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Laparoscopic findings of reddish markings predict hepatocellular carcinoma in patients with hepatitis B virus-related liver disease

Bon Shoji · Fusao Ikeda · Shin-ichi Fujioka · Haruhiko Kobashi · Tetsuya Yasunaka · Yasuhiro Miyake · Hidenori Shiraha · Akinobu Takaki · Kazuhiro Nouso · Yoshiaki Iwasaki · Kazuhide Yamamoto

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Abstract
Background For patients with chronic hepatitis due to hepatitis B virus (HBV), factors predicting hepatocellular carcinoma (HCC) other than high levels of HBV-DNA and alanine aminotransferase (ALT) are needed to prevent HCC development, as many patients with chronic HBV infection fulfill these conditions. The purpose of this study was to clarify factors predictive of HCC development for those patients.
Methods The study was a systematic cohort analysis of 303 consecutive patients with hepatitis B e-antigen, receiving laparoscopic examination for assessment of liver disease. Laparoscopic, histological, and clinical characteristics were investigated as related to HCC development.
Results HCC occurred in 27 patients during a mean follow-up of 8.0 ± 5.0 years, at the age of 37–72 years. Significant associations with HCC development were shown for liver cirrhosis, histological activity grade, reddish markings, and older age. Multivariate analysis revealed that HCC development was strongly associated with older age and male gender ($P = 0.002$ and $P = 0.043$, respectively). HCC occurred more frequently in patients of age ≥30 years even with early stage than in patients of age <30 years ($P = 0.031$). Severe reddish markings, a laparoscopic finding of widespread parenchymal destruction, were highly associated with HCC development in patients of age ≥30 years at diagnosis (odds ratio = 1.67, $P = 0.034$), while histological activity grade and ALT level were not ($P = 0.075$ and $P = 0.69$, respectively).
Conclusions HCC development is associated with older age, male gender, and liver cirrhosis. Reddish markings, rather than histological activity or ALT level, can be useful to predict HCC for HBV patients of age ≥30 years.

Keywords Hepatitis B virus · Hepatocellular carcinoma · Laparoscopy

Abbreviations

HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
ALT	Alanine aminotransferase
HCV	Hepatitis C virus
AST	Aspartate aminotransferase

Introduction

Hepatitis B virus (HBV) is distributed worldwide, and 400 million people suffer from chronic hepatitis B infection [1]. Hepatocellular carcinoma (HCC) and liver failure are frequent among patients with HBV infection. The incidence

B. Shoji · F. Ikeda (✉) · H. Kobashi · T. Yasunaka · Y. Miyake · H. Shiraha · A. Takaki · K. Nouso · Y. Iwasaki · K. Yamamoto
Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Okayama 700-8558, Japan
e-mail: fikeda@md.okayama-u.ac.jp

F. Ikeda · Y. Miyake · K. Nouso · K. Yamamoto
Department of Molecular Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

S. Fujioka
Department of Internal Medicine, Okayama Saiseikai General Hospital, Okayama, Japan

of HCC development is estimated at 0.8% annually, approximately 100-fold higher than the rate among uninfected people. Half a million patients die of liver-related causes every year [2]. Several studies of the prognosis of HBV have shown that persistent elevation of HBV-DNA and alanine aminotransferase (ALT) in serum are highly associated with rapid disease progression and HCC development [3, 4]. Host factors such as age, gender, and alcohol intake, and viral factors including hepatitis B e-antigen (HBeAg) and HBV genotype have been implicated as important contributors to disease progression. In Japan, HBV genotype C is predominant over other genotypes, and most HBV patients with chronic hepatitis have been infected perinatally or during early childhood [5]. Recent reports have indicated that HBV genotype C is related to poor outcome of slower HBeAg seroconversion [6], earlier disease progression, and more frequent HCC development [7].

Good control of viral replication with nucleoside analogues can decrease liver inflammation and reduce the risk of poor outcomes [8]. Such drugs may work, in the short term at least, for most patients in the immune-active phase of chronic HBV infection. However, benefits for long-term survival have not been well defined. Some patients in young or middle age hesitate to use these drugs due to the possibility of drug resistance and the high cost for medication for life-long use. The presence of HBeAg often indicates active viral replication, and high levels of ALT in the immune-active phase; many patients with HBeAg are thus suitable candidates for use of nucleoside analogues. Predictors for rapid progression to liver cirrhosis and high risk of HCC development should be more clearly defined, to facilitate the selection of HBeAg-positive patients who should be treated immediately with nucleoside analogues.

Laparoscopy provides wide and precise observation of the liver surface. Kalk [9, 10] reported morphological progression from acute hepatitis to cirrhosis. Laparoscopic observation with liver biopsy is considered the most accurate method of evaluating liver cirrhosis [11–14]. Besides usefulness in evaluating present disease progression, direct observation of the liver surface can provide a large amount of information on disease activity, capsular structural changes, and small lesions on the surface, which can be difficult or impossible to detect on ultrasonography (US) or computed tomography (CT). Studies of patients with hepatitis C virus (HCV) have proposed the importance of laparoscopic examination and have noted that irregular regenerative nodules, degree of regenerative nodules, and atrophic right lobe can be observed clearly by laparoscopy, and also that those findings represent independent risk factors for HCC development [15, 16]. Associations with laparoscopic features have not been well defined for HBV patients with regard to HCC development.

The purpose of this study was to clarify useful predictive factors of HCC development for HBV patients with HBeAg, by evaluating laparoscopic features, clinical characteristics, and histology with regard to the development of HCC. We reveal that liver cirrhosis, older age, male gender, and a laparoscopic feature of reddish markings were strongly associated with HCC development, and propose the importance of laparoscopic examination to evaluate the risk of HCC development.

Patients and methods

Patients

This study was a systematic cohort analysis of 303 consecutive patients with HBeAg, and who underwent laparoscopic examination and liver biopsy for the assessment of chronic liver injury at Okayama University Hospital between 1982 and 2002. Presence of HCC was excluded in all patients by imaging examinations with abdominal ultrasonography and computed tomography and by showing normal values of alpha-fetoprotein in serum at the time of diagnosis. Patients suffering from acute hepatitis due to HBV, those with serum positivity for anti-HCV antibodies, and those with daily ethanol intake >75 g were excluded from the study. The study was performed in accordance with the Helsinki Declaration, and all protocols were approved by the ethics committees of the involved institutes. All patients provided informed consent before enrolment into the study.

Scoring of liver function by using laboratory parameters

In order to estimate the usefulness of laboratory parameters to assess liver function, we selected five conventional parameters, and evaluated the score based on these values with histological fibrosis stage. These parameters were scored according to the normal ranges in our institutes as follows: prothrombin time (0, >80%; 1, ≤80%); platelet count (0, $>15 \times 10^4/mm^3$; 1, $\leq 15 \times 10^4/mm^3$); serum level of albumin (0, >3.9 g/dl; 1, ≤3.9 g/dl); serum level of total bilirubin (0, <1.2 mg/dl; 1, ≥1.2 mg/dl); and the ratios of aspartate aminotransferase (AST) and ALT (0, <1.0; 1, ≥1.0).

Histological evaluation

Stage of histological fibrosis and grade of activity were assigned by two pathologists according to the criteria of Desmet et al. [17]. All biopsy specimens were obtained under laparoscopic guidance and were more than 1.5 cm

long and 2 mm wide. The amount of obtained material was therefore adequate for histological evaluation.

Laparoscopic examination

We selected the following six features for analysis, because these are routinely used for evaluation of disease progression and activity: surface irregularity, whitish markings, vascular proliferation, reddish markings, patchy markings, and fat deposition [18–21]. Surface irregularity was evaluated, based on depression and nodular formation, and classified into three stages: S1, normal or early stage; S2, advanced, pre-cirrhotic stage; and S3, cirrhotic stage. Reddish markings were scored according to location, distribution, and color tone of the markings. Whitish markings were defined with their location. These features were assessed as mild or severe based on the total scores as in Table 1. As for vascular proliferation, dilated peripheral portal veins are often observed on liver surface of the patients with chronic hepatitis, and small arteries may become visible when the disease has progressed. We graded dilated peripheral portal veins as mild and proliferation of small arteries as severe for vascular proliferation. These classifications have been used since Shimada et al. [18] reported their usefulness in 1971 to evaluate disease activity and to predict disease progression for chronic hepatitis. Several reports from different institutes have proposed similar classifications by using these features, and revealed their importance for evaluation of disease progression [16, 22, 23]. Final laparoscopic findings were evaluated independently by three experienced hepatologists (S.F., B.S., and K.Y.), and discussed for final diagnosis. Figure 1 shows typical laparoscopic features of the liver surface.

Table 1 Laparoscopic evaluations of reddish markings and whitish markings

Item	Definition	Score
Reddish markings		
Location	Periportal	1
	Pericentral	1
	Multilobular	2
Distribution	Localized	1
	Sparse	2
	Dense	3
Tone of color	Indistinct	1
	Common	2
	Hemorrhagic	3
Diagnostic classification		
None		0 points
Mild reddish marking		<5 points
Severe reddish marking		≥5 points
Whitish markings		
Location	Spotted	1
	Asteroidal	2
	Network-like	2
Diagnostic classification		
None		0 points
Mild whitish marking		<2 points
Severe whitish marking		≥2 points

Follow-up

All patients received medical check-ups with blood examinations every 2–3 months, and abdominal US or CT every 6 months at least as recommended [24, 25]. Patients who had not visited our hospital in the previous 6 months were contacted by letter or telephone and asked to provide details of recent medications by questionnaires. If they visited other hospitals, we also asked them about the results of any imaging studies. For cases in which the patient had died, the date and cause of death were recorded. No patients were treated with nucleoside analogues during follow-up.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) or median (range). Patient laboratory data and laparoscopic findings were compared with histological findings using the Kruskal–Wallis test and canonical correlation analysis. Proportional hazards models were utilized to estimate the effects of patient characteristics on HCC development. Incidence rates of HCC were estimated by using the Kaplan–Meier method, and compared with the log-rank test. A value of $P < 0.05$ was considered significant. Statistical analysis was performed with JMP software (SAS Institute, Cary, NC).

Results

Patient characteristics

Table 2 lists the clinical characteristics of patients enrolled in this study. Mean age of patients was 34 ± 11 years, and 232 patients were male (76.6%). Of the patients, 71.6% had some family history of liver disease. In order to estimate the usefulness of laboratory parameters to assess liver function, we selected five conventional parameters, and compared the scores based on these values with histological fibrosis stage (Fig. 2a). Surprisingly, only half of the patients with a total score of 0 (49.1%), representing completely normal in this scoring system, were histologically defined as early stage

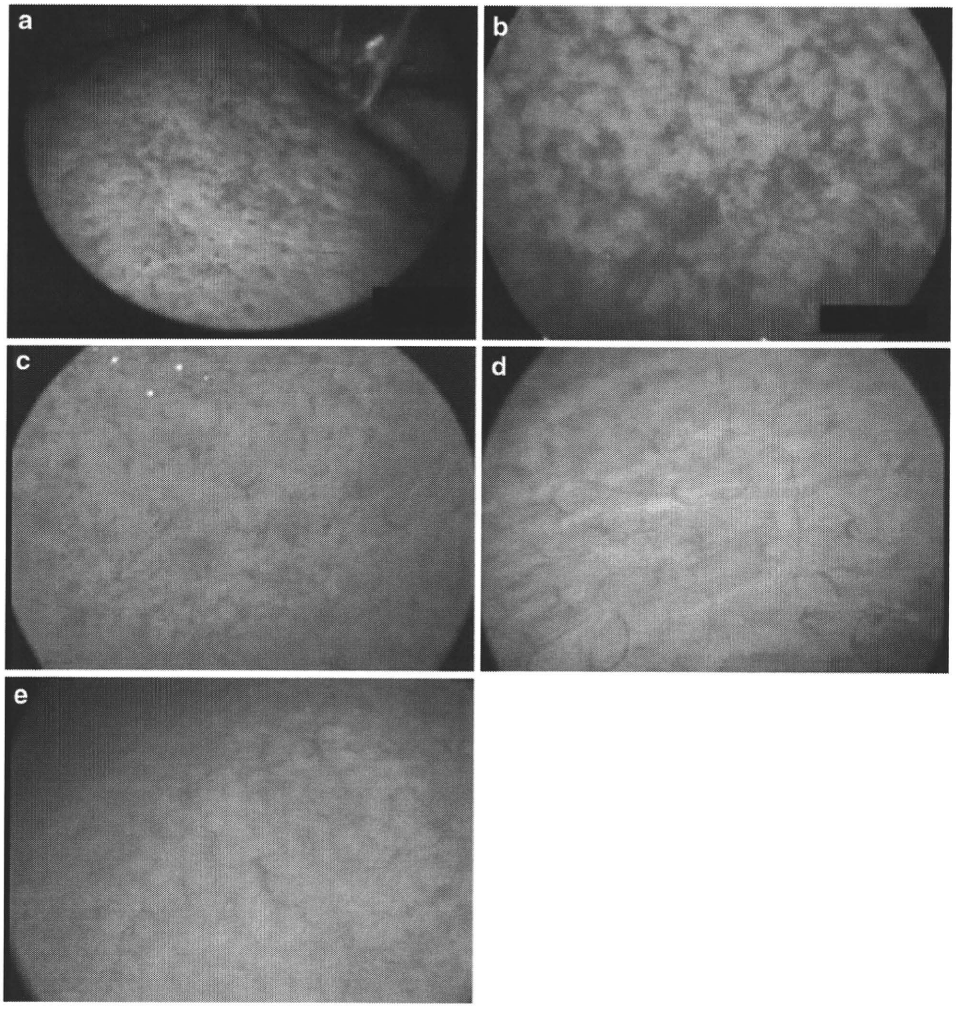

Fig. 1 Laparoscopic features of the patients with chronic viral hepatitis. Figures show typical pictures of laparoscopic features; laparoscopy of severe reddish markings, showing advanced surface irregularity with densely distributed reddish markings (**a**), closer view of severe reddish markings in hemorrhagic color which are multilobularly located (**b**), closer view of mild reddish markings, showing common redness in periportal areas (**c**), laparoscopy of vascular proliferation (**d**), and laparoscopy of normal liver (**e**)

Table 2 Patient characteristics at the time of diagnosis ($N = 303$)

Age at diagnosis (years)	34 ± 11[b]
Gender (female/male)	71/232
Family history of liver disease	217 (71.6%)
History of blood transfusion	14 (4.6%)
Liver histology	
Fibrosis stage (1/2/3/4)[a]	92/90/101/20
Activity grade (1/2/3)[a]	104/135/64
Laboratory data at diagnosis	
AST (IU/l)	91 ± 73[b]
ALT (IU/l)	156 ± 142[b]
Total bilirubin (mg/dl)	0.87 ± 0.53[b]
Albumin (g/dl)	4.2 ± 0.4[b]
Platelet count ($\times 10^4$/mm^3)	18 ± 6[b]

AST aspartate aminotransferase, *ALT* alanine aminotransferase

[a] Histological stage classified according to Desmet et al. [17]

[b] Mean ± SD

(fibrosis stage 0 or 1), and 20.5% were advanced, at the pre-cirrhotic or cirrhotic stage (fibrosis stage 3 or 4). These results indicate the necessity for liver biopsy, as conventional laboratory parameters cannot distinguish patients in the early stage from those in the advanced stages, although total scores of laboratory data correlated significantly with stages of histological fibrosis ($R = 0.46$, $P < 0.0001$, canonical correlation analysis). In terms of activity grades, mean ALT levels in patients were very high (156 ± 142 IU/l), and 51.9% of patients with histological grade A1 showed ALT levels ≥80 IU/l (Fig. 2b). ALT levels displayed weak associations with histological activity grade ($R = 0.14$, $P = 0.013$).

Laparoscopic findings at the time of diagnosis

Table 3 provides a summary of laparoscopic features. Frequencies were calculated for each group of surface

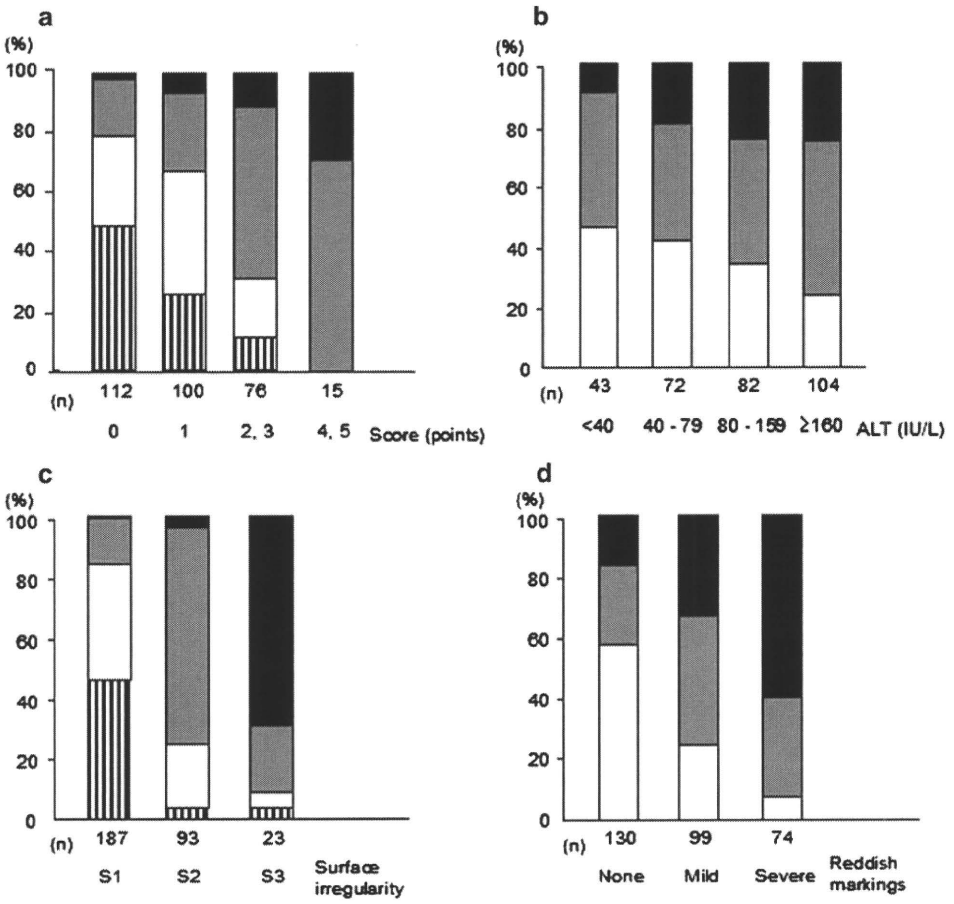

Fig. 2 Comparisons of histology, laboratory parameters, and laparoscopic findings. Histological fibrosis stage was compared with total scores of the five conventional parameters related to liver function with significant correlations ($R = 0.46$, $P < 0.0001$, canonical correlation analysis, **a**): fibrosis stage 1, *striped*; stage 2, *open*; stage 3, *gray*; and stage 4, *black*. Significantly high correlations were also shown between histological fibrosis stage and laparoscopic surface irregularity ($R = 0.66$, $P < 0.0001$, **c**): fibrosis stage 1, *striped*; stage 2, *open*; stage 3, *gray*; and stage 4, *black*. As for the activity, alanine aminotransferase (ALT) levels were divided into four groups and compared with histological activity grade, showing significant associations ($R = 0.14$, $P = 0.013$, **b**): A1, *open*; A2, *gray*; A3, *black*. Correlations between histological activity grade and reddish markings were significant as shown in **d** ($R = 0.45$, $P < 0.0001$): A1, *open*; A2, *gray*; and A3, *black*

Table 3 Summary of laparoscopic features of HBV patients

	Surface irregularity[a]		
	S1 ($n = 187$)	S2 ($n = 93$)	S3 ($n = 23$)
Reddish markings	89 (48%)	69 (74%)	15 (65%)
Severe reddish markings	34 (18%)	34 (37%)	6 (26%)
Whitish markings	51 (27%)	22 (24%)	3 (13%)
Severe whitish markings	34 (18%)	12 (13%)	2 (9%)
Vascular proliferation	160 (86%)	68 (73%)	20 (87%)
Severe vascular proliferation	110 (59%)	57 (61%)	15 (65%)
Patchy markings	28 (15%)	67 (72%)	2 (9%)
Fat deposition	46 (25%)	25 (27%)	11 (48%)

[a] Surface irregularity, classified in three stages: S1, normal or early stage; S2, advanced, pre-cirrhotic stage; and S3, cirrhotic stage

irregularity. Reddish markings and patchy markings were frequently observed in S2 (74 and 72%, respectively, $P < 0.001$ each). Vascular proliferation was observed less in S2 (73%) than in S1 (86%) or S3 (87%, $P = 0.018$, Kruskal–Wallis tests). Severe vascular proliferation, reflecting proliferation of small arteries, was more frequently observed in S3 than S1 or S2, although this increase was not statistically significant ($P = 0.84$). Whitish markings tended to be less frequent, and fat deposition more frequent in S3 than in S1 or S2, but no significant differences were identified ($P = 0.31$ and $P = 0.061$, respectively). Correlations between histological fibrosis stage and laparoscopic surface irregularity were significantly strong ($R = 0.71$, $P < 0.0001$, canonical correlation analysis; Fig. 2c). Reddish markings were significantly associated with histological activity grade as shown in Fig. 2d ($R = 0.45$, $P < 0.0001$).

Risks of HCC development

HCC development was evaluated for 250 patients who were observed for ≥1 year. The accumulated observation was 1991 person-years, accounting for 80% of the total potential follow-up. HCC developed in 27 patients during a mean follow-up period of 8.0 ± 5.0 years, at the age of 37–72 years. The incidence of HCC development was estimated as 5.7% at 5 years of follow-up, 13.5% at 10 years, and 20.6% at 15 years (Fig. 3a). Figure 3b shows cumulative rates of HCC development by age, estimated as 1.3% at 40 years old, 12.3% at 50 years old, and 27.2% at

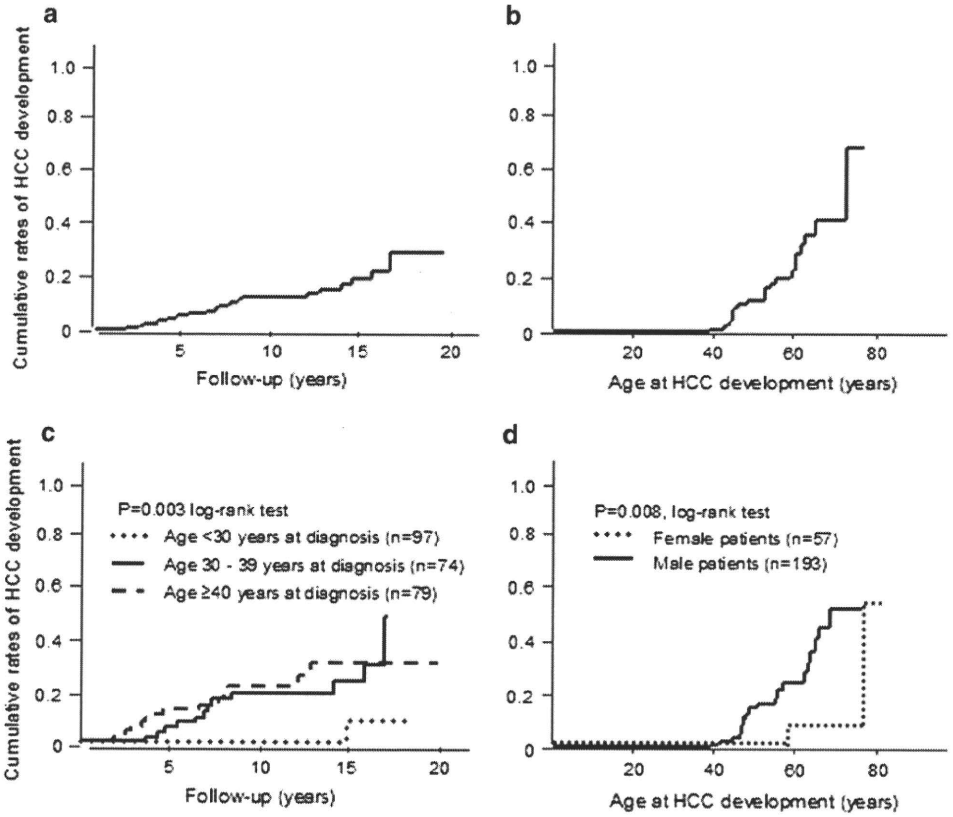

Fig. 3 Cumulative rates of hepatocellular carcinoma (HCC) development. **a** Shows cumulative rate of HCC development as a function of the follow-up period, estimated by the Kaplan–Meier method. The incidence of HCC development was estimated as 5.7% at 5 years of follow-up, 13.5% at 10 years, and 20.6% at 15 years. **b** Shows cumulative rates of HCC development by age, estimated as 1.3% at 40 years old, 12.3% at 50 years old, and 27.2% at 60 years old. When the patients were divided into three groups according to age at diagnosis (<30, 30–39, ≥40 years), there were significant differences in cumulative rates of HCC development among the groups ($P = 0.003$, log-rank test, **c**). Furthermore, **d** shows significant difference in cumulative rates of HCC development between the female patients and the male patients ($P = 0.008$, log-rank test)

60 years old. When the patients were divided into three groups according to age at diagnosis (<30, 30–39, ≥40 years), there were significant differences in cumulative rates of HCC development among the groups ($P = 0.003$, log-rank test; Fig. 3c), especially between the age groups <30, and ≥30 years ($P = 0.0009$, log-rank test). The patient groups of age 30–39 years and age ≥40 years were estimated to have similar risks of HCC occurrence ($P = 0.57$, log-rank test). Furthermore, male patients showed a higher risk of HCC development than females ($P = 0.008$, log-rank test; Fig. 3d), as previously reported [1–7]. Table 4 shows evaluations of clinical characteristics, histology, and laparoscopic features, with regard to HCC development using proportional hazards models. Significant associations with HCC development were shown for liver cirrhosis according to histological fibrosis and laparoscopic surface irregularity, high histological activity grade, laparoscopic severe reddish markings, and older age at diagnosis in univariate analysis. Cumulative risks of HCC development were also estimated by the Kaplan–Meier method (Fig. 4). Severity of reddish markings correlated significantly with risk of HCC development ($P = 0.036$, log-rank test), while histological activity grade did not ($P = 0.054$), suggesting some difference between these two parameters. Multivariate analysis, adjusted with a logistic likelihood ratio test, revealed that HCC development was strongly associated with older age and male gender ($P = 0.002$ and $P = 0.043$, respectively). Laparoscopic surface irregularity was not used for multivariate analysis, due to high correlations of laparoscopic surface irregularity with histological fibrosis stage as shown in Fig. 2c.

Subgroup analysis for HCC development

Next, we studied age difference by dividing patients according to age at diagnosis (<30, and ≥30 years), and our results in proportional hazards models showed that advanced stages according to histological fibrosis stage and surface irregularity were significantly associated with HCC development for patients of age ≥30 years at diagnosis ($P = 0.040$ and $P = 0.016$, respectively; Table 5). Severe

Table 4 Analysis of factors predicting HCC development with the proportional hazards model

Factors	Univariate analysis		Multivariate analysis	
	Odds ratio (range[a])	*P*	Odds ratio (range[a])	*P*
Age at diagnosis (years)	1.06 (1.03–1.10)	<0.001	1.06 (1.02–1.11)	0.002
Gender (male)	3.32 (0.78–14.0)	0.10	4.53 (1.05–19.6)	0.043
Blood transfusion	2.40 (0.56–10.2)	0.24		
Family history of liver disease	1.46 (0.67–3.18)	0.35		
Interferon therapy	0.65 (0.29–1.45)	0.29		
Histological fibrosis stage	1.80 (1.18–2.76)	<0.001	1.21 (0.71–2.07)	0.49
Histological activity grade	1.82 (1.06–3.14)	0.031	1.16 (0.58–2.34)	0.68
AST (≥80 IU/l)	1.32 (0.62–2.83)	0.47		
ALT (≥80 IU/l)	1.06 (0.48–2.37)	0.88		
Surface irregularity	2.45 (1.46–4.09)	<0.001		
Whitish markings	0.77 (0.31–1.90)	0.57		
Vascular proliferation	1.27 (0.48–3.36)	0.64		
Reddish markings	1.66 (1.04–2.65)	0.036	1.45 (0.54–3.90)	0.46
Patchy markings	2.04 (0.96–4.36)	0.065	1.38 (0.57–3.32)	0.48
Fat deposition	1.28 (0.48–3.37)	0.62		

AST aspartate aminotransferase, *ALT* alanine aminotransferase

[a] 95% confidence interval

inflammatory activity with reddish markings also affected HCC development ($P = 0.034$). Therefore we estimated cumulative rates of HCC development, by using the Kaplan–Meier method. Among patients of age ≥30 years at diagnosis, cumulative rates of HCC development were higher in more advanced disease, according to surface irregularity (Fig. 5b, $P = 0.043$, log-rank test). Cumulative rates of HCC development were 37.1% at the 10-year follow-up among the patients in cirrhotic S3 stage, 25.6% among those in pre-cirrhotic S2 stage, and 10.1% among those in S1 stage. Interestingly, the risk of HCC occurrence was significantly higher for those in as early as S1 stage, compared with the patients of age <30 years (Fig. 5c, $P = 0.031$, log-rank test). Actually, none of the patients of age <30 years experienced HCC during the 10-year follow-up. Further subgroup analysis in those of age ≥30 years in each laparoscopic stage could not find any significant factors contributing to HCC development. As for the effects of inflammatory activity on HCC development, significant differences in cumulative rates of HCC development were observed among the patients of age ≥30 years at diagnosis when stratified by reddish markings (Fig. 6, $P = 0.025$, log-rank test), but not by histological activity ($P = 0.087$) or ALT levels ($P = 0.69$).

Discussion

Persistent elevation of HBV-DNA and ALT are associated with rapid disease progression and HCC development [3, 4]. Most patients with HBeAg might be candidates for treatment with nucleoside analogues, as the presence of HBeAg often indicates active viral replication and high levels of ALT in an immune-active state of chronic infection. However, due to drug resistance and the high cost of life-long medication, predictors for HCC development should be more clearly defined so that patients can judge the necessity of immediate treatment using nucleoside analogues. We hypothesized that laparoscopic observation of the liver surface might work for this purpose. The present study retrospectively evaluated long-term outcomes for a large systematic cohort of HBeAg-positive patients, focusing on HCC development, using laparoscopic, histological, and clinical characteristics.

In the present study, half of patients with early-stage (S1) disease were <30 years old at diagnosis. Cumulative rate of HCC development was 0.0% during the following 10 years, partly because some patients showed seroconversion to negative HBeAg in the following 10 years with cessation of hepatitis. Conversely, the patients who were ≥30 years old in the early stage showed a significantly higher risk of HCC, compared with the patients of age <30 years. Treatment with nucleoside analogues may be worth considering in such patients, although incidence rates were less than those of patients in the pre-cirrhotic or cirrhotic stage. Age differences in disease progression have been reported with other chronic liver diseases, including chronic hepatitis C [26], autoimmune hepatitis [27], and primary biliary cirrhosis [28]. Our results suggest that age difference plays some role in HCC development among

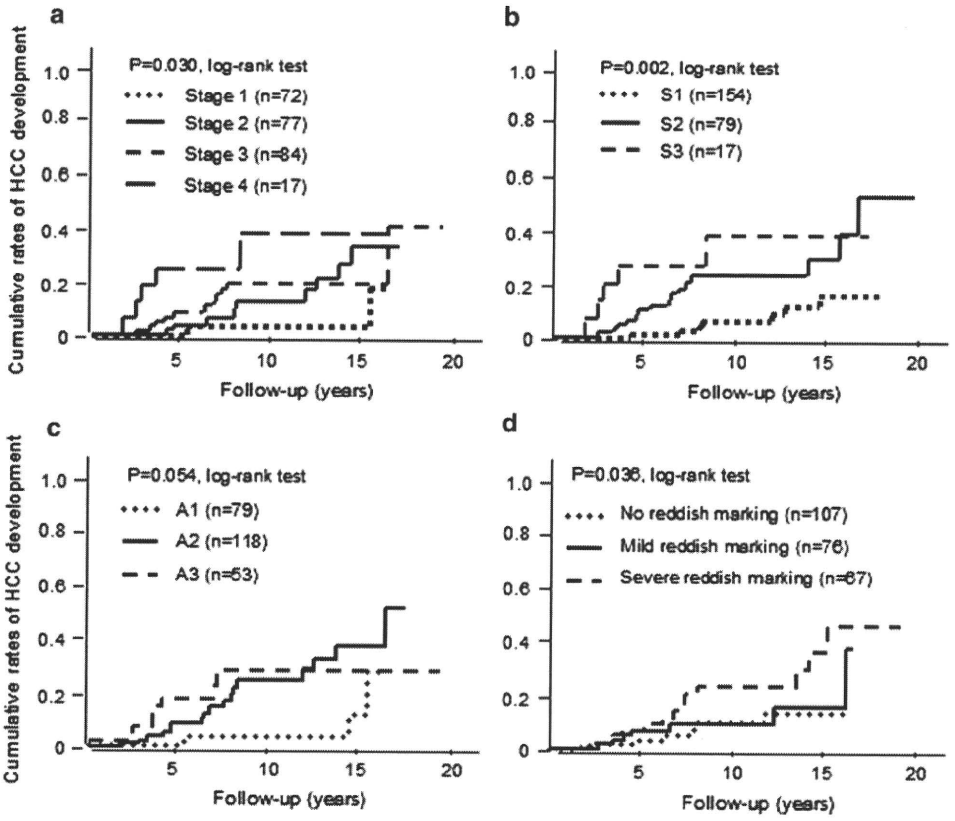

Fig. 4 Cumulative rates of hepatocellular carcinoma (HCC) development, stratified by histology and laparoscopic findings. Figures show cumulative rates of HCC development estimated by the Kaplan–Meier method, stratified by histological fibrosis stage (**a**), laparoscopic surface irregularity (**b**), histological activity grade (**c**), and laparoscopic reddish markings (**d**). Significant associations with HCC development were shown for liver cirrhosis according to histological fibrosis ($P = 0.030$, log-rank test) and laparoscopic surface irregularity ($P = 0.002$). Severity of laparoscopic reddish markings was significantly associated with HCC development ($P = 0.036$), while that of histological activity grade was not ($P = 0.054$, log-rank test)

Table 5 Analysis of factors predicting HCC development for patients of age ≥30 years with the proportional hazards model

Factors	Univariate analysis	
	Odds ratio (range[a])	*P*
Histological fibrosis stage	1.57 (1.02–2.40)	0.04
Histological activity grade	1.67 (0.95–2.95)	0.075
AST (≥80 IU/l)	0.72 (0.33–1.57)	0.41
ALT (≥80 IU/l)	1.18 (0.53–2.66)	0.69
Surface irregularity	1.93 (1.13–3.31)	0.016
Reddish markings	1.67 (1.04–2.70)	0.034
Patchy markings	1.54 (0.68–3.48)	0.30

AST aspartate aminotransferase, *ALT* alanine aminotransferase

[a] 95% confidence interval

HBV patients, and that patients of age <30 years should be re-evaluated with liver biopsy within 10 years if HBV-DNA and ALT levels remain elevated.

Interestingly, our analysis of the patients of age ≥30 years revealed that a laparoscopic finding of reddish markings correlated significantly with HCC development. Reddish markings were significantly correlated with histological activity, but these parameters showed different influences on HCC development. This was suspected to arise from differences in the origins of these parameters. Ohta et al. performed precise histological analysis of reddish markings with histological reconstruction using serial sections of liver biopsy specimens from cases with reddish markings [24]. They revealed that reddish markings correspond to widespread necrosis of hepatocytes, and proposed this finding as a useful index of activity in chronic hepatitis. Shibayama et al. [16, 23] showed that reddish markings did not appear in the early stage of chronic hepatitis with piecemeal necrosis around the portal area, instead appearing only after hepatic parenchymal destruction subjacent to the liver capsule due to prolonged active hepatitis or repeated acute exacerbations of chronic hepatitis. Reddish markings as an index of laparoscopic activity are not equivalent to piecemeal necrosis as an index of histological activity. Progression to liver cirrhosis may occur after the appearance of reddish markings unless the activity of chronic hepatitis can be reduced, because hepatic parenchymal destruction may change the pattern of blood flow in the liver to an increasingly cirrhotic pattern. Reddish markings might be useful not for early detection of HCC, but as a warning of transition to liver cirrhosis prior to HCC development. Our results indicate reddish markings as a useful predictor of HCC development.

In terms of liver cirrhosis, our results are consistent with previous reports, showing that liver cirrhosis in histological fibrosis or laparoscopic surface irregularity is strongly associated with HCC development [14]. This strong association might explain the results of subgroup analysis

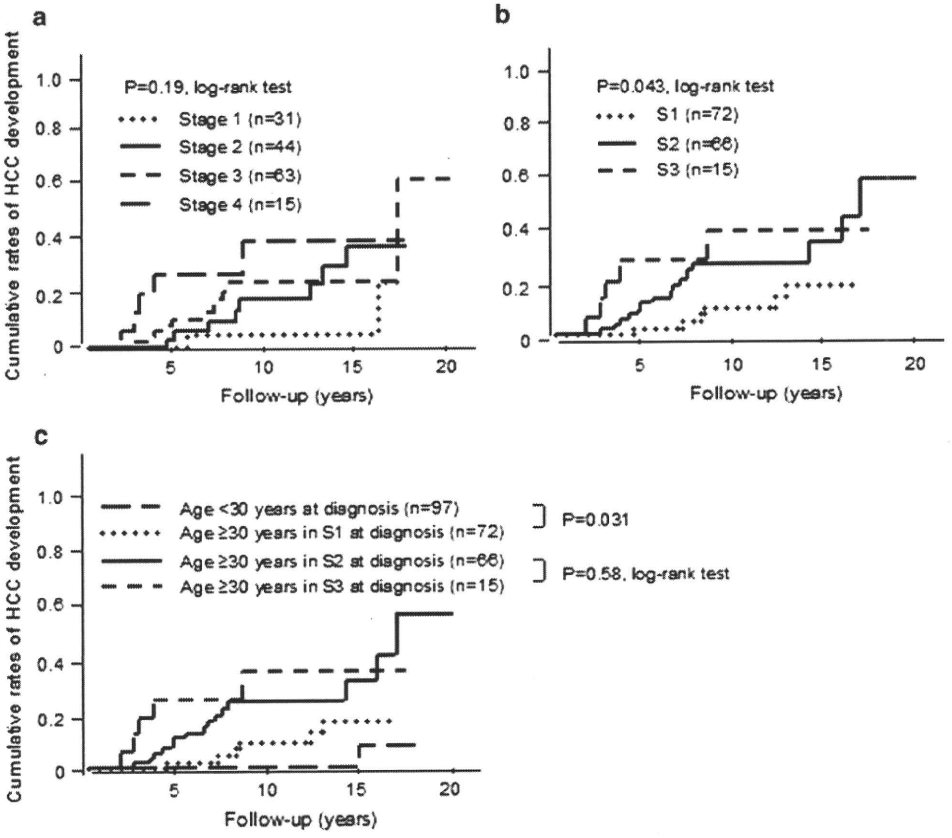

Fig. 5 Cumulative rates of hepatocellular carcinoma (HCC) development, for the patients of age ≥30 years at diagnosis, stratified by disease progression. Figures show cumulative rates of HCC development, for the patients of age ≥30 years at diagnosis, stratified by histological fibrosis stage (**a**) and surface irregularity (**b**, **c**). Cumulative rates of HCC development were significantly higher in more advanced diseases, according to surface irregularity (**b** $P = 0.043$, log-rank test), but not to histological fibrosis stage (**a** $P = 0.19$). The risk of HCC development was significantly higher among the patients of age ≥30 years even in laparoscopic S1 stage at diagnosis, compared with the patients of age <30 years (**c** $P = 0.031$, log-rank test)

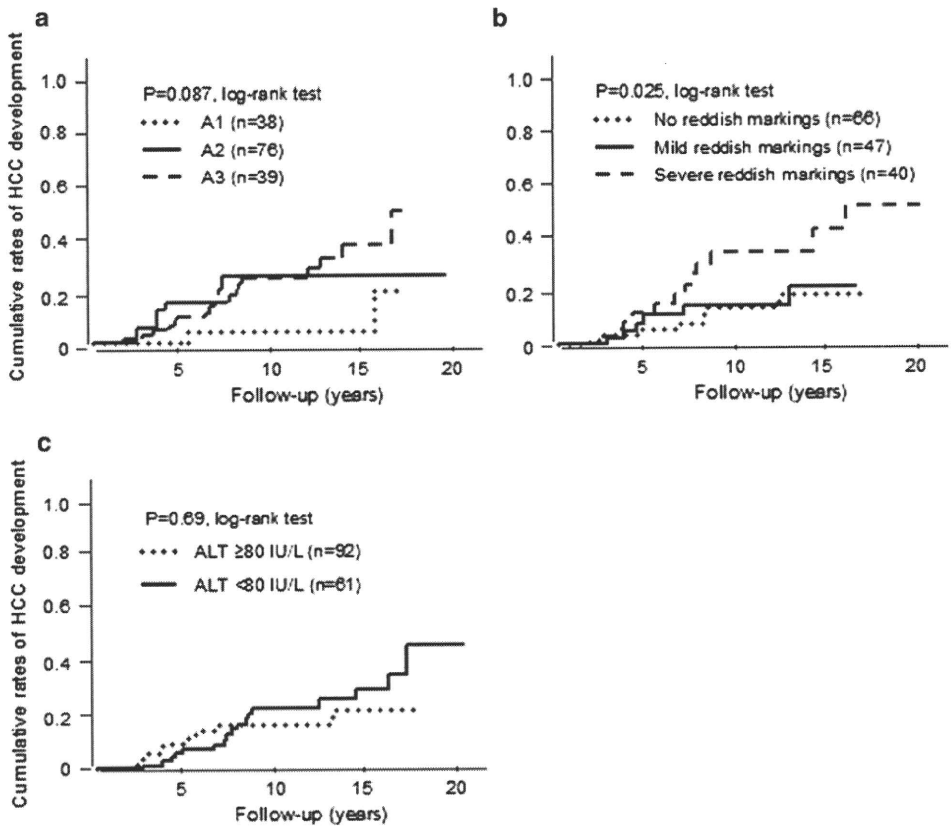

Fig. 6 Cumulative rates of hepatocellular carcinoma (HCC) development, for the patients of age ≥30 years at diagnosis, stratified by inflammatory activity. Cumulative rates of HCC development are shown for patients of age ≥30 years at diagnosis, stratified by histological activity (**a**), laparoscopic reddish markings (**b**), and ALT levels (**c**). Cumulative rates of HCC development showed significant differences when stratified by reddish markings ($P = 0.025$, log-rank test), but not by histological activity ($P = 0.087$) or ALT levels ($P = 0.69$)

among cirrhotic patients, in which no significant predictive factors could be found for HCC development. This reveals that HCC might occur irrespective of other conditions such as liver inflammation, once liver disease has progressed to cirrhosis. Actually, the role of antiviral therapy with nucleoside analogues has not been well defined for cirrhotic patients with regard to reduced HCC development. We have previously reported that cumulative recurrence rates of HCC after initial and complete treatment for HCC did not differ between lamivudine-treated and control groups [29]. Kuzuya et al. [30] supported this finding and suggested that antiviral therapy may improve remnant liver function and increase the chances of receiving available treatment modalities for recurrent HCC.

Completely normal values from routine laboratory tests of liver function might suggest a normal liver or only early-stage liver disease, but our analysis showed that only half of patients with such completely normal values were in the early stage. Several investigators have reported noninvasive approaches for quantitative diagnosis of liver fibrosis, using routine laboratory tests, serum fibrosis markers, radiological imaging, and elastography [31], all of which have been in practical use for hepatitis C. Prolonged active hepatitis or repeated acute exacerbations may occur frequently in HBV patients, and might disturb the accuracy of noninvasive quantitation of liver fibrosis [32]. Liver biopsy appears warranted for precise evaluation of disease progression, and further examination with laparoscopy would be ideal, even if liver function tests continue to yield normal results.

In conclusion, HCC development is associated with older age, male gender, and liver cirrhosis. Reddish markings, rather than histological activity or ALT level, can be useful to predict HCC for HBV patients of age $\geq$30 years at diagnosis. Patients of age $\geq$30 years even in the early stage may consider treatment with nucleoside analogues because of the relatively high risk of HCC development.

References

1. Beasley RP, Hwang LY, Lin CC, et al. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. Lancet. 1981;2:1129–33.
2. Liaw YF, Tai DI, Chu CM, et al. Early detection of hepatocellular carcinoma in patients with chronic type B hepatitis. A prospective study. Gastroenterology. 1986;90:263–7.
3. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol. 2008;48:335–52.
4. Tong MJ, Hsien C, Hsu L, et al. Treatment recommendations for chronic hepatitis B: an evaluation of current guidelines based on a natural history study in the United States. Hepatology. 2008; 48:1070–8.
5. Lai CL, Lok AS, Lin HJ, et al. Placebo-controlled trial of recombinant alpha 2-interferon in Chinese HBsAg-carrier children. Lancet. 1987;2:877–80.
6. Orito E, Ichida T, Sakugawa H, et al. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology. 2001;34:590–4.
7. Sumi H, Yokosuka O, Seki N, et al. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. Hepatology. 2003;37:19–26.
8. Yang HI, Lu SN, Liaw YF, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med. 2002;347:168–74.
9. Kalk H. Dienchronishen Verlaufsformen der Hepatitis epidemica in Beziehung zu ihren anatomischen Grudlagen. DMW. 1947;72:308–13.
10. Kalk H, Wildhirt E. Lehrbuch und Atlas der Laparoskopie and Leberpunction. Stuttgart: Georg Thieme Verlag; 1962.
11. Poniachik J, Bernstein DE, Reddy KR, et al. The role of laparoscopy in the diagnosis of cirrhosis. Gastrointest Endosc. 1996;43:568–71.
12. Cardi M, Muttillo IA, Amadori L, et al. Superiority of laparoscopy compared to ultrasonography in diagnosis of widespread liver diseases. Dig Dis Sci. 1997;42:546–8.
13. Regev A, Berho M, Jeffers LJ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol. 2002;97:2614–8.
14. Arase Y, Suzuki F, Suzuki Y, et al. Potential of laparoscopy in chronic liver disease with hepatitis B and C viruses. Hepatol Res. 2008;38:877–85.
15. Shiraki K, Shimizu A, Takase K, et al. Prospective study of laparoscopic findings with regard to the development of hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis. Gastrointest Endosc. 2001;53:449–55.
16. Shibayama T, Mori S, Ohtake H, et al. Risk factors of hepatocellular carcinoma in chronic hepatitis C and cirrhosis: special reference to laparoscopic findings. Dig Endosc. 1999;11:24–31.
17. Desmet VJ, Gerber M, Hoofnagle JH, et al. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology. 1994;19:1513–20.
18. Shimada N, Itoshima T, Ohta W, et al. A new classification of liver surface pictures by peritoneoscopic photography. Gastroenterol Endosc. 1971;13:68–76.
19. Watanabe M, Hirakawa H, Ikeda S, et al. Peritoneoscopy as an aid in intravenous injection of indocyanine green (ICG). Endoscopy. 1985;17:149–52.
20. Ito T, Itoshima T, Ukida M, et al. Peritoneoscopy of the liver stained by intravenous injection of indocyanine green-experimental and clinical studies. Gastroenterol Jpn. 1983;18: 593–8.
21. Michitaka K, Onji M, Horiike N, et al. Hepatitis B virus replication in dark reddish patchy markings appearing on the hepatic surface. Gastrointest Endosc. 1990;36:15–8.
22. Kudo M. Imaging diagnosis of hepatocellular carcinoma and premalignant/borderline lesions. Semin Liver Dis. 1999;19:297–309.
23. Peterson MS, Baron RL. Radiologic diagnosis of hepatocellular carcinoma. Clin Liver Dis. 2001;5:123–44.
24. Ohta W. Significance of reddish asteroidal and meshwork markings on the liver surface of chronic hepatitis observed by peritoneoscopy-observation of liver cell necrosis in relation to the portal and hepatic veins. Acta Hepatol Jpn. 1975;16:448–63.
25. Shibayama T, Ohtake H, Hishima T. Formation and clinical significance of reddish markings on the liver surface: activity of chronic liver disease. Dig Endosc. 2003;15:100–7.
26. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC,

METAVIR, CLINIVIR, and DOSVIRC groups. Lancet. 1997; 349:825–32.
27. Al-Chalabi T, Boccato S, Portmann BC, et al. Autoimmune hepatitis (AIH) in the elderly: a systematic retrospective analysis of a large group of consecutive patients with definite AIH followed at a tertiary referral centre. J Hepatol. 2006;45:575–83.
28. Kubota J, Ikeda F, Terada R, et al. Mortality rate of patients with asymptomatic primary biliary cirrhosis diagnosed at age 55 years or older is similar to that of the general population. J Gastroenterol. 2009;44:1000–6.
29. 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.
30. Kuzuya T, Katano Y, Kumada T, et al. Efficacy of antiviral therapy with lamivudine after initial treatment for hepatitis B virus-related hepatocellular carcinoma. J Gastroenterol Hepatol. 2007;22:1929–35.
31. Koda M, Matunaga Y, Kawakami M, et al. FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. Hepatology. 2007;23:297–306.
32. Ngo Y, Benhamou Y, Thibault V, et al. An accurate definition of the status of inactive hepatitis B virus carrier by a combination of biomarkers (FibroTest-ActiTest) and viral load. PLoS ONE. 2008;3:e2573.

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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

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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

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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.

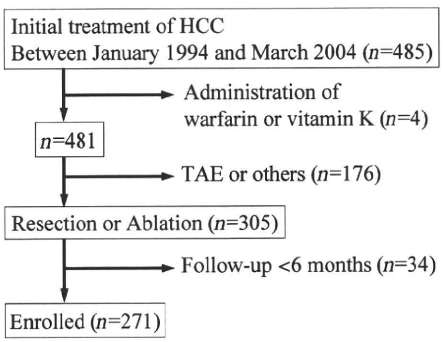

Fig. 1. Flow chart of consecutive 271 patients who received curative treatments. HCC, hepatocellular carcinoma; TAE, transcatheter arterial embolization.

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.

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.

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.

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

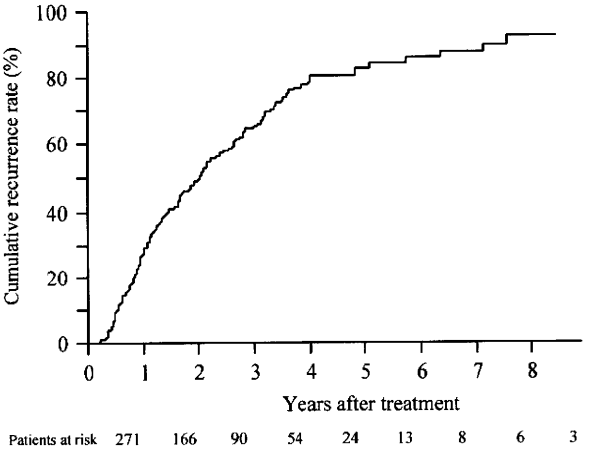

Fig. 2. Kaplan–Meier estimation of the cumulative recurrence rate of consecutive 271 patients which were 27.9, 65.1 and 86% at 1, 3 and 5 years respectively.

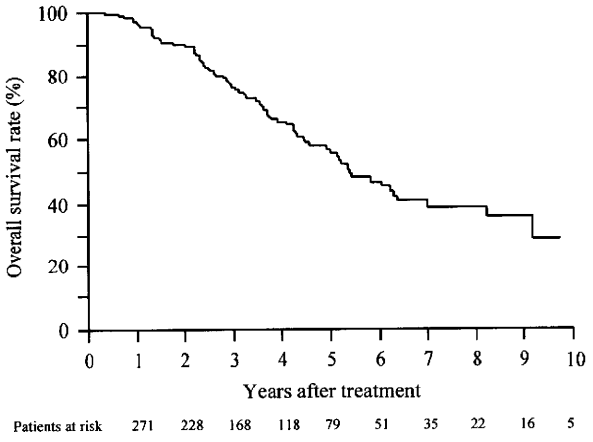

Fig. 3. Kaplan–Meier estimation of the overall survival rate of consecutive 271 patients which were 97.3, 76.4 and 55.6% at 1, 3 and 5 years respectively.

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

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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 extrahepatic 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%)

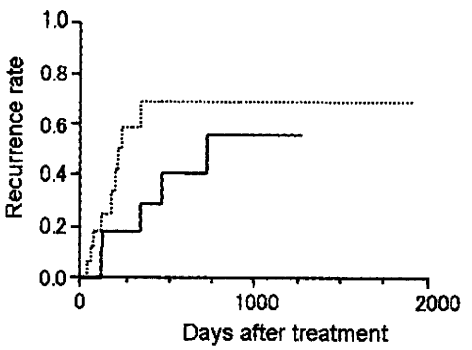

FIGURE 1 Intrahepatic distant recurrence (recurrence outside of the RFA–treated liver segment) of metastatic liver cancers. The recurrence rate of colon cancer (solid line) was lower than that of non–colon cancer (dotted line); however, the difference was not statistically significant (p=0.09). The rates 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.

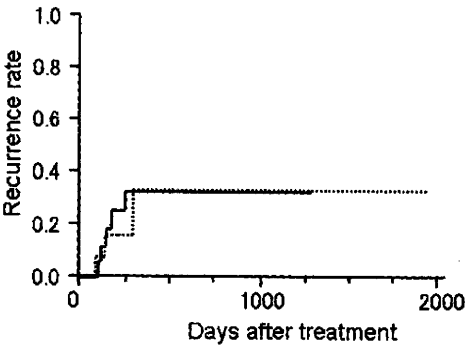

FIGURE 2 Local recurrence (recurrence at the RFA–treated segment) of metastatic liver cancers. No significant difference was observed between colon cancer (solid line) and non–colon cancer (dotted line, p=0.88). All local recurrences were observed in the first year after treatment, and the rates for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