CLINICAL STUDY

Placement of a Sodium Hyaluronate Solution onto the Liver Surface as a Supportive Procedure for Radiofrequency Ablation of Hepatocellular Carcinomas Located on the Liver Surface: A Preliminary Report

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ABSTRACT

Purpose: To evaluate safety and efficacy of the placement of sodium hyaluronate solution onto the liver surface as a supportive procedure for radiofrequency (RF) ablation of hepatocellular carcinomas (HCCs) located on the liver surface as a possible alternative to RF ablation via laparoscopic approach or with the creation of artificial ascites.

Materials and Methods: Changes in temperature of a sodium hyaluronate layer placed onto an egg white were measured during coagulation of the egg white by an RF ablation needle. A phase I study was performed to evaluate the safety of intraperitoneal injection of a maximum of 20 mL of sodium hyaluronate solution into humans by observing for the occurrence of intraperitoneal inflammation and adhesion. After these studies, RF ablation with ultrasound-guided injection of sodium hyaluronate onto the liver surface was performed, targeting 28 HCC nodules located on the liver surface. Treatment outcomes and complications of this procedure were investigated.

Results: In the in vitro experiment, the maximum temperature of sodium hyaluronate solution was 41°C during RF ablation. No intraperitoneal inflammation or adhesions were observed after intraperitoneal injection of sodium hyaluronate in the phase I study. HCC was completely ablated with sufficient margins after one session of RF ablation, without any burn injuries to the abdominal wall or adjacent organs. Local recurrence was observed in one of 28 patients (3.6%) during 30.1 months of follow-up.

Conclusions: RF ablation can be safely and effectively performed on HCCs located close to the liver surface with placement of sodium hyaluronate onto the liver surface, thereby preventing burn injuries to abdominal wall or adjacent organs.

ABBREVIATIONS

CRP = C-reactive protein, HCC = hepatocellular carcinoma, RF = radiofrequency, WBC = white blood cell

Radiofrequency (RF) ablation is currently one of the standard treatment modalities for hepatocellular carcinoma (HCC) and, along with hepatic resection, is potentially curative (1–3). However, problems have been reported with the use of RF ablation for HCCs that are located close to the liver surface; these adverse effects include damage to the abdominal wall or to adjacent organs such as the gallbladder and gastrointestinal tract (4–6). RF ablation via laparoscopic approach (7–9) and RF ablation with the creation of artificial ascites (10,11) have been employed in the ablation of tumors located near the liver surface. These supportive procedures for RF ablation are used to prevent damage to the abdominal wall or adjacent organs by separating the ablation area from adjacent structures.

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This article includes Figures E1 and E2, which are available online at *www.jvir.org*.

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However, RF ablation via laparoscopic approach usually requires general anesthesia. In addition, RF ablation via laparoscopic approach or creation of artificial ascites may cause vascular and respiratory complications as a result of increased intraperitoneal pressures (12,13).

Sodium hyaluronate solutions have been used in various clinical fields. In orthopedics, a sodium hyaluronate solution is used in intraarticular injections for osteoarthritis (14,15). In ophthalmology, the solution is used in intralenticular injections during cataract surgery (16). In gastroenterology, the solution is used as a submucosal fluid cushion during the endoscopic resection of mucosal neoplasms in the stomach or colon (17–20). Because sodium hyaluronate has a high viscosity (limiting viscosity of 11.8–19.5 dL/g) and remains at the injection site for a considerable length of time, the solution can effectively continue to separate the two objects between which it is injected. The compound creates a durable separation between the submucosal and muscle layers during endoscopic mucosal resections. Therefore, it may be possible to create a continuous separation between the liver surface and the abdominal wall or adjacent organs by injecting sodium hyaluronate onto the liver surface. Moreover, it may be possible to perform RF ablation of HCCs that are located close to the liver surface without using the laparoscopic approach or creating artificial ascites while still preventing damage to the abdominal wall or adjacent organs.

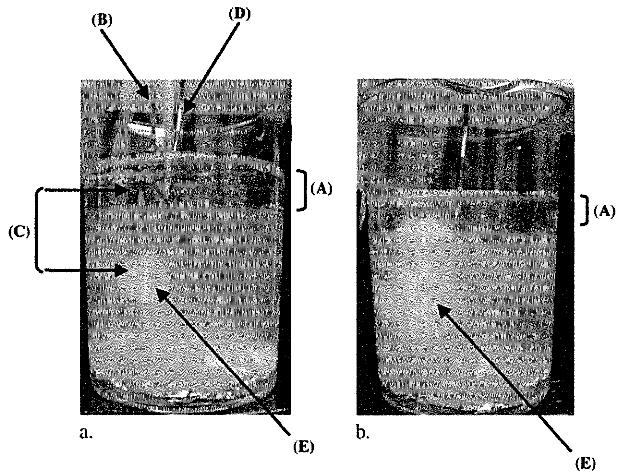

Figure 1. In vitro determination of temperature increases in the sodium hyaluronate solution associated with thermal ablation by an RF ablation needle. A 0.4% sodium hyaluronate solution layer was placed onto a layer of egg whites in a beaker (*A*). Subsequently, an RF ablation needle electrode for thermal ablation (*B*) was inserted with the tip in the egg white layer and the proximal end of the exposed tip (*C*) in the sodium hyaluronate layer. Another electrode to monitor the temperature of the sodium hyaluronate solution (*D*) was inserted into the sodium hyaluronate solution layer. The images display the results after 3 minutes (**a**) and 8 minutes (**b**) of thermal ablation. The volume of the coagulated egg whites increased with thermal ablation (*E*). (Available in color online at *www.jvir.org*.)

MATERIALS AND METHODS

In Vitro Experiment: Sodium Hyaluronate Temperature Determination during RF Ablation

The change in temperature of sodium hyaluronate solution during the RF ablation procedure was initially investigated in an in vitro experiment. In a beaker, 20 mL of a 0.4% sodium hyaluronate solution (MucoUp; Johnson and Johnson, Tokyo, Japan) was placed on the surface of raw egg whites, creating an approximately 1.8-cm layer of the sodium hyaluronate solution. An RF electrode that was equipped with a 3-cm exposed metallic tip (Cool-tip; Covidien, Mansfield, Massachusetts) was inserted into this beaker. The electrode that was used for thermal ablation was positioned with its tip in the egg white layer and part of its exposed portion in the sodium hyaluronate layer. Temperature changes in the sodium hyaluronate layer were continuously monitored by using another RF electrode, the tip of which was placed in the middle of the sodium hyaluronate solution when the egg white was heated (**Fig 1**). This procedure was continued until the heated egg white coagulated. The experiment was repeated three times.

Phase I Study: Safety of Intraperitoneal Sodium Hyaluronate Injection

After obtaining approval from the institutional ethics committee and written informed consent from each individual, a phase I study was performed to evaluate the safety of intraperitoneal injection of the sodium hyaluronate solution into humans. A 0.4% sodium hyaluronate solution (MucoUp; Johnson and Johnson) was injected percutaneously onto the liver surface under ultrasound (US) guidance with a 21-gauge needle (PEIT needle; Hakko, Nagano, Japan) that is usually used for ethanol injection therapy. The sodium hyaluronate solution was injected into six patients in a dose-escalating manner, ie, 5 mL, 10 mL, and 20 mL in two patients each. Laboratory tests were performed on day 3 after the injection to determine whether white blood cell (WBC) counts and C-reactive protein (CRP) values would increase as a result of inflammation caused by intraperitoneal injection of the sodium hyaluronate solution. The US examination was performed 5–7 days after the injection to determine whether ileus would occur as a result of peritoneal adhesions caused by the intraperitoneal injection.

RF Ablation of HCC with Sodium Hyaluronate Solution on the Liver Surface

After obtaining approval from the institutional ethics committee (based on the in vitro experiment and the phase I study) and written informed consent from each subject, RF ablation of the HCCs was performed with the placement of a sodium hyaluronate solution onto the liver surface. Between July 2009 and December 2011, 294 patients underwent RF ablation as a treatment of primary or recurrent HCCs. Patients with no more than three ($\leq$ 3) HCC tumors at largest 3 cm in maximum diameter were considered to be candidates for RF ablation. Patients with Child–Pugh Class C cirrhosis and patients with platelet counts of less than 50×10^3 were not considered for RF ablation. HCC tumors were located on the liver surface or close to the liver surface in 28 patients who were treated with RF ablation with placement of sodium hyaluronate solution onto the liver surface. For all patients, the diagnosis of HCC was made by observing the appropriate imaging characteristics based on criteria in the guidelines issued by the American Association for the Study of Liver Diseases (21).

All patients were placed in the supine position for treatment. A sodium hyaluronate solution was placed onto the liver surface where the HCC was located by injecting the solution percutaneously through a 21-gauge needle (PEIT needle; Hakko) under real-time US guidance. The sodium hyaluronate solution was injected until the thickness of the sodium hyaluronate layer reached approximately 1–2 cm. The needle was withdrawn after the sodium hyaluronate solution was injected and before RF ablation was performed.

After placement of the sodium hyaluronate solution onto the liver surface, RF ablation was performed with use of a 20-cm-long, 17-gauge RF electrode equipped with a 2-cm or 3-cm exposed metallic tip and connected to a 500-kHz RF generator (Cool-tip; Covidien). The electrode was inserted percutaneously under real-time US guidance and positioned accurately within the tumor. The length of the exposed metallic tip (2 or 3 cm) was determined based on the HCC tumor size. RF ablation durations were 8 minutes with a 2-cm exposed tip and 12 minutes with a 3-cm exposed tip, according to the manufacturer's recommendations. If there were fewer than four occurrences of power roll-off during these ablation periods, the duration of ablation was increased until four power roll-offs occurred. The distance between the tumor and adjacent structures was monitored by real-time US throughout the RF ablation procedure.

The occurrence of abdominal pain or fever was prospectively surveyed by clinical examination during the 3 days after RF ablation, and WBC counts and CRP levels were measured on the first day after RF ablation to determine whether there were signs or symptoms of burn injuries to the abdominal wall or other organs adjacent to the tumor treated by RF ablation. Evaluation of treatment response was performed 1–3 days after the RF ablation procedure by imaging examinations, including contrast-enhanced US and either contrast-enhanced computed tomography (CT) or magnetic resonance (MR) imaging (22–28). All patients were followed for the recurrence of HCCs after RF ablation for a median of 9.7 months (range, 2.0–30.1 mo) at our institution with US every 3 months and CT or MR imaging every 6 months until March 2012. Regular monitoring of serum tumor markers (α-fetoprotein, *Lens culinaris* agglutinin–reactive fraction of α-fetoprotein, and des-γ-carboxy prothrombin) was performed every 3 months. If an increase in tumor marker levels was detected, an additional imaging examination (usually CT or MR imaging) was performed to check for HCC recurrence.

The entire protocol was approved by the institutional ethics committee and was performed in compliance with the Declaration of Helsinki. Informed consent to treatment and participation in the study was obtained in writing from all subjects.

Statistical Analyses

Continuous variables are expressed as mean ± standard deviation or as median and range. Categoric variables are expressed by using absolute numbers and percentages. The JMP statistical software package (version 4.0; SAS, Cary, North Carolina) was used for all statistical analyses.

RESULTS

In Vitro Experiment: Sodium Hyaluronate Temperature Determination

The temperature of the sodium hyaluronate solution layer was monitored during the heating of the egg white by an RF electrode with a 3-cm exposed tip. The egg white was heated and coagulated (**Fig 1**). The temperature of the egg white, which was measured at the tip of the RF electrode that was placed in the egg white layer, increased to a maximum of 86°C at 5 minutes after heating was initiated. The procedure was completed in 8 minutes with the coagulation of the egg white. The temperature of the sodium hyaluronate solution increased 2 minutes after the heating was initiated, reached the maximum temperature of 41.3°C at 6 minutes, and remained constant until the end of the procedure.

Phase I Study: Safety of Intraperitoneal Sodium Hyaluronate Injection

No difficulties were encountered while injecting the sodium hyaluronate solution via the 21-gauge needle. Based on the real-time US observation, the sodium hyaluronate solution created a layer (emitting hyperechoic and hypoechoic signals) that maintained a space between the abdominal wall and liver surface until 2 hours after the injection. The layer was not detectable by US on the following morning, which was approximately 15–18 hours after the injection.

None of the patients reported any symptoms, such as fever or abdominal pain, during or after the injection of the sodium hyaluronate solution. **Table 1** presents the WBC and CRP results obtained before and 3 days after the injection. No increase in WBC or CRP values that would indicate marked inflammation was observed in any patient regardless of the dose of sodium hyaluronate solution injected. In addition, no findings suggestive of ileus were observed based on abdominal US.

RF Ablation of HCC with Sodium Hyaluronate Solution on the Liver Surface

Table 2 displays the characteristics of the patients and HCC tumors. The treated HCCs were recurrent in 75% of

Table 1. Demographic Details of Peritoneal Injection of Sodium Hyaluronate Solution (N = 6)

		WBC Count (/μL)		CRP (mg/dL)	
Pt. No./Age (y)/Sex	**Dose Injected (mL)**	**Baseline**	**After Injection***	**Baseline**	**After Injection***
1/64/F	5	3,710	3,970	0.06	0.58
2/74/M	5	4,340	3,540	0.69	2.26
3/78/M	10	4,260	4,320	0.14	0.77
4/71/M	10	7,180	6,780	0.12	0.63
5/64/M	20	6,240	6,280	0.25	1.96
6/64/F	20	2,550	3,150	0.06	0.03

CRP = C-reactive protein, WBC = white blood cell.
* Tested 3 d after injection of sodium hyaluronate solution.

patients. Tumors were smaller than 3 cm in all cases. All tumors were located on the liver surface in various sections of the liver except for segments I and VIII. The HCCs were located adjacent to the gallbladder in three patients, the gastrointestinal tract in one patient, and the right kidney in one patient based on US imaging. HCC thermal ablation was performed for 8 minutes with an electrode with a 2-cm exposed tip in 19 patients and for 12 minutes with an electrode with a 3-cm exposed tip in nine patients. None of the patients required a prolonged duration of thermal ablation. In 23 of 28 patients, the proximal end of the exposed metallic tip was located within the sodium hyaluronate solution layer above the liver surface rather than in the tumor or liver tissue.

During the RF ablation procedure, the sodium hyaluronate solution layer on the liver surface did not flow outside of the injection area (**Fig 2**). No changes in the thickness or in the real-time US appearance of the sodium hyaluronate layer were observed throughout the RF ablation procedure. Based on the clinical evaluations and laboratory tests, no patients experienced clinically significant burn injuries of the abdominal wall or other organs, including those patients who underwent RF ablation for HCCs that were adjacent to the gallbladder (**Fig 3**) or gastrointestinal tract (**Fig E1**, available online at *www.jvir.org*). Based on the imaging examinations, the HCCs were ablated and necrotized with sufficient margins with only one session of RF ablation in all cases (**Figs E1 and E2**, available online at *www.jvir.org*). Local recurrence was observed in one of 28 patients (3.6%) during a follow-up of 30.1 months after treatment. The time interval between RF ablation and recurrence was 14.8 months. No patients experienced tumor seeding after RF ablation.

Table 2. Characteristics of Patients and HCC Tumors

Detail	Value
Age (y)	
Mean ± SD	70.8 ± 6.5
Range	58–83
Sex	
Male	23 (82.1)
Female	5 (17.9)
Etiology	
HBV	4 (14.3)
HCV	21 (75.0)
Non-HBV/non-HCV	3 (10.7)
ALT (IU/L)	52.6 ± 38.7
Platelet count (×10^3/mL)	120 ± 52
Prothrombin (%)	82.5 ± 14.2
AFP (ng/mL)	
Median	9.8
Range	0.6–521.7
DCP (mAU/mL)	
Median	101.0
Range	6.0–763.0
History of HCC	
Primary	7 (25.0)
Recurrence	21 (75.0)
Tumor location	
Segment II	2 (7.2)
Segment III	6 (21.4)
Segment IV	2 (7.2)
Segment V	7 (25.0)
Segment VI	9 (32.0)
Segment VII	2 (7.2)
Tumor size (mm)	
Mean ± SD	14.8 ± 4.8
Range	8.0–26.0
Exposed electrode tip length	
2 cm	19 (67.9)
3 cm	9 (32.1)

AFP = α-fetoprotein, ALT = alanine aminotransferase, DCP = des-γ-carboxy prothrombin, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, SD = standard deviation.
Values presented as means ± SD where applicable. Values in parentheses are percentages.

DISCUSSION

The injection of sodium hyaluronate solution into the human body is performed in clinical practice (including intraarticular injections, intralenticular injections, and submucosal injections in the gastrointestinal tract), and the safety of the use of this material in the human body has been established (14–20). In the United States, the use of sodium hyaluronate solution has been approved by the

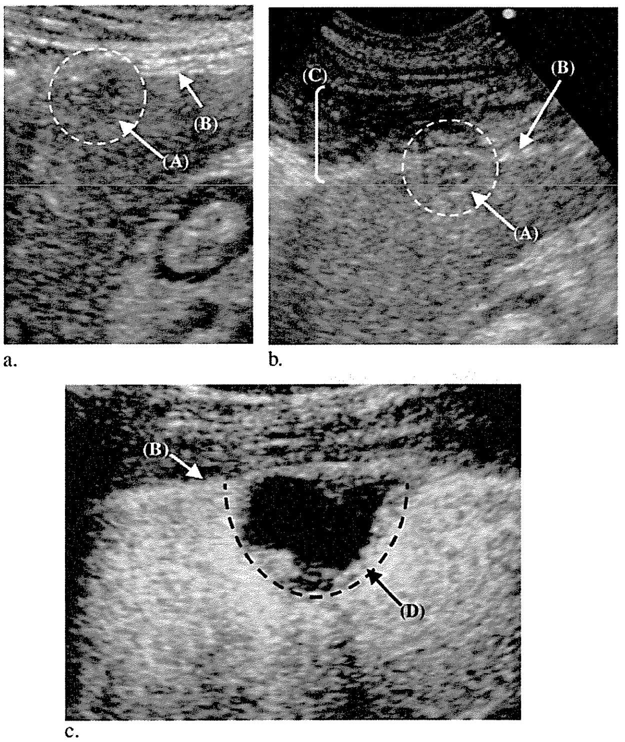

Figure 2. RF ablation of HCC (*A*) located on the liver surface (*B*) with the injection of a sodium hyaluronate solution onto the liver surface (*C*). **(a)** Plain US before injection of sodium hyaluronate solution. **(b)** Plain US after injection of sodium hyaluronate solution. **(c)** Evaluation 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

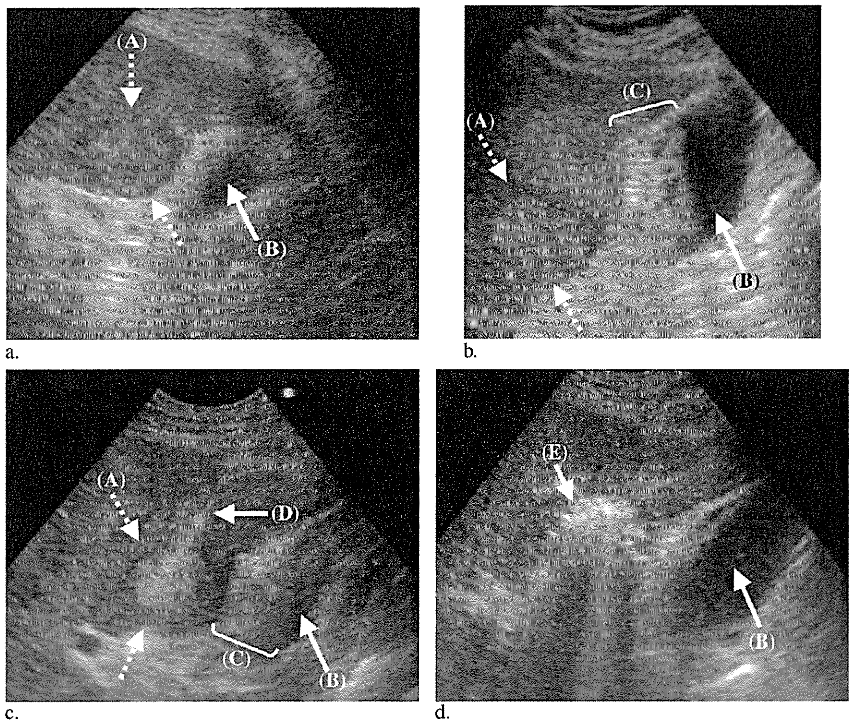

Figure 3. RF ablation of HCC (*A*, dotted arrow) located on the liver surface and adjacent to the gallbladder (*B*) with the injection of a sodium hyaluronate solution. **(a)** Before the injection of the sodium hyaluronate solution, the gallbladder was adjacent to the HCC tumor. **(b)** The injection 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*).

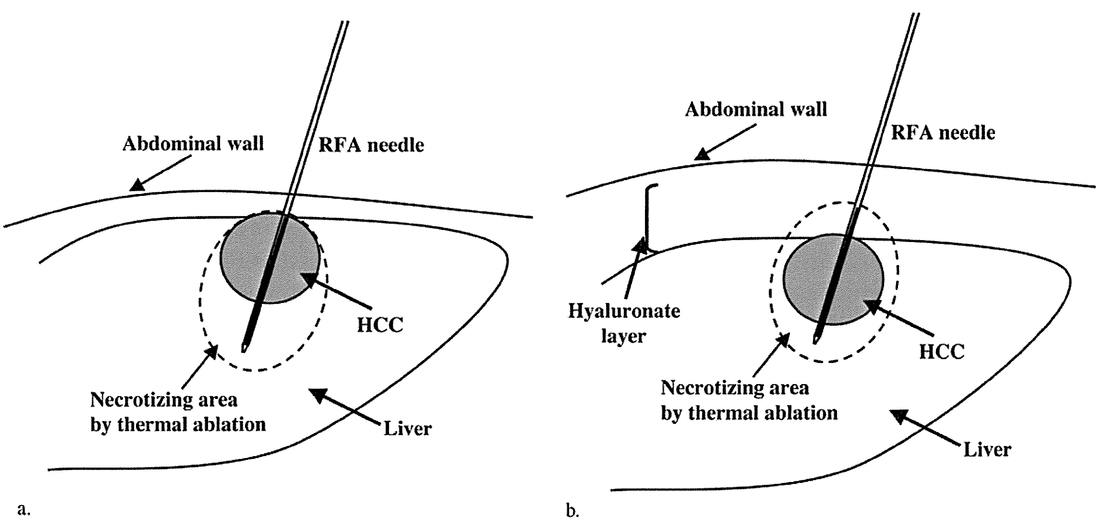

Figure 4. Schematic representation of RF ablation of HCC located on the liver surface. **(a)** The margin around the HCC was small in certain parts if the exposed metallic tip was completely inserted into the tumor or the adjacent liver tissue to prevent burn injuries to the abdominal wall. **(b)** Thermal 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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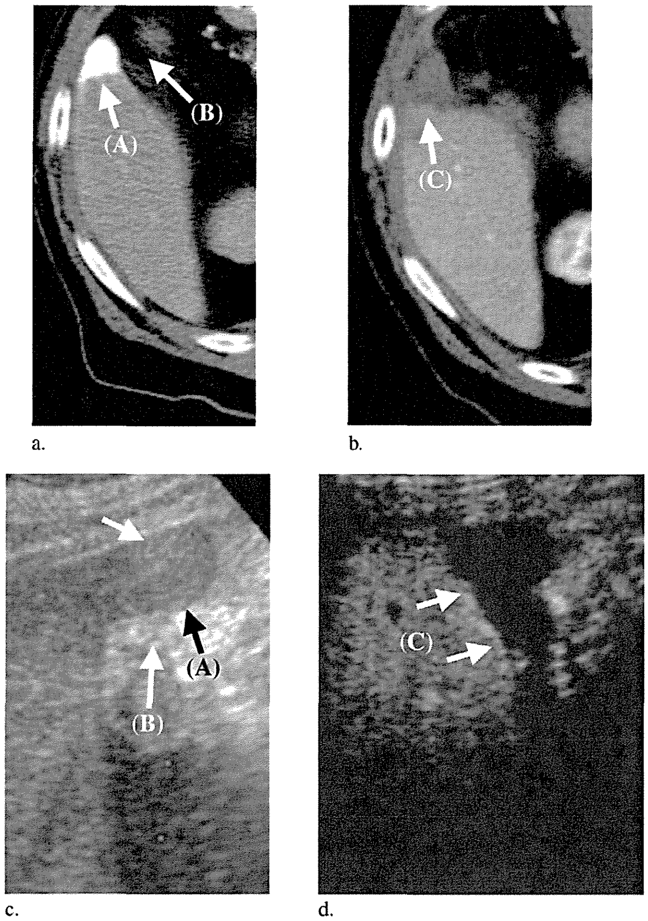

Figure E1. Radiofrequency (RF) ablation of a hepatocellular carcinoma (HCC; *A*) located on the liver surface and adjacent to the intestinal tract (*B*). Computed tomography before **(a)** and after **(b)** RF ablation. The HCC located on the edge of the liver was necrotized completely (*C*). **(c)**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

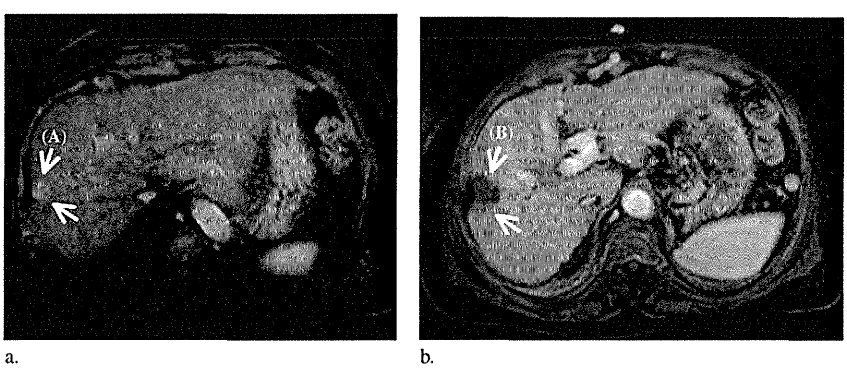

Figure E2. Dynamic magnetic resonance imaging results of RF ablation of HCC located on the liver surface. **(a)** Before RF ablation, an enhancing HCC tumor (*A*) was observed. **(b)** After RF ablation, the HCC and adjacent liver tissue were ablated in an oval shape (*B*).

Research Article

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

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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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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

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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 (x10³/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 (x10³/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 (x10³/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

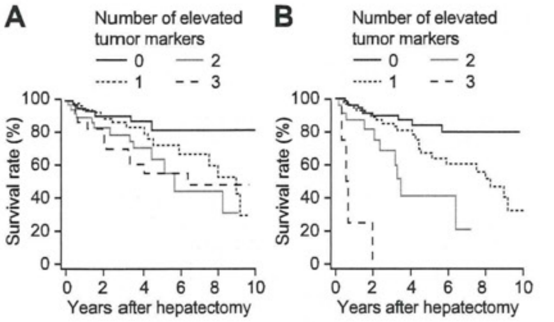

Fig. 1. Survival rates after hepatectomy as a function of the number of elevated tumor markers measured before and after hepatectomy. (A) Survival rates according to the number of elevated tumor markers measured before hepatectomy (zero *vs.* one, $p = 0.0907$; one *vs.* two, $p = 0.1542$; two *vs.*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

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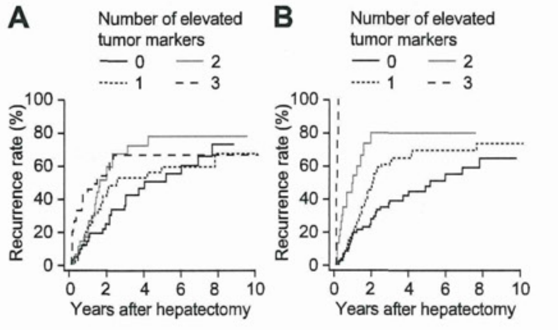

Fig. 2. Recurrence rates after hepatectomy as a function of the number of elevated tumor markers measured before and after hepatectomy. (A) Recurrence rates according to the number of elevated tumor markers measured before hepatectomy (zero *vs.* one, $p = 0.4966$; one *vs.* two, $p = 0.1756$; two *vs.* 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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Usefulness of the Multimodality Fusion Imaging for the Diagnosis and Treatment of Hepatocellular Carcinoma

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Key Words

US fusion imaging · CT fusion imaging · Fusion imaging · Volume Navigation System · Hepatocellular carcinoma · Radiofrequency ablation · Gd-EOB-DTPA · Sonazoid

Abstract

A multimodality fusion imaging system has been introduced for the clinical practice of diagnosis and treatment of hepatocellular carcinoma (HCC), especially for loco-regional treatment. An ultrasonography (US) fusion imaging system can provide a side-by-side display of real-time US images and any cross-sectional images of multiplanar reconstruction of CT or MRI that synchronize real-time US. The US fusion imaging system enables us to perform radiofrequency ablation (RFA) for HCCs difficult to detect on conventional US safely. Besides, we can evaluate the treatment effects of RFA easily at the bedside by combining the contrast-enhanced US and the US fusion imaging system. Fusion images of pre- and post-RFA CT have been utilized for the assessment of the treatment effects of RFA. Although the treatment effects of RFA have been conventionally evaluated, comparing pre- and post-RFA CT side-by-side, the evaluation tends to be inaccurate. On CT fusion images, the tumor and the ablation zone are overlaid and we can grasp the positional relation easily, leading to quantitative and more accurate evaluation. The multimodality fusion imaging system has become quite an important tool for loco-regional treatment of HCC because of its usefulness for both the guidance during the RFA procedure and the evaluation of its treatment effects.

Introduction

Recently, imaging technology in CT, MRI and ultrasonography (US) for the diagnosis of hepatocellular carcinoma (HCC) has dramatically progressed. In addition, contrast media such as Sonazoid (Daiichi-Sankyo, Tokyo, Japan), one of the second-generation US contrast agents, and gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) (Primovist; Bayer HealthCare, Osaka, Japan), a liver-specific contrast MR agent have become available [1–9]. As a result, the diagnosis of HCC has come to be made at an earlier stage. However, it is occasionally difficult to perform needle-based loco-re-

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gional treatment such as radiofrequency ablation (RFA) and percutaneous ethanol injection therapy for early HCCs, since some of them are not clearly visualized on grayscale US [10].

Along with the progress in diagnostic imaging, a multimodality fusion imaging system has been developed for the clinical practice of treatment of HCC, particularly for the assistance of loco-regional treatment. It has been reported that HCCs hardly detectable on conventional grayscale US could be detected with subsequent loco-regional treatment by virtue of the US fusion imaging system [1, 10–15]. In addition to the US fusion imaging system, a CT fusion imaging system has been reported to be useful for accurate assessment of treatment effects of RFA [16–19]. This report aims to review the usefulness of the multimodality fusion imaging system for percutaneous loco-regional treatment of HCC.

Outline of the US Fusion Imaging System

The US fusion imaging system, such as the Volume Navigation System (GE Healthcare Japan, Tokyo, Japan) [1, 15] and Real-time Virtual Sonography (Hitachi Medico, Co., Tokyo, Japan) [11–14] enables the synchronized display of real-time US images and multiplanar reconstruction (MPR) images of CT or MRI corresponding to the cross section of real-time US. The MPR images are reconstructed based on the volume data of CT or MR images and displayed as a reference, side-by-side, with real-time US images on a single screen.

The US fusion imaging system is useful for the accurate diagnosis and treatment of HCC with safety, in particular at the time of percutaneous loco-regional procedures. It has been reported that the US fusion imaging system is helpful in the detection of HCCs which are difficult to recognize on grayscale US [1, 14, 15]. Therefore, even if the diagnosis of HCC is made at an early stage and the tumor is not detected on US, percutaneous loco-regional treatments can be conducted using the US fusion imaging system. Besides the guidance of loco-regional treatments, the US fusion imaging system can be applied to the evaluation of treatment effects of RFA [13].

The Volume Navigation System, one of the multimodality fusion imaging systems commercially available since 2009 in Japan, is equipped with an ultrasound unit LOGIQ E9 (GE Healthcare Japan) (fig. 1). In Volume Navigation System, the following steps are needed for the synchronized display of real-time US images and CT or MR images.

First, the volume data of CT or MR images for reference should be imported into the system in the digital imaging and communication in medicine (DICOM) format, through a network or a recording media such as CD-ROM or USB-HDD. After the volume data is imported, US images are displayed on the left side of the screen and CT or MR images on the right side, as a reference. In order to synchronize real-time US images to the reference, the cross section of US images approximately parallel to the axial image of the reference has to be registered. Next, two magnetic positioning sensors attached to the probe of an ultrasound scanner (a in fig. 1) detect the magnetic field radiated from a magnetic field transmitter (b in fig. 1) and transmit the information of spatial location and orientation of the probe to a magnetic position-detecting unit (c in fig. 1) equipped with LOGIQ E9. In this way, a magnetic position-detecting unit integrates the positional information of two magnetic positioning sensors and reconstructs MPR images which match the 3D information of the sensors. Subsequently, real-time US and reference images are synchronously displayed in accordance with the movement of the probe. However, since these two image sets are not exactly matched at this time yet, further positional registration of real-time US and reference images is needed. A common point on US and reference images should be visualized for the registration. Practically, we have to visualize a characteristic landmark on US images and mark the point. After marking the landmark point, the US image is fixed and we should operate the probe to seek the corresponding landmark on reference images, by comparing it with the fixed US image. When the landmark point on the reference image is marked, the registration is completed. Then, US and reference images are matched and simultaneously displayed, side by side, on the same screen.

In addition to the simultaneous display of real-time US images and a reference, the Volume Navigation System is equipped with the global positioning system (GPS) function. After positional registration, when GPS markers are indicated on the target on reference images, corresponding sites are pinpointed on real-time US images. This GPS function provides several advantages: firstly, since the site where the target should be visible is pinpointed on real-time US images, it is helpful to detect the target on US, even if it is difficult to perceive on conventional US, and secondly, once the GPS markers are indicated on a target, the target can easily be detected by anyone and from any scanning section, by referring to the GPS markers. Thirdly, it can be applied to the evaluation of treatment effects of RFA, as described later.

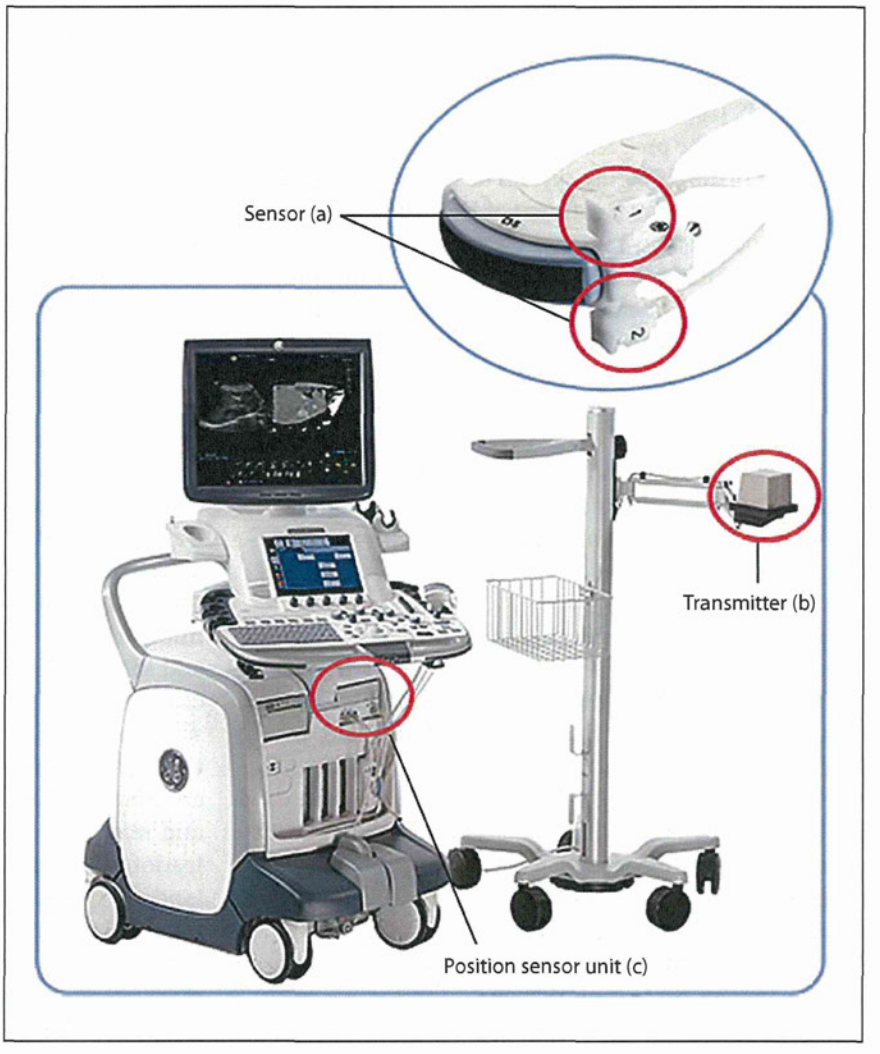

Fig. 1. An outline of the Volume Navigation System. Two magnetic positioning sensors (a), a magnetic field transmitter (b), and a position sensor unit installed in the body of an ultrasound system (c) are needed for the Volume Navigation System. A magnetic field transmitter can be set anywhere at the bedside. Two magnetic positioning sensors are attached to the US probe and the positional information of each sensor is compared in order to evaluate the accuracy of it. The positional information of magnetic positioning sensors is sent to a magnetic position-detecting unit and MPR images which synchronize real-time US images are reconstructed based on this.

Application of the US Fusion Imaging System to the Guidance of Percutaneous Loco-Regional Treatments

Since Gd-EOB-DTPA-enhanced MRI has been clinically available, the diagnosis of HCC tends to be made at an earlier stage than before [1–5]. Accordingly, HCCs difficult to recognize on grayscale US have become detectable as hypointense nodules on hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI. The US fusion imaging system effectively serves as assistance in the diagnosis and percutaneous loco-regional treatment of such HCCs.

Figure 2 shows a case of HCC in which needle core tumor biopsy and RFA using US fusion imaging system were carried out. Although the hypovascular nodule in segment III was depicted as a hypointense nodule on the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI, it was undetectable either on dynamic CT, grayscale US, or Sonazoid-enhanced US (fig. 2a–c). With the Volume Navigation System using the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI as a reference, we performed US-guided target biopsy of the site corresponding to the nodule on reference images, using intrahepatic vessels and hepatic contours as landmarks (fig. 2c). The pathological diagnosis was well-differentiated HCC (fig. 2d). After the diagnosis of HCC, we conducted RFA

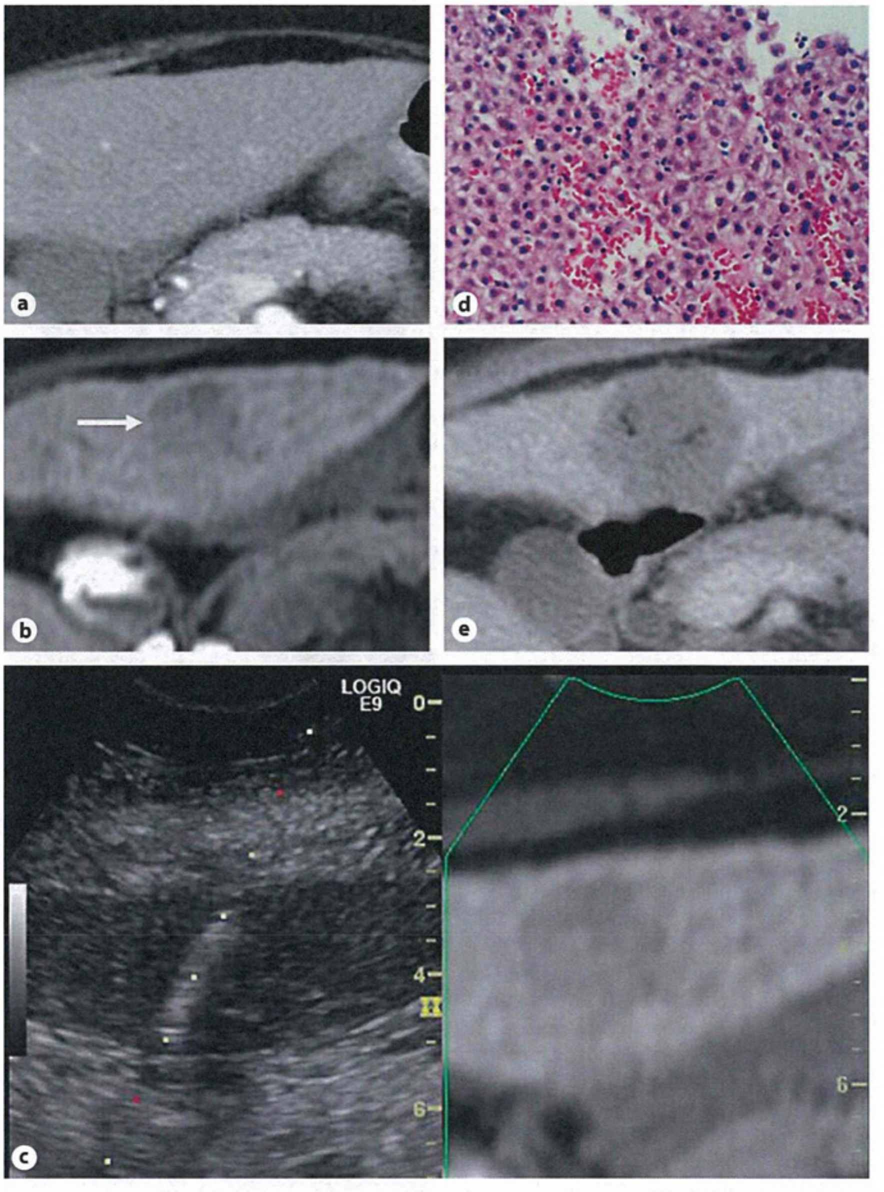

Fig. 2. Needle core biopsy and RFA with guidance from an US fusion imaging system in an 80-year-old woman with hypovascular HCC. **a** Arterial phase of pre-RFA dynamic CT. **b** Hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI. **c** Fusion images of pre-RFA grayscale US and hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI using the Volume Navigation System. **d** Histology of the biopsy specimen of the nodule in segment III (HE staining). **e** Portal phase of post-RFA dynamic CT. The nodule in segment III was detected on neither grayscale US, Sonazoid-enhanced US, or dynamic CT. Only the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI could identify the hypointense nodule 27 mm in diameter (arrow), which was suspected of being a hypovascular HCC. Therefore, using the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI as a reference, needle core biopsy was performed with the Volume Navigation System and the nodule was proven to be a well-differentiated HCC. Subsequently, RFA was carried out with the Volume Navigation System in the same way, and the tumor was completely ablated.

in the same way with the guidance of the Volume Navigation System. The dynamic CT at 3 days after RFA revealed complete ablation of the tumor (fig. 2e). In this way, percutaneous target biopsy or loco-regional treatment can be performed with the assistance of US fusion imaging system, even if the nodule is undetectable either on conventional grayscale US or contrast-enhanced US.

The utility of the US fusion imaging system has also been reported in several studies [10–15]. Kunishi et al. [15] reported that US fusion imaging combining conventional US and the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI was more sensitive than conventional US or contrast-enhanced US for the detection of HCCs, especially small or atypical HCCs, which was quite similar to our results.

Thus, the indication of percutaneous loco-regional treatment seems to have been greatly extended by virtue of the US fusion imaging system.

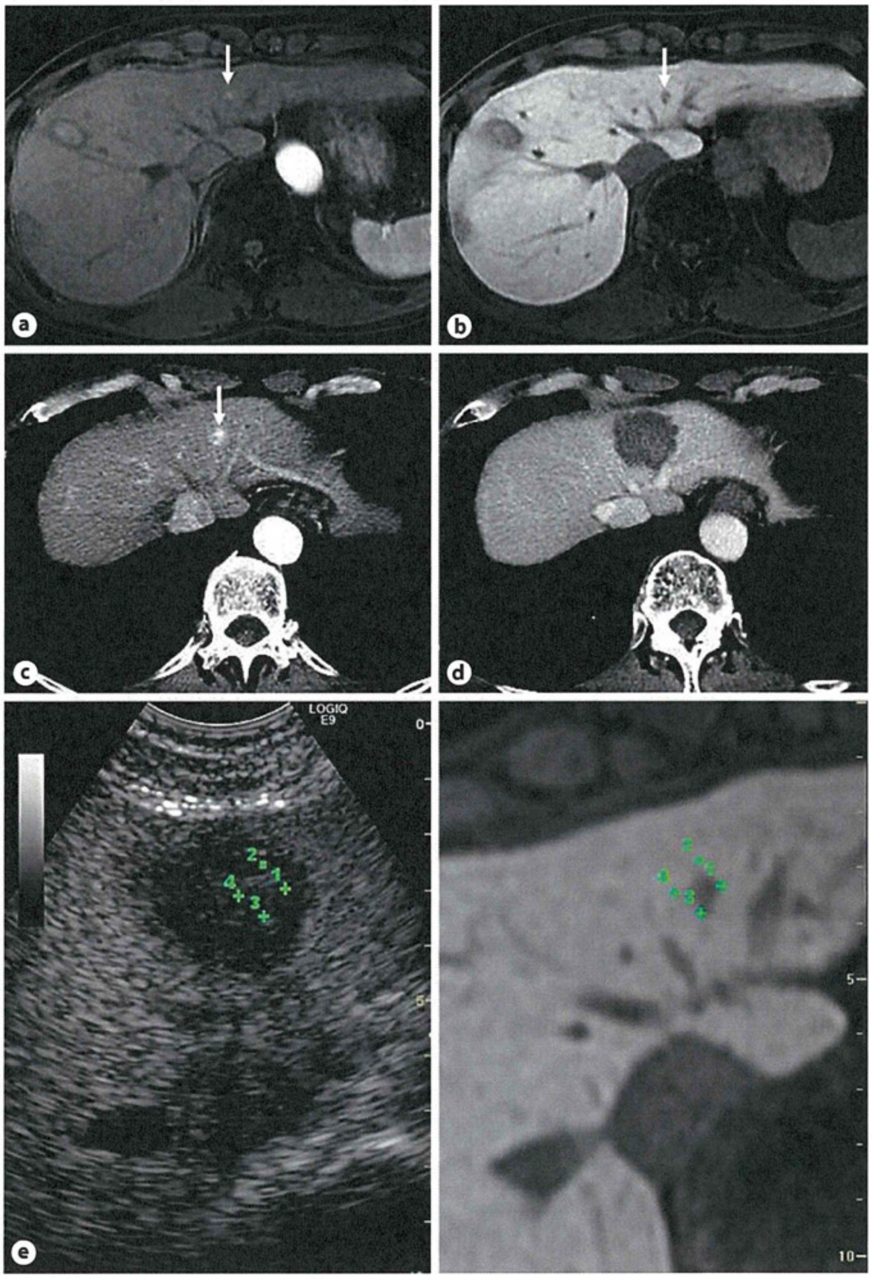

Fig. 3. US fusion imaging system for the evaluation of treatment effects of RFA in a 57-year-old man with hypervascular HCC. **a** Arterial phase of pre-RFA Gd-EOB-DTPA-enhanced MRI. **b** Hepatobiliary phase of pre-RFA Gd-EOB-DTPA-enhanced MRI. **c** Arterial phase of pre-RFA dynamic CT. **d** Portal phase of post-RFA dynamic CT. **e** Fusion images of post vascular phase of contrast-enhanced US (left side) and hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (right side). The tumor was visualized as an early enhancing nodule on both the arterial phase of Gd-EOB-DTPA-enhanced MRI and dynamic CT, and as a hypointense nodule on the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI in segment III (arrow). Following RFA, contrast-enhanced US using Sonazoid was performed at the bedside to evaluate the treatment effects with the Volume Navigation System using the GPS function. The hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI was used as reference. After marking four points on the margin of the tumor on the reference image (**e**; right side), the corresponding four points were displayed on real-time US (**e**; left side). In the post-vascular phase of Sonazoid-enhanced US, these four points were fully encompassed within the hypoechoic area of the ablation zone, indicating complete ablation. Complete ablation was also confirmed on dynamic CT after RFA.

Application of the US Fusion Imaging System in the Evaluation of the Treatment Effects of RFA

The US fusion imaging system is useful not only for the guidance of loco-regional treatment, but also for the assessment of the treatment effects of RFA [13]. Since an ablated tumor becomes obscure on US during RFA due to surrounding high echoic bubbles, it is difficult to evaluate the treatment effects of RFA using conventional US. However, by using the GPS function of the US fusion imaging system described above, the treatment effects can be easily evaluated at the bedside by comparing the ablated area depicted as a low echoic area on the post-vascular phase of Sonazoid-enhanced US with the