in cirrhotic patients with small hepatocellular carcinoma. *Cancer* 1996; **77**: 1050–5.
10. Makuuchi M, Kokudo N, Arii S, *et al.* Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. *Hepatol Res* 2008; **38**: 37–51.
11. Brown JE, Cook RJ, Major P, *et al.* Bone turnover markers as predictors of skeletal complications in prostate cancer, lung cancer, and other solid tumors. *J Natl Cancer Inst* 2005; **97**: 59–69.
12. Preston DM, Bauer JJ, Connelly RR, *et al.* Prostate-specific antigen to predict outcome of external beam radiation for prostate cancer: Walter Reed Army Medical Center experience, 1988–1995. *Urology* 1999; **53**: 131–8.
13. Fisher B, Anderson S, Fisher ER, *et al.* Significance of ipsilateral breast tumour recurrence after lumpectomy. *Lancet* 1991; **338**: 327–31.
14. Gail MH. Evaluating serial cancer marker studies in patients at risk of recurrent disease. *Biometrics* 1981; **37**: 67–78.
15. Nouso K, Matsumoto E, Kobayashi Y, *et al.* Risk factors for local and distant recurrence of hepatocellular carcinomas after local ablation therapies. *J Gastroenterol Hepatol* 2008; **23**: 453–8.
16. Makuuchi M, Kokudo N. Clinical practice guidelines for hepatocellular carcinoma: the first evidence based guidelines from Japan. *World J Gastroenterol* 2006; **12**: 828–9.
17. Okuda K, Ohtsuki T, Obata H, *et al.* Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. *Cancer* 1985; **56**: 918–28.
18. The Cancer of the Liver Italian Program (CLIP) investigators. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients. *Hepatology* 1998; **28**: 751–5.
19. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329–38.
20. Kudo M, Chung H, Haji S, *et al.* Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. *Hepatology* 2004; **40**: 1396–405.
21. Tateishi R, Yoshida H, Shiina S, *et al.* Proposal of a new prognostic model for hepatocellular carcinoma: an analysis of 403 patients. *Gut* 2005; **54**: 419–25.
22. Jorgensen B, Fischer E, Ingeberg S, *et al.* Decreased blood platelet volume and count in patients with liver disease. *Scand J Gastroenterol* 1984; **19**: 492–6.
23. Pohl A, Behling C, Oliver D, *et al.* Serum aminotransferase levels and platelet counts as predictors of degree of fibrosis in chronic hepatitis C virus infection. *Am J Gastroenterol* 2001; **96**: 3142–6.
24. Tarao K, Takemiya S, Tamai S, *et al.* Relationship between the recurrence of hepatocellular carcinoma (HCC) and serum alanine aminotransferase levels in hepatectomized patients with hepatitis C virus-associated cirrhosis and HCC. *Cancer* 1997; **79**: 688–94.
25. Peng YC, Chan CS, Chen GH. The effectiveness of serum alpha-fetoprotein level in anti-HCV positive patients for screening hepatocellular carcinoma. *Hepatogastroenterology* 1999; **46**: 3208–11.
26. Liaw YF, Tai DI, Chen TJ, Chu CM, Huang MJ. Alpha-fetoprotein changes in the course of chronic hepatitis: relation to bridging hepatic necrosis and hepatocellular carcinoma. *Liver* 1986; **6**: 133–7.
27. Bellet DH, Wands JR, Isselbacher KJ, Bohuon C. Serum alpha-fetoprotein levels in human disease: perspective from a highly specific monoclonal radioimmunoassay. *Proc Natl Acad Sci USA* 1984; **81**: 3869–73.
28. Ikeda K, Saitoh S, Tsubota A, *et al.* Risk factors for tumor recurrence and prognosis after curative resection of hepatocellular carcinoma. *Cancer* 1993; **71**: 19–25.
29. Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. *Hepatology* 1994; **19**: 61–6.
30. Aoyagi Y, Oguro M, Yanagi M, *et al.* Clinical significance of simultaneous determinations of alpha-fetoprotein and des-gamma-carboxy prothrombin in monitoring recurrence in patients with hepatocellular carcinoma. *Cancer* 1996; **77**: 1781–6.
31. Ikeda K, Saitoh S, Arase Y, *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; **29**: 1124–30.
32. Fujiyama S, Tanaka M, Maeda S, *et al.* Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma. *Oncology* 2002; **62**: 57–63.
33. Morimoto M, Numata K, Nozaki A, Tanaka K. Prognosis following non-surgical second treatment in patients with recurrent hepatocellular carcinoma after percutaneous ablation therapy. *Liver Int* 2009; **29**: 443–8.
34. Hosaka T, Ikeda K, Kobayashi M, *et al.* Predictive factors of advanced recurrence after curative resection of small hepatocellular carcinoma. *Liver Int* 2009; **29**: 736–42.
35. Tarao K, Rino Y, Takemiya S, *et al.* Close association between high serum ALT and more rapid recurrence of hepatocellular carcinoma in hepatectomized patients with HCV-associated liver cirrhosis and hepatocellular carcinoma. *Intervirology* 2000; **43**: 20–6.
36. Chen LS, Yen MF, Wu HM, *et al.* Predictive survival model with time-dependent prognostic factors: development of computer-aided SAS Macro program. *J Eval Clin Pract* 2005; **11**: 181–93.
37. Liao CS, Yang KC, Yen MF, Hsiu-Hsi Chen T. Time-varying predictors for clinical surveillance of small hepatocellular carcinoma. *Cancer J* 2005; **11**: 226–33.

Original Paper

Application of Radiofrequency Ablation for the Treatment of Metastatic Liver Cancers

Kazuhiro Nouso[1,2], Yoshiyuki Kobayashi[2], Shinichiro Nakamura[2], Shuji Uematsu[3], Kunihiro Shiraga[3], Shouta Iwadou[3], Yasuyuki Araki[3], Hideaki Taniguchi[4], Hironori Tanaka[4], Nobuyuki Toshikuni[5], Toshihiko Kaneyoshi[5], Hiroshi Ikeda[5], Shinichi Fujioka[6], Toshiya Osawa[6], Yoshiaki Iwasaki[2], Hidenori Shiraha[2], Kazuhide Yamamoto[2]

[1]Department of Molecular Hepatology; [2]Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences; [3]Department of Internal Medicine, Hiroshima City Hospital; [4]Department of Internal Medicine, Tsuyama Central Hospital; [5]Department of Internal Medicine, Kurashiki Central Hospital; [6]Department of Internal Medicine, Okayama Saiseikai General Hospital. Japan

Corresponding Author: Kazuhiro Nouso, MD, PhD, 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
Tel: +0862357219, Fax: +0862255991, E-mail: nouso@cc.okayama-u.ac.jp

ABSTRACT

Background/Aims: The aim of this study is to elucidate the effectiveness of radiofrequency ablation (RFA) for the treatment of metastatic liver cancers.

Methodology: From 74 patients with metastatic liver cancers treated by RFA, 40 patients including 23 colon cancer who had received curative resection of the primary tumor were analyzed.

Results: Recurrence of the tumor was observed in 29 (72.5%) patients. The most prevalent site of recurrence was the liver in both colon cancer (10/15, 66.7%) and non-colon cancer patients (12/14, 85.7%). Among the recurrence in the liver, the rate of intrahepatic distant recurrence (recurrence outside of the RFA-treated segment) was high in both colon cancer (55.6%) and non-colon cancer patients (69.0%). Local recurrence (recurrence at the RFA-treated segment) rate was low (32.6% and 32.9%, respectively) and none of single tumor less than 2cm in diameter showed local recurrence. The intrahepatic recurrence was single in 67.6% of the patients and 59.1% of the patients were re-treated by RFA.

Conclusions: RFA is a less-invasive method for the treatment of metastatic liver tumors and can be performed repetitively. Although the rate of intra-hepatic distant recurrence and extra-hepatic recurrence was high, good local control can be achieved by RFA.

KEY WORDS: Liver neoplasm; Metastasis; Prognosis; Therapeutics

ABBREVIATIONS: Radiofrequency Ablation (RFA); Hepatocellular Carcinoma (HCC); Ultrasonography (US); Computed Tomography (CT); Magnetic Resonance Imaging (MRI)

INTRODUCTION

Radiofrequency ablation (RFA) is a standard therapy for the treatment of small hepatocellular carcinoma (HCC) (1-3). The application criteria of RFA for the treatment of HCC is HCC less than or equal to 3 cm and less than or equal to 3 tumors. Recently, RFA has been applied to the treatment of metastatic liver tumors (4-15). Although the gold standard for the treatment of metastatic liver tumors is surgical resection (16), it is sometimes avoided because the patient selects not to receive polysurgery, because complications in the patient were too severe to perform an operation, or because the effectiveness of resection of metastatic lesions has not been verified in some cancers. Moreover, efficacy of RFA for the treatment of small metastatic colon cancer has been reported (9, 14, 15), so the less-invasive RFA tends to be applied more frequently for the treatment of metastatic liver tumors. However, recurrence of metastatic liver cancers after local ablation therapy is known to be higher than for that of HCC (10). The recurrence pattern of metastatic liver cancers after RFA might be different from that of HCC and this information is important for planning a treatment strategy; however, few reports have been published. In this study, we analyzed the clinical course of metastatic liver cancers, especially focusing on the recurrence pattern after RFA and evaluated the effectiveness of RFA.

METHODOLOGY

From 74 consecutive patients with metastatic liver cancers treated by RFA between June 2001 and November 2007, 34 patients were excluded because of the presence of residual primary tumor, distant metastasis other than in the liver, or incomplete ablation of the liver tumor. The remaining 40 patients were enrolled in this study. They consisted of 23 colon cancers and 17 non-colon cancers (7 gastric cancers, 3 gastrointestinal stromal cell tumors, 2 esophageal cancers, 2 ovarian cancers, 1 hemangiopericytoma, 1 uterine cancer, and 1 maxillary sinus cancer). Informed consent was obtained from

Hepato-Gastroenterology 2010; 57:117-120

all patients for the use of their clinical data. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki.

Treatments: RFA was performed percutaneously according to the procedure for the treatment of HCC previously described (17). We used a 17-gauge, cooled-tip RF electrode (20cm long with a 2 or 3-cm-long exposed metallic tip; Covidien, Mansfield, MA, USA) under the guidance of ultrasonography (US). Ablation was evaluated by dynamic computed tomography (CT) or magnetic resonance imaging (MRI) after each session and the treatments were deemed to be finished when the size of the ablated area was large enough to cover the pretreatment lesions within a week of the therapy. In cases of incomplete ablation, RFA was repeated until the ablated area met the criteria for complete ablation described above.

Follow up: US, dynamic CT, or MRI were performed at least every 3 months after RFA except in 1 case that was followed up every 6 months. When there were feasible tumor markers such as carcinoembrionic antigen for colon cancer or gastric cancer, the markers were measured simultaneously. A chest X-ray was performed in cases in which recurrence was observed in the liver or otherwise at 6 to 12 months intervals. Local recurrence in the liver was diagnosed via the emergence of a tumor in the same segment where RFA was performed. Intrahepatic distant recurrence was diagnosed by the emergence of a new tumor in the liver that did not meet the criteria for local recurrence.

Statistical analysis: The cumulative survival and recurrence rates after RFA were compared by the Kaplan–Meier method and the differences were evaluated by the log-lank test. The Mann-Whitney test or Fisher's exact test was performed for a comparison of two groups. JMP (version 5.0.1) software packages (SAS Institute, Cary, NC, USA) were used for the analyses and $p < 0.05$ was considered statistically significant.

TABLE 1 Clinical Characteristics of the Patients

	Colon Cancer	Non-colon cancers	Total
Patient number	23	17	40
Sex (male)	13(56.5%)	8(47.1%)	21(52.5%)
Age (years)	67 (43-80)	63 (48-83)	64.5(43-83)
Tumor number (single)	10 (43.5%)	11 (64.7%)	21(52.5%)
Tumor size (mm)	23 (11-45)	15 (10-41)	21(10-45)
Simultaneous occurrence of liver metastasis	4(17.4%)	3(17.6%)	7(17.5%)
Adjuvant Chemotherapy (present)	16 (69.6%)	10 (58.8%)	26 (65.0%)
Mean observation period (days)	492	742	599

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

RESULTS

Clinical Characteristic of the patients: The study was comprised of 21 men and 19 women with a median age of 64.5 years. The characteristics of all patients are reported in **Table 1.** Simultaneous occurrences of liver metastasis with primary tumors were observed in 7 patients (17.5%), and the rest of the liver metastasis was found during follow up. The median period from the treatment of primary tumors to RFA of liver metastasis was 399 days. The median tumor size was 21 mm and 21 patients (52.5%) had a single tumor. The clinical backgrounds were similar between the colon cancer and non-colon cancer patients and no statistical difference was observed. Adjuvant chemotherapy was performed in 26 patients (65.0%) and 7 out of 23 colon cancer patients (30.4%) were treated by FOLFOX or FOLFILI (18, 19). The mean observation period was 599 days. The overall survival rates for the patients were 90.8%, 78.0%, and 57.8% at 1, 2, and 3 years after RFA, respectively. Intraperitoneal bleeding and subcapsular bleeding were both observed in one patient each; however, no other severe complications of RFA were observed among the rest of the patients.

Reasons for choosing RFA: The main reason for choosing RFA was patients' desire to avoid surgical resection or to receive RFA despite the condition of the patient being good enough to receive the operation (n=14, 35.0%). There were some cases in which RFA was chosen by the physician: when the tumor was small enough to be treated by RFA completely and it was better to avoid the risk of surgical resection (n=8, 20.0%), when multiple tumors that were hard to remove curatively by surgery were present (n=7, 17.5%), when the effectiveness of surgical treatment was not established (n=6, 15.0%), or when patient's complications such as chronic respiratory failure or chronic renal failure were too severe to perform surgical resection (n=6, 15.0%).

Recurrence pattern: Recurrence was observed in 29 patients (72.5%, **Table 2**). The major site of recurrence was the liver (n=22: 75.9%), followed by the lungs (n=6: 20.7%), lymph nodes (n=4: 13.8%), and bone (n=3: 10.3%). The liver was the most prevalent site of recurrence in both colon cancers (n=10: 66.7%) and non-colon cancers (n=12: 85.7%).

Most of the recurrences in the liver were observed at a different segment from the primary RFA site (intrahepatic distant recurrence: 63.6%) and the local recurrence rate was 31.8%. None of single tumor less than 2cm in diameter showed local recurrence (0/5, 0%). Simultaneous recurrence (local and distant) in the liver was observed in one patient (4.6%). The distant recurrence rate of colon cancer (55.6% at 3 years) seemed to be lower than that of the non-colon cancers (69.0% at 3 years); however, the difference was not statistically significant (**Figure 1**, p=0.09). The local recurrence rate was almost the same in colon cancers (32.6% at 3 years) and non-colon cancers (32.9% at 3 years) (**Figure 2**, p=0.88). The number of recurrent tumors in the liver was 1 in 14 out of 22

patients (63.6%) and 13 patients (59.1%) were able to be re-treated by RFA.

DISCUSSION

RFA can be used for the treatment of metastatic liver cancer as well as for that of HCC (4-15). RFA is a good method for the treatment of liver cancers because the risks for RFA are lower than those for surgical resection, and the feasibility of repetitive treatment is high. In this study, RFA was performed safely in all patients and two thirds of the recurrent liver tumors could be re-treated by RFA. However, there are several problems for the treatment of metastatic tumors by RFA. As we demonstrated, 31.9% of the recurrences in the liver were observed at the same segment where the primary metastatic tumor treated by RFA was located. If the segment was surgically removed, the recurrence could theoretically be avoided. Enough of a safety margin during ablation might overcome the local recurrence of RFA; however, little information is available concerning this effect. Therefore, we should be careful to choose RFA because of its easy applicability alone.

Meanwhile, one quarter of the recurrences after RFA were observed in distant organs and over two thirds of the recurrences in the liver were intrahepatic distant metastases. This result indicates that the recurrences could not be avoided by achieving good local control alone and this was true even in the colon cancer cases, which are known as a good target for surgical resection (16). From this point of view, adjuvant chemotherapies are mandatory. Recently, new promising regimens for the treatment of colon cancers such as FOLFOX, FOLFIRI, and Bevacizumab have been reported(18, 19). Although the effectiveness of these chemotherapies was examined only in advanced cancers, their effect could be proved in neo-adjuvant or adjuvant chemotherapies in the near future. Consequently, the recurrence rate should decrease and this would also change the advantages and disadvantages of RFA and surgical resection.

Although, there are several reports of RFA treatment for metastatic colon cancers in the liver, application of RFA for the treatment of other metastatic liver cancers has not been well studied except in some rare cases such as neuroendocrine diseases (4). In this study, the recurrence patterns of non-colon cancers were similar to those of colon cancer and no significant difference was observed. Although some cancers were highly metastatic and it is clear that they cannot be a candidate for interventional treatment, there must be a certain population of non-colonic metastatic liver cancers that can be treated by RFA effectively. Further examination is needed to understand the effective target metastatic cancers of RFA.

In conclusion, RFA is a useful method for the treatment of metastatic liver tumors. Although intra-hepatic distant recurrence as well as extra-hepatic recurrence was frequently observed and adjuvant chemotherapy should be considered mandatory, good local control can be achieved by RFA.

TABLE 2 Recurrence Pattern in the Liver

	Colon Cancer	Non-colon	Total
Recurrence rate	15/23 (65.2%)	14/17 (82.4%)	29/40 (72.5%)
Site of recurrence			
Liver	10 (66.7%)	12 (85.7%)	22 (75.9%)
Lung	5 (33.3%)	1 (6.1%)	6 (20.7%)
Lymph node	2 (13.3%)	2 (12.2%)	4 (13.8%)
Bone	1 (6.7%)	2 (12.2%)	3 (10.3%)
Others	1 (6.7%)	3 (21.4%)	4 (13.8%)
Recurrence pattern in the liver			
Local recurrence	4 /10 (40.0%)	3 /12(25.0%)	7 /22(31.8%)
Distant recurrence	5 /10(50.0%)	9 /12(75.0%)	14/22 (63.6%)
Simultaneous (local and distant)	1/10 (10.0%)	0	1 /22(4.6%)
Number of liver tumor (single)	7 (70.0%)	7 (58.3%)	14 (63.6%)
RFA for the recurrence	6 (60.0%)	7 (58.3%)	13 (59.1%)

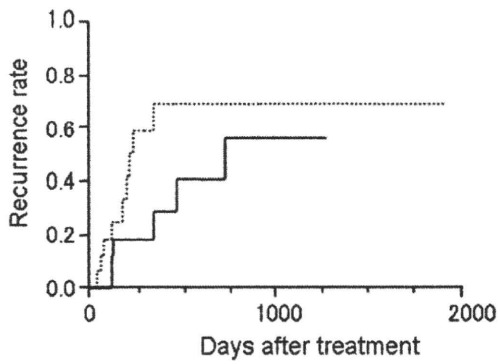

FIGURE 1 Intrahepatic distant recurrence (recurrence outside of the RFA–treated liver segment) of metastatic liver cancers. The recurrence rate of colon cancer (solid line) was lower than that of non–colon cancer (dotted line); however, the difference was not statistically significant (p=0.09). The rates of colon cancer were 28.9%, 40.8, and 55.6% at 1 year, 2, and 3 years, respectively, and the rate of non–colon cancers was 69.0% at 1 year and remained constant until 3 years.

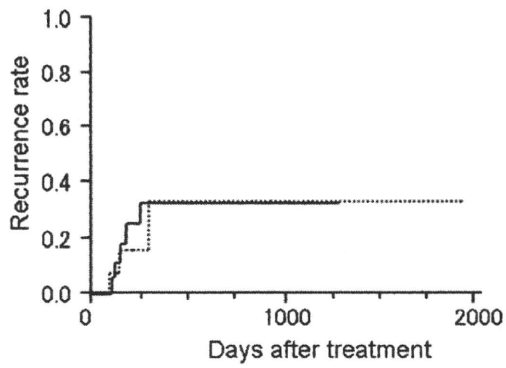

FIGURE 2 Local recurrence (recurrence at the RFA–treated segment) of metastatic liver cancers. No significant difference was observed between colon cancer (solid line) and non–colon cancer (dotted line, p=0.88). All local recurrences were observed in the first year after treatment, and the rates for colon cancer and non–colon cancers at one year were 32.6% and 32.9%, respectively.

REFERENCES

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

Alimentary Pharmacology & Therapeutics

Evolution of prognostic factors in hepatocellular carcinoma in Japan

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.

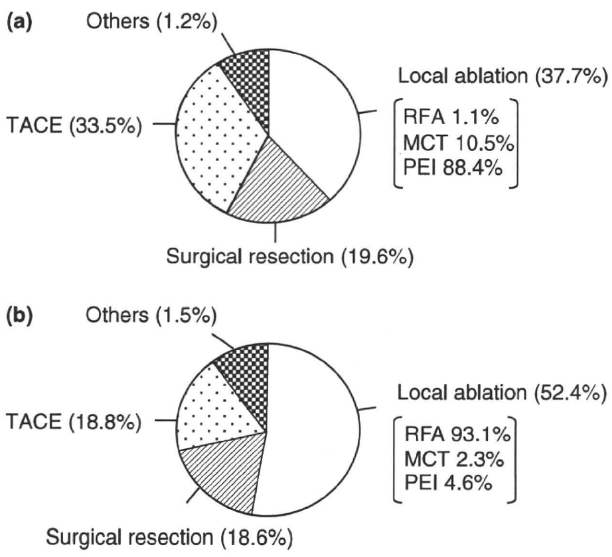

Figure 1. Changes in treatment modalities with time. The percentage of local ablation was 37.7% before December 2000 (a) and increased to 52.4% after January 2001 (b). PEI was popular before 2000; however, RFA was chosen as the standard therapy after 2001. Abbreviations: RFA, radiofrequency ablation;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

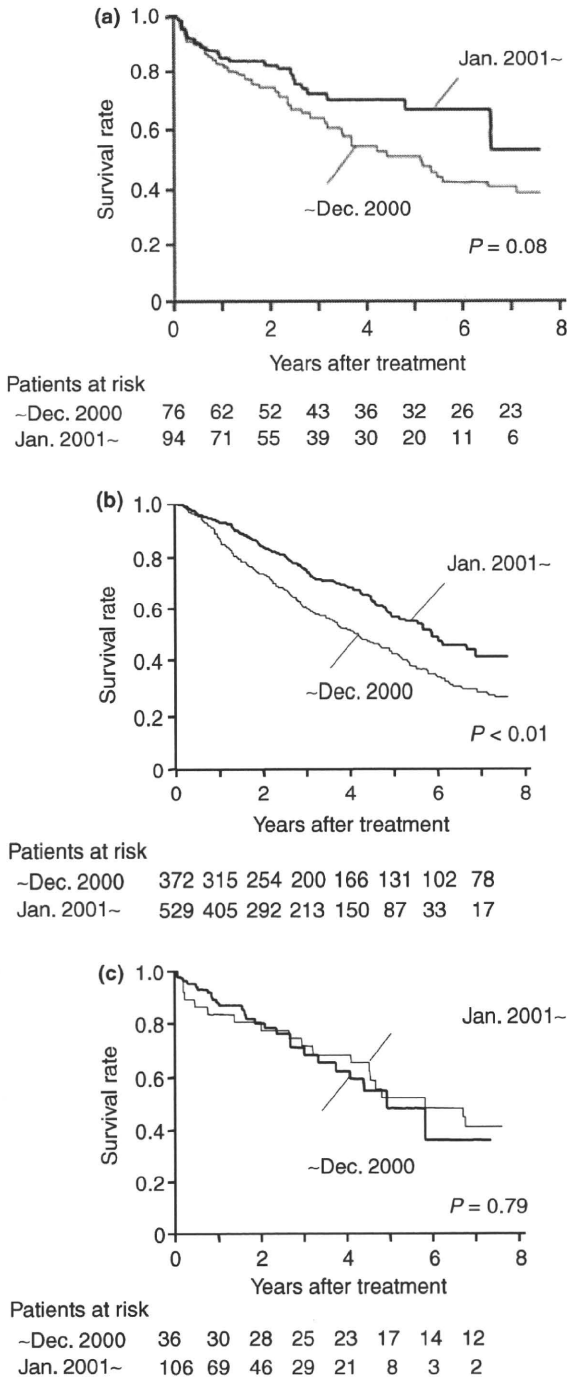

Figure 2. Survival curves of B-HCC (a), C-HCC (b) and NBNC-HCC (c). Note that the survival of C-HCC improved ($P < 0.01$) and a tendency towards improvement was observed in B-HCC ($P = 0.08$); however, no difference was observed for NBNC-HCC ($P = 0.79$). Thin line, HCC patients treated before December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

Int J Clin Oncol
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

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

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

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

Published online: 09 December 2010

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).