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

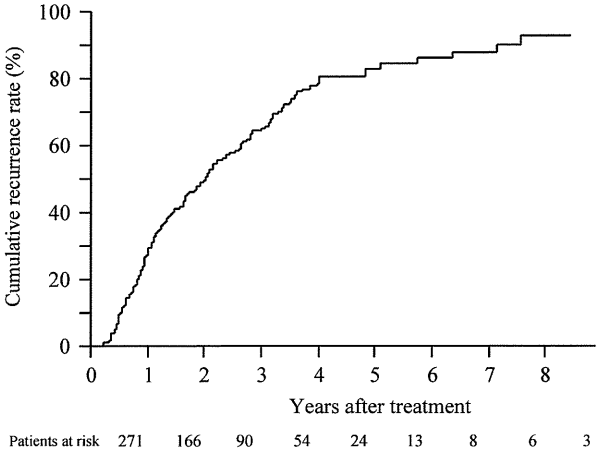

Fig. 2. Kaplan–Meier estimation of the cumulative recurrence rate of consecutive 271 patients which were 27.9, 65.1 and 86% at 1, 3 and 5 years respectively.

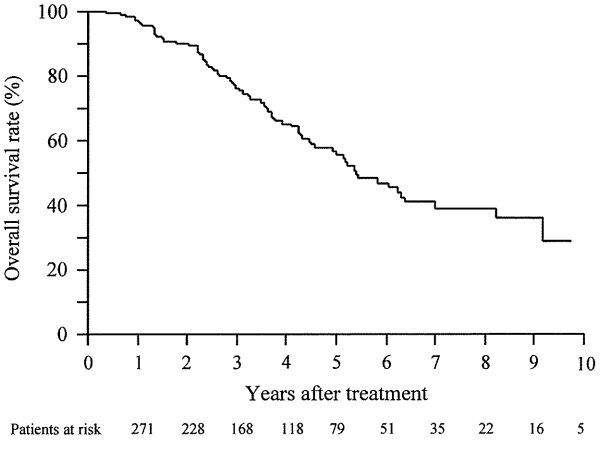

Fig. 3. Kaplan–Meier estimation of the overall survival rate of consecutive 271 patients which were 97.3, 76.4 and 55.6% at 1, 3 and 5 years respectively.

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 ($\leq 100 \times 10^9$ 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.

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European Journal of Radiology 73 (2010) 385–390

Contents lists available at ScienceDirect

European Journal of Radiology

journal homepage: www.elsevier.com/locate/ejrad

Prevention of biliary complication in radiofrequency ablation for hepatocellular carcinoma—Cooling effect by endoscopic nasobiliary drainage tube

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ARTICLE INFO

Article history:
Received 9 August 2008
Received in revised form 22 October 2008
Accepted 23 October 2008

Keywords:
Prevention of biliary complication
Radiofrequency ablation
Hepatocellular carcinoma
Endoscopic nasobiliary drainage tube

ABSTRACT

Background and study aims: Biliary stricture after radiofrequency ablation (RFA) for nodules of hepatocellular carcinoma (HCC) close to major bile ducts sometimes causes septic complications and liver failure. Therefore, it may require interventional drainage for decompression during the follow-up period. The purpose of this study is to clarify the feasibility and safety of bile duct cooling using an endoscopic nasobiliary drainage (ENBD) tube in RFA for HCC close to major bile ducts.

Patients and methods: Between August 2003 and July 2007, 14 consecutive patients (14 nodules) undergoing RFA with cooling by an ENBD tube for HCCs close to major bile ducts were enrolled in this study. We infused chilled saline solution via the ENBD tube at 1 ml/s to prevent heat damage during RFA. As controls, 11 patients (13 nodules) undergoing RFA without cooling close to major bile ducts between April 2001 and August 2003 were reviewed. The major outcomes for evaluation were biliary complications and the secondary outcome was local tumor recurrence.

Results: There were no significant differences in tumor recurrence between the two groups. However, the rate of biliary complications was significantly lower in the cooling group than in the non-cooling group (0% vs. 39%, $P = 0.02$).

Conclusions: Cooling of bile ducts via an ENBD tube can prevent biliary complications induced by RFA of HCC close to major bile ducts without increasing local recurrence. This technique increases indication of RFA in difficult cases.

1. Introduction

In patients with hepatocellular carcinoma (HCC), radiofrequency ablation (RFA) has recently established as a promising and safe percutaneous technique [1–3]. However, a variety of complications related to RFA have been reported. These include early complications, such as bleeding, infection, hepatic vascular damage, and visceral damage, as well as late complications, such as bile duct injury and tumor seeding along the electrode track [4,5]. Of these, bile duct injuries by thermal damage, including biliary stricture, abscess, biloma, bilioperitoneum, hemobilia, and both biliovenous and biliocutaneous fistula have been reported [5]. Furthermore, biliary stricture after RFA for HCC nodules close to major bile ducts sometimes causes septic complications, liver failure and death. Therefore, it may require interventional drainage (percutaneous transhepatic biliary drainage or endoscopic drainage) for decompression during the follow-up period.

Recently, prophylactic placement of a biliary stent and radiofrequency ablation during biliary duct cooling with saline have been used to prevent biliary damage when treating tumors close or adjacent to major bile ducts [6–8,16].

Endoscopic nasobiliary drainage (ENBD) is usually adopted for the treatment of obstructive jaundice. Injecting contrast materials via an ENBD tube, we can obtain cholangiogram repeatedly. Therefore, it is hypothesized that the use of an ENBD tube to cool the major bile ducts can reduce the rates of these complications. We evaluated the feasibility and safety of percutaneous sonographically guided RFA with cooling by ENBD tube for HCC close to major bile ducts.

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doi:10.1016/j.ejrad.2008.10.021

2. Patients and methods

2.1. Patient eligibility

RFA was performed on 439 patients with 1253 HCC nodules in the Department of Gastroenterological Internal Medicine at Okayama University Hospital between April 2001 and July 2007. Of 380 patients with 1055 HCCs undergoing RFA between August 2003 and July 2007, 14 consecutive patients with 14 HCCs close to the major bile ducts underwent RFA with cooling by ENBD tube.

The inclusion criteria for cooling RFA was the presence of an RFA-treated tumor that, at its edge, located no more than 5 mm from a major bile duct, as revealed by computed tomography (CT) or ultrasonography (US). Major bile ducts were defined as follows: on the left side, the primary duct up to the origin of segment 3; on the right side, the primary duct and its two second branches. Patients with Child-Pugh grade C, severe chronic respiratory disease, heart disease, or renal disease were excluded. Tumor diameters were determined as the largest dimension measured by CT and/or US.

As controls, we retrospectively reviewed 11 patients with 13 HCCs undergoing RFA without cooling close to the major bile ducts (<5 mm), of 121 patients with 222 HCCs undergoing RFA between April 2001 and August 2003, who were selected in the above-mentioned eligibility criteria from HCC database in our institute. The primary end point of the study was biliary complication after RFA. The secondary end point was local tumor recurrence. In this study, all patients were treated by means of transarterial chemoembolization (TACE) before RFA. Informed consent to undergo the procedure was obtained from all patients.

2.2. Diagnosis of HCC

Pretreatment examination of HCC lesions included US, helical CT, and CT during arterial portography and hepatic arteriography. The diagnosis of HCC was based on elevated serum levels of α-fetoprotein and/or des-γ-carboxy-prothrombin, and was made also by the presence of typical tumor features, using CT and/or CT during arterial portography and hepatic arteriography, such as hyperattenuations in the arterial phase and hypoattenuations in the portal phase [9,10]. Hepatic impairment was classified as Child-Pugh Class A, B, or C.

2.3. TACE

In all cases, TACE was performed 1 week before RFA. The catheter was introduced by using the Seldinger approach through the right femoral artery with a 5-Fr catheter. After CT during arterial portograpy and CT arteriography, the catheter tip was advanced at the site as close as possible to the feeding artery. An emulsion of 30 mg of epirubicin hydrochloride and lipiodol followed by gelatin sponge particles was injected by hand under the fluoroscopy.

2.4. Endoscopic procedure

ERCP was performed by using Olympus JF-240 side-viewing endoscopes (Olympus Optical Corporation, Tokyo, Japan). Patients underwent conscious sedation with diazepam (5–10 mg) and pethidine hydrochloride (35–70 mg). After a diagnostic cholangiogram was obtained, a 6-Fr ENBD tube (Wilson-Cook Medical, Winston-Salem, NC, USA) was inserted. The tip of the ENBD tube was inserted deeply into the peripheral branch of the target bile duct close to the nodule. Sphincterotomy was not performed. The ENBD tube was removed after confirmation of the hypoattenuated area, including the whole tumor, on a contrast-enhanced CT scan, which was performed 5–7 days after the last RFA session. The definitions of individual complications after ERCP were similar to those of Cotton et al. [11].

2.5. Radiofrequency ablation with or without bile duct cooling by ENBD

If no severe adverse effects, such as pancreatitis or cholangitis, followed ENBD, RFA was performed 1–3 days after. A 17 G cooled-tip electrode with a 2- or 3-cm exposed tip (Radionics, Burlington, MA, USA) was attached to a radiofrequency generator (CC-1 Cosman Coagulator; Radionics) and inserted into the tumor nodule under US guidance. Temperature and tissue impedance were monitored during ablation. A 4 °C saline solution, 10–20 ml, was infused into an ENBD tube at 1 ml/s and then was evacuated by manual suctioning. This procedure was repeated during RFA. Radio frequency energy was delivered for 6–12 min per session. For large lesions, the electrode was inserted into additional sites of the tumor. The procedure was repeated 1–2 weeks later until the hypoattenuated area was confirmed on a contrast-enhanced CT scan.

2.6. Assessing the effect of RFA

The short-term effects of ablation were assessed using helical CT for 4 weeks after treatment. If nodular enhancement was noted in the treated tumor under CT, then the same treatment was given as part of another course of treatment. CT was repeated 2–6 months after treatment. Complete tumor necrosis was defined as persistent hypoattenuation of the tumor on CT for 4 months after the most recent ablation therapy. In addition to CT, follow-up examinations, such as the monitoring of serum tumor markers (α-fetoprotein, des-γ-carboxy-prothrombin, and α-fetoprotein lectin-binding 3 fraction) and US, were performed every 2 months. Local tumor recurrence was defined as the presence of an enhanced tumor on CT, corresponding to the initial target tumor. Additional new tumor recurrence was defined as the development of an enhanced tumor on CT in a different segment from that of the original tumor. RFA-related complications were also evaluated at the same time. One example case is demonstrated in (Fig. 1).

2.7. Statistical analysis

Either Fisher's exact test or the Mann–Whitney *U*-test was used to compare the cooling and non-cooling groups. Local tumor recurrence was estimated using the Kaplan–Meier method, and significant differences were determined by the log-rank test. A *P* value of less than 0.05 was considered statistically significant.

3. Results

3.1. Preprocedural findings

Clinical characteristics of patients are shown in Table 1. There were no significant differences between the two groups in background characteristics, such as gender, age, Child-Pugh class, viral marker, tumor size, and tumor location.

3.2. ENBD

In the cooling group, the insertion of an ENBD tube was possible for all cases during the first attempt. Post-ERCP pancreatitis occurred in three cases (21%: mild 1, moderate 2). All patients were treated conservatively and had no necrosis, pseudocyst, or abscess requiring surgical or percutaneous debridement or drainage. RFA was performed after ERCP at 2, 5, and 6 days. The ENBD tube was inserted for a median of 7 days (range, 3–16).

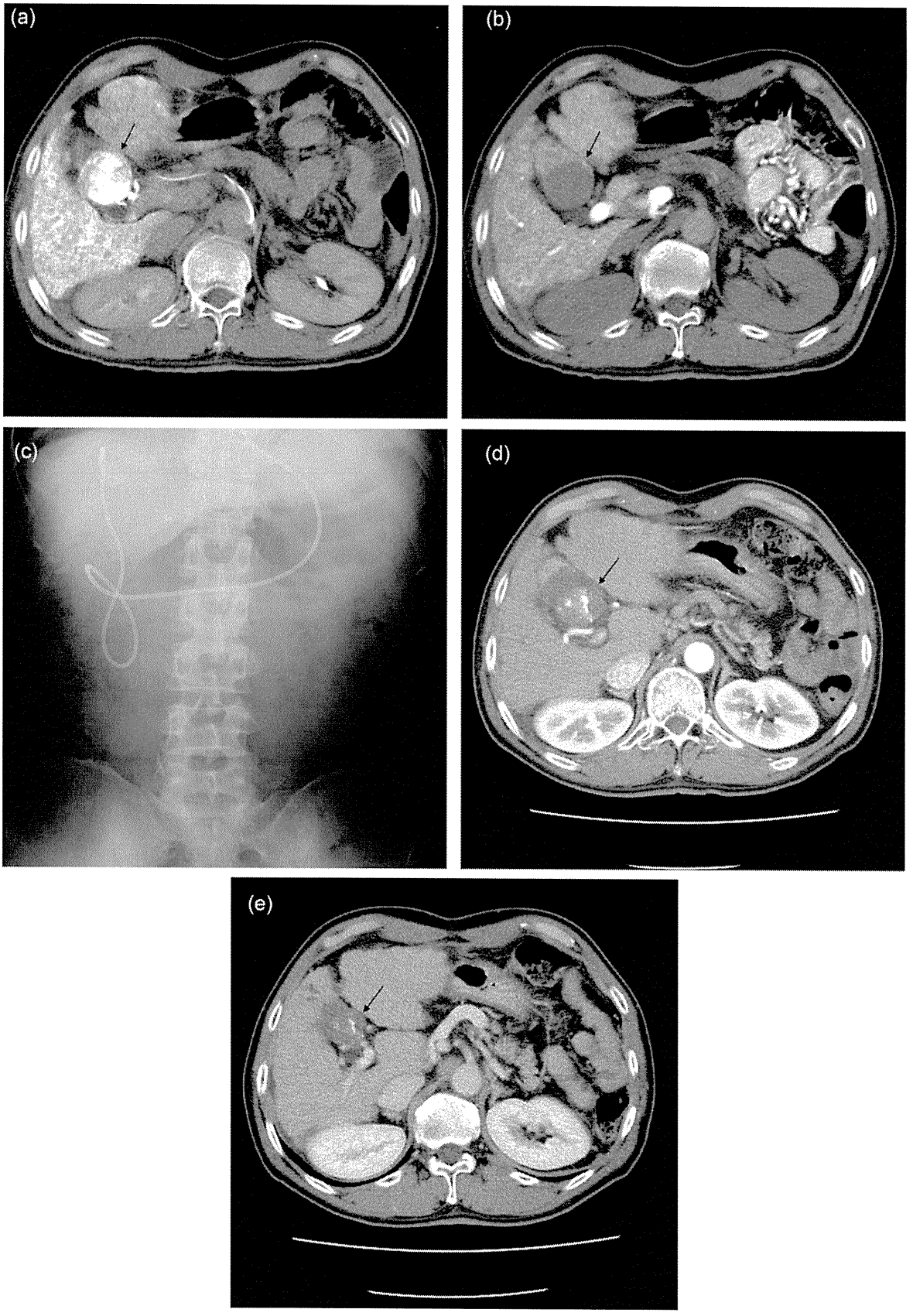

Fig. 1. A 32 mm hepatocellular carcinoma in the S4 segment of the liver of a 59-year-old man. (a and b) CT angiography (CTA) and CT arterial portography (CTAP) shows a tumor (arrow) close to the biliary confluence. (c) Nasobiliary drainage tube was endoscopically inserted into the left hepatic bile duct. (d) After RFA with bile duct cooling, dynamic CT scan shows a 40 mm ablated area. (e) Biliary complications and local recurrence were not detected in a CT scan taken 12 months after RFA.

Table 1
Clinical characteristics of HCC patients who underwent RFA with or without bile duct cooling by ENBD.

	Non-cooling RFA (11 patients, 13 nodules)	Cooling RFA (14 patients, 14 nodules)	*P*
Age (years)	75 (59–79)	68 (52–81)	0.12
Gender (male/female)	7/4	7/7	0.69
HCV Ab (+)	11	13	
Size of tumor (mm)	18 (14–24)	20 (11–39)	0.72
Site of bile duct near the nodule			
Right or left primary duct	5	10	0.13
Two second branch (anterior or posterior) or UP	8	4	
Total bilirubin (mg/dl)	0.9 (0.6–2.5)	0.9 (0.4–1.7)	0.94
Albumin (g/dl)	3.9 (3.3–4.5)	3.6 (2.5–4.6)	0.58
Prothrombin time (%)	89 (50–105)	98 (59–145)	0.12
Platelet count ($\times 10^4/mm^3$)	9.2 (3.9–20)	9.8 (4.9–30.7)	0.34
Child-Pugh class			
A	12	11	0.6
B	1	3	
Follow-up (days)	1198 (42–1703)	781 (118–1472)	0.27

Statistical analysis was performed by Fisher's exact test or Mann–Whitney's *U*-test.

3.3. Treatment efficacy

In all cases, tumor lesions that were enhanced before treatment were not enhanced after. For 11 nodules (79%), treatment was successful after one session of cooling RFA. One additional session of RFA or percutaneous ethanol injection (PEI) was performed for three nodules (21%), because the necrotic area was smaller than the tumor size.

3.4. Complications

In the non-cooling group, 3 patients (23%) presented segmental dilatation of bile ducts above the site of RFA 2–4 months after treatment, and one patient presented biloma 1 week after RFA. No treatment was given to that patient, and there was no further complication. Portal thrombus was observed in one patient 3 weeks after, but it disappeared immediately after administration of an anticoagulant.

On the other hand, in the cooling group, no complication after RFA was observed even up to the time of analysis. The complication rate after RFA was significantly lower with cooling than without (Table 2, Fig. 2).

3.5. Local recurrence and survival

Three patients (21%) in the cooling group presented a local recurrence of HCC on the RFA site 13–35 months (13, 30, and 35 months) after treatment. On the other hand, in the non-cooling group, 4 patients (31%) had a local recurrence 7–24 months (7, 13, 22, and 24 months) after treatment (Fig. 3). All patients were treated with RFA, PEI, or TACE again.

During follow-up, 3 patients (21%) in the cooling group died of local and/or distant progression of new liver lesions, and 2 patients (14%) died of unrelated causes (liver failure, acute myocardial infarction). In the non-cooling group, 3 patients (27%) died of local and/or distant progression, and 4 patients (36%) died of unrelated causes (liver failure, acute myocardial infarction, esophageal

Table 2
Complications and local recurrence.

	Non-cooling RFA (13 nodules)	Cooling RFA (14 nodules)	*P*
Complications			
Segmental dilatation of bile duct	3 (23%)	0	
Biloma	1 (8%)	0	
Portal thrombus	1 (8%)	0	
Liver abscess	0	0	
Cholangitis	0	0	
Total	5 (39%)	0	0.02
Local recurrence	4 (31%)	3 (21%)	0.68

Statistical analysis was performed by Fisher's exact test.

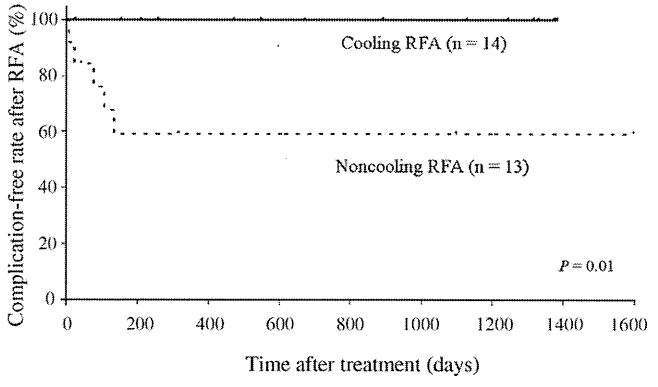

Fig. 2. Kaplan–Meier estimates of complications in 14 nodules assigned to RFA with intraductal cooling by ENBD and 13 nodules assigned to RFA without cooling ($P = 0.01$ by the log-rank test).

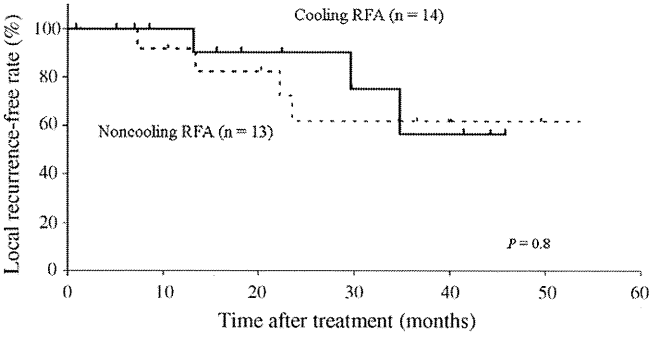

Fig. 3. Kaplan–Meier estimates of local recurrence in 14 nodules assigned to RFA with intraductal cooling by ENBD and 13 nodules assigned to RFA without cooling ($P = 0.8$ by the log-rank test).

varix rupture, sudden death). There was no death associated with RFA complications.

4. Discussion

RFA has widely been used as a minimal invasive treatment for hepatocellular carcinomas [1–3]. However, a variety of complications related to RFA have been reported. Of these, bile duct injury by thermal damage is one of the severe complications to cause cholangitis, liver failure and death. The incidence of bile duct stricture after RFA was reported 0.3–0.5% [4,5]. On the other hand, Kim et al. reported that although bile duct changes were frequent after RFA of HCC (17%), most were of no clinical significance, and major complications requiring additional treatment were rare [12]. However, biliary stricture had no clinical significance when it was located peripherally, but was sometimes dangerous when located centrally in the major bile ducts. Teratani et al. reported that there was a significant difference in the incidence of bile duct injury between nodules near a large portal vein (7.6%) and other nodules (1.6%), and the incidence of bile duct dilation differed significantly according to the distance between the targeted tumor and intrahepatic bile duct, as surrogated by neighboring portal vein [13]. In fact, it was reported that the subsequent stenosis of major bile ducts as a result of thermal damage and the progression of bile duct dilatation caused liver failure and liver abscess requiring interventional drainage [4,6,14]. Such damage led to the contraindication of RFA to ablate tumors that were less than 15–20 mm to the major bile ducts.

Three procedures prevented biliary stricture: the endoscopically placed internal stent, as reported by Bilchik in 2001 [15], bile duct intraoperative cooling with catheters introduced after the common bile duct is opened, as reported by Elias et al. in 2004 [7], and intraductal chilled saline perfusion through a nasobiliary tube as reported by Ohnishi et al. in 2008 [16]. In this study, we employed an ENBD tube for intraductal cooling which was recently reported by Ohnishi. ENBD is an external drainage procedure performed on patients with cholestasis and/or severe acute cholangitis by placing a 6-Fr tube over the guidewire. Intraductal cooling by ENBD tube has some advantages over intraoperative cooling: (i) there is little invasion; (ii) RFA can be performed repeatedly; (iii) the tube can be placed correctly under fluoroscopy by endoscopic retrograde cholangiography. On the other hand, due to the nasal discomfort, there is jeopardy that the patients withdraw the ENBD tube inadvertently. Fortunately, all the patients in our series tolerated it well. However, the thin ENBD tube (5-Fr) and short custody period are mandatory to lessen the discomfort. Furthermore, some patients in this series had poor hepatic reserve due to liver cirrhosis. Accordingly, it is imperative to avoid biliary complications in these patients through this procedure. We could demonstrate almost the same result relevant to the biliary complication as reported by Ohnishi et al. (0% vs. 39%, 2.5% vs. 46%). However, they do not mention about local recurrence in their study.

Local recurrence should be mentioned as well as prevention of biliary complications. Because this procedure has a potentially cooling effect on tumor cells near the cooled bile duct. This means that the tumor cells are secured from heat during RFA. With regard to local recurrence rate from the start of RFA treatment, the Kaplan–Meier curves showed no significant differences between the cooling and non-cooling groups ($P = 0.8$). However, only one case recurred in less than 2 years and other 2 cases recurred in more than 2 years in the cooling group (median 888 days, 394–1044), whereas all cases recurred in less than 2 years in non-cooling group (median 534 days, 218–716). The reason is uncertain and requires additional investigation.

To achieve adequate bile duct protection and complete ablation of the target lesion including the surrounding liver tissue, the manner with which the bile ducts are cooled is important. Low-temperature saline could improve the cooling effect [18]. In previous studies, 6–8 °C saline was used by Raman et al. and 4 °C Ringer's lactate was used by Elias et al. We used saline at 4 °C. Chilled saline at a rate of 1 ml/s was infused and expelled through the ENBD tube during RFA repeatedly, based on the cholangiography. In this study, biliary complication was not observed and the local recurrence rate was not so high. However, we cannot deny the local recurrence after more long-term observation in the future, because there is a possibility that we cannot secure an ablated enough area due to excessive cooling, as previously described.

Vascular flow is also considered to protect the bile ducts from the thermal effects of RFA by dissipating the heat generated in the ablated area [19–21], but with a potentially high risk of tumor recurrence [6]. Blood flow is a strong predictor of all RFA lesion dimensions in porcine liver in vivo [19,21,22]. Clinical studies have revealed that reduction of blood flow by occluding the hepatic artery with balloon catheter or feeding arteries with gelatin sponge particles could enlarge the tumor necrosis area obtained by RFA [23–25]. In this study, we performed TACE before RFA by bile duct cooling in all cases. This procedure is reasonable for decreasing heat dispersion enforced by blood flow and for expanding the area of necrosis. Therefore, it is necessary to prevent RFA-induced bile duct injury.

Post-ERCP pancreatitis occurred in three cases. The incidence was higher than we anticipated, but all patients were treated conservatively. There is no report of ENBD inducing or worsening pancreatitis. However, the ENBD tube itself may obstruct the pancreatic duct orifice and induce pancreatitis if it is placed through the normal papilla. In addition, left lobe hypertrophy by liver cirrhosis does not allow straightening of a duodenoscope to obtain a short scope position, resulting in a poor view of the papilla and difficult cannulation. The pancreatic duct stent, especially in the case of difficult cannulation, might be useful for preventing ERCP pancreatitis [17].

There are several limitations in this study, because it is retrospective nature and study size is small. Furthermore, irrespective of no significant differences in the backgrounds between two groups, the proportion of HCCs close to the major bile duct in the cooling group was significantly smaller than that in the non-cooling group. In the cooling group, we prospectively selected HCCs close to the major bile duct on the strict criteria. To the contrary, in non-cooling group we collected the patients retrospectively from our data base. This is why this discrepancy happened in this study. Probably, the indication rate of cooling is not so high in the clinical setting. However, it is noteworthy that there was no biliary complication in the strictly selected cooling group.

5. Conclusions

In conclusion, the cooling of bile ducts by an ENBD tube can prevent biliary complications induced by RFA of HCC close to major bile ducts without increasing local recurrence. This technique is well tolerated and decreases the contraindication of RFA without reducing its efficacy. However, special care should be taken to prevent post-ERCP pancreatitis.

Conflict of interest

The article is original and the text has not been published. The material submitted is not under consideration for publication elsewhere. All authors have read and approved the manuscript. There are no conflicts of interest for all authors.

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J Gastroenterol (2010) 45:1172–1182
DOI 10.1007/s00535-010-0266-9

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

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

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Received: 25 January 2010 / Accepted: 18 May 2010 / Published online: 12 June 2010

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

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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

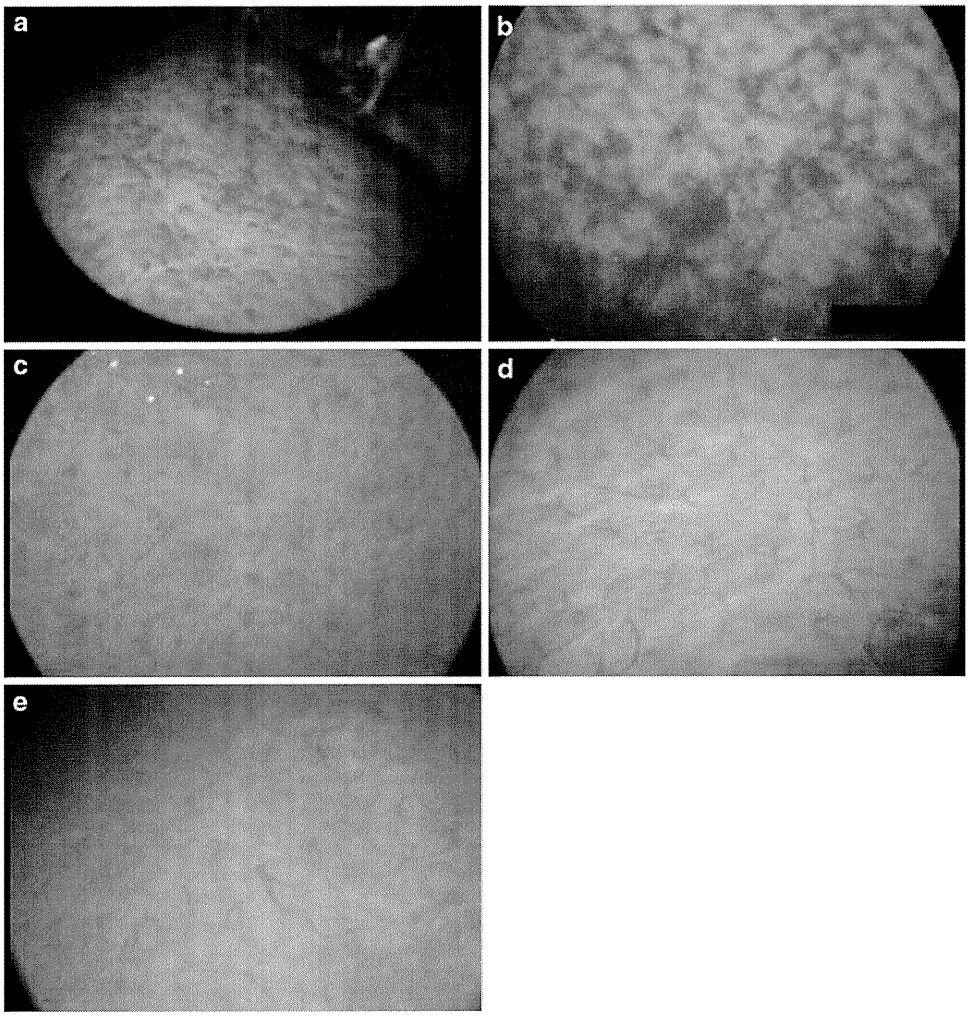

Fig. 1 Laparoscopic features of the patients with chronic viral hepatitis. Figures show typical pictures of laparoscopic features; laparoscopy of severe reddish markings, showing advanced surface irregularity with densely distributed reddish markings (**a**), closer view of severe reddish markings in 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

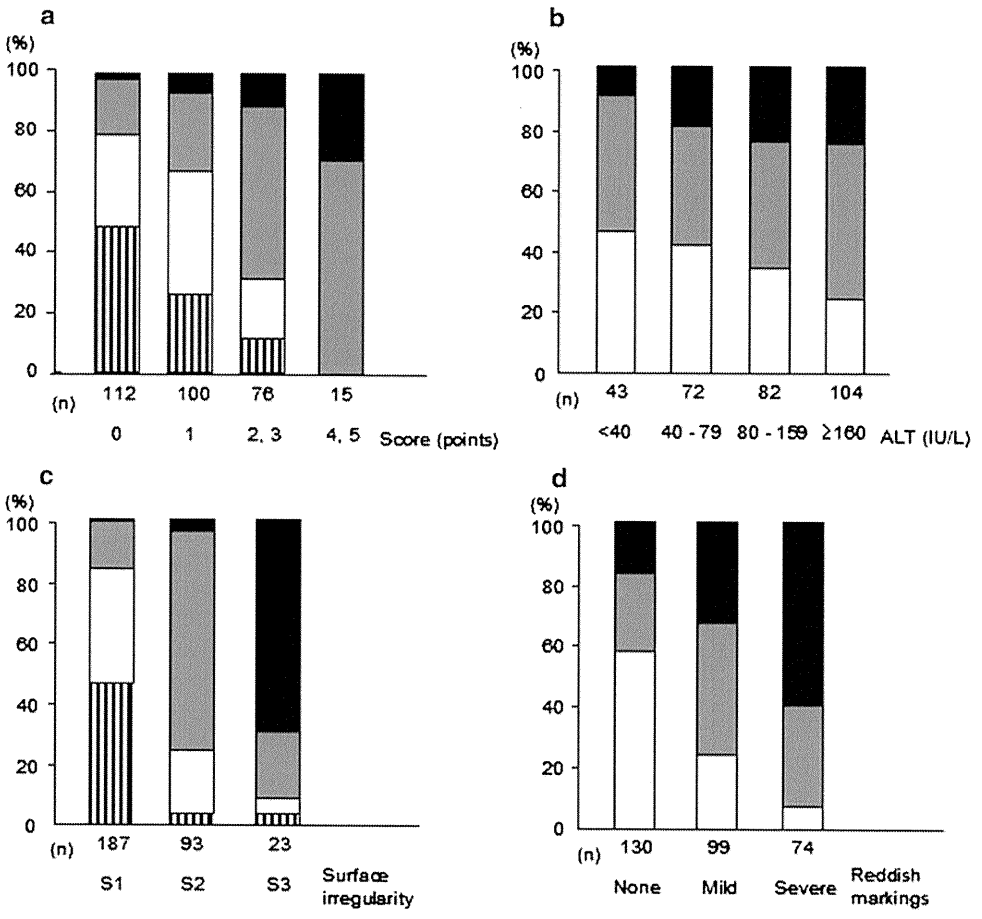

Fig. 2 Comparisons of histology, laboratory parameters, and laparoscopic findings. Histological fibrosis stage was compared with total scores of the five conventional parameters related to liver function with significant correlations ($R = 0.46$, $P < 0.0001$, canonical correlation analysis, **a**): 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

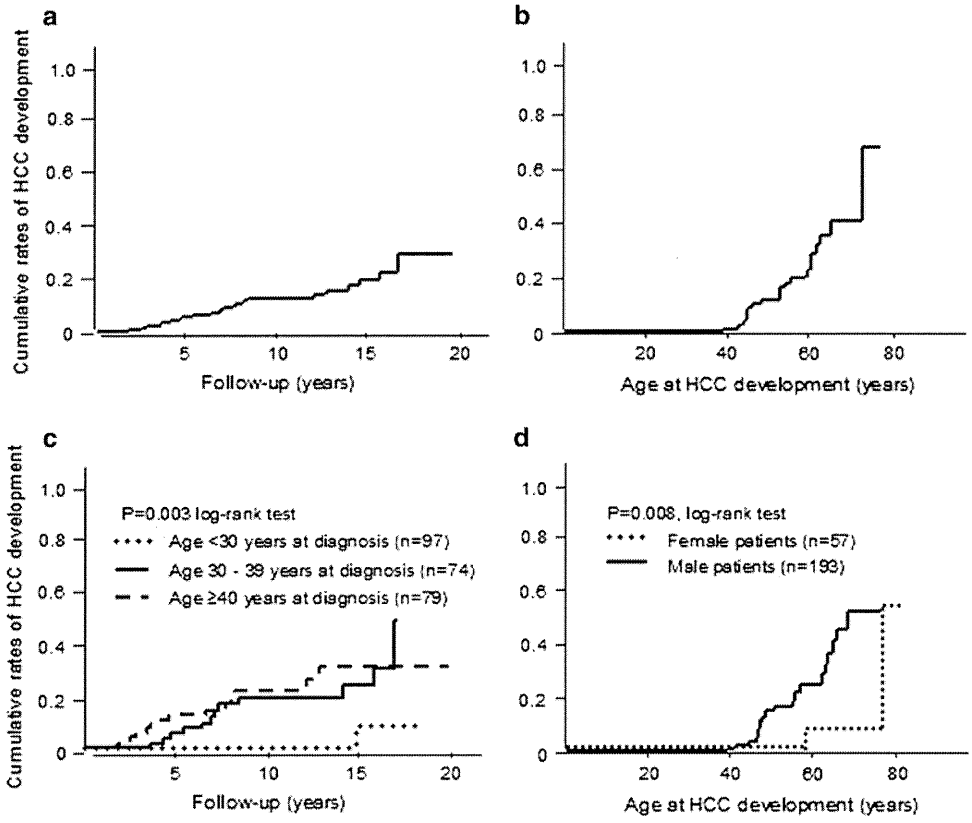

Fig. 3 Cumulative rates of hepatocellular carcinoma (HCC) development. **a** Shows cumulative rate of HCC development as a function of the follow-up period, estimated by the Kaplan–Meier method. The incidence of HCC development was estimated as 5.7% at 5 years of follow-up, 13.5% at 10 years, and 20.6% 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

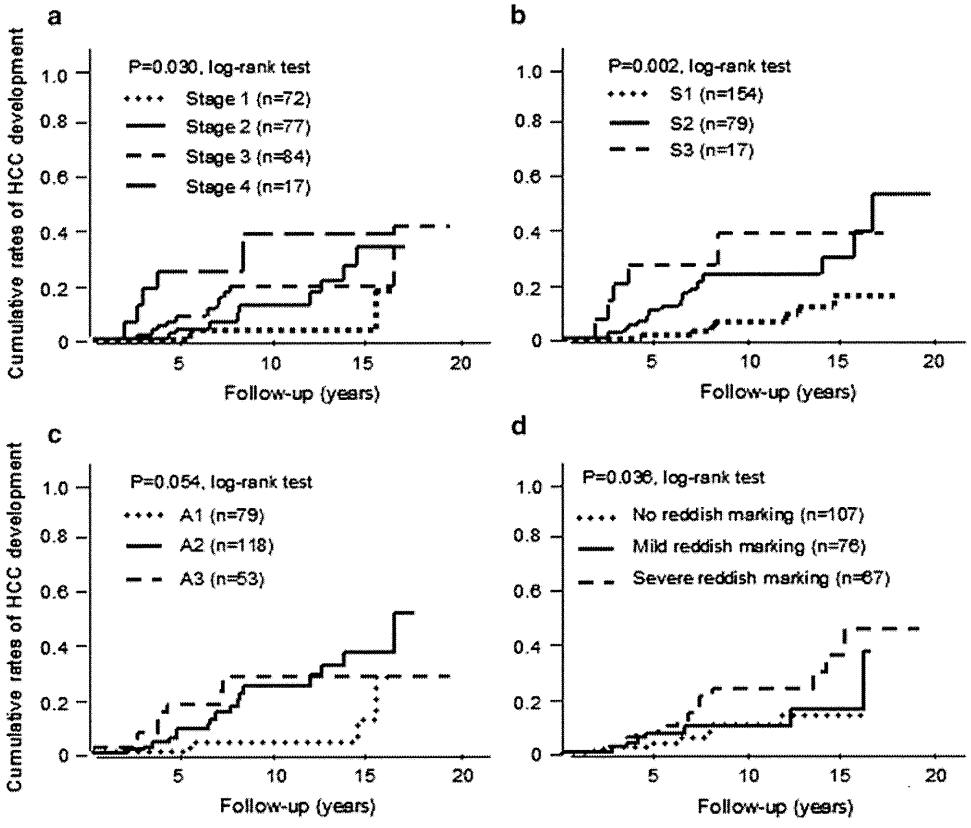

Fig. 4 Cumulative rates of hepatocellular carcinoma (HCC) development, stratified by histology and laparoscopic findings. Figures show cumulative rates of HCC development estimated by the Kaplan–Meier method, stratified by histological fibrosis stage (**a**), laparoscopic surface irregularity (**b**), 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

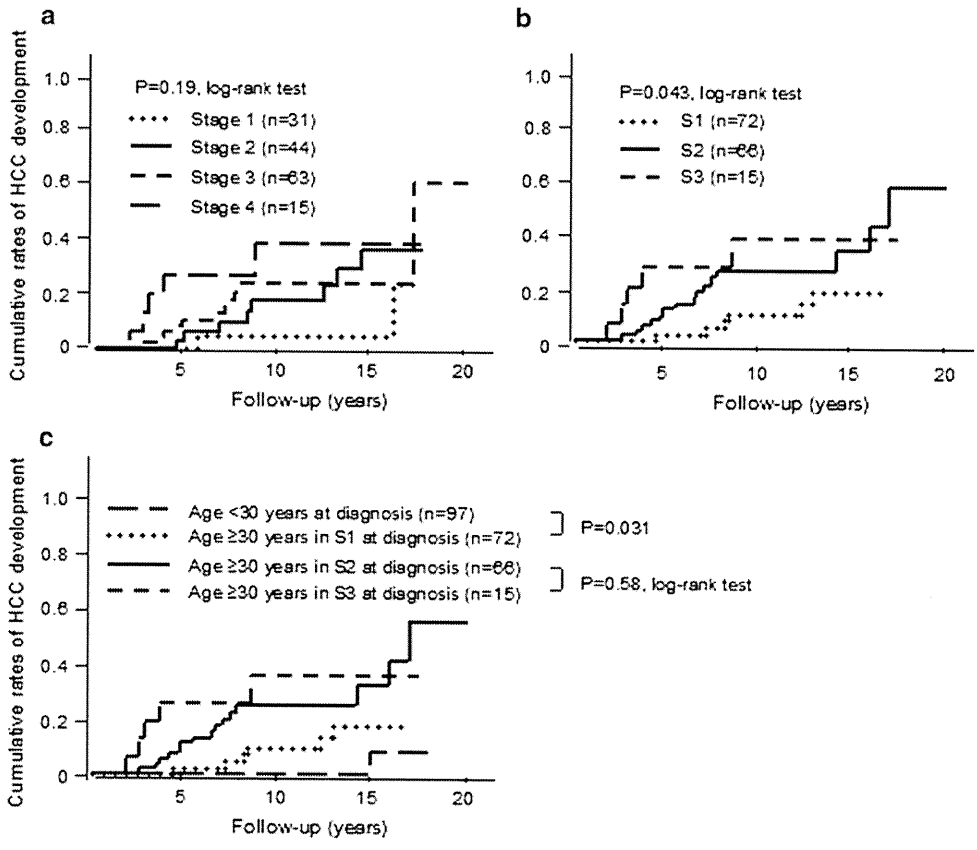

Fig. 5 Cumulative rates of hepatocellular carcinoma (HCC) development, for the patients of age ≥30 years at diagnosis, stratified by disease progression. Figures show cumulative rates of HCC development, for the patients of age ≥30 years at diagnosis, stratified by histological fibrosis stage (**a**) 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)

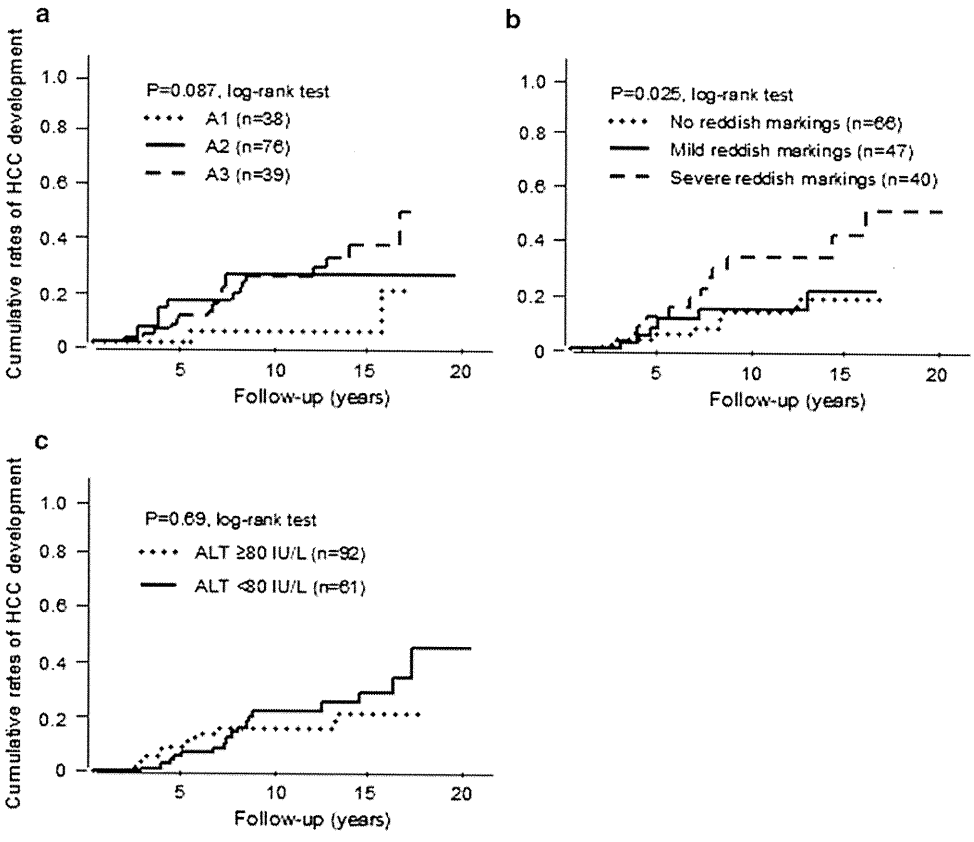

Fig. 6 Cumulative rates of hepatocellular carcinoma (HCC) development, for the patients of age ≥30 years at diagnosis, stratified by inflammatory activity. Cumulative rates of HCC development are shown for patients of age ≥30 years at diagnosis, stratified by histological activity (**a**), laparoscopic 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.

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