



High-intensity focused ultrasound combined with transarterial chemoembolization for unresectable hepatocellular carcinoma: Long-term follow-up and clinical analysis

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ABSTRACT

Objective: High-intensity focused ultrasound (HIFU) combined with transarterial chemoembolization (TACE) has been used to treat unresectable HCC, but its long-term effects and major prognostic factors remain to be determined. The purpose of this study was to assess its long-term effects and find major prognostic factors to help us select eligible patients in the future.

Methods: 73 patients with unresectable HCC received follow-up after HIFU + TACE. The variables of sex, age, AFP level, liver function, tumor location, tumor number, tumor size, TNM staging (5th edition), TNM staging (6th edition), portal vein invasion, ultrasonic pathway of HIFU, TACE session and ablation response were evaluated by univariate analysis. Those variables with significant difference were assessed by multivariate analysis.

Results: The mean follow-up time was 11.7 ± 11.1 months (range, 1–60 months). The median survival time and overall survival rates of 1, 2, 3-year were 12 months, 49.1%, 18.8%, 8.4%, respectively. 45.2% patients achieved complete ablation. At the end of follow-up, 51 patients (69.9%) died from tumor progression (27 patients), liver function failure (18 patients), hemorrhage of upper digestive tract (3 patients) and infection (3 patients). 1 with liver abscess, 2 with serious skin burns and 2 with rib fracture were observed after HIFU. On univariate analysis, age ($P=0.017$), tumor size ($P=0.000$), tumor number ($P=0.039$), the 5th edition of TNM staging ($P=0.023$), portal vein invasion ($P=0.02$) and ablation response ($P=0.000$) had significant difference. On multivariate analysis, ablation response ($P=0.001$) and tumor size ($P=0.013$) were major prognostic factors.

Conclusion: HIFU combined with TACE is a safe method with a low rate of severe complications. As major prognostic factors, ablation response and tumor size may help us predict the survival and select eligible patients clinically.

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1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world [1]. Only 30% of patients with HCC diagnosed at early stage are suitable for curative resection, liver transplantation and local ablation [2,3]. For these early patients, beneficial survival can be received from these treatments. In the remaining 70% of patients with HCC diagnosed at intermediate or advanced stages, a large majority of patients are technically unresectable owing to the presence of tumors involving major vascular structures, multiple, bilateral tumors or underlying hepatic dysfunction. Although

tumors in few patients with HCC at late stage can be resected successfully, rapid recurrence will be occurred after resection because of the advanced nature of these tumors, and the long-term prognosis of these patients is poor. Similar to resection, the long-term prognosis of patients undergoing local ablation is poor. Owing to the presence of high vascular fed primarily by hepatic artery in HCC, embolization of the feeding arteries may be one possible treatment option for unresectable patients. Even though no effective standard forms of therapy have been established up to now, transarterial chemoembolization (TACE) is becoming one treatment option of unresectable hepatocellular carcinomas in many countries in the Western world and Asia.

TACE is one major technique to slow tumor progression on the basis of effective targeted chemotherapy and ischemic necrosis caused by hepatic arterial embolization [4]. Although prior several

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studies showed TACE has no significant survival benefit [5–7], two randomized controlled trials of TACE for unresectable HCC showed a significant survival advantage was found in selected patients treated by chemoembolization compared with those by embolization and conservative treatment [8,9]. Unfortunately, a recent systematic review and meta-analysis of transarterial treatments for HCC showed that there was no significant difference in survival between embolization and chemoembolization [10].

As a noninvasive method, high-intensity focused ultrasound (HIFU) is being promoted to treat certain solid malignancy such as liver and breast [11]. Ultrasound beam can travel through the body and reach area of targeted tissue which is deep within the body. With the use of focusing, the acoustic energy at the focal region is much higher compared with the region outside the focus. As a result, the energy deposition in the tissue along the ultrasound beam path which locates outside the focal region is so low that be difficult to induce the tissue damage. Accordingly, the tissues including tumors at the focal region would absorb enough energy of ultrasound beam to induce tissues damage by producing biologic effects. During HIFU exposure, heat generation due to absorption of acoustic energy in the targeted tissue occurred. If the temperature in the tissue is rapidly raised to 60 °C or higher for 1 s or longer, coagulation necrosis will be induced. In fact, the temperature at a focal region may rapidly arrive at above 80 °C due to HIFU exposure [12,13]. Therefore, coagulation necrosis in the target tissue is the major pathological result of biologic effects induced by HIFU exposure [14]. Heat effect may be a major mechanism of HIFU for ablating tumor [13].

Because blood supply of HCC origins from the blood of both hepatic artery and portal vein, it is difficult to receive complete necrosis of HCC after arterial embolization of TACE treatment alone. Nonetheless, TACE may reduce blood flow of HCC. For ablative therapies such as radiofrequency ablation, heat loss caused by blood flow may reduce the efficacy. Therefore, TACE combined with ablative therapies has been used in HCC patients in order to possibly achieve complete necrosis of HCC [15]. A randomized controlled clinical trial on comparisons of therapeutic effect between HIFU combined with TACE and TACE alone in the treatment of advanced HCC showed a significant survival advantage was found in the HIFU combined with TACE group as compared with the TACE group [16]. However, 26 patients with unresectable HCC were treated by HIFU combined with TACE in this trial. In addition, this trial was limited to short-term follow-up with a maximal time of 24 months (range, 3–24 months) and no clinical analysis. The long-term effects and major prognostic factors remain to be determined. In this study, 73 patients with unresectable HCC (stage IIIA 25, IVA 48; TNM staging, 5th edition) treated by HIFU combined with TACE achieved long-term follow-up with a maximal time of 60 months. Meanwhile, the variables of sex, age, AFP level, liver function, tumor location, tumor number, tumor size, TNM staging (5th edition), TNM staging (6th edition), portal vein invasion, ultrasonic pathway of HIFU, TACE session and ablation response were evaluated by univariate analysis and multivariate analysis. The purpose of this study was to assess the long-term effects of HIFU combine with TACE for treating unresectable HCC and find major prognostic factors by clinical analysis to help us select eligible patients in the future.

2. Materials and methods

2.1. Patients

From January 1999 to February 2005, 73 consecutive patients with HCC who met the inclusion criteria were eligible for a prospective study that was approved by the ethics committee at Chongqing University of Medical Sciences. All patients understood every

Table 1

Clinical characteristics of the patients with unresectable HCC.

Characteristic	TACE + HIFU
Total of patients	73
Sex: M/F	60/13
Age (mean ± SD)	51.2 ± 12.3
Tumor size (mean ± SD)	8.9 ± 3.1
AFP (≥400/<400 ng/ml)	49/24
HBV infection (+/–)	64/9
Child-Pugh class (A/B)	65/8
No. of tumors(1/2–3/≥4)	31/18/24
Portal vein invasion (trunk/branch/non)	10/15/48
TNM stage (III/IV)	25/48
Tumor location (lobe, right/left/both)	4/42/27

procedure in this study and agreed to participate. Before every procedure, an informed consent form was obtained from all patients and their family members. The diagnosis of HCC in all patients was confirmed by either ultrasound-guided fine-needle biopsy or by the characteristic findings of a hepatic mass with arterial hypervascularization on contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) and a serum α -fetoprotein level of 400 ng/ml or higher [17]. The inclusion criteria were: (1) patients were unresectable surgically; (2) five or fewer lesions were presented at CT or MRI; (3) the value of Karnofsky performance scale score was 70% or higher; (4) patients had liver function of Child-Pugh class A or B; (5) few or no ascites was detected by Doppler ultrasonography; (6) patients had no history of hepatic encephalopathy. The exclusion criteria were: (1) patients with the metastases of lymph nodes or extrahepatic organs; (2) patients with active infection; (3) tumors undetectable by ultrasound scanning; (4) patients with the liver function of Child-Pugh class C or with severe coagulation disorders (prothrombin activity under 60%). On the basis of these criteria, 73 patients with unresectable HCC (stage IIIA 25, IVA 48; TNM staging, 5th edition) were enrolled in this study. Among them, the diagnosis of 24 with a serum level of <400 ng/ml were conformed by the pathological findings. There were 60 males and 13 females. The mean age was 51.2 ± 12.3 years (range, 30–78 years). 31 patients (42.5%) had solitary lesion; 18 (24.7%) had two or three lesions; and the remaining 24 (32.8%) had four or five lesions. The average size of all lesions in diameter was 8.9 ± 3.1 cm (range, 3.2–18 cm). The lesions of the liver located at the left lobe in 4 patients (5.5%), at the right lobe in 42 patients (57.6%) and at both lobes in 27 patients (36.9%). 48 patients (65.8%) with no portal vein invasion, 15 (20.5%) with invasion of the branch and 10 (13.7%) with invasion of the trunk were presented on CT or MRI. All patients received TACE followed by HIFU ablation. The characteristics of 73 patients are shown in Table 1.

2.2. TACE procedures

All patients received TACE before HIFU ablation. TACE was performed with the use of the Seldinger technique of arterial embolization described previously [18]. A 5-F pigtail catheter was introduced through the femoral artery firstly. Then hepatic arteriography was performed to assess the size and location of liver lesions. According to the size, location, and arterial blood supply of the tumors, either the right or left hepatic artery was catheterized selectively guided by digital subtraction angiography. A 3–5-F tracker catheter was catheterized to the feeding arteries of tumors for superselective embolization. The feeding arteries of all tumors were embolized with the use of the suspension mixed with either 80–120 mg of cisplatin (Qilu Pharmaceutical Factory, Jinan, China) or 40–60 mg of adriamycin (Main Luck Pharmaceutical, Shenzhen, China) and 10–20 ml of iodized oil (Lipiodol; Huaihai Pharmaceutical Factory, Shanghai, China). 9 patients received

chemoembolization with the suspension including adriamycin between January 1999 and December 1999. The remaining 64 patients received chemoembolization with the suspension including cisplatin after December 1999 because of potential serious side effects of adriamycin such as cardiotoxic and marrow depression. Each patient received TACE ranged from 1 to 2 sessions (mean: 1.25 ± 0.43 sessions) before HIFU ablation. 55 patients (75.3%) received 1 session of TACE, and the remaining 18 patients (24.7%) received 2 sessions.

2.3. Preparation before HIFU treatment

Plain CT was performed to evaluate the lipiodol deposition of the lesions in all patients 7–10 days after each TACE. Conventional serum chemical tests including liver biochemical tests, complete blood cell counts, prothrombin time and α -fetoprotein were detected before ablation. In addition, chest radiography, abdominal ultrasonography and electrocardiogram (ECG) were assessed before HIFU ablation. MRI was applied to evaluate the information of each tumor including its size, location, number and enhancement before treatment and periodical period after HIFU ablation.

2.4. Partial rib resection

37 patients received partial rib resection after TACE because they met the following criteria. The criteria were: (1) the tumor location in the right lobe of the liver; (2) the tumor mass shielded by ribs and the interspaces smaller than 1.5 cm. The remaining 36 patients did not undergo rib resection due to reasons as follows: (1) the treated lesion was located in the left lobe of the liver with no overlying ribcage; (2) the right lesion was close to the liver capsule, and easy to be treated with HIFU through the intercostal space; (3) the width of the intercostal space was more than 1.5 cm, and the right lesion concealed by the ribs could be moved into the intercostal space by using lung ventilation (either inspiration or expiration) under general anesthesia, so that the concealed lesion could be ablated. The procedure of partial rib resection was described previously [19]. Before rib resection, the rib removal range had been defined by the combination of the HIFU treatment position depending on MRI and ultrasonography under the treatment position. All 34 patients received resection under general anesthesia. The incision in an arc was designed to avoid the acoustic field and reduce the possibility of side effects during HIFU. At first, the skin flap was detached along the subcutaneous fascia of the superficial lamella of chest wall. Then, the periosteum of the rib was dissected followed by disassociating and cutting it. Finally, after smoothing the ends of the rib, the medullary cavity of bone was blocked by bone wax followed by filling the rib bed with the use of gel foam, and the incision was sutured slice by slice. The ribs removal ranged from the right midclavicular line and posterior auxiliary line and from rib 6 to 10. In general, the ribs 7–9 were removed partially. In this study, no serious complications related to rib resection were found.

Suture thread for the skin incision was removed 1 week after rib resection, and HIFU procedure was usually performed at 2 weeks postoperatively when the wound had contraction and epithelialization in the proliferative phase of wound healing. No obvious influences of the surgical bed were observed on the surgery-created acoustic window where the focused ultrasound beams passed through. There were no bleeding and gaping of the wound, as well as subsequent infection in all patients after HIFU ablation. Furthermore, due to rib removal within the adequately acoustic window, the potential damage of overlying chest wall tissues including muscles was significantly reduced by heat deposition caused previously by the rib reflection of ultrasound energy.

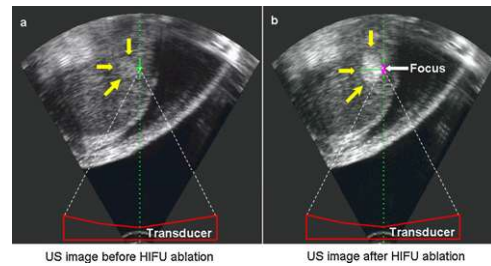


Fig. 1. Schematic diagram of US-guided HIFU ablation shows the real-time ultrasound-guided HIFU in the treatment of HCC. (a) A sagittal US image before HIFU shows tumor mass in the liver (yellow arrow); (b) a sagittal US image of the nearly similar location shows grey scale in the target volume increased obviously after HIFU ablation with the use of linear scan.

Ribs overlying hepatic lesions could attenuate acoustic power deposited in the target tumors, leading to partial ablation. It was also difficult to ablate the target behind the ribs. In addition, the ribs' reflection of ultrasound beams could cause an increased temperature of the bones, resulting in damage to the bone, muscle, subcutaneous tissue and skin. In this study, rib resection was a relatively minor procedure, which could reduce the noninvasive nature of HIFU ablation. But it allowed potentially curative ablation of tumors that otherwise would not be amenable to this form of therapy. With no rib reflection of ultrasound, it might provide the potential to ablate the tumor completely, and decrease the possibility of HIFU complications related to the ribs.

2.5. HIFU treatment procedure

The device used for HIFU procedure was a Model-JC HIFU system (Chongqing Haifu (HIFU) Tech Co., Ltd., Chongqing, China). It consisted of ultrasound therapy transducers with an ultrasound generator, a real-time diagnostic ultrasound device, a six-direction movement system, computer units for automated control, a treatment bed, and a degassed water circulation unit. A 12-cm diameter PZT-4 piezo-ceramic transducer was employed to produce therapeutic ultrasound energy. The frequency of the transducers was 0.8 MHz, with various focal lengths ranging from 135 to 155 mm. An ultrasound imaging device (Esaote DU3, Genova, Italy) was used as a real-time imaging guidance in the HIFU system, with a 2.5–3.5 MHz probe. This diagnostic probe was situated in the center of the HIFU transducer, and the integrated transducer was then placed in the bag filled with degassed water.

HIFU procedure was performed 2–3 weeks after TACE or 2 weeks after rib resection. All patients always received either general or epidural anesthesia for HIFU treatment, which could prevent individual patient's discomfort and mobilization. After suitable anesthesia was achieved, the patient was positioned either prone or on his or her right side, so that the skin overlaying the targeted lesion would be easily put in contact with the degassed water. With a six-direction movement of the integrated transducer, the targeted liver tumor was clearly identified on ultrasound imaging, and the targeted volume was divided into parallel slices of 5-mm separation. One operator outlined the margin of the treated region in each of the slices, including the tumor and at least 1-cm normal tissue surrounding the tumor. The range of the target of the each slice was automatically recorded by the computer in three orthogonal directions.

A schematic diagram of HIFU ablation was shown in Fig. 1. After a detailed planning session was finished, a linear scanning track of HIFU exposure was selected as an ablative scheme. Using provisional therapeutic parameters based on the depth and vascular supply of the target region, the tumor on each slice was completely ablated from the deep to shallow regions, and this process was

repeated slice by slice to achieve entire tumor treatment. Dissimilar to MR-guided HIFU device, which used temperature mapping technique for evaluating the extent of coagulation necrosis, ultrasound imaging in this HIFU system was not able to measure temperature changes within the focus immediately after each exposure. However, gray-scale changes obtained on the diagnostic images within the focus after each HIFU exposure were used during ablative procedure to identify and monitor the extent of treatment. In this study acoustic power used for ablation ranged from 160 to 350 W, and HIFU exposure time varied from 58 to 242 min, which was dependent on the size of targeted tumors. The number of HIFU sessions was 1.64 ± 0.90 (range, 1–4 sessions) per patient.

2.6. Follow-up

All 73 patients were followed up after HIFU ablation, and contrast-enhanced MRI was used as a main follow-up radiological assessment in all patients. The first MRI examination was performed 2 weeks after HIFU treatment while inflammatory edema disappeared completely at the marginal area of the ablated tumor. Compared to MR images before HIFU, an obvious absence of contrast enhancement was found in the treated tumor after HIFU, which was indicative of coagulation necrosis. In addition, a thin contrast-enhancement ring observed between the treated and untreated regions was very useful to assess whether there would be tumor cells remained after HIFU ablation. If the residual viable tumor was demonstrated on the follow-up MRI, a subsequent HIFU ablation was carried out for the destruction of the remaining tumor 1–4 weeks after MRI examination. Every patient was followed up every 3 months for 2 years and every 6 months thereafter. Serum biochemistry and clinical examination were also performed during follow-up period.

2.7. Statistical analysis

All data are reported as the mean $\pm$ standard deviation. Statistical analysis was performed by a statistical software package (SPSS version 10.0). The period of follow-up was defined as the time from the first session of HIFU ablation to death or last follow-up. The Kaplan–Meier method was used to assess overall survival. Age, sex, AFP level, liver function, tumor location, tumor number, tumor size, TNM staging (5th edition), TNM staging (6th edition), portal vein invasion, ultrasonic pathway of HIFU, TACE session and ablation response were evaluated by univariate analysis using the Kaplan–Meier method and the log-rank test. The potentially significant variables assessed by univariate analysis were evaluated by multivariate analysis using Cox regression analysis. *P* values were judged significant if they were less than 0.05.

3. Results

3.1. Overall survival

All patients achieved follow-up. The mean period of follow-up was 11.7 ± 11.1 months (range, 1–60 months). At the end of follow-up, 51 patients (69.9%) had died from tumor progression (27 patients) and other diseases including liver function failure (18 patients), hemorrhage of upper digestive tract (3 patients) and infection (3 patients). Overall survival was assessed using the Kaplan–Meier method. The median survival time was 12 months (95% CI: 9–15 months), and the survival rates of 1, 2, 3-year were 49.1%, 18.8% and 8.4%, respectively (Table 2). The survival curve of patients in this study was shown in Fig. 2.

Table 2

Survival analysis of patients with HCC treated by HIFU combined with T ACE.

N	Follow-up (months)		
	12	24	36
73	49.1%	18.8%	8.4%

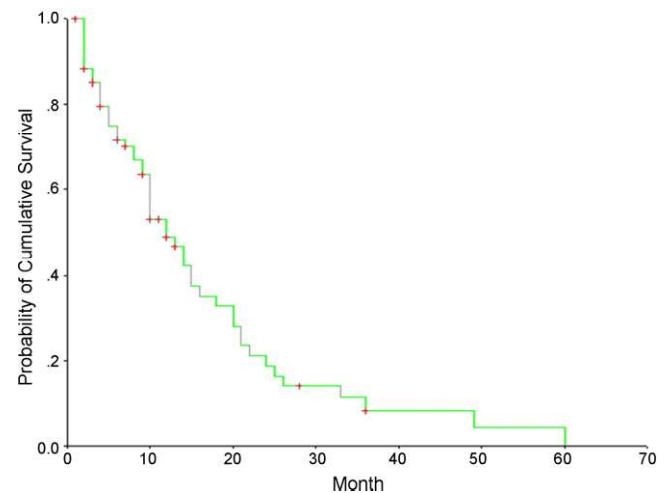


Fig. 2. Survival curve of 73 patients with unresectable HCC treated by HIFU combined with TACE. The graph shows the survival probability of patients after a mean period of 11.7 ± 11.1 months (range, 1–60 months) of follow-up.

3.2. Tumor response

Contrast-enhanced MRI was performed before and after HIFU ablation. The short-term effectiveness of HIFU ablation was assessed by MRI at 2 weeks after HIFU. The disappearance of the enhancement within the treated tumor was found on MRI images after HIFU as compared with before HIFU (Fig. 3). This was presented as an indication of complete ablation. In this study, contrast-enhanced MRI at 2 weeks after HIFU ablation showed all tumors in the same patient were completely ablated in 33 patients (45.2%). The median survival time in patients with complete ablation and in patients with partial ablation was 20 months (95% CI: 14–26 months) and 7 months (95% CI: 3–11 months), respectively. There was significant difference in survival between two groups ($P=0.000$).

3.3. Severe complications

A low rate of severe complications was found in this study (Table 3). One patient developed liver abscess with a temperature of $>39^\circ\text{C}$ for 5 days after HIFU ablation. This patient recovered 2 weeks after abscess drainage and antibiotics injection. Serious skin burns induced by HIFU ablation was found in 3 patients (4.1%). Coagulation necrosis with white in color was observed in the serous skin burns. 3 patients recovered 10 days after the removal of the skin burns and incision suturation. Among 36 patients without rib resection, follow-up MRI showed that 61% (22) patients had a potential HIFU-induced damage on the ribs within acoustic window. It presented a small area of non-contrast enhancement of the ribs and

Table 3

Severe complications after HIFU ablation.

Type	No. of cases
Liver abscess	1 (1.4%)
Serious skin burn	3 (4.1%)
Rib fracture	2 (5.6%)

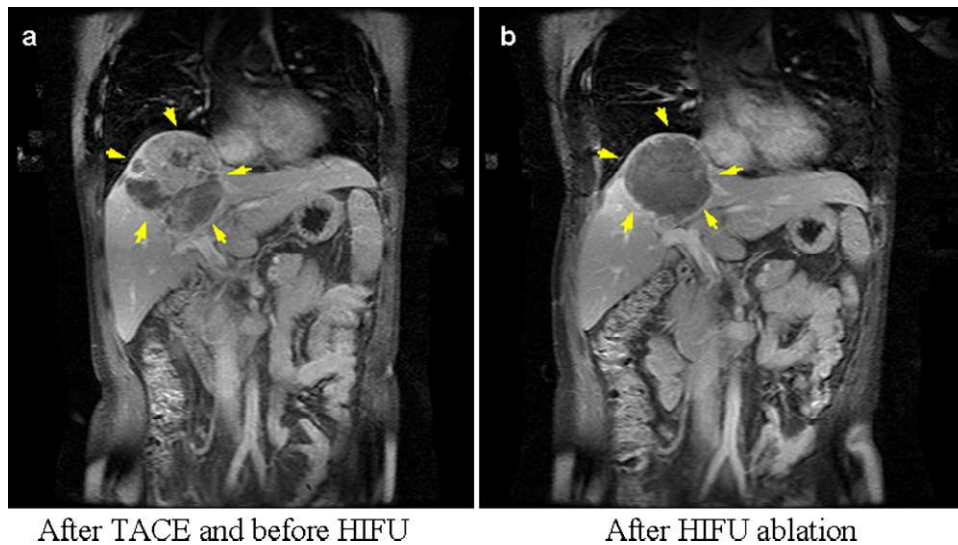


Fig. 3. The changes of enhanced MRI at 1 week after TACE and at 2 weeks after HIFU in the large HCC of the right lobe of liver. (a) A coronary image of enhanced MRI after TACE and before HIFU ablation shows partial absence of blood supply in the large HCC at the right lobe of liver; (b) a coronary image of enhanced MRI at 2 weeks after HIFU ablation shows complete absence of blood supply of the same tumor in this patient without rib resection.

pre-rib tissues within acoustic window 2 weeks after HIFU, but disappeared in subsequent follow-up MR images. One year after HIFU, rib fracture was accidentally found within the acoustic window in 2 (5.6%) patients with positive rib-damage images observed previously by MRI. The fracture of rib 7 and rib 8 at the right chest was found in one patient by chest radiography, and the fracture of rib 8 and rib 9 was found by physical examination and chest radiography. Mild pain of the right chest was presented in these two patients and palliated by oral non-steroid analgesics. Neither tumor bleeding nor large blood vessel rupture was found during follow-up. Acute hepatic failure or renal dysfunction caused directly by either TACE or HIFU ablation was not detected.

3.4. Clinical analysis

The Kaplan–Meier method and the log-rank test were used for univariate analysis. Significant difference in survival between stage IIIA patients and IVA patients (TNM staging, 5th edition) was observed ($P=0.023$). All 73 patients in this study were classified into the group of stage IIIA (17 patients) and the group of stage IVA (56 patients) using the 6th edition of TNM staging. The difference in survival between two groups was not significant ($P=0.124$). The median survival time in patients aged >60 years was increased by 5 months compared with that in patients aged ≤ 60 years, which had significant difference in survival ($P=0.017$). There was no significant difference in survival between males and females ($P=0.297$). The median survival durations in patients with the largest tumor of ≤ 5 cm, 5–8 cm, 8–10 cm and 10 cm in diameter were 32, 20, 10 and 6 months, respectively, which had significant difference ($P=0.000$). Significant difference in survival was observed between patients with solitary lesion, 2–3 lesions and ≥ 4 lesions ($P=0.039$). The median survival durations of patients with no portal vein invasion, invasion of the main branch and invasion of the trunk were 15, 9 and 4 months, respectively, which had significant difference ($P=0.02$). The difference in survival between patients with hepatic tumors located at the right lobe, left lobe and both was not significant ($P=0.342$). No significant difference in survival was observed between patients with the liver function of Child–Pugh class A and B ($P=0.137$). There was no significant difference in survival between patients with an AFP level of <400 ng/ml and ≥ 400 ng/ml ($P=0.388$). As compared with patients without rib resection, the median survival time of patients with rib resection was increased by 1 month,

with no significant difference in survival ($P=0.835$). Comparison of the survival between patients with one session of TACE and 2 sessions showed no significant difference ($P=0.745$). All results obtained from univariate analysis were shown in Table 4.

The variables with significant difference evaluated by univariate analysis were assessed by multivariate analysis using Cox regression analysis. As shown in Table 4, the variables of the 5th edition of TNM staging, tumor size, portal vein invasion, tumor number and ablation response had potentially significant difference in survival. The results obtained from Cox regression analysis showed tumor size ($P=0.013$) and ablation response ($P=0.001$) had significant difference. All results assessed by multivariate analysis using Cox regression analysis were shown in Table 5.

4. Discussion

The prognosis of patients with unresectable HCC is poor with the short-term period of median survival in patients without specific treatment. TACE is one of the most common therapies in the treatment of unresectable HCC. Since blood supply of HCC is obtained from both hepatic artery and portal vein, it is difficult to receive complete necrosis of HCC after arterial embolization alone. Viable tumor cells are still found in the tumor after TACE by the embolization of the hepatic artery alone [20]. However, TACE may reduce blood flow of HCC by arterial embolization. It has been reported that thermal ablation therapies combined with TACE may increase the efficacy in patients with HCC because heat loss induced by blood flow may be reduced by TACE [15]. As a thermal ablation therapy, ablation response of HIFU may be improved by the lipiodol deposition caused by TACE except the reduction of blood flow [21]. A randomized controlled clinical trial on comparisons of therapeutic effect between HIFU combined with TACE and TACE alone in the treatment of advanced HCC showed a significant survival benefit in patients treated by HIFU ablation combined with TACE [16]. The short-term effects in this trial showed a median survival time of 11.3 months and a 1-year survival rate of 42.9% in the combined group (26 subjects). In the present study, a median survival time of 12 months and a 1-year survival rate of 49.1% were observed after long-term follow-up, which were similar to previous study. In addition, we observed that the survival rates of 2- and 3-year were 18.8% and 8.4%, respectively. The survival rates of 2- and 3-year were so low that we speculate that it is difficult to improve the

Table 4

Univariate analysis of factors affecting overall survival after HIFU ablation combined with TACE.

Type	n (%)	Median (95% CI) (months)	Survival rate (%)			χ^2	P
			1-y	2-y	3-y		
Total	73 (100)	12 (9–15)	49.1	18.8	8.4		
TNM staging (5th edition)							
IIIA	25 (34.2)	20 (12–28)	54.0	30.1	16.0	5.19	0.023
IVA	48 (65.8)	11 (6–16)	42.5	11.7	3.8		
TNM staging (6th edition)							
II	17 (23.3)	16 (6–26)	54.2	31.0	15.6	2.37	0.124
IIIA	56 (76.7)	10 (6–14)	44.4	10.2	5.2		
Age (years)							
≤60	54 (74.0)	10 (8–12)	41.2	10.3		5.73	0.017
>60	19 (26.0)	15 (3–27)	62.8	39.2	23.5		
Sex							
Male	60 (82.2)	13 (8–18)	50.2	20.8	11.1	1.09	0.297
Female	13 (17.8)	10 (4–16)	33.0	10.9			
Tumor size (cm)							
≤5 cm	8 (10.9)	32 (16–48)	68.6	51.4	34.3	21.2	0.000
5–8 cm	23 (31.5)	20 (15–25)	67.9	27.2	10.2		
8–10 cm	18 (24.7)	10 (5–15)	34.5	11.5			
>10 cm	24 (32.9)	6 (4–8)	21.4				
No. of tumors							
Solitary	31 (42.5)	12 (9–15)	49.5	11.8		6.48	0.039
2–3	18 (24.7)	21 (10–32)	62.2	26.7	13.3		
≥4	24 (32.8)	6 (1–11)	34.2	8.6	–		
Tumor location (lobe)							
Left	4 (5.5)					2.14	0.342
Right	42 (57.6)	13 (9–17)	53.5	22.6	11.0		
Both	27 (36.9)	10 (7–13)	38.5	11.9	5.9		
Portal vein invasion							
Non	48 (65.8)	15 (11–19)	58.5	22.1	11.7	12.49	0.02
Branch	15 (20.5)	9 (7–11)	24.5				
Trunk	10 (13.7)	4 (0–8)					
Child-Pugh class							
A	65 (89.0)	13 (9–17)	52.1	19.2	6.9	2.21	0.137
B	8 (11.0)	2					
AFP level (ng/ml)							
<400	24 (32.9)	12 (3–21)	48.1	6.0	–	0.74	0.388
≥400	49 (67.1)	12 (9–15)	49.4	22.9	10.6		
Rib resection							
Non	36 (49.3)	12 (8–16)	49.7	15.7	–	0.04	0.835
Resection	37 (50.7)	13 (6–20)	50.9	21.2	6.4		
TACE session							
1	55 (75.3)	14 (9–19)	47.4	25.5	9.1	0.11	0.745
2	18 (24.7)	12 (8–16)	46.5	13.3	–		
Ablation response							
Complete	33 (45.2)	20 (14–26)	69.7	31.0	13.9	18.76	0.000
Partial	40 (54.8)	7 (3–11)	20.8				

long-term overall survival in patients with unresectable advanced HCC by this combination therapy. Nonetheless, the median survival time and the survival rates of 1-, 2-, and 3-year in patients with complete ablation achieved 20 months and 69.7%, 31.0%, 13.9%, respectively. Furthermore, the patients with tumor size of <5 cm in diameter achieved a median survival time of 32 months and a

3-year survival rate of 34.3%. Thus, we believe that the long-term survival benefit may be probably observed in the selected patients according to the prognostic factors.

Although TACE may reduce blood flow of HCC, a previous study on a combined approach of arterial embolization and thermal ablation demonstrated that thermal ablation could destroy the blood supply of residual tumor after TACE more efficiently than repeated TACE courses [22]. Furthermore, the greatest reduction of blood flow in the tumor may be achieved in the first one or two sessions of TACE. In this study, 55 patients (75.3%) achieved 1 session of TACE, and the remaining 18 patients (24.7%) achieved 2 sessions. There was no significant difference in survival between patients with 1 and 2 sessions of TACE. The result indicates that one course of successful TACE is enough to improve the efficacy of HIFU ablation.

The ribcage is a critical problem in the HIFU treatment of HCC located in the right lobe of the liver. The rib can conceal the lesion on

Table 5

Multivariate analysis of factors using Cox regression analysis.

Variable	β	SE	Wald	Df	Sig.	exp(β)
Age	−0.562	0.403	1.949	1	0.163	0.570
TNM staging (5th edition)	0.208	0.364	0.325	1	0.569	1.231
Tumor size	0.530	0.212	6.220	1	0.013	1.699
Portal vein invasion	0.257	0.265	0.937	1	0.333	1.293
No. of tumors	0.073	0.180	0.165	1	0.685	1.076
Ablation response	1.333	0.387	11.855	1	0.001	3.791

ultrasound images, making the tumor beyond the ribs untreatable. The ribs absorb focused ultrasound beams, resulting in a significant decrease of acoustic energy deposited at the focus. This may cause therapeutic energy insufficient enough to induce coagulation necrosis of the targeted tumor. On the other hand, ultrasound energy absorbed by the ribs can obviously increase local temperature, leading to the heat damage of the ribs and surrounding tissues such as muscles. In this study, 37 patients were selected to receive partial rib resection in order to improve the deposition of acoustic energy at the focus and to reduce potential damage of the rib through the acoustic window. However, the remaining 36 patients did not undergo the rib resection before HIFU procedure as focused ultrasound beams can pass through the intercostal space to form a focus. Compared to the patients with rib resection, similar ablative results are achieved in the patients with no surgery due to following reasons. First, the lesion is located in the left liver and there is no overlying ribcage. Secondly, using general anesthesia with lung ventilation (inspiration or expiration), the lesion beyond the ribs can be moved into the intercostal space, thus ablated. Thirdly, the lesion is close to the liver capsule, and deposited energy is good enough to ablate the targeted tumor via the intercostal space, though there is energy attenuation by the ribs. Finally, iodized oil (Lipiodol) located in the lesion after TACE procedure can efficiently increase the deposition of ultrasound energy at the focus, resulting in temperature rise in the treated lesion. This strengthens the destructive effects of HIFU on deep tumors where huge energy attenuation occurs. Our results suggest that with no rib resection, HIFU could induce successful ablation of the targeted tumor in the selected patients with unresectable HCC.

Using univariate analysis, the prognostic factors are evaluated in this study for the HIFU-treated patients with unresectable HCC. We found that the variables of age, tumor size and number, portal vein invasion and ablation response had significant differences in the HIFU-treated patients. The median survival time in patients aged >60 years was increased by 5 months while compared with patients aged ≤60 years, suggesting that tumors could be less aggressive in the elderly patients. The increased number and size of HCC lesions, as well as vein invasion could potentially cause the worse survival in the HIFU-treated patients. However, the tumor invasion of blood vessel, a predominant prognostic factor in the 6th edition of TNM staging classification standard, is not used in the study because the patients do not undergo surgery after HIFU procedure [23]. Without any histological examination for the identification of tumor invasion of blood vessels in tissue samples, it would be inappropriate to employ this prognostic factor for clinical evaluation of the prognosis in the study.

The variables of liver function, tumor location, AFP level and sex had no significant difference evaluated by univariate analysis. The result of no significant difference in survival between patients with liver function of Child-Pugh class A and B indicates that all patients with liver function of class A or B could endure the treatment of our combined approach well to achieve the scheduled effects and acquire the similar survival. Since TACE may improve the efficacy of HIFU ablation discussed above, HIFU may effectively ablate the tumor located at the left lobe or right lobe or both although the energy deposition of HIFU may be influenced by different acoustic window. So tumor location may not be a prognostic factor in this study as indicated by our results. Higher AFP levels may reflect a more advanced stage and greater malignant potential of tumors because the high level of AFP is correlated with big tumor burden and poor differentiation in HCC patients [24,25]. However, in the present study, all subjects with unresectable HCC had bad life expectancy, which implies AFP level correlated with tumor burden and differentiation maybe not directly assess their survival. Contrary to previous studies, our results suggest that AFP level is not

probably selected as one of the major prognostic factors in patients with unresectable HCC.

The variables of the 5th edition of TNM staging, tumor size, portal vein invasion, tumor number and ablation response with potential influence of survival evaluated by univariate analysis were assessed by multivariate analysis using Cox regression analysis. Our results show ablation response and tumor size with significant difference are substantially associated with the influence of survival in patients with unresectable HCC treated by HIFU combined with TACE. Therefore, ablation response and tumor size may be selected as major prognostic factors to predict the survival in unresectable HCC patients followed by the treatment of our combined approach. Furthermore, the results provide us how to select eligible patients to receive the therapy using our combined approach. According to the results, the patients with smaller size of tumor could be eligible to receive the combined therapy. Similarly, we may predict the survival depending on the ablation response.

At the end of follow-up, our results showed the main causes of death were tumor progression and liver function failure. Thus, survey of tumor situations and liver function are important during follow-up. We speculate that it is possible to improve the survival by the therapies of ablating the viable tumor and improving the live function when the follow-up shows tumor progression or liver function disorder. However, this study did not investigate it. We will develop it in the future study.

As shown in Table 3, a low rate of severe complications was found in this study. Rib fracture, which is not reported before, was observed 1 year after HIFU ablation in 2 out of 36 patients (5.6%). This result indicates that ribs on the acoustic window could be damaged by rib-overheating during HIFU ablation. Fortunately, the probability of rib fracture occurred with a low rate of 5.6%, and no serious symptoms were observed in the patients. Recent studies showed that rib-overheating problem during HIFU ablation could be solved by a segmented transducer using switching off its edge segments for rib sparing or by an independent array-element activation scheme using switching off the transducer elements obstructed by the ribs based on feedback anatomical medical imaging [26,27]. Therefore, HIFU ablation will be applied for HCC therapy with lower rates of adverse effects along with the development of HIFU technology.

Our study has some weaknesses. Several preparative therapies before HIFU may weaken the assertion that HIFU ablation is effective, safe, and feasible in the treatment of patients with unresectable HCC. One is that rib resection was performed in some patients, which can detract from the status of HIFU as a noninvasive therapy, though this surgery is relatively minor. Another is that pre-procedural TACE is performed in the patients. We acknowledge that this is not a randomized clinical trial and that TACE can have an independent effect on tumor volume. Thus, it will be necessary to perform large randomized clinical trials in the future to assess this further. However, we are currently undertaking technical improvements in HIFU device especially in the therapeutic transducer, which will solve the problems we had in this study.

5. Conclusions

Our long-term follow-up indicates that a combined approach of HIFU ablation and TACE is a safe method with a low rate of severe complications in the treatment of patients with unresectable HCC. By means of univariate analysis and multivariate analysis, ablation response and tumor size are major prognostic factors for predicting the survival in patients with unresectable HCC treated by HIFU ablation combined with TACE. Although the overall survival rates of 2- and 3-year indicates the long-term survival benefit is not obvious, we believe that the benefit of the long-term survival may be

probably observed in the selected patients according to the prognostic factors such as patients with smaller tumor.

Conflict of interest

The authors declare that there is no conflict of interest.

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