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FULL PAPER

Ablative safety margin depicted by fusion imaging with post-treatment contrast-enhanced ultrasound and pre-treatment CECT/CEMRI after radiofrequency ablation for liver cancers

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Objective: To evaluate the value of fusion imaging with post-treatment contrast-enhanced ultrasound (CEUS) and pre-treatment contrast-enhanced CT/MRI (CECT/CEMRI) in evaluating ablative safety margin after percutaneous ultrasound (US)-guided radiofrequency ablation (RFA) for liver cancers.

Methods: 34 consecutive patients with 47 liver lesions who had undergone RFA were included. Fusion imaging with post-treatment CEUS and pre-treatment CECT/CEMRI was carried out to evaluate local treatment response and ablative safety margin within 1–3 days after RFA. The minimal ablative safety margins of the ablation zones were recorded. The complete response (CR) rate was calculated with reference to CECT/CEMRI results 1 month after RFA. The local tumour progression (LTP) was also recorded.

Results: Of the 47 ablation zones, 47 (100%) were clearly depicted with CEUS-CECT/CEMRI fusion imaging, 36 (76.6%) with US-CECT/CEMRI fusion imaging and 21 (44.7%) with conventional US (both $p < 0.001$). The minimal ablative safety margins were greater than or equal to 5 mm in 28 ablation zones, between 0 and 5 mm in 15, and less than 0 mm in 4. For the four lesions without

enough ablative safety margin, three were referred to follow-up because CEUS showed larger ablation zones than pre-treatment lesions and the remaining lesion was subject to additional RFA 5 days after the first RFA. The CR rate was 95.7% (45/47) with reference to CECT/CEMRI results 1 month after RFA. During 2 to 34 months follow-up, LTP was found in two (4.4%) of 45 lesions with CR. Insufficient ablative safety margin was more commonly found in those lesions with LTP than those without LTP (1/4 vs 1/43, $p < 0.001$).

Conclusion: Fusion imaging with post-treatment CEUS and pre-treatment CECT/CEMRI can depict the ablative safety margin accurately after RFA. Inadequate ablative safety margin is associated with LTP. Depiction of ablative safety margin by fusion imaging after ablation might be considered as a routine procedure to assess the treatment response of RFA.

Advances in knowledge: Fusion imaging with post-treatment CEUS and pre-treatment CECT/CEMRI is an effective method to evaluate the ablative safety margin early after RFA. Therefore, it should be recommended to be used as a routine procedure after RFA for liver cancers.

INTRODUCTION

Ultrasound (US)-guided percutaneous radiofrequency ablation (RFA) has been widely accepted as an effective and minimally invasive treatment method for liver tumour.^{1–5} However, the 5-year local tumour progression (LTP) rate is as high as 12.2–26% no matter what kind of RFA equipment is used.^{6–9} How to exactly evaluate the local treatment response after RFA and reduce the LTP rate are

the important issues for people concerned. An insufficient ablative safety margin is a major risk factor for tumour LTP.^{10,11} The ablative safety margin (Figure 1) is defined as the distance from the border of pre-treatment lesion to the border of the post-treatment ablation zone.⁴ A 5–10 mm ablative safety margin is needed in order to obtain successful ablation.⁴ Currently, the ablative safety margin is usually evaluated by post-treatment contrast-enhanced CT/MRI

Figure 1. Graph shows the minimal ablative safety margin (light blue) after RFA (ablative safety margin = 5 mm). (a) Margin <0 mm, indicating the pre-treatment lesion (light green) is not completely ablated because the ablation zone (red) doesn't cover the lesion (light green) and ablative safety margin (light blue) in some directions. (b) Margin 0–5 mm, indicating margin is not enough in that the lesion (light green) although are entirely covered by the ablation zone (red) the margin is only 0–5 mm in some directions. (c) Margin >5 mm, indicating the lesion (light green) is completely ablated because the ablation zone (red) covers the ablative safety margin (light blue) and the lesion (light green) and the margin is above 5 mm in all directions.

(CECT/CEMRI) since the post-treatment images of the ablated zone and pre-treatment lesion can be easily overlapped for evaluation of the ablative safety margin with CECT/CEMRI. However, in clinical practice, 1 month has to be waited to evaluate the local treatment response using CECT/CEMRI to avoid the influence of peritumoral hyperemia after RFA. In cases of incomplete ablation, the patients have to be re-admitted to hospitalization, which increases the patients' cost and anxiety. In addition, repeat CECT/CEMRI is always limited due to the associated increasing cost, radiation exposure, allergy to contrast agent, insufficient kidney function, or inadequate medical resources.

Contrast-enhanced ultrasound (CEUS) is a favourable imaging modality for treatment response evaluation, which can avoid the above-mentioned shortcomings of CECT/CEMRI. CEUS can be carried out immediately or 24 h after RFA and many studies have confirmed its efficacy in evaluating treatment response after RFA is equivalent to CECT/CEMRI.^{12–15} Nevertheless, CEUS alone is seldom used for evaluation of ablative safety margin since the post-treatment CEUS images are hard to be overlapped with the pre-treatment CEUS images plane by plane in that the scanning planes for CEUS are usually not fixed. Therefore, even sometimes the post-treatment ablation zones are larger than pre-treatment lesions on CEUS, an adequate ablative safety margin is still hard to be guaranteed since it is difficult to judge whether or not the ablated zone covers the lesion at all directions.

Fusion imaging technique is a new technique that can display US and CT/MRI images simultaneously on the same US screen. After the DICOM volume CT/MRI data are set into the US scanner, the transmitter and the magnetic sensors are connected to the position-sensing unit on US transducer, the

lesions displayed on US machine will be targeted following the CECT/CEMRI cross-sectional multiplanar images on the basis of the magnetic navigation system.^{16–18} Conversely, if the lesions can be displayed on CECT/CEMRI, the lesions that are invisible on conventional US or CEUS can be detected through image fusion. Fusion imaging has been widely used for targeting tumours that are inconspicuous on conventional US before RFA and guiding RFA.^{19–21} Min et al²² reported that fusion imaging (92%, 46/50) detected more HCCs with LTP than conventional US (70%, 35/50) after RFA at follow-up period. Furthermore, the ablative safety margin can be measured on post-treatment CEUS comparing with the size of the pre-treatment lesion on CECT/CEMRI through image fusion. Comparing with CEUS or CECT/CEMRI alone, the ablative safety margin measured on fusion imaging with post-treatment CEUS and pre-treatment CECT/CEMRI seems to be more accurate. To our knowledge, few studies have reported the value of post-treatment CEUS and pre-treatment CECT/CEMRI fusion imaging after RFA to assess the ablative safety margin.^{23–25} Accordingly, the purpose of our study was to assess its value in assessing the ablative safety margin and to evaluate the association between ablative safety margin and local treatment response after RFA.

METHODS AND MATERIALS

Patients

This study was performed as a retrospective analysis of a prospective database obtained during planning US and percutaneous RFA. This retrospective study was approved by the institutional review board and informed consent from the patients was waived whereas all the patients provided written informed consent before RFA and CEUS. Inclusion criteria for the study were as follows: (1) all the patients underwent CECT/CEMRI before RFA and all the lesions were visualized clearly on CECT/CEMRI; (2) patients who underwent CEUS-CECT/CEMRI fusion imaging within 1–3 days after RFA. Those patients who were lost to follow-up were excluded. Therefore, between January 2014 and November 2016, 34 consecutive patients (22 males, 12 females; mean age, 58.1 years $\pm$ 8.9; range, 33–74 years) with 47 lesions were finally included in the study excluding one patient who was lost to follow-up. Table 1 shows the patients' clinical characteristics. Finally, there were 23 patients with 29 HCCs (10 primary HCC lesions; 19 recurrent HCC lesions) and 11 patients with 18 MLCs (seven lesions from colon cancer, four lesions from breast cancer, five lesions from esophageal cancer, one lesion from pancreas cancer, one lesion from gallbladder cancer). In the 34 patients, 25 patients had 1 lesion, 7 had 2 lesions and 2 patients had 4 lesions. Of the 47 lesions, 32 were less than or equal to 2 cm and 15 ranged from 2 to 5 cm. The mean maximal diameter of these lesions measured on CEUS-CECT/CEMRI fusion imaging was 2.0 cm $\pm$ 1.0 cm (range, 1.0–5.0 cm). When the distance between the lesion and intrahepatic major vessel (e.g. intrahepatic blood vessel with diameter greater than 0.3 cm, such as intrahepatic portal vein or hepatic vein) was equal to or less than 1.0 cm, the lesion was regarded as proximity to major vessel. In addition, when the distance between the lesion and liver surface was equal to or less than 1.0 cm, the lesion was regarded as proximity to liver surface.

Table 1. Baseline clinical characteristics of the patients

Characteristics	Values
Age (years) ^a	58.1 ± 8.9 (33–74)
Sex	
Male/female	22/12
Liver function	
Child–Pugh classification A/B/C	34/0/0
Lesion nature	
HCC/MLC	29/18
Previous treatment	
TACE	4
Tumour number	
Single/two/three/four	25/7/0/2
Tumour segment	
S1/S2/S3/S4/S5/S6/S7/S8	0/1/4/9/7/8/9/9

^aMean ± standard deviation. Numbers in parentheses are ranges.

HCC, hepatocellular carcinoma; MLC, metastatic liver cancer; TACE, transcatheterarterial chemoembolization.

RFA procedures

RFA was performed with percutaneous US guidance and general anesthesia for all patients. Real-time US with a 1–5 MHz curved-array transducer (GE LogiQ E9, Milwaukee, WI) were chosen as the guidance modality in all patients. RFA devices included a bipolar 470 kHz radiofrequency generator (CelonLab Power; Olympus-Celon, Teltow, Germany) and a commercially available internally cooled monopolar radiofrequency generator (StarMed, Goyang, South Korea). The maximal output power of bipolar RFA system was 250W. Electrodes were cooled by a triple peristaltic pump (CelonAquaflow, Olympus-Celon, Teltow, Germany). The electrode of T30 (the conducting part of the applicator is 30 mm, including both the insulator and the tip) or T40 (the conducting part of the applicator is 40 mm, including both the insulator and the tip) was used according to the sizes of the lesions. The electrodes selected and the geometric configurations of their insertions were depended on the tumour size and location as previously described.^{26–28} The output energy and power were set according to the electrode types used. The ablation procedure would end when the impedance increases to lead to shutting down between each electrode combination according to a given arrangement of electrodes. The power (200W) and ablation time (12 min) of the monopolar RFA system were set following the manufacturer's recommended protocol. The 15 or 20 cm long, 15G electrodes with a 3 cm or 4 cm active tip were used for appropriate patients. For lesions 2 cm or smaller, a single ablation (3 cm or 4 cm active tip electrode was used) was performed. For lesions larger than 2 cm, multiple overlapping ablations were performed with protocols according to previous studies.^{29,30} All electrodes were inserted into the bottom of the lesions firstly and were retreated to the shallow portion of the lesions when needed. After ablation, electrodes were withdrawn and the puncture routes were cauterized to prevent tumour seeding and minimize the risk of bleeding.

CEUS, CTCT/CEMRI

CEUS was performed with 1.5 ml of US contrast agent (SonoVue, Bracco, Milan, Italy) injected into the antecubital vein through a 20 G cannula, followed by of 5 ml normal saline flush. We adjusted a low mechanic index of less than 0.2 to avoid disrupting the microbubbles in the circulation. The index ablation zone was continuously observed up to 6 min after the injection. Three enhancement phases (arterial phase: 10–30 s after contrast administration, portal venous phase, 31–120 s, late phase: 121–360 s) were recorded and reviewed later. All CT images were obtained using the multi-detector row CT scanner (Light Speed VCT, GE Medical Systems, Milwaukee, WI). The liver was scanned 35, 65 and 125 s (hepatic arterial, portalvenous and late phases) after injection of intravenous non-ionic contrast. The non-ionic contrast material containing 300 mgI ml⁻¹ was injected into the antecubital vein with an automatic power injector at a rate of 2–3 ml s⁻¹. MR imaging were performed with a 3.0-Tesla unit (Verio3.0T, Siemens Medical Systems, Berlin, Germany) for the sequential acquisition of 5–8 mm sections. The unenhanced *T*₁ weighted and *T*₂ weighted spin-echo, three-dimensional gradient echo *T*₁ weighted pulse sequence that was enhanced with gadopentetate dimeglumine in the hepatic arterial, portalvenous, and delayed phases was performed.

Conventional US and fusion imaging after RFA

All conventional US, CEUS and fusion imaging procedures were performed with the same US machine that guided RFA. Fusion imaging is carried out on the basis of the hardware and software in the US machine. The hardware includes a magnetic field transmitter and two receivers (*i.e.* position sensors; GE Healthcare). The distance of the magnetic field transmitter and the position sensors should be less than 40 cm so that the sensors can receive the signals generated from the magnetic field generator. After the magnetic field transmitter and position sensors were connected with the US machine and the US transducer, the image fusion could be performed.

Prior to image fusion, pre-treatment CECT/CEMRI DICOM format data were transferred into the US system. After the hepatic arterial, portal venous phase and delayed phase CECT/CEMRI images were uploaded, the operator started the plane registration. The sagittal part of the left branch of portal vein image on CECT/CEMRI was often used as the first reference plane. The arterial or portal phase CECT/CEMRI was often applied for coordination with US images. When the US image and the CECT/CEMRI image matched well for the visualization of the sagittal part of the left branch of portal vein, the images were locked and the US image and the CECT/CEMRI image would display in same size and move simultaneously on the US screen depending on the direction of US scanning. The portal phase, right portal vein or the hepatic vein was used to adjust the plane registration. Thereafter, point registration was performed. The obvious anatomic landmarks (*i.e.* hepatic cysts, calcifications or the prior ablation zones) on US and CECT/CEMRI images were usually selected to coordinate the point registration. Then the US transducer was moved slowly to find the ablation zone on conventional US along with CECT/CEMRI images. The anatomic landmarks around the ablation zone was also further registered simultaneously on

Figure 2. Graphs show the minimal ablative safety margin measurement methods. (a) Margin >0 mm (arrows) measured using dual caliper mode on CEUS-CECT fusion imaging. (b) Margin >0 mm (arrows) measured using GPS markers function on CEUS-CECT fusion imaging.

conventional US and CECT/CEMRI. On the basis of these registrations, the location of the ablation zone was finally confirmed on the US image. The “overlay” function was used to adjust and confirm the registrations. The patient was asked to hold breath when the operator performed the registrations.

Afterwards, CEUS was applied to observe the ablation zone and record its size. The ablation zone on CEUS was compared with pre-treatment lesions on CECT/CEMRI using dual caliper or dual trace function on US machine, which guaranteed that calipers or traces were corresponding to each other on both images. The minimal ablative safety margin was measured through the dual imaging mode (dual caliper or dual trace) or the point-navigation function (*i.e.* GPS markers) (Figure 2). All the procedures were performed by one operator who has more than 5 years' experience in RFA and fusion imaging. The conspicuousness, size of the ablation zone and the minimal ablative safety margin were all evaluated and recorded by two experienced observers with consensus.

Assessment of the ablation zones after RFA and follow-up protocol

The size of the ablation zone calculated by CEUS-CECT/CEMRI fusion imaging within 1–3 days after RFA was compared with the size calculated with pre-treatment CECT/CEMRI before RFA. The minimal ablative safety margin was measured and recorded. If the minimal ablative safety margin of ablation zone was greater than 0 mm, the lesion was considered as successful ablation.²² 1-month post-treatment CECT/CEMRI was used to assess the local therapeutic response. Complete response (CR) was defined as no enhanced area in the ablation zone. In the follow-up period, CECT/CEMRI or CEUS and laboratory tests, including liver function and serum tumour marker tests every 3 months were performed. LTP was defined as arterial hyper-enhancement in

the ablation zones at a follow-up CECT/CEMRI. Distant recurrence (DR) was defined as any new intrahepatic and extrahepatic recurrent lesions outside the ablation zone.

Statistical analysis

All continuous data were expressed as mean $\pm$ standard deviation if normal distribution was achieved. The conspicuity of ablation zones on conventional US, US-CECT/CEMRI fusion imaging and CEUS-CECT/CEMRI fusion imaging were compared with McNemar test. The sizes of the lesions and the ablation zones calculated with CEUS-CECT/CEMRI fusion imaging were compared with student's *t* test. The sensitivity, specificity and accuracy of CEUS-CECT/CEMRI fusion imaging were calculated in reference to the results of the CECT/CEMRI 1 month after RFA. The comparisons of proportion of the lesions with sufficient and insufficient ablative safety margins were performed using Fisher exact test in terms of lesions in the right lobe of liver, lesions proximity to major vessel or liver surface, lesions with LTP. $p < 0.05$ was considered to indicate a significant difference. Statistical analysis was performed by using SPSS 20.0 statistical software (SPSS, Chicago, IL).

RESULTS

Of the 47 ablation zones, 47 (100%) were clearly depicted with CEUS-CECT/CEMRI fusion imaging, 36 (76.6%) with US-CECT/CEMRI fusion imaging, and 21 (44.7%) with conventional US (both $p < 0.001$ in comparison with conventional US). The minimal ablative safety margins were great than or equal to 5 mm in 28 ablation zones, between 0 and 5 mm in 15 (Figure 3), and less than 0 mm in four. Therefore, four (8.5%) lesions had no sufficient safety margins in the ablation zone. In addition, there were no significant differences between the lesions with sufficient and insufficient ablative safety margins in terms of the proportion of the lesions in the right lobe of liver, lesions proximity to major vessel or liver surface (all $p > 0.05$) (Table 2).

CEUS-CECT/CEMRI fusion imaging within 1–3 days after RFA suggested that 43 (91.5%) lesions were successfully ablated. For the three lesions without enough ablative safety margin, three were referred to follow-up because CEUS showed larger ablation zones than pre-treatment lesions and the remaining lesion was subject to additional RFA 5 days after the first RFA. For the 43 lesions with successful ablation, the maximum and minimum diameters of the ablation zones on CEUS-CECT/CEMRI fusion imaging were significantly greater than those of the pre-treatment lesions, with the maximum diameter, 3.8 cm $\pm$ 1.0 (range, 2.1–6.8 cm) *vs* 2.0 cm $\pm$ 1.0 (range, 1.0–5.0 cm); and minimum diameter, 3.0 cm $\pm$ 0.9 (range, 1.8–6.3 cm) *vs* 1.6 cm $\pm$ 0.7 (range, 0.8–3.8 cm) (both $p < 0.001$).

The CR rate was 95.7% (45/47) in reference to the CECT/CEMRI 1 month after RFA and two lesions in two patients showed residual viable tumour on CECT/CEMRI. One patient with one residual lesion was subjected to radiotherapy and the other patient chose chemotherapy for subsequent treatment. Comparing with the results of CECT/CEMRI 1 month after RFA, CEUS-CECT/CEMRI fusion imaging within 1–3 days after RFA achieved sensitivity, specificity, positive predictive value,

Figure 3. A 54-year-old male with two MLCs adjacently from rectal (maximum diameter = 5.0 cm) in segment IV of the liver and the patient underwent chemotherapy 2 months before. (a) The lesion (arrows) is conspicuous with the help of US-CE-MRI fusion imaging. (b) The lesion (arrows) shows peripheral ring enhancement during the arterial phase of CEUS. (c) CEUS-CEMRI fusion imaging obtained two days after RFA shows the minimal ablative safety margin of the ablation zone is great than 0 mm (arrows) in the arterial phase of CEUS, indicating successful ablation. (d) The ablation zone (arrows) shows non-enhancement in the arterial phase of CEMRI 2 months after ablation, indicating complete response.

negative predictive value and overall accuracy of 66.7% (2/3), 93.3% (42/45), 50.0% (2/4), 97.7% (42/43) and 93.6% (44/47) respectively for detection of unsuccessfully treated lesions.

No procedure-related death happened after RFA. One patient experienced hepatic infarction after RFA and recovered after medication and hospitalization for ten days after RFA. One patient experienced large amount of pleural effusion 2 weeks after RFA and the pleural effusion disappeared after drainage and

Table 2. The comparisons of proportion of the lesions with sufficient and insufficient ablative safety margins

Characteristics	Sufficient ablative safety margins (n = 43)	Insufficient ablative safety margins (n = 4)	p-value
Lesions in the right lobe of liver	31 (72.1)	2 (50.0)	0.572
Lesions proximity to major vessels	6 (13.9)	1 (25.0)	0.488
Lesions proximity to liver surface	11 (25.6)	3 (75.0)	0.073
Lesions with LTP	1 (2.3)	1 (25.0)	<0.001

LTP, local tumour progression.

Note. Numbers in parentheses are in percentages.

symptomatic treatment. Upper abdomen pain (67.6%, 23/34), slight pleural effusion (32.4%, 11/34) and fever more than 38°C (20.6%, 7/34) were found several days after RFA and disappeared without special treatment.

During 2 to 34 months follow-up, LTP (Figure 4) was found in two (4.4%) of 45 lesions with CR. DR (all intrahepatic recurrent lesions) occurred in eight (23.5%) of 34 patients. The two lesions in two patients with LTP were subject to second session of RFA and chemotherapy respectively. Inadequate ablative safety margin was more commonly found in those lesions with LTP (1/4, 25.0%) than those without LTP (1/43, 2.3%) ($p < 0.001$) (Table 2). Seven patients with intrahepatic DR lesions were treated with RFA or TACE thereafter. One patient died of hepatic failure during the follow-up period. One patient died of tumour progression with extrahepatic recurrent lesions outside the liver.

DISCUSSION

Accurate localization of the ablation zone is the first step for treatment response evaluation after RFA. It is noteworthy that in the present study, the ablation zones were more easily visualized with CEUS-CECT/CEMRI fusion imaging than US-CECT/CEMRI fusion imaging or conventional US. Because of the low spatial resolution of conventional US, echoic change after RFA, interference of lung or gastric gas, liver background of hepatocirrhosis or hepatic adipose infiltration, some ablation zones show inferior conspicuity on conventional US. CEUS, on the other hand, although improves visualization of the ablation zone due to increased signal-to-noise, is limited to evaluate the whole ablation zone since the arterial phase only lasts several seconds after contrast administration. In comparison with CEUS alone, CEUS-CECT/CEMRI fusion imaging is more accurate in terms of targeting ablation zone. Accordingly, image fusion of CEUS and CECT/CEMRI can be considered as a routine procedure to observe the ablation zone after RFA.

Figure 4. A 61-year-old male with a HCC (maximum diameter = 1.5 cm) in segment VII of the liver. (a) The hypoechoic lesion (arrows) is conspicuous on conventional ultrasound. (b) CEUS-CEMRI fusion imaging obtained three days after RFA shows the minimal ablative safety margin of the ablation zone is great than 0 mm (arrows) in the arterial phase of CEUS, indicating successful ablation. (c) LTP (arrows) is detected in the arterial phase of CEUS seven months after RFA. (d) The ablation zone (arrows) shows non-enhancement in the arterial phase of CEMRI 1 month after second session of RFA and an internal haemorrhage (arrowhead), indicating complete response.

In addition, an adequate ablative safety margin of ablation zone is of paramount important, which guarantees that not only the tumour itself but also the possible small satellite foci around the tumour are completely eradicated. Therefore, theoretically, adequate ablative safety margin after RFA may reduce the possibility of LTP in the follow-up period. Our study demonstrated that the lesions that had no adequate safety margin were more likely to emerge LTP in the follow-up period in that inadequate ablative safety margin was found in

25.0% of those with LTP whereas 2.3% of those without LTP. Some previous studies^{23,24,31} also found that the scope of the ablation zone or safety margin is an important risk factor for LTP after RFA. Therefore, accurate assessment of the ablative safety margin is relevant in clinical practice, which might determine the following treatment or follow-up strategy. For instance, for those with adequate safety margin, regular follow-up is enough, while for those with inadequate safety margin, re-treatment or intense follow-up might be necessary.

Traditionally, local treatment response evaluation by CEUS mainly relies on measurement of the non-enhanced areas, however, it is not enough for accurate safety margin evaluation. In some cases, even though the size of ablation zone is greater than pre-treatment lesion, the minimal ablative safety margin might be insufficient in some directions. Therefore, only relying on size of ablation zone might lead to false negative results. Fusion imaging of post-treatment CEUS and pre-treatment CECT/CEMRI is an ideal solution for this problem. Through image fusion, post-treatment CEUS images and pre-treatment CECT/CEMRI images can be overlapped plane by plane, thus the ablative safety margin is able to be visualized and measured at all directions since both CEUS and CECT/CEMRI images are retrieved from volume dataset. The post-treatment CEUS images and pre-treatment CECT/CEMRI images can also be displayed side by side on the same screen and both can move simultaneously. In our study, CEUS-CECT/CEMRI fusion imaging within 1–3 days after RFA was used to evaluate the ablative safety margin. CEUS-CECT/CEMRI fusion imaging found four lesions with inadequate safety margin. Among them, one was subject to additional RFA five days after the first RFA during the same hospitalization, thus CEUS-CECT/CEMRI fusion imaging changed the patient management in some cases.

Because of the hyperemia reaction around the ablation zone, CEUS-CECT/CEMRI fusion imaging within 1–3 days after RFA can only observe the scope of the ablation zone instead of the viable tumour. A previous study²² reported that successful ablation can be considered if the ablation zone on CEUS immediately after RFA covers the pre-treatment lesion. Meloni et al¹⁴ suggested that immediate CEUS and 24 h CEUS following thermal ablation could be used to detect and re-treat the residual viable tissue in the same ablation session. In the current study, CEUS-CECT/CEMRI fusion imaging within 1–3 days after RFA was used to observe the ablation zone. With reference to 1 month post-treatment CECT/CEMRI, the sensitivity, specificity and accuracy of CEUS-CECT/CEMRI fusion imaging within 1–3 days after RFA were 66.7%, 93.3%, respectively, for detection viable tumour tissue after RFA, which were similar with previous studies^{14,32,33} (sensitivity, 20–100%; specificity, 20–97%) with CEUS immediately or 24 h after RFA. Quick detection of viable tumour tissue after first RFA is helpful for management of the lesions during the same hospitalization, which would reduce medical cost, facilitate subsequent treatment, and reduce the overall recurrence rate. Mauri et al³⁴ also stated that intraprocedural use of CEUS could reduce the number of re-treatments and the related costs per patient. Moreover, fusion of CEUS and CECT/CEMRI allows three-dimensional depiction of ablation zone, which is especially

helpful for non-experienced operator to build up his confidence in evaluating ablation zone and improve his RFA skills.

However, one false negative and two false positive patients appeared in this study. The lesion size of the false negative patient was bigger than 3 cm and it was located to segment VIII. This may cause the lesion difficultly to be completely ablated. The two false positive patients were completely ablated in reference to 1 month CEMRI. This may due to the registration error of fusion imaging. The possible reasons for the registration error of fusion imaging are as follows. First, the operator is unskillful. Second, the patient had a liver resection before RFA and even some anatomic landmarks disappear so that the registration becomes more difficult. Third, the lesion is located to the right lobe of liver so that the operator needs speed more time to adjust the point and plane registration. Finally, some patients experience pleural effusion after RFA so that the position of liver changed and then the registration error of fusion imaging may emerge. In addition, the partially damaged cell in inflammatory reaction zone might transition to non-viability 1 month after RFA, like a previous experimental study³⁵ in rat liver mode. In other words, the ablation zone might reach a maximum 1 month after RFA. However, the sizes of the two ablation zones on CEUS were bigger than pre-treatment lesions. The second session of RFA was not arranged thereafter. As to the two HCC lesions with LTP, one lesion was located to segment VII and the other had close relationship with live capsule. Furthermore, the size of one lesion was more than 3 cm. The location and size make it hard to achieve sufficient ablative safety margin, which might lead to LTP in the follow-up. Although the ablative safety margin of another lesion was between 0 and 5 mm, it was also not enough in terms of safety margin. However, the lesions in the right lobe of liver and proximity to major vessel or liver surface don't affect the ablative safety margin because there were no significant differences between the lesions with sufficient and insufficient ablative safety margins

in terms of the proportion of the lesions in the right lobe of liver, lesions proximity to major vessel or liver surface.

The study had some limitations. First, the patient number was small and the study only reflected the experience of one center, thus further prospective and randomized controlled multi-center studies are required in the future to investigate the usefulness of CEUS-CECT/CEMRI fusion imaging within 1–3 days after RFA application. In addition, the follow-up period was not long enough and the longest follow-up period was 35 months. Second, the fusion imaging was operated by one operator, thus the interobserver repeatability and registration error of this technique could not be analyzed. There must be a learning curve for mastering this technique, however, the conspicuity, sizes of the ablation zones and the minimal ablative safety margins were all evaluated by two observers with consensus.

CONCLUSION

In conclusion, fusion imaging with post-treatment CEUS and pre-treatment CECT/CEMRI can depict the ablative safety margin accurately after RFA. Inadequate ablative safety margin is associated with LTP in follow-up. Depiction of ablative safety margin by fusion imaging after ablation might be considered as a routine procedure to assess the treatment response of RFA.

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