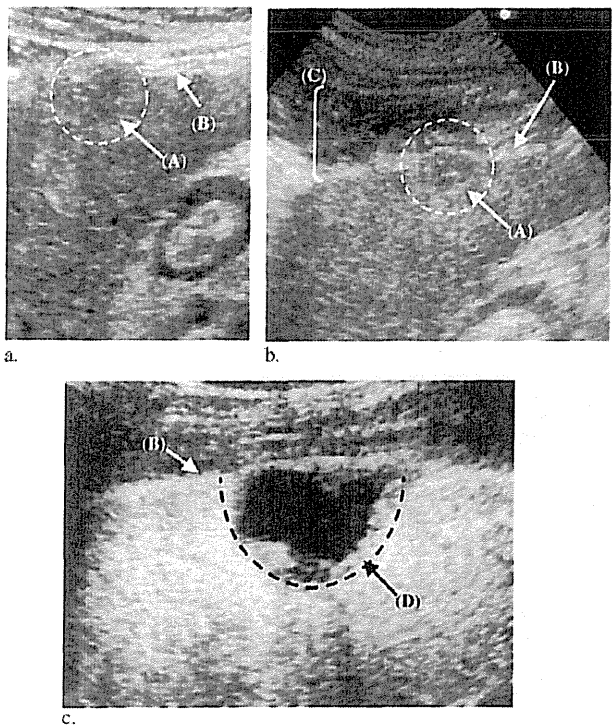

a. b. c.

Figure 2. RF ablation of HCC (*A*) located on the liver surface (*B*) with the injection of a sodium hyaluronate solution onto the liver surface (*C*). **(a)** Plain US before injection of sodium hyaluronate solution. **(b)** Plain US after injection of sodium hyaluronate solution. **(c)** Evaluation of the treatment response by contrast-enhanced US. The shape of the ablated area (*D*) was oval instead of round.

Food and Drug Administration for intraarticular injections (Supartz; Smith and Nephew, London, United Kingdom). Although the intraperitoneal injection of sodium hyaluronate is not approved by the Food and Drug Administration, a material containing sodium hyaluronate (Seprafilm; Genzyme, Framingham, Massachusetts) has been approved to be placed into the peritoneum for the prevention of intraperitoneal adhesions. A previous study in animals (29) investigated the concentration of carbon 14–labeled sodium hyaluronate in the plasma, spleen, liver, adrenal gland, fat, expired air, and urine after an intraperitoneal injection. According to this study (29), sodium hyaluronate is absorbed into the blood, and the plasma concentration of sodium hyaluronate peaks 16 hours after injection. The plasma sodium hyaluronate is metabolized mainly in the liver and is excreted into the expired air as CO_2 and into the urine as N-acetyl-D-glucosamine or D-glucosamine until 120 hours after administration. Another study in animals (30) investigated the effects of intravenous injection of sodium hyaluronate on respiration, blood pressure, heart rate, and blood flow, and revealed no changes after the intravenous injection. Because sodium hyaluronate dissolves into the blood (29,30), the inadvertent injection of this solution into a blood vessel would not pose a potential risk for embolization.

The present in vitro experiment to determine the temperature of the sodium hyaluronate solution revealed that the thermal ablative procedure did not increase the temperature of the sodium hyaluronate solution to a degree that could cause burn injuries. A previous study (31) also reported that viscoelastic substances, including sodium hyaluronate, protect against the temperature increase that occurs during phacoemulsification for cataract surgeries. In addition, a phase I study of the safety of the intraperitoneal injection of a sodium hyaluronate solution did not reveal any marked intraperitoneal inflammation or adhesions. Indeed, a material containing sodium hyaluronate (Seprafilm; Genzyme) is routinely placed into the peritoneum to prevent the formation of adhesions; in addition, an animal study (32) demonstrated that a cross-linked hyaluronate hydrogel reduces the reformation of postsurgical adhesions.

In the present study, the sodium hyaluronate solution separated the liver surface from the abdominal wall or other organs adjacent to the HCC for 2 hours. This separation prevented burn injuries to the adjacent structures and allowed completion of the thermal ablation of HCCs located on the liver surface. Based on the evaluation of the treatment response, each HCC was sufficiently ablated and necrotized by one session of RF ablation. Because the shape of the area necrotized by thermal ablation is usually oval (especially with the Cool-tip needle), some parts of an HCC could have remained unablated if the exposed metallic tip was completely inserted into the HCC or the adjacent liver tissue to prevent a burn injury to the abdominal wall (**Fig 4a**). By contrast, with a sodium hyaluronate layer on the liver surface, it would be possible to perform the thermal ablation with the proximal end of the exposed tip placed outside of the liver, thereby ablating the entire HCC tumor with a sufficient margin while preventing burn injuries to the adjacent structures (**Fig 4b**).

Based on the evaluation of treatment response and follow-up, all HCCs were successfully treated in one session, which yielded adequate margins. In addition, the local recurrence rate of HCCs after treatment was comparable to that of all HCCs treated by RF ablation, including those that are not located close to the liver surface (33–35), and was also comparable to that of HCCs located on the liver surface and treated with RF ablation via laparoscopic approach or with the creation of artificial ascites (9,11,36). Based on these results, the placement of a sodium hyaluronate solution onto the liver surface might be an additional supportive procedure for RF ablation of HCCs located on the liver surface.

There are limitations to the present study. First, the results are preliminary and based on a small series of patients. Larger studies would be necessary to confirm the safety and efficacy of this procedure. In addition, the assessment of the intraperitoneal inflammation and adhesion associated with the intraperitoneal injection of sodium hyaluronate, as well as the assessment of the burn injuries

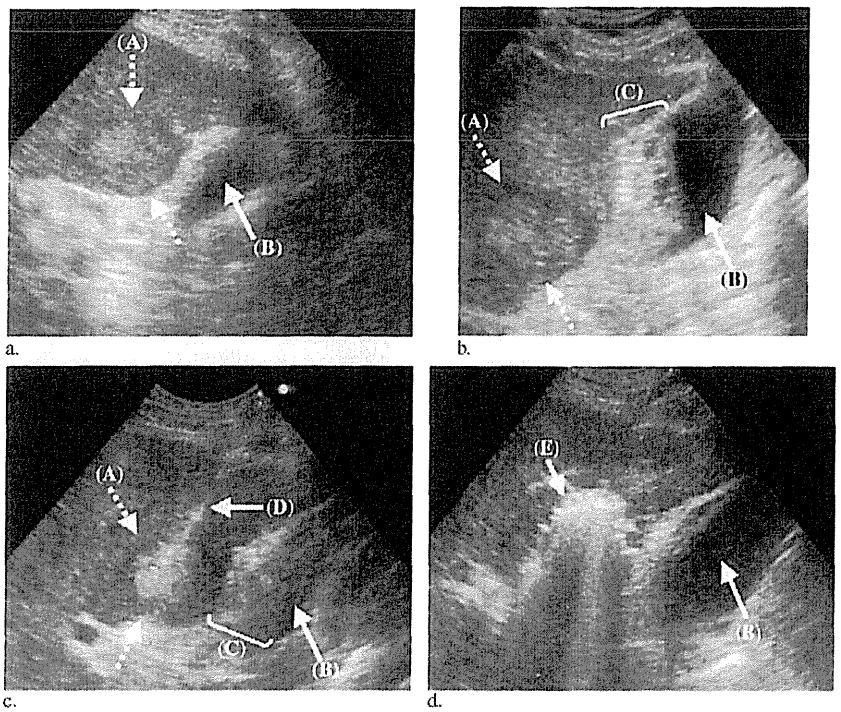

Figure 3. RF ablation of HCC (*A*, dotted arrow) located on the liver surface and adjacent to the gallbladder (*B*) with the injection of a sodium hyaluronate solution. **(a)** Before the injection of the sodium hyaluronate solution, the gallbladder was adjacent to the HCC tumor. **(b)** The injection of sodium hyaluronate solution created a separation (*C*) between the HCC tumor and the gallbladder. **(c)** Insertion of RF ablation needle (*D*). **(d)** Thermal ablation with bubbles (*E*).

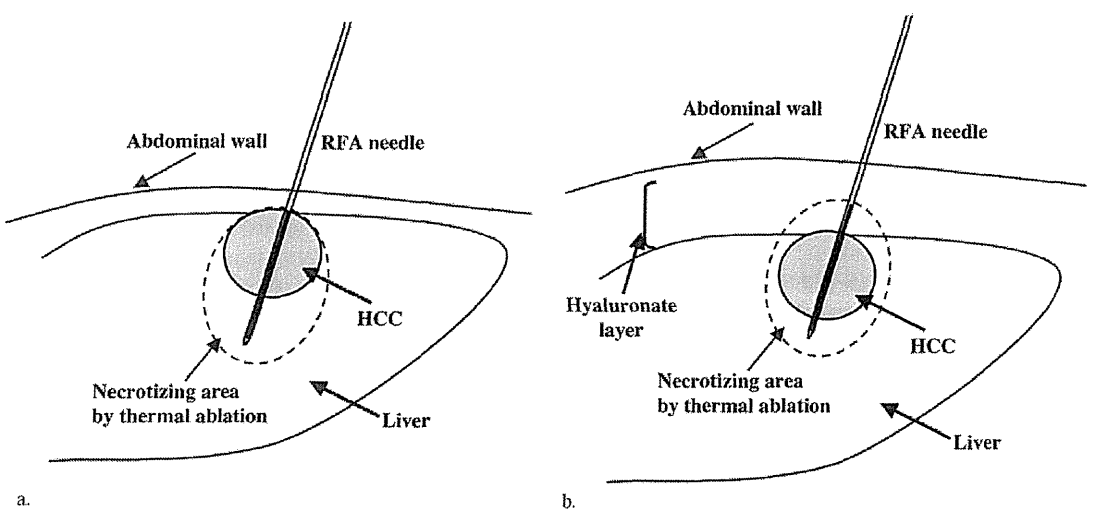

Figure 4. Schematic representation of RF ablation of HCC located on the liver surface. **(a)** The margin around the HCC was small in certain parts if the exposed metallic tip was completely inserted into the tumor or the adjacent liver tissue to prevent burn injuries to the abdominal wall. **(b)** Thermal ablation could be performed with the proximal end of the exposed tip placed outside of the liver and within the sodium hyaluronate layer, allowing the safe ablation of the entire HCC and yielding a uniform margin.

associated with RF ablation, might not have been sufficient; these assessments were based on clinical symptoms and laboratory data. It was not possible to investigate the occurrence of intraperitoneal inflammation, adhesions, or burn injuries directly, and further evaluations would be required to assess the intraperitoneal complications associated with RF ablation and intraperitoneal injection of a sodium hyaluronate solution. US images of the injected sodium hyaluronate layer revealed a mixture of hyperechoic and hypoechoic signals that may cause a reduction in the quality of the HCC visualization. This phenomenon could be caused by microbubbles in the

sodium hyaluronate solution that may disturb the permeability of the US waves. The development of sodium hyaluronate solutions that produce no microbubbles might resolve this problem. In addition, the placement of sodium hyaluronate solution onto the liver surface distal to the body surface or onto the liver surface in patients with ascites was not fully attempted. Therefore, further attempts to apply this material in various patients and to various sites on the liver surface will be necessary to establish this method as a supportive procedure for RF ablation to treat HCCs located close to the liver surface.

In conclusion, the placement of a sodium hyaluronate solution onto the liver surface appears to be a safe and effective supportive procedure for RF ablation of HCCs located on or close to the liver surface. Use of a sodium hyaluronate solution would allow the entire HCC to be necrotized while preventing burn injuries to the adjacent abdominal wall or organs.

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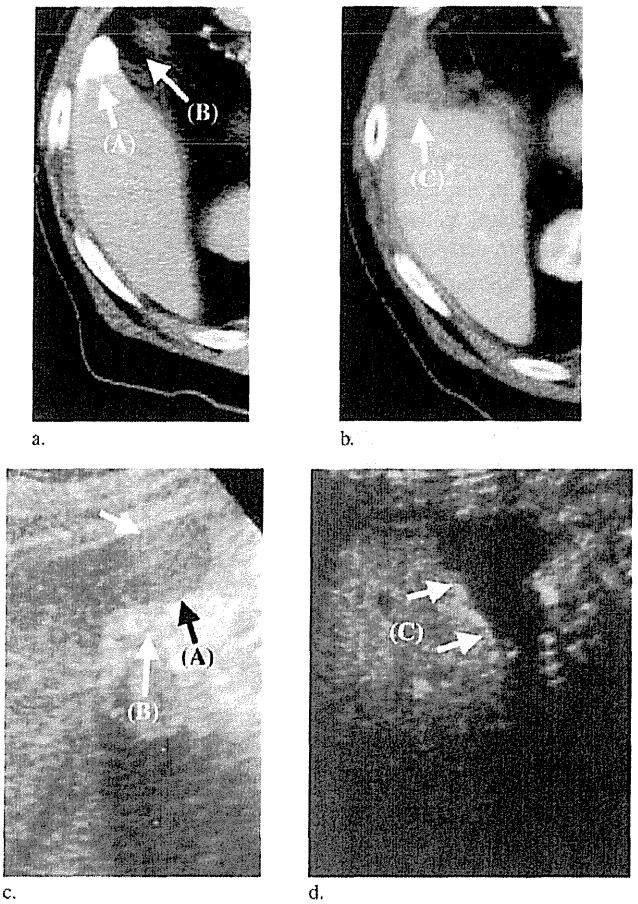


Figure E1. Radiofrequency (RF) ablation of a hepatocellular carcinoma (HCC; *A*) located on the liver surface and adjacent to the intestinal tract (*B*). Computed tomography before **(a)** and after **(b)** RF ablation. The HCC located on the edge of the liver was necrotized completely (*C*). **(c)** Plain ultrasound (US) before RF ablation. **(d)** Contrast-enhanced US after RF ablation shows necrotizing area (*C*).

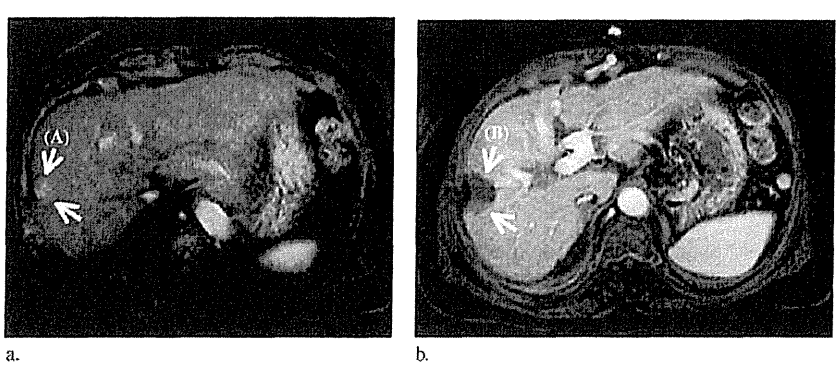
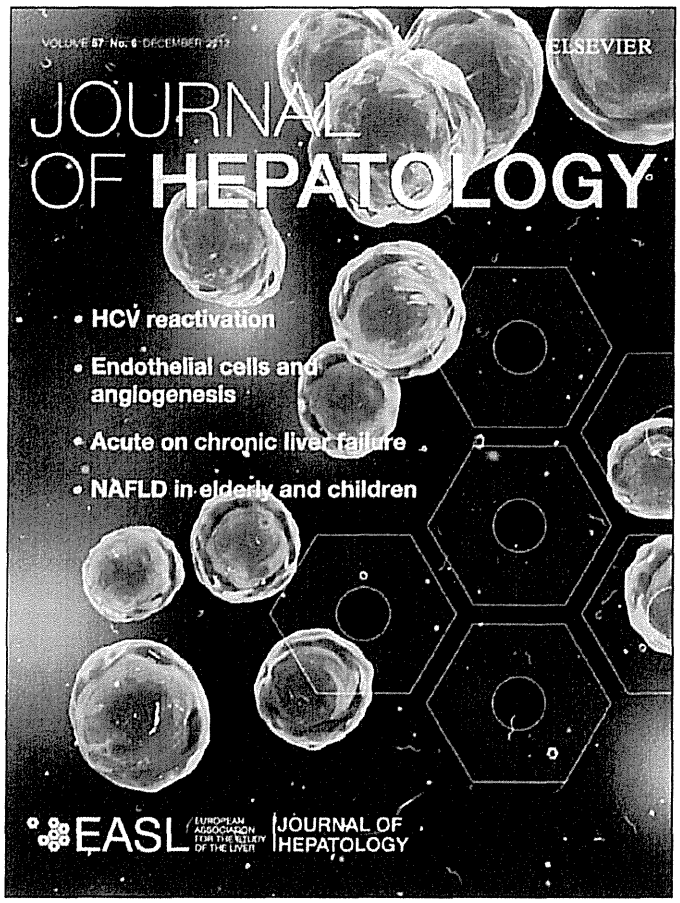


Figure E2. Dynamic magnetic resonance imaging results of RF ablation of HCC located on the liver surface. **(a)** Before RF ablation, an enhancing HCC tumor (*A*) was observed. **(b)** After RF ablation, the HCC and adjacent liver tissue were ablated in an oval shape (*B*).



Research Article

EASL JOURNAL OF HEPATOLOGY

Prognostic significance of a combination of pre- and post-treatment tumor markers for hepatocellular carcinoma curatively treated with hepatectomy

Hidenori Toyoda[1,*], Takashi Kumada[1], Toshifumi Tada[1], Takuro Niinomi[1], Takanori Ito[1], Yuji Kaneoka[2], Atsuyuki Maeda[2]

[1]*Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan;* [2]*Department of Surgery, Ogaki Municipal Hospital, Ogaki, Japan*

Background & Aims: Previous studies reported that the combination of three tumor markers for hepatocellular carcinoma (HCC), alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive AFP (AFP-L3), and des-gamma-carboxy prothrombin (DCP), has the ability to discriminate survival among patients with HCC. In those studies, however, the study population included all patients with various treatment modalities, and tumor markers were measured only before treatment. We investigated the prognostic value of a combination of these tumor markers for HCC, measured before and after treatment, on survival and recurrence in patients treated with hepatectomy.

Methods: A total of 173 patients who underwent hepatectomy for primary, non-recurrent HCC were analyzed. Tumor characteristics, postoperative survival, and recurrence rates were compared according to the number of elevated tumor markers measured before and after treatment.

Results: The correlation between the number of elevated tumor markers before treatment and tumor size, rate of portal vein invasion, and tumor differentiation, respectively, was stronger than that between the number of elevated tumor markers after treatment. In contrast, the number of elevated tumor markers after treatment displayed an excellent ability to discriminate post-treatment survival and recurrence rates compared to that before treatment, and was an independent factor associated with survival and recurrence in multivariate analysis.

Conclusions: The combination of tumor markers measured after hepatectomy has a better discriminatory ability for postoperative survival and recurrence in HCC patients treated with hepatectomy in comparison to the combination of tumor markers measured before treatment.

Keywords: Hepatocellular carcinoma; Tumor markers; Survival; Recurrence; Curative hepatectomy.

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* Corresponding author. Address: Department of Gastroenterology, Ogaki Municipal Hospital, 4-86 Minaminokawa, Ogaki, Gifu 503-8502, Japan. Tel.: +81 584 81 3341; fax: +81 584 75 5715.

E-mail address: hmtoyoda@spice.ocn.ne.jp (H. Toyoda).

Abbreviations: HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin; PIVKA-II, vitamin K absence/antagonist-II; CT, computed tomography; US, ultrasound; MRI, magnetic resonance imaging.

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of cancer-related death [1,2]. In Japan, HCC currently represents the third and fifth most common cause of death from cancer in men and women, respectively, [3]. Presently, three tumor markers specific for HCC are used clinically: alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3), and des-gamma-carboxy prothrombin (DCP), which is also known as protein induced by vitamin K absence/antagonist-II (PIVKA-II). The clinical utility of these tumor markers for detection and diagnosis of HCC, for evaluation of tumor progression, and for determination of prognosis has been reported [4–7]. In addition, the combination of these three tumor markers has been indicated as a useful predictor of patient outcome. We previously reported the prognostic significance of the combination of three tumor markers measured at diagnosis on the survival of all patients with HCC [8]. An increase in the number of elevated tumor markers, consisting of AFP, AFP-L3, and DCP, was clearly associated with a decreased survival rate in patients with HCC. In addition, an increase in the number of elevated tumor markers was well correlated with indicators of HCC progression, including the size and number of tumors, and the rate of portal vein invasion. More recently, Kim *et al.* have reported less progression of HCC without the elevation of AFP and DCP, with higher survival rates [9].

However, in these studies, all patients with HCC who underwent various treatment modalities had been included into the study. In addition, the levels of tumor markers were measured at diagnosis and before treatment. The predictive ability of post-treatment *vs.* pretreatment tumor markers has not been evaluated and compared. In the present study, we measured the levels of these three tumor markers both before and after treatment, in patients who underwent hepatectomy with curative intent. We analyzed their relationship with tumor progression, survival, and recurrence after treatment.

Materials and methods

Patients

A total of 828 patients were diagnosed with primary, non-recurrent HCC, between January 2001 and December 2010 at Ogaki Municipal Hospital. Of these

patients, 264 were treated with hepatectomy. Stored serum samples were available for measurement of the levels of three tumor markers, AFP, AFP-L3, and DCP, before and after hepatectomy in 173 patients. Decisions regarding each patient's course of treatment were made based on the treatment guidelines for HCC in Japan [10]. Anatomical hepatectomy was performed in all 173 patients. HCC tumors were resected with ample margins and enucleation of tumors without adequate margins was not performed. The diagnosis of HCC was confirmed by pathologic examination of resected specimens.

One month after hepatectomy, all patients underwent computed tomography (CT) examination of thorax and abdomen to confirm the absence of residual HCC. All patients were followed-up, for a median of 34.2 months (range, 4.3–122.8 months) until March 2012 at our institution, with ultrasound (US) and CT, or US and magnetic resonance imaging (MRI) every 3–6 months. Regular monitoring of the three tumor markers was performed every 3 months. When an elevation of tumor markers was detected, additional imaging examinations (usually CT or MRI) were performed to check for recurrence. If the presence of recurrence was confirmed, patients underwent treatment for recurrent HCC based on treatment guidelines.

The entire protocol was approved by the hospital institutional review board and carried out in compliance with the Helsinki Declaration.

Measurement of hepatocellular carcinoma tumor markers

Pretreatment tumor markers were measured within 1 week before hepatectomy. Post-treatment tumor markers were measured in the serum sample obtained at the first visit, between 1 and 2 months after hepatectomy. The reported half-lives of AFP and AFP-L3 are 4 days [11] and the half-life of DCP is 60 h [12]. Therefore, the values of post-treatment tumor markers were not influenced by pretreatment tumor marker elevations. Measurements of AFP, AFP-L3, and DCP levels were performed with a microchip capillary electrophoresis and liquid-phase binding assay on the μTASWako i30 auto analyzer (Wako Pure Chemical Industries, Ltd., Osaka, Japan) [13]. The cut-off value of 20 ng/ml was used to establish positivity for AFP, as proposed by Oka *et al.* and Koda *et al.* [14,15]. The cut-off value used to establish positivity for AFP-L3 was 5%, based on our previous study [16]. The cut-off value used to establish positivity for DCP was 40 mAU/ml, as proposed by Okuda *et al.* [17]. The number of tumor markers above the cut-off values was calculated as the number of elevated tumors markers, and survival and recurrence rates were analyzed according to the number of elevated tumor markers.

Statistical analyses

Differences in percentages between groups were analyzed with the Chi-square test. Differences in mean quantitative values were analyzed with the Mann–Whitney *U* test. Changes in percentages and quantitative values with the increase in the number of elevated tumor markers were analyzed with the Cochran–Armitage test and the Jonckheere–Terpstra test, respectively. Receiver-operating characteristics analyses were performed to determine the cut-offs of the number of elevated tumor markers in order to evaluate the accuracy of prediction of 1-, 3-, and 5-year survivals and recurrences and compare them with the accuracy of elevation of each tumor marker. The date of hepatectomy was defined as time zero for the calculation of survival and recurrence rates. In the analysis of survival rates, patients who died were non-censored and patients who survived were censored. In the analysis of recurrence rates, patients in whom HCC recurred were non-censored, and those in whom HCC did not recur were censored. The Kaplan–Meier method [18] was used to calculate survival and recurrence rates, and the log-rank test [19] was used to analyze differences in survival and recurrence.

The Cox proportional hazards model [20] was used for multivariate analyses of factors related to survival and recurrence. Variables analyzed included age, sex, Child-Pugh class (A/B), tumor size, number of tumors, differentiation of HCC (well-moderately or poorly), growth pattern (expansive growth/infiltrative growth), macroscopic and microscopic portal vein invasion (absent/present), and number of elevated tumor markers (zero, one, two, or three). Data analyses were performed using JMP statistical software, version 6.0 (Macintosh version; SAS Institute, Cary, NC, USA). All p values were derived from two-tailed tests, with $p < 0.05$ considered to indicate statistical significance.

Results

Characteristics of patients and hepatocellular carcinoma

Table 1 summarizes the pretreatment characteristics of the study patients. This population comprised 136 males and 37 females with a mean age of 67.0 ± 8.8 years. Most (95.4%) patients belonged to Child-Pugh class [21] A. Multiple tumors were present in 16.8% of patients. HCC was well differentiated in 37.0% and portal vein invasion was observed in 23.1% of patients, based on the pathologic examination of resected HCC specimens. Pretreatment AFP, AFP-L3, and DCP were above the specified cut-off levels in 34.7%, 44.5%, and 52.0% of patients, respectively.

Clinical and pathologic characteristics of hepatocellular carcinoma based on a combination of three tumor markers measured before and after hepatectomy

At pretreatment, there were 47 (27.2%) patients with no elevated tumor markers and 57 (32.9%) patients with one, 38 (22.0%) with two, and 31 (17.9%) with three elevated tumor markers. After hepatectomy, 75 (43.3%) patients had no elevated tumor markers, 70 (40.5%) patients had one, 24 (13.9%) had two, and 4 (2.3%) had three elevated tumor markers. Tables 2 shows pretreatment clinical characteristics and pathologic characteristics of the resected HCC specimens according to the number of elevated tumor markers measured before and after hepatectomy. An increase in tumor size was associated with an increase in the number of elevated tumor markers before treatment ($p < 0.0001$). This gradual increase in tumor size according to the number of elevated tumor markers was not observed with post-treatment values ($p = 0.5836$). On pathologic examination, there was a gradual decrease in the rate of well-differentiated HCC ($p < 0.0001$) and of HCC with expansive growth ($p = 0.0010$), and a gradual increase in the rate of HCC with portal vein invasion ($p < 0.0001$) based on the number of elevated tumor markers before treatment. These are not significant when compared with postoperative values (the rate of well-differentiated HCC, $p = 0.3962$, of HCC with expansive growth, $p = 0.3036$, and the rate of HCC with portal vein invasion, $p = 0.0898$).

Post-operative survival rates based on the combination of three tumor markers measured before and after hepatectomy

The survival rates were compared by the elevation of each tumor marker. By comparing tumor markers measured before treatment, we found significant differences in survival rates by elevated AFP, and AFP-L3 levels, but not DCP (Supplementary Fig. 1). By comparing tumor markers measured after treatment, survival rates were significantly lower in patients with elevated AFP, AFP-L3, and DCP levels, respectively (Supplementary Fig. 2). We determined the survival rates of patients after hepatectomy as a function of the number of elevated tumor markers before and after hepatectomy (Fig. 1). The number of elevated tumor markers after treatment provided a better discrimination of survival rates than the number of elevated tumor markers before treatment. The survival rates were higher in patients without elevated tumor markers after treatment, followed by patients with one, two, and three elevated tumor markers, in this order.

We next compared the accuracy of death prediction between each individual tumor marker and the combination of the three (Supplementary Table 1). Higher accuracy in predicting death within 1, 3, and 5 year(s), respectively, was found by combining the three markers than by each individual marker alone.

In multivariate analysis, the number of elevated tumor markers was not associated with specific survival after hepatectomy, when tumor markers measured before treatment were used (Supplementary Table 2). In contrast, it was an independent

Table 1. Characteristics of the study patients (n = 173).

Age (yr); (range)	67.0 ± 8.8 (21-83)
Sex (female/male)	37 (21.4)/136 (78.6)
Etiology (HBV/HCV/HBV+HCV/non-HBV, non-HCV)	29 (16.8)/116 (67.0)/2 (1.2)/26 (15.0)
Child-Pugh class (A/B)	165 (95.4)/8 (4.6)
Albumin (g/dl)	4.02 ± 0.42
Total bilirubin (mg/dl)	0.78 ± 0.34
15-min retention rate of ICG (%)	15.4 ± 7.4
Prothrombin (%)	92.7 ± 14.3
Platelet (x10^3/ml)	144 ± 71
Tumor size (cm); (range)	3.28 ± 2.59 (0.8-16.4)
Number of tumors (n); (range)	1.24 ± 0.56 (1-3)
Single/multiple	144 (83.2)/29 (16.8)
Macroscopic portal vein invasion (absent/present)*	167 (96.5)/6 (3.5)
Microscopic portal vein invasion (absent/present)	133 (76.9)/40 (23.1)
Differentiation (well-/moderately or poorly)	64 (37.0)/109 (63.0)
Growth pattern (expansive growth/infiltrative growth)	151 (87.3)/22 (12.7)
AFP (ng/ml); median (range)	10.9 (0.8-27,242.8)
≥20 ng/ml/<20 ng/ml	60 (34.7)/113 (65.3)
AFP-L3 (%); median (range)	3.9 (0.0-89.7)
≥5%/<5%	77 (44.5)/96 (55.5)
DCP (mAU/ml); median (range)	43.0 (5.0-60,030.0)
≥40 mAU/ml/<40 mAU/ml	90 (52.0)/83 (48.0)

Values are mean ± SD, unless otherwise indicated.
Percentages are given in parentheses, unless otherwise indicated.
HBV, hepatitis B virus; HCV, hepatitis C virus; ICG, indocyanine green test; AFP, alpha-fetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive AFP; DCP, des-gamma-carboxy prothrombin.
*Evaluated based on imaging findings.

factor associated with survival when the number of elevated tumor markers was replaced by those measured after treatment (Table 3).

Post-treatment recurrence rates based on the combination of three tumor markers measured before and after hepatectomy

The rates of recurrence were compared by the elevation of each tumor marker. In comparisons of tumor markers measured before treatment, we did not find significant differences in recurrence rates by the elevation of AFP and DCP (Supplementary Fig. 3). In comparisons of tumor markers measured after treatment, recurrence rates were significantly higher in patients with elevated AFP, AFP-L3, and DCP levels, respectively (Supplementary Fig. 4). We determined the rates of recurrence in patients, after hepatectomy with curative intent, based on the number of elevated tumor markers before and after hepatectomy (Fig. 2). We did not find any difference in the recurrence rates based on the number of elevated tumor markers measured before treatment. In contrast, higher recurrence rates were associated with an increasing number of elevated tumor markers measured after treatment. Moreover, higher accuracy in prediction of recurrence within 1, 3, and 5 year(s), was found with the combination of these three markers than with each individual marker alone (Supplementary Table 3).

In multivariate analysis, the number of elevated tumor markers was not associated with recurrence after hepatectomy, when tumor markers measured before treatment were used (Supplementary Table 4). In contrast, it was an independent factor associated with recurrence when the number of elevated tumor markers was replaced by those measured after treatment (Table 4).

Discussion

In the present study, we investigated the significance of a combination of three tumor markers for HCC (AFP, AFP-L3, and DCP) measured after treatment *vs.* before treatment, in predicting outcome in patients undergoing hepatectomy with curative intent. Our results demonstrated that the number of elevated tumor markers after treatment had a better discriminatory ability for both survival and recurrence rates after hepatectomy. A gradual decrease in survival rates and an increase in recurrence rates were observed as the number of post-treatment elevated tumor markers increased.

In our previous study of 685 patients with HCC [8], the number of elevated tumor markers measured before treatment was well associated with the progression of HCC and survival rates. In contrast, in the present study, the number of elevated pretreatment tumor markers did not predict survival and recurrence rates, although it was associated with the progression of HCC. This could be due to the fact that the patients analyzed in our previous study underwent various types of treatment according to HCC progression and liver function, while in the present study we focused on HCC patients who underwent hepatectomy with

Table 2. Clinical characteristics of patients with HCC based on the number of positive tumor markers measured (A) before hepatectomy and (B) after hepatectomy (n = 173).

A

	Number of positive tumor markers before treatment			
	0 (n = 47)	1 (n = 57)	2 (n = 38)	3 (n = 31)
Age (yr)	67.1 ± 7.6	67.8 ± 8.0	68.2 ± 8.4	64.2 ± 12.0
Sex (female/male)	6 (12.8)/41 (87.2)	16 (28.1)/41 (71.9)	10 (26.3)/28 (73.7)	5 (16.1)/26 (83.9)
Child-Pugh class (A/B)	46 (97.9)/1 (2.1)	55 (96.5)/2 (3.5)	35 (92.1)/3 (7.9)	29 (93.5)/2 (6.5)
Albumin (g/dl)	4.03 ± 0.32	4.04 ± 0.45	3.96 ± 0.48	4.06 ± 0.43
Total bilirubin (mg/dl)	0.75 ± 0.34	0.77 ± 0.28	0.81 ± 0.39	0.80 ± 0.36
15-min retention rate of ICG (%)	14.9 ± 6.2	16.6 ± 9.1	16.1 ± 6.7	13.1 ± 5.6
Prothrombin (%)	93.1 ± 13.9	90.8 ± 15.6	91.3 ± 11.9	97.2 ± 14.9
Platelet (x10^3/ml)	149 ± 89	140 ± 62	138 ± 70	150 ± 59
Tumor size (cm)[1]	2.25 ± 1.09	2.96 ± 2.02	3.75 ± 2.88	4.87 ± 3.74
Number of tumors (single/multiple)	40 (85.1)/7 (14.9)	50 (87.7)/7 (12.3)	30 (78.9)/8 (21.1)	24 (77.4)/7 (22.6)
Differentiation (well-/moderately or poorly)[2]	26 (55.3)/21 (44.7)	27 (47.4)/30 (52.6)	9 (23.7)/29 (76.3)	2 (6.5)/29 (93.5)
Growth pattern (expansive/infiltrative)[3]	44 (93.6)/3 (6.4)	53 (93.0)/4 (7.0)	34 (89.5)/4 (10.5)	20 (64.5)/11 (35.5)
Capsular formation (absent/present)*	24 (54.5)/20 (45.5)	25 (47.2)/28 (52.8)	10 (29.4)/24 (70.6)	3 (15.0)/17 (85.0)
Capsular infiltration (absent/present)**	11 (55.0)/9 (45.0)	15 (53.6)/13 (46.4)	8 (33.3)/16 (66.7)	6 (35.3)/11 (64.7)
Portal vein invasion (absent/present)***[,4]	44 (93.6)/3 (6.4)	52 (91.2)/5 (8.8)	27 (71.1)/11 (28.9)	10 (32.3)/21 (67.7)

B

	Number of positive tumor markers after treatment			
	0 (n = 75)	1 (n = 70)	2 (n = 24)	3 (n = 4)
Age (yr)	67.7 ± 9.3	66.8 ± 7.9	65.1 ± 10.3	72.0 ± 4.5
Sex (female/male)	14 (18.7)/61 (81.3)	17 (24.3)/53 (75.7)	6 (25.0)/18 (75.0)	0/4 (100.0)
Child-Pugh class (A/B)	73 (97.3)/2 (2.7)	64 (91.4)/6 (8.6)	24 (100.0)/0	4 (100.0)/0
Albumin (g/dl)	4.10 ± 0.41	3.99 ± 0.44	3.88 ± 0.35	4.13 ± 0.39
Total bilirubin (mg/dl)	0.82 ± 0.36	0.73 ± 0.31	0.74 ± 0.31	0.80 ± 0.35
15-min retention rate of ICG (%)	14.6 ± 6.2	16.3 ± 8.8	15.7 ± 6.4	15.5 ± 4.6
Prothrombin (%)	94.4 ± 13.3	90.4 ± 16.1	93.7 ± 12.8	94.8 ± 6.2
Platelet (x10^3/ml)	157 ± 87	136 ± 58	121 ± 52	161 ± 39
Tumor size (cm)	3.33 ± 2.58	2.86 ± 1.93	3.71 ± 3.11	6.90 ± 6.44
Number of tumors (single/multiple)	63 (84.0)/12 (16.0)	61 (87.1)/9 (12.9)	18 (75.0)/6 (25.0)	2 (50.0)/2 (50.0)
Differentiation (well-/moderately or poorly)	30 (40.0)/45 (60.0)	25 (35.7)/45 (64.3)	9 (37.5)/15 (62.5)	0/4 (100.0)
Growth pattern (expansive/infiltrative)	66 (88.0)/9 (12.0)	64 (91.4)/6 (8.6)	18 (75.0)/6 (25.0)	3 (75.0)/1 (25.0)
Capsular formation (absent/present)*	23 (34.8)/43 (65.2)	33 (51.6)/31 (48.4)	6 (33.3)/12 (66.7)	0/3 (100.0)
Capsular infiltration (absent/present)**	20 (46.5)/23 (53.5)	17 (54.8)/14 (45.2)	3 (25.0)/9 (75.0)	0/3 (100.0)
Portal vein invasion (absent/present)***	59 (78.7)/16 (21.3)	59 (84.3)/11 (15.7)	14 (58.3)/10 (41.7)	1 (25.0)/3 (75.0)

[1]$p < 0.0001$ (Jonckheere–Terpstra test); [2,4]$p < 0.0001$; [3]$p = 0.0010$ (Cochran–Armitage test).
ICG, indocyanine green test.
*Evaluated only in HCC with expansive growth.
**Evaluated only in HCC with capsular formation.
***On pathologic evaluation.
Unless otherwise indicated, values are mean ± SD and percentages are indicated in parentheses.
ICG, indocyanine green test.

curative intent. Recently, Kiriyama *et al.* have investigated the utility of the combination of these three tumor markers measured at diagnosis (before treatment) in predicting outcomes in HCC patients treated with hepatectomy [22]. They have reported that elevation of all three tumor markers (triple positive tumor markers) is associated with invasive tumor growth, and patients

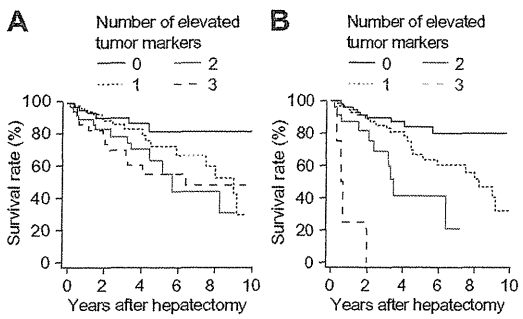

Fig. 1. Survival rates after hepatectomy as a function of the number of elevated tumor markers measured before and after hepatectomy. (A) Survival rates according to the number of elevated tumor markers measured before hepatectomy (zero *vs.* one, $p = 0.0907$; one *vs.* two, $p = 0.1542$; two *vs.* three, $p = 0.8772$). (B) Survival rates according to the number of elevated tumor markers measured after hepatectomy (zero *vs.* one, $p = 0.0322$; one *vs.* two, $p = 0.0085$; two *vs.* three, $p = 0.0006$).

with triple positive tumor markers have significantly lower recurrence-free and disease-specific survival rates after hepatectomy. Their study has included patients who underwent non-anatomical hepatectomy (35.7%). This treatment with insufficient curativity might have increased the impact of triple positive pretreatment tumor markers and associated progression of HCC on the prognosis of patients after hepatectomy. In contrast to their results, we did not find any difference in survival and recurrence rates between patients with triple positive tumor markers and other patients in the pretreatment evaluation, for patients who underwent anatomical hepatectomy. In addition, they failed to find any differences in the rates between patients with one or two positive tumor markers and those without positive tumor markers, measured before treatment. With respect to the post-treatment evaluation in our study, decreased survival rates and increased recurrence rates were observed, not only in patients with elevation of all three tumor markers but also in patients with one or two elevated tumor markers, when compared to those with no elevated tumor markers. Thus, the number of elevated tumor markers after treatment was well associated with prognosis after curative hepatectomy and categorized patients into 4 groups by the likelihood of survival and recurrence.

An increase in tumor size and the rate of portal vein invasion, and a decrease in the rate of well-differentiated HCC and of HCC

Table 3. Univariate and multivariate analyses for factors associated with postoperative survival using a combination of three tumor markers measured after hepatectomy (n = 173).

	Univariate analyses		Multivariate analyses	
Factor	*p* value	Risk ratio (95% CI)	*p* value	Risk ratio (95% CI)
Age	0.0518	1.0358 (0.9997-1.0766)	0.1032	1.0320 (0.9939-1.0753)
Sex				
Male		1		
Female	0.3682	0.8371 (0.5350-1.2138)		
Child-Pugh class				
A		1		
B	0.2379	0.6038 (0.6039-1.2951)		
Tumor size	0.0012	1.1625 (1.0671-1.2497)	0.2049	1.0639 (0.9650-1.1651)
Number of tumors	0.0006	2.2953 (1.4681-3.4226)	0.0370	1.7047 (1.0345-2.7230)
Differentiation				
Well-		1		
Moderately/poorly	0.0009	1.7532 (1.2453-2.6068)	0.0554	1.4437 (0.9918-2.1943)
Growth pattern				
Expansive		1		
Infiltrative	0.0142	1.6225 (1.1114-2.2602)	0.3353	1.2306 (0.7984-1.8370)
Macroscopic portal vein invasion				
Absent		1		
Present	0.2168	1.5151 (0.7443-2.5195)		
Microscopic portal vein invasion				
Absent		1		
Present	0.0083	1.5829 (1.1327-2.1604)	0.5730	1.1162 (0.7554-1.6191)
Number of positive tumor markers				
0		1		
1	0.0315	1.4807 (1.0344-2.1980)	0.0194	1.5534 (1.0720-2.3312)
2	0.0004	2.3463 (1.4928-3.6906)	0.0172	1.8241 (1.1157-2.9683)
3	<0.0001	6.0824 (3.0998-10.9708)	0.0018	3.6788 (1.7020-7.3886)

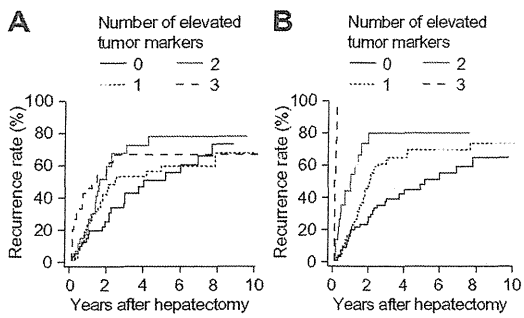

Fig. 2. Recurrence rates after hepatectomy as a function of the number of elevated tumor markers measured before and after hepatectomy. (A) Recurrence rates according to the number of elevated tumor markers measured before hepatectomy (zero *vs.* one, $p = 0.4966$; one *vs.* two, $p = 0.1756$; two *vs.* three, $p = 0.6227$). (B) Recurrence rates according to the number of elevated tumor markers measured after hepatectomy (zero *vs.* one, $p = 0.0352$; one *vs.* two, $p = 0.0050$; two *vs.* three, $p < 0.0001$).

with expansive growth were found in association with the number of elevated tumor markers before treatment, as in our previous report [8] and the report by Kiriyama *et al.* [22]. These associations lacked with the number of elevated tumor markers after treatment. Although pretreatment elevations of HCC tumor markers, especially AFP-L3 and DCP, have been reported to indicate advanced characteristics of HCC with poor prognosis [23,24], the effects markedly decreased when patients underwent hepatectomy [25]. Hepatectomy appears to effectively treat HCC with high malignant potential associated with a pretreatment elevation of tumor markers, if hepatectomy is performed anatomically with curative intent. Indeed, the number of elevated tumor markers markedly decreased after treatment in patients who underwent hepatectomy compared to patients treated with locoregional ablative therapies (radiofrequency ablation or ethanol injection) or transcatheter arterial chemoembolization [8]. Therefore, the elevation of tumor markers after hepatectomy may indicate the residual minute HCC cells that cannot be identified during hepatectomy and imaging examination after treatment. Patients in whom all three tumor markers remained positive even after hepatectomy had markedly low survival and high recurrence

Table 4. Univariate and multivariate analyses for factors associated with postoperative recurrence using a combination of three tumor markers measured after hepatectomy (n = 173).

	Univariate analyses		Multivariate analyses	
Factor	*p* value	Risk ratio (95% CI)	*p* value	Risk ratio (95% CI)
Age	0.1167	1.0185 (0.9957-1.0437)		
Sex				
Male		1		
Female	0.5384	0.9220 (0.6971-1.1841)		
Child-Pugh class				
A		1		
B	0.7533	1.0701 (0.6675-1.5515)		
Tumor size	<0.0001	1.1745 (1.0994-1.2431)	0.1180	1.0764 (0.9815-1.1826)
Number of tumors	0.0023	1.8096 (1.2539-2.5105)	0.0329	1.5975 (1.0409-2.3558)
Differentiation				
Well-		1		
Moderately/poorly	0.0222	1.2817 (1.0354-1.6045)	0.2196	1.1620 (0.9149-1.4852)
Growth pattern				
Expansive		1		
Infiltrative	0.2978	1.1830 (0.8512-1.5707)		
Macroscopic portal vein invasion				
Absent		1		
Present	0.0123	1.8793 (1.1690-2.7380)	0.1300	1.6593 (0.8519-2.9452)
Microscopic portal vein invasion				
Absent		1		
Present	0.0504	1.3051 (0.9995-1.6620)	0.4978	1.1115 (0.8121-1.4897)
Number of positive tumor markers				
0		1		
1	0.0413	1.2716 (1.0095-1.6088)	0.0069	1.4017 (1.0967-1.8044)
2	0.0001	1.9214 (1.3999-2.5862)	0.0001	2.0143 (1.4590-2.7324)
3	<0.0001	11.8230 (5.5814-25.0773)	<0.0001	8.3969 (3.4707-19.7694)

rates; elevation of all three tumor markers after treatment was a strongest indicator of poor survival. These patients should be considered to have received insufficient resection despite anatomical hepatectomy with curative intent and the absence of residual HCC tumors on CT examination after hepatectomy.

The recurrence rates were high regardless of the number of post-treatment tumor markers; recurrence was detected in more than 40% of patients, even in patients without elevated tumor markers after hepatectomy (Fig. 2). This is partly due to the high rate of recurrence of HCC even after curative treatment including multicentric occurrence [26]. Therefore, the sensitivity of the elevated post-treatment tumor markers in predicting recurrence is not high and, conversely, the absence of elevated tumor markers after treatment does not necessarily indicate a low risk of recurrence. However, the time to recurrence after treatment is an important factor for prognosis, and the number of elevated tumor markers after hepatectomy well discriminated recurrence rates when the time to recurrence was taken into account.

This study has several limitations. All three post-treatment tumor markers were measured at one time in the same serum sample despite differences in the half-lives between AFP/AFP-L3 and DCP, and was not strictly based on the half-lives of each marker. It was difficult to obtain serum samples at multiple occasions in a short period, after hepatectomy in clinical settings. We, therefore, measured all tumor markers between 1 and 2 months after hepatectomy, considering that the values of post-treatment tumor markers were not influenced by the pretreatment elevation during this period. Since none of our patients were treated with liver transplantation, we do not have data on the changes in the number of elevated tumor markers when patients undergo liver transplantation. Such analysis should be performed in the future. In addition, the association between number of elevated post-treatment tumor markers and status of the remnant liver after hepatectomy, including the residue of minute HCC cells undetected by imaging modalities, remains unknown. However, we hope this will be investigated in the future to shed light on the mechanisms behind the elevation of tumor markers after hepatectomy.

In conclusion, in our examination of 173 patients treated with hepatectomy with curative intent, the combination of three tumor markers measured after treatment had high discriminatory ability for survival and recurrence after hepatectomy. Further studies are warranted to confirm this association in other populations.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2012.07.018.

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Letter to the Editor

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Lower incidence of hepatocellular carcinoma in patients with transient virologic response to peginterferon and ribavirin combination therapy: Is it really the effect of the therapy?

To the Editor:

Ogawa *et al.* reported interesting and important findings, based on a large prospective cohort, regarding the effect of combination therapy with peginterferon and ribavirin on the incidence of hepatocellular carcinoma (HCC) [1]. They reported lower incidence of HCC after treatment in patients with transient virological response (TVR, defined as relapse or breakthrough), as well as in patients with sustained virological response (SVR), relative to patients with non-virological response (NVR). The suppressive effect of this antiviral therapy on the development of HCC in patients with SVR has been established by several reports and can be explained by the eradication of hepatitis C virus (HCV), resulting in the release of inflammation and improvement of liver fibrosis [2]. However, it is unclear why the incidence of HCC after treatment was also lower in patients with TVR than in those with NVR, despite the persistence of viremia after treatment. Ogawa *et al.* attributed this observation to the preventive effect of complete HCV suppression during therapy on the development of HCC.

Previously reported viral and host factors that are strongly associated with response to antiviral therapy with peginterferon and ribavirin [3,4] may also be associated with the pathogenesis of HCC. Amino acid substitutions in the HCV core region, a viral factor reportedly associated with the response to peginterferon and ribavirin therapy in patients with HCV genotype 1b [3] (i.e., the vast majority of subjects in the study by Ogawa *et al.*), are also associated with the development of HCC [5]. Regarding host factors associated with the response to combination therapy [4], genetic polymorphisms near the *IL28B* gene are reportedly associated with hepatic steatosis [6] and interact with amino acid substitutions in the HCV core region [5,7]. Both hepatic steatosis and amino acid substitutions in the HCV core region are associated with the development of HCC [5,8]. In addition, amino acid substitutions in the HCV core region are reportedly associated with the development of HCC, even in patients who achieved SVR [9]. Ogawa *et al.* reported, without providing detailed data, a higher incidence of HCC in patients bearing the non-TT genotype of rs8099917 near the *IL28B* gene, which is unfavorable to response to the combination therapy; this observation is also consistent with our previous report [10].

These results suggest that differences in HCC incidence based on the outcome of antiviral combination therapy are mainly attributable to these viral and host factors. It is possible that Ogawa *et al.* simply classified patients based on the likelihood of developing HCC upon observing the response to the combination therapy (i.e., TVR and NVR). It would be interesting if the authors were to analyze the incidence of HCC in relation to the outcome of combination therapy based on these host and viral factors. In addition, it would be interesting to investigate genetic polymorphisms near the *IL28B* gene and amino acid substitutions at residue 70 of the HCV core region, in the 13 patients who developed HCC despite the achievement of SVR.

Given the existence of factors associated with both therapeutic response and incidence of HCC, one should be cautious in drawing the conclusion that lower incidence of HCC in patients with TVR, relative to those with NVR, actually reflects the "suppressive effect" of peginterferon and ribavirin combination therapy on hepatocarcinogenesis.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Letter to the Editor

Hidenori Toyoda*
Takashi Kumada
Toshifumi Tada
Department of Gastroenterology, Ogaki Municipal Hospital, Japan
*Corresponding author.
E-mail address: hmtoyoda@spice.ocn.ne.jp (H. Toyoda)

Research Article

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Non-hypervascular hypointense nodules detected by Gd-EOB-DTPA-enhanced MRI are a risk factor for recurrence of HCC after hepatectomy

Hidenori Toyoda[1,*], Takashi Kumada[1], Toshifumi Tada[1], Takuro Niinomi[1], Takanori Ito[1], Yasuhiro Sone[2], Yuji Kaneoka[3], Atsuyuki Maeda[3]

[1]*Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan;* [2]*Department of Radiology, Ogaki Municipal Hospital, Ogaki, Japan;* [3]*Department of Surgery, Ogaki Municipal Hospital, Ogaki, Japan*

Background & Aims: The gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging (MRI) often depicts non-hypervascular hypointense hepatic nodules during the hepatobiliary phase in patients with hepatocellular carcinoma (HCC). It is unclear whether the presence of these nodules is associated with HCC recurrence after hepatectomy. We conducted a prospective observational study to investigate the impact of the presence of non-hypervascular hypointense hepatic nodules on the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI on the recurrence of HCC after hepatectomy.

Methods: A total of 77 patients who underwent hepatectomy for primary, non-recurrent, hypervascular HCC were prospectively followed up after hepatectomy. Post-operative recurrence rates were compared according to the presence of non-hypervascular hypointense nodules on preoperative Gd-EOB-DTPA-enhanced MRI.

Results: Recurrence rates after hepatectomy were higher in patients with non-hypervascular hypointense nodules (risk ratio 1.9396 [1.3615–2.7222]) and the presence of non-hypervascular hypointense nodules was an independent factor associated with postoperative recurrence (risk ratio 2.1767 [1.5089–3.1105]) along with HCC differentiation and portal vein invasion. While no differences were found in the rate of intrahepatic metastasis recurrence based on the preoperative presence of non-hypervascular hypointense hepatic nodules, the rate of multicentric recurrence was significantly higher in patients with preoperative non-hypervascular hypointense hepatic nodules.

Conclusions: Patients with preoperative non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI are at higher risk of HCC recurrence after hepatectomy, mainly due to multicentric recurrence.

Keywords: Hepatocellular carcinoma; Gd-EOB-DTPA-enhanced MRI; Non-hypervascular hypointense nodule; Hepatobiliary phase; Hepatectomy; Recurrence.
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* Corresponding author. Address: Department of Gastroenterology, Ogaki Municipal Hospital, 4-86 Minaminokawa, Ogaki, Gifu 503-8502, Japan. Tel.: +81 584 81 3341; fax: +81 584 75 5715.
E-mail address: hmtoyoda@spice.ocn.ne.jp (H. Toyoda).
Abbreviations: Gd-EOB-DTPA, gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid; MRI, magnetic resonance imaging; HCC, hepatocellular carcinoma; US, ultrasonography; MDCT, multidetector-row computed tomography; TFE, turbo field echo; CTHA, computed tomography during hepatic arteriography.

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of cancer-related death [1,2]. In Japan, HCC is the third and fifth most common cause of death from cancer in men and women, respectively [3]. Tremendous efforts have been made to improve various imaging techniques, including ultrasonography (US), multidetector-row computed tomography (MDCT) [4,5], and magnetic resonance imaging (MRI) [6], for the detection of hepatic nodules, including small early-stage HCC tumors in high-risk patients under surveillance.

The liver-specific contrast agent gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA), which is taken up by hepatocytes, has been in clinical use for dynamic MRI studies since February 2008 in Japan. Gd-EOB-DTPA provides both dynamic and liver-specific hepatobiliary MR images [7–10]. In the hepatobiliary phase, hepatic lesions that lack normally functioning hepatocytes are imaged as an absence of hepatocyte-selective enhancement as compared with normal parenchyma [10,11]. The use of Gd-EOB-DTPA-enhanced MRI increases detection of concurrent non-hypervascular hepatic nodules as hypointense nodules during the hepatobiliary phase in patients with HCC. It is controversial whether the presence of these non-hypervascular hepatic nodules detected in patients with typical hypervascular HCC lesions has an impact on the recurrence of HCC after treatment.

In the present study, we attempted to evaluate the impact of concurrent non-hypervascular hepatic nodules detected as hypointense nodules during the hepatobiliary phase of Gd-EOB-

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Table 1. Comparison of clinical characteristics of study patients based on the presence of non-hypervascular hypointense nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (n = 77).

	Non-hypervascular hypointense nodule (+) (n = 18)	Non-hypervascular hypointense nodule (-) (n = 59)	*p* value
Age (mean ± SD, yr) (range)	65.8 ± 9.0 (46-76)	69.1 ± 7.0 (53-82)	0.2727
Sex (female/male)	3 (16.7)/15 (83.3)	18 (30.5)/41 (69.5)	0.3921
Etiology (HBV/HCV/non-HBV, non-HCV)	2 (11.1)/11 (61.1)/5 (27.8)	9 (15.3)/39 (66.1)/11 (18.6)	0.6796
Child-Pugh class (A/B)*	17 (94.4)/1 (5.6)	58 (98.3)/1 (1.7)	0.9474
Albumin (mean ± SD, g/dl)	3.91 ± 0.51	4.08 ± 0.32	0.1664
Total bilirubin (mean ± SD, mg/dl)	0.88 ± 0.36	0.84 ± 0.33	0.7296
15-minute ICG retention rate (%)	18.1 ± 5.4	16.0 ± 6.7	0.2405
Prothrombin (%)	95.3 ± 15.6	95.1 ± 11.2	0.9105
Platelet count (x10^3/ml)	132 ± 47	152 ± 66	0.5433
Tumor size (mean ± SD, cm) (range)	2.52 ± 0.99 (1.3-4.7)	2.84 ± 1.54 (1.0-8.6)	0.6600
Number of tumors (single/multiple)	15 (83.3)/3 (16.7)	53 (89.8)/6 (10.2)	0.7358
Portal vein invasion (absent/present)**	17 (94.4)/1 (5.6)	50 (84.7)/9 (15.3)	0.4989
Differentiation (well-/moderately or poorly)**	7 (38.9)/11 (61.1)	21 (35.6)/38 (64.4)	0.9999
Growth pattern (expansive/infiltrative)**	14 (77.8)/4 (22.2)	52 (88.1)/7 (11.9)	0.4718
Follow-up period (months) (median, range)	31.3 (9.4-53.9)	34.9 (8.5-55.4)	0.4200

Percentages are in parentheses.
HBV, hepatitis B virus; HCV, hepatitis C virus; ICG, indocyanine green test.
* Child-Pugh class A includes patients without cirrhosis.
** Evaluated by pathologic examination based on resected specimens.

DTPA-enhanced MRI on postoperative recurrence in patients who underwent hepatectomy with curative intent for HCC.

Cancer

Materials and methods

Patients, treatment and follow-up

This prospective study was conducted after the approval by the hospital institutional review board and carried out in compliance with the Helsinki Declaration. Patient enrollment was carried out between February 2008 and December 2011. A total of 102 patients underwent hepatectomy as a curative treatment for primary, non-recurrent HCC during the study period at Ogaki Municipal Hospital. Gd-EOB-DTPA-enhanced MRI could not be performed prior to hepatectomy in 25 patients, including 11 patients who had been referred from another institution only for hepatectomy and 14 patients who could not receive examination due to metal implants, history of allergy to contrast medium, tattoos, or claustrophobia. The remaining 77 patients who underwent Gd-EOB-DTPA-enhanced MRI within 2 weeks prior to hepatectomy were studied. The initial diagnosis of HCC before treatment was based on appropriate imaging characteristics according to criteria of the guidelines by the American Association for the Study of Liver Diseases [12,13]. The final diagnosis of HCC was confirmed by pathologic diagnosis of resected specimens.

Decisions regarding individual treatments were based on Japanese treatment guidelines for HCC [14]. In all patients, HCC tumors were resected with ample margins; enucleation of tumors without margins was not performed.

After hepatectomy, all patients were prospectively followed from 8.5 months to 55.4 months (median follow-up, 34.1 months) until the end of September 2012 at our institution, with US and either MDCT or MRI every 3–6 months. Regular monitoring of serum tumor markers (alpha-fetoprotein, *Lens culinaris* agglutinin-reactive alpha-fetoprotein, and des-gamma-carboxy prothrombin) was performed every 3 months. When an elevation in tumor markers was detected, additional imaging (usually MDCT or MRI) was performed to check for HCC recurrence. Recurrence was diagnosed by pathologic examination of resected specimens when patients underwent re-hepatectomy. In the remaining patients, HCC was diagnosed by appropriate imaging characteristics according to criteria of the guidelines by the American Association for the Study of Liver Diseases [12,13]. Recurrent HCC was categorized into two groups prior to the study, as intrahepatic metastasis recurrence or multicentric recurrence according to a previous study [15,16]. Intrahepatic metastasis recurrence was defined as recurrent tumors consisting of moderately or poorly differentiated HCC with the same or lower degree of differentiation than the primary tumors on pathologic examination or hypervascular tumor without non-hypervascular peripheral regions in a same hepatic segment on imaging examination. Multicentric recurrence was defined according to previously reported criteria with some modifications [17,18] as follows: (i) the recurrent tumor consists of well-differentiated HCC occurring in a different hepatic segment, than moderately or poorly differentiated pre-existing HCCs; (ii) both the primary and recurrent tumors are well-differentiated HCCs; and (iii) the recurrent tumor contained regions of dysplastic nodules in peripheral areas based on pathologic examination or contained non-hypervascular regions in peripheral areas of hypervascular tumor on imaging examination.

Preoperative imaging examinations of liver nodules by gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced MRI and confirmation of non-hypervascular hypointense hepatic nodules

All patients underwent Gd-EOB-DTPA-enhanced MRI within 2 weeks of hepatectomy. MRI was performed using a 1.5-T whole-body MRI system (Intera Achieva 1.5T NOVA; Philips Medical Systems) with a phased-array body coil as the receiver coil. T1-weighted sequences were acquired with the following parameters: T1-weighted turbo field echo (TFE) in-phase and opposed-phase transverse (TE, opposed-phase 2.3, in-phase 4.6; flip angle, 12°; matrix size, 256 × 512; scan percentage, 70) with 3.5-mm section thickness, a 0-mm intersection gap, and a 38-cm field of view. After intravenous injection of Gd-EOB-DTPA (Primovist; Bayer Schering Pharma, Osaka, Japan), T1-weighted transverse gradient-echo sequences (high-resolution isotropic volume examination [THRIVE] with spectral presaturation with inversion recovery [SPIR], 4/1.8; flip angle, 12°; matrix size, 256 × 512; scan percentage, 78.54) with 3.5-mm section thickness, a 0-mm intersection gap, and a 38-cm field of view were obtained. Gd-EOB-DTPA was administered intravenously as a bolus at a rate of 2 ml/s (0.1 ml/kg, maximum dose of 10 ml) through an intravenous cubital line (20–22 gauge), which was flushed with 20 ml of saline using a power injector (Sonic Shot; Nemoto Kyorindo, Tokyo, Japan). The timing for dynamic arterial phase imaging was determined using

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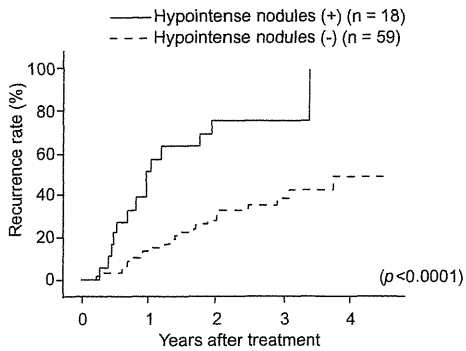

Patients at risk					
Hypointense nodules (+)	18	17	14	7	2
Hypointense nodules (-)	59	58	47	29	16

Fig. 1. Overall recurrence rate after hepatectomy in patients with or without concurrent non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of preoperative Gd-EOB-DTPA-enhanced MRI.

MR fluoroscopic bolus detection of the descending aorta (Bolus Trak; Philips Medical Systems). The mean delay times (time interval between the start of bolus administration and the start of image acquisition) for the arterial, portal, and delayed phases were 20, 60, and 180 s, respectively. Immediately after the dynamic study, a respiration-triggered single-shot T2-weighted sequence, with a reduction factor of 4 (1200/100; flip angle, 90°; matrix size, 400 × 512) with 7-mm section thickness, a 1-mm intersection gap, and a 38-cm field of view, was obtained with SPIR. The 20-min-delayed hepatobiliary phase [19] was obtained with a T1-weighted TFE sequence (TR/TE, 4/1.8; flip angle, 12°; matrix size, 256 × 512) with 3.5-mm section thickness, a 0-mm intersection gap, and a 38-cm field of view. All the sequences were obtained with parallel imaging (SENSE). Hypointense hepatic nodules during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI were nodules greater than 3.5 mm with low-intensity.

Prior to hepatectomy, all patients underwent CT during hepatic arteriography (CTHA) [20–22] to evaluate the intranodular blood supply, and to confirm the hypervascularity of HCC lesions and the lack of hypervascularity of non-hypervascular hepatic nodules.

All imaging findings were evaluated by a radiologist (Y.S.) and a hepatologist (H.T.) independently, blind to the clinical data. When the imaging assessment was discordant between two reviewers, consensus was made through the discussion.

Statistical analyses

Differences in percentages between groups were analyzed using the Chi-square test. Differences in mean quantitative values were analyzed by the Mann–Whitney *U* test. The date of hepatectomy was defined as time zero for calculations of recurrence rates. In the analysis of the overall recurrence rate, patients in whom HCC did not recur were censored, and those in whom HCC recurred were not censored. In the analysis of the intrahepatic metastasis recurrence rate, patients in whom HCC did not recur or patients with multicentric HCC recurrence were censored, and those in whom HCC recurred as intrahepatic metastases were not censored. In the analysis of the multicentric recurrence rate, patients in whom HCC did not recur were censored and patients with multicentric HCC recurrence were not censored, while those in whom HCC recurred as intrahepatic metastases were excluded from the analysis. The Kaplan–Meier method [23] was used to calculate recurrence rates, and the log-rank test [24] was used to analyze differences.

The Cox proportional hazards model [25] was used for univariate and multivariate analyses of factors related to recurrence. Variables analyzed included patient age and sex, Child-Pugh class (A/B), tumor size, number of tumors (single/multiple), differentiation of resected HCC (well-differentiated/moderately or

Table 2. Univariate and multivariate analyses of factors associated with post-operative recurrence in HCC patients (n = 77).

Factor	Univariate analysis		Multivariate analysis	
	Risk ratio (95% CI)	*p* value	Risk ratio (95% CI)	*p* value
Age	0.9943 (0.9535-1.0396)	0.7974	-	
Sex				
Male	1			
Female	1.0068 (0.6818-1.4290)	0.9711	-	
Child-Pugh class*				
A	1			
B	0.0428 (0.0198-1.5669)	0.2068	-	
Tumor size	0.9376 (0.7179-1.1700)	0.5935	-	
Number of tumors				
Single	1			
Multiple	1.0419 (0.5669-1.6643)	0.8792	-	
Differentiation**				
Well-	1		1	
Moderately/poorly	1.5871 (1.0958-2.4354)	0.0134	1.6536 (1.1381-2.5445)	0.0073
Growth pattern**				
Expansive	1			
Infiltrative	1.1101 (0.6798-1.6625)	0.6487	-	
Portal vein invasion**				
Absent	1		1	
Present	1.5659 (1.0161-2.2813)	0.0428	1.7818 (1.1388-2.6597)	0.0134
Non-hypervascular hypointense nodules				
Absent	1		1	
Present	1.9396 (1.3615-2.7222)	0.0004	2.1767 (1.5089-3.1105)	0.0001

CI, confidence interval.
* Child-Pugh class A includes patients without cirrhosis.
** Evaluated by pathologic examination of resected specimens.

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poorly differentiated), growth pattern of resected HCC (expansive growth/infiltrative growth), portal vein invasion of resected HCC (absent/present), and presence of non-hypervascular hypointense nodules on the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (absent/present). Data analyses were performed using JMP statistical software, version 6.0 (Macintosh version; SAS Institute, Cary, NC). All p values were derived from 2-tailed tests, with $p < 0.05$ accepted as statistically significant.

Results

Patients characteristics and imaging findings

Patients consisted of 56 males and 21 females with a mean age of 68.3 ± 7.6 years (range, 46–82 years). A total of 40 non-hypervascular hypointense hepatic nodules were identified during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI in 28 of 77 patients (36.4%). The size of non-hypervascular hypointense nodules was 1.17 ± 0.38 cm (range, 0.4–2.1 cm). Two of 40 non-hypervascular hypointense hepatic nodules (5.0%) were identified by T2-weighted sequence as high-intensity nodules. The other 38 non-hypervascular hypointense hepatic nodules were not identified either by T1- and T2-weighted sequences. Two nodules were located in segment II of the liver, 7 in III, 1 in IV, 10 in V, 6 in VI, 4 in VII, and 10 in VIII, respectively. Among 28 patients with non-hypervascular hypointense nodules, 19 patients had one non-hypervascular hypointense nodule, 6 patients had 2 nodules, and the remaining 3 patients had 3 nodules. Non-hypervascular hypointense nodules were resected along with HCC lesions during hepatectomy in 10 patients, because they were included within the intended area of resection. Therefore, we categorized these 10 patients and the 49 patients in whom non-hypervascular hypointense nodules were not detected by preoperative Gd-EOB-DTPA-enhanced MRI as the hypointense nodule (−) group and the remaining 18 patients who had residual hypointense nodules after hepatectomy as the hypointense nodule (+) group. Of 13 hypointense nodules in 10 patients resected along with HCC at hepatectomy, 3 nodules were diagnosed as well-differentiated HCC and the remaining 10 nodules were diagnosed as dysplastic nodules on pathologic examination.

Table 1 compares the preoperative characteristics of the study patients. No differences were found in patient age and sex, etiology, liver function, and tumor progression as evaluated by preoperative imaging examinations and by post-operative pathologic examinations. Multiple HCC nodules were resected in 6 patients (10.2%) of the hypointense nodule (−) group and 3 patients (16.7%) of the hypointense nodule (+) group, without difference in proportions. No difference was observed in the length of follow-up period.

Recurrence rate after hepatectomy according to the presence of non-hypervascular hypointense nodules detected during preoperative gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced MRI

We determined the recurrence rate in patients after hepatectomy with curative intent based on the presence of non-hypervascular hypointense hepatic nodules identified during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (Fig. 1). The recurrence rate was significantly higher in patients in the hypointense nodule (+) group than in the hypointense nodule (−) group ($p < 0.0001$). In univariate analysis, HCC differentiation and portal vein invasion were identified as factors associated with the rate of recurrence after hepatectomy along with preoperative non-hypervascular hypointense nodules by Gd-EOB-DTPA-enhanced MRI. In multivariate analysis, these factors were confirmed to be independently associated with the rate of recurrence (Table 2). Among 18 patients in the hypointense nodule (+) group, recurrence was observed in 7 of 11 patients with one non-hypervascular hypointense nodule, whereas recurrence was observed in all 7 patients with multiple non-hypovascular hypointense nodules.

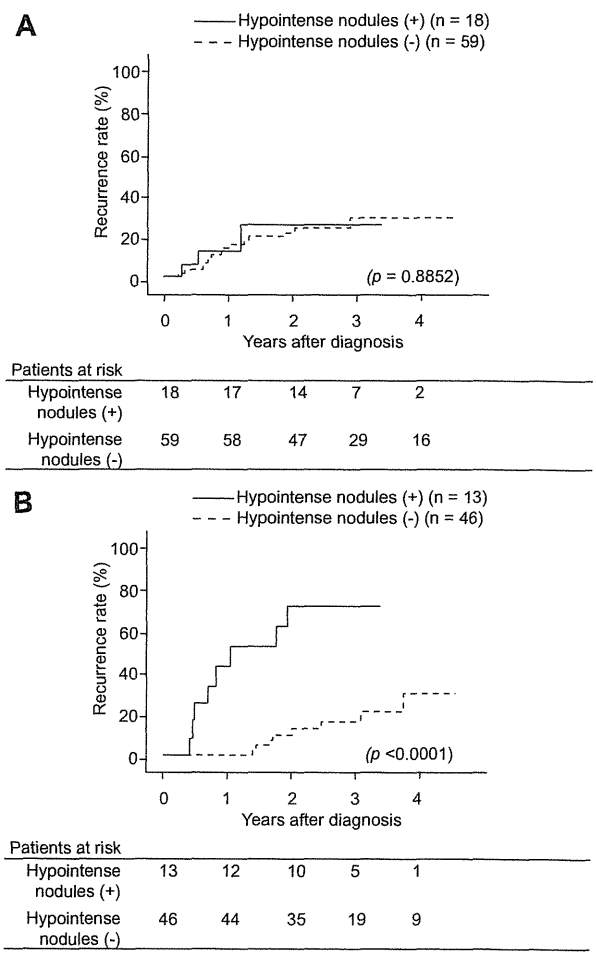

Fig. 2. Recurrence rate after hepatectomy according to the patterns of recurrence. (A) Rates of intrahepatic metastasis recurrence after hepatectomy in patients with or without concurrent non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of preoperative Gd-EOB-DTPA-enhanced MRI. (B) Rates of multicentric recurrence after hepatectomy in patients with or without concurrent non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of preoperative Gd-EOB-DTPA-enhanced MRI, among 59 patients, excluding 16 patients with intrahepatic metastasis recurrence.

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Table 3. Univariate and multivariate analyses of factors associated with post-operative intrahepatic metastasis recurrence in HCC patients (n = 77).

Factor	Univariate analysis		Multivariate analysis	
	Risk ratio (95% CI)	*p* value	Risk ratio (95% CI)	*p* value
Age	0.9825 (0.9265-1.0470)	0.5743	-	
Sex				
Male	1			
Female	0.9022 (0.4784-1.5192)	0.7148	-	
Child-Pugh class*				
A	1			
B	0.0242 (0.0059-2.1819)	0.3573	-	
Tumor size	1.0051 (0.6929-1.3406)	0.9755	-	
Number of tumors				
Single	1			
Multiple	0.7038 (0.1655-1.5643)	0.4504	-	
Differentiation**				
Well-	1		1	
Moderately/poorly	1.7843 (1.0185-3.7176)	0.0424	1.6742 (0.9520-3.4993)	0.0769
Growth pattern**				
Expansive	1			
Infiltrative	0.9266 (0.3678-1.7453)	0.8365	-	
Portal vein invasion**				
Absent	1		1	
Present	2.1224 (1.2405-3.4608)	0.0079	2.0041 (1.1672-3.2828)	0.0138
Non-hypervascular hypointense nodules				
Absent	1			
Present	1.0474 (0.5012-1.8442)	0.8864	-	

CI, confidence interval.
* Child-Pugh class A includes patients without cirrhosis.
** Evaluated by pathologic examination of resected specimens.

Patterns of recurrence after hepatectomy according to the presence of non-hypervascular hypointense nodules detected during preoperative gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced MRI

Among 30 patients with HCC recurrence after hepatectomy, 16 patients (53.3%) had intrahepatic metastasis recurrence and 14 patients (46.7%) had multicentric recurrence. There was no difference in the rate of intrahepatic metastasis recurrence between patients in the hypointense nodule (+) group and those in the hypointense nodule (−) group ($p = 0.8852$). In contrast, patients in the hypointense nodule (+) group had a significantly higher rate of multicentric recurrence than patients in the hypointense nodule (−) group ($p < 0.0001$, Fig. 2). Univariate and multivariate analyses revealed that portal vein invasion was independently associated with intrahepatic metastasis recurrence but preoperative non-hypervascular hypointense nodules detected by Gd-EOB-DTPA-enhanced MRI was not associated with intrahepatic metastasis recurrence (Table 3). The presence of preoperative non-hypervascular hypointense nodules detected by Gd-EOB-DTPA-enhanced MRI was the only factor associated with multicentric recurrence in univariate and multivariate analyses (Table 4). Among 8 HCCs that recurred multicentrically in the hypointense nodule (+) group, 6 nodules (75.0%) had existed as non-hypervascular hypointense hepatic nodules on Gd-EOB-DTPA-enhanced MRI before hepatectomy and progressed to hypervascular HCC tumors (Fig. 3), while the other 2 nodules (25.0%) newly occurred as multicentric recurrence after hepatectomy.

Discussion

Although one study reported that dysplastic nodules and early, well-differentiated HCC can be differentiated based on findings on Gd-EOB-DTPA uptake [26], differentiation of early, non-hypervascular HCC from dysplastic nodules within hypointense nodules is not actually feasible and controversial [27]. In addition, it is nearly impossible to characterize these hepatic nodules specifically using US or MDCT. Therefore, a histological diagnosis should be obtained with percutaneous liver biopsy under US guidance. However, this is not always possible due to the need for multiple samples and its invasive nature. Therefore, we did not resect these hepatic nodules during hepatectomy, except for nodules located within the hepatectomy field.

This study demonstrates a higher rate of recurrence of HCC in patients in whom non-hypervascular hypointense hepatic nodules were identified during the hepatobiliary phase of preoperative Gd-EOB-DTPA-enhanced MRI. This large difference in the recurrence rates indicated that the presence of non-hypervascular hypointense nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI is a risk factor for recurrence of HCC after hepatectomy. Although we did not find differences in the rate of intrahepatic metastasis recurrence according to the non-hypervascular hypointense hepatic nodule status, we found a significantly higher rate of multicentric recurrence in patients with preoperative concurrent non-hypervascular hypointense hepatic nodules. In addition, the majority of multicentric recurrences involved the hypervascularization of non-hypervascular

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Table 4. Univariate and multivariate analyses of factors associated with post-operative multicentric recurrence in HCC patients (n = 59).

Factor	Univariate analysis		Multivariate analysis	
	Risk ratio (95% CI)	*p* value	Risk ratio (95% CI)	*p* value
Age	1.0047 (0.9359-1.0823)	0.8985	-	
Sex				
Male	1			
Female	1.0701 (0.5999-1.7781)	0.8038	-	
Child-Pugh class*				
A	1			
B	0.0664 (0.0176-5.7947)	0.7029	-	
Tumor size	0.9517 (0.6300-1.2943)	0.7801	-	
Number of tumors				
Single	1			
Multiple	1.1331 (0.4469-2.1714)	0.7510	-	
Differentiation**				
Well-	1			
Moderately/poorly	1.5198 (0.8959-2.8769)	0.1249	-	
Growth pattern**				
Expansive	1			
Infiltrative	1.3486 (0.7124-2.2884)	0.3270	-	
Portal vein invasion**				
Absent	1			
Present	1.2908 (0.5077-2.4730)	0.5312	-	
Non-hypervascular hypointense nodules				
Absent	1		1	
Present	2.8436 (1.6900-4.8407)	0.0002	2.8436 (1.6900-4.8407)	0.0002

CI, confidence interval.
* Child-Pugh class A includes patients without cirrhosis.
** Evaluated by pathologic examination of resected specimens.

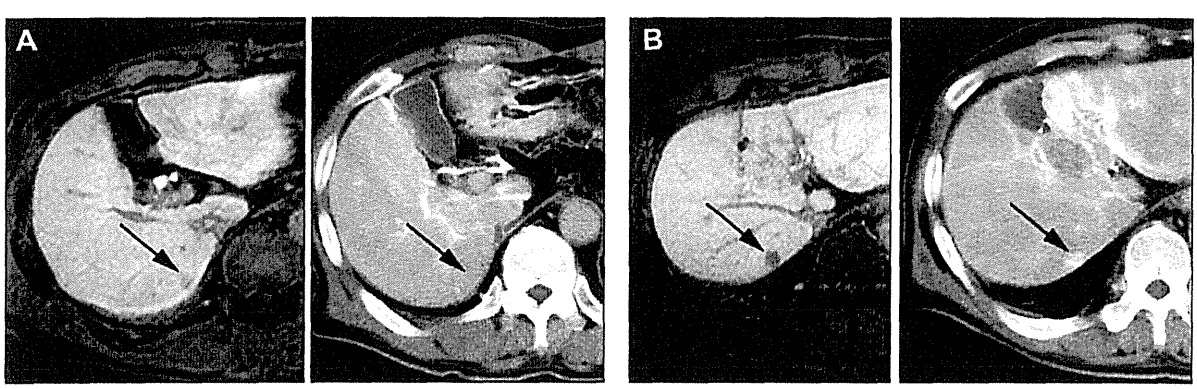

Fig. 3. Development of multicentric hepatocellular carcinoma in patients with preoperative non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI. (A) Hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (left panel) and computed tomography during hepatic arteriography (CTHA, right panel) before hepatectomy for hepatocellular carcinoma (HCC). In addition to the typical HCC located in segment VIII, a hypointense hepatic nodule was detected in segment VI during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (arrow). No hypervascular nodule was detected at this site by CTHA (arrow). (B) Hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (left panel) and CTHA (right panel) 10 months after hepatectomy for HCC. The nodule detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI showed minute growth in size, with clearer margin, compared to preoperative image (arrow). The hypervascularity of this nodule was identified by CTHA (arrow). This nodule was resected by re-hepatectomy and was diagnosed as HCC pathologically.

hypointense hepatic nodules observed preoperatively with Gd-EOB-DTPA-enhanced MRI. It is controversial whether all non-hypervascular hypointense nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI have the potential to progress to typical, hypervascular HCC. However, 26.5% of non-hypervascular hypointense nodules showed hypervascular spots with a long-term follow-up in our previous study [28]. In addition to the likelihood of non-hypervascular hypointense nodules

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