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Original Article

Clinical value of combined contrast-enhanced ultrasound and elasticity imaging in evaluation of efficacy of radiofrequency ablation for hepatocellular carcinoma

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Abstract: The goal of this study was to investigate the clinical value of combined contrast enhanced ultrasound (CEUS) and elasticity imaging (EI) in the evaluation of efficacy of radiofrequency ablation (RFA) for hepatocellular carcinoma (HCC). Thirty patients (36 lesions) with a final diagnosis of HCC were enrolled. CEUS, real-time elastography (RTE), and virtual touch tissues quantization (VTQ) were conducted in all patients before RFA and at 1-2 days, 1 month, and 3 months after RFA. Results were compared with findings obtained from enhanced CT or enhanced magnetic resonance imaging (MRI). Before RFA, all 36 lesions showed obvious enhancement in the arterial phase and indicated regression or enhancement in the portal and delayed phases. Compared with enhanced CT or MRI, the coincidence rate of CEUS was 100% before RFA treatment and at 1-2 days and 1 month after RFA treatment. The coincidence rate of CEUS was 97.2% at 3 months after RFA treatment. VTQ value obtained before RFA was significantly different from those obtained at 1-2 days, 1 month, and 3 months after RFA. CEUS could evaluate the therapeutic efficacy of RFA. RTE was capable of reliably displaying the form and the circumscription of the RFA-treated lesions and thus might be a useful noninvasive method for evaluation and quantification of such lesions. VTQ values were increased significantly after RFA and it could also contribute to the evaluation of treated lesions. The combination of CEUS and EI presented to be effective for accurate evaluation of RFA efficacy in treating HCC.

Keywords: Contrast enhanced ultrasound (CEUS), real-time elastography (RTE), virtual touch tissues quantification (VTQ), hepatocellular carcinoma (HCC), radiofrequency ablation (RFA)

Introduction

Hepatocellular carcinoma (HCC), as the fifth most common tumor, has caused nearly 10,000 newly diagnosed cases and one million deaths each year worldwide. Among different treatment modalities, radiofrequency ablation (RFA) has emerged as a safe, minimally invasive, and effective option which is an optional method to replace surgery and even achieve radical cure [1]. Evaluation of the therapeutic effect of RFA is extremely important, especially for confirmation of residual cancer. Contrast-enhanced ultrasound (CEUS) can help to identify residual tumor or tumor relapse by accurately revealing the tissue perfusion [2], and has been regarded as an indirect measurement for inactivation degree of the tumor. Real-time elastography (RTE), using conventional ultrasound probes, can clearly demonstrate the extent of a liver lesion. Additionally, virtual touch tissues quantification (VTQ) makes quantitative evaluation of the efficacy of RFA [3]. To the best of our knowledge, there is little literature about the combination of CEUS and elasticity imaging (EI) for evaluating the efficacy of RFA.

Therefore, this study was designed to evaluate the clinical value of combined CEUS and EI in evaluating the efficacy of RFA for HCC.

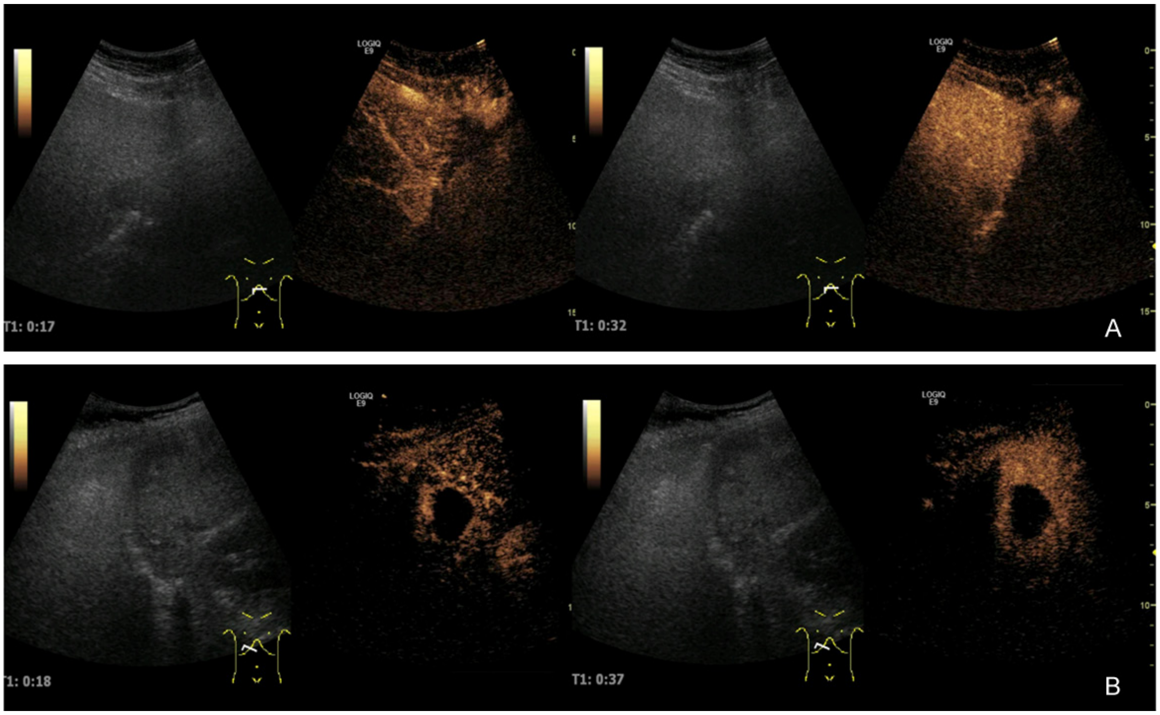

Figure 1. CEUS before RFA and after RFA.

Table 1. Comparison of lesion's features between examinations of CEUS and enhanced CT/MRI

	CT/MRI			CEUS		
	Before RFA	1 month after RFA	3 months after RFA	Before RFA	1 month after RFA	3 months after RFA
Strengthening, enhancement	36	0	0	36	0	0
No strengthening, enhancement	0	36	34	0	36	35
Local strengthening, enhancement	0	0	2	0	0	1

The differences between examinations of CEUS and CT/MRI were not statistically significant.

Materials and methods

This study was approved by the Ethics Committee of The First Affiliated Hospital and Zhejiang Chinese Medical University. Informed consents were obtained from all enrolled patients in this study.

Patients

A total of 30 patients (36 lesions) diagnosed with HCC by liver biopsy were enrolled in this study. All the patients had undergone RFA with cool-tip electrode guided by ultrasound from September 2015 to January 2016. Patients were selected for RFA treatment according to the criteria specified by the expert consensus on RFA for liver cancer, 2011 [4]. The final study group was comprised of 24 males and 6 females of ages ranging from 46 to 74 years old (mean age, 58.27 ± 8.13 years). The mean lesion diameter was 2.25 ± 1.01 cm (range 1.32 cm-5.22 cm).

Procedure

Ultrasound examination was performed four times in each patient: before RFA, at 1-2 days, 1 month, and 3 months after RFA. The examinations were performed by using the LOGIQ E9 color Doppler ultrasonographic machine (GE Healthcare) with a convex array probe (1-5 MHz), or the ACUSON S2000 color Doppler ultrasonographic machine (Siemens Medical solutions) with a 1-4 MHz probe (4C1).

During the examination, patients were in the supine or left lateral position, with the right hand raised over the head to widen the intercostal space if necessary. The probe was

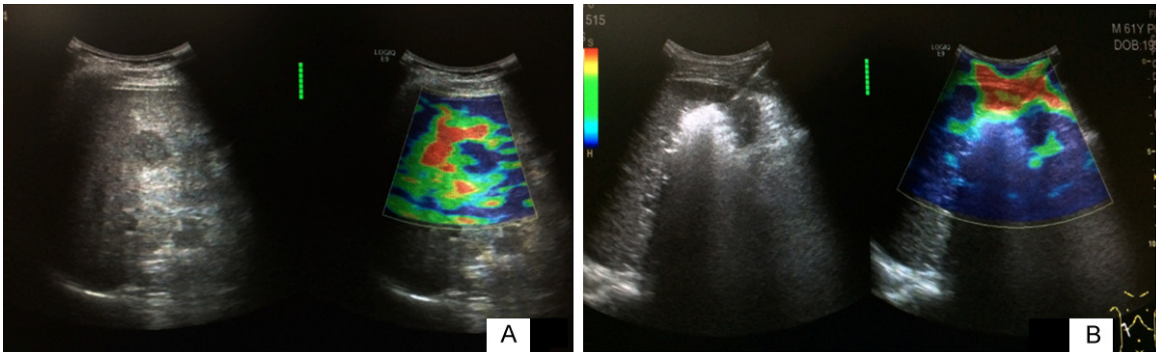

Figure 2. RTE before RFA and after RFA.

super-selectively placed in the chest wall or below the xiphoid. Routine ultrasound examination was performed first. The lesion was fully displayed in 2D mode and lesion size (the maximum diameter) was measured on the maximal section. The internal echo and borders were also observed. Second, the internal blood flow in the target lesion was observed by the color Doppler mode and power Doppler mode. The RTE mode was subsequently used and the operator alternately applied and released slight pressure on the probe 2-3 times until a stable elasticity image was obtained. The elasticity score was recorded and the lesion borders were observed on the obtained elasticity images. The size of the target lesion (the maximum diameter) was measured three times, and then the average value was calculated [5]. The patient was asked to hold his or her breath and the update button was clicked to calculate immediate VTQ (in m/s) when the sampling frame was inserting into the lesion being cautious to ensure that the sampling depth was no more than 5.5 cm from the liver surface. The VTQ value was calculated three times and the mean value was then recorded. In order to reduce errors, all attempts were made to select a section without any large intrahepatic vessels. Finally, contrast imaging was performed after the adjustment of mechanical index (<0.15) and the optimization of system settings. The second-generation acoustic contrast agent SonoVue (Bracco, Milan, Italy) was injected through the median cubital vein. The timer was activated with the start of contrast agent injection. Continuous imaging and recording for the target lesion was performed for 3-6 mins, with the arterial phase (8 to 30 seconds), the portal phase (31 to 120 seconds), and the delayed phase (121 to 360 seconds) included. The enhancement level, the enhanced morphology of the target lesion, and changes in enhancement with time were accessed. All procedures, including 2D, RTE and contrast imaging were executed by an ultrasound operator with over five years of experience. All findings were then compared with the results obtained from the enhanced CT/MRI after RFA.

Statistical analysis

Statistical analyses were performed using the SPSS 21.0 software (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± SD (standard deviation). Parametric variables were compared among groups by using single factor analysis of variance after applying the Bonferroni method analysis for multiple comparisons. Non-parametric variables, with the median (four percentile) table showed, compared between groups using the independent Mann-Whitney U test; $P<0.05$, according to the inspection standards, was considered to be statistically significant.

Results

CEUS

In 36 lesions, the preoperative CEUS showed high enhancement in the arterial phase and low or equal enhancement in the portal phase (**Figure 1A**). All these changes were typically malignant. Therefore, CEUS presented 100% sensitivity for the detection of HCC lesions. At 1-2 days and 1 month after RFA, CEUS showed that there was no significant blood perfusion in 36 ablated lesions (**Figure 1B**). At 3 months after RFA, however, local enhancement was found in the edge of the treated lesion in one case, while the other 35 cases did not show any significant blood perfusion. In addition, the comparison of lesion's features between exam-

Table 2. Comparison of cross-sectional area of lesion measured by 2D ultrasound, elastography, and enhanced CT/MRI at different time points

	2D Ultrasound			RTE			CT/MRI		
	Before RFA	1 month after RFA	3 months after RFA	Before RFA	1 month after RFA	3 months after RFA	Before RFA	1 month after RFA	3 months after RFA
Median (25%, 75%) (cm^2)	2.09 (1.29, 4.28)*	4.75 (3.4, 6.79)△	4.01 (2.75, 5.28)☆	2.54 (1.67, 4.60)	10.8 (9.01, 14.73)	8.66 (6.18, 11.78)	2.50 (1.64, 4.57)	10.66 (8.60, 14.39)	8.54 (6.02, 11.67)
Maximum (cm^2)	16.36	14.89	11.57	17.79	31.48	24.39	17.59	31.22	24.14
Minimum (cm^2)	0.91	2.07	1.61	1.08	4.82	3.89	1.05	4.64	3.60
Z	-3.052	-5.631	-5.597	-2.072	-2.557	-2.433			
P	0.002	<0.001	<0.001	0.058	0.065	0.077			

1. The cross-sectional area measured by 2D ultrasound and enhanced CT/MRI before RFA was statistically different from those measured at 1 month and 3 months after RFA (*Z=-3.052, P<0.05; △Z=-5.631, P<0.001; ☆Z=-5.597, P<0.001). 2. The cross-sectional area measured by RTE and enhanced CT/MRI before RFA was not statistically different from those measured at 1 month and 3 months after RFA (P>0.05).

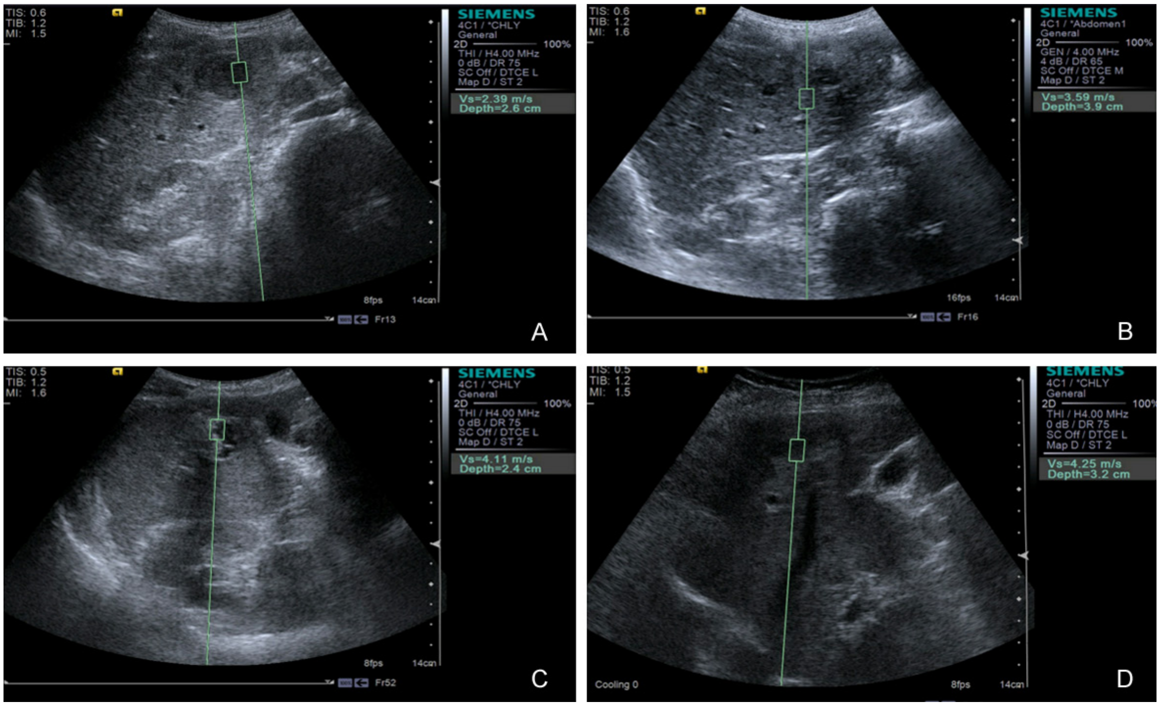

Figure 3. The VTQ values of lesions before RFA and at 1-2 days, 1 month and 3 months after RFA.

inations of CEUS and enhanced CT/MRI were also stated (**Table 1**).

RTE

Under the examination of RTE before RFA, 22/36 lesions (61.1%) had elasticity score of 3 (**Figure 2A**), and the remaining lesions (14/36; 38.9%) had a score of 2-3. Furthermore, the examinations at 1-2 days, 1 month, and 3 months after RFA showed that elasticity scores of all 36 lesions were more than 3 (**Figure 2B**).

Lesion size

Under the measurement through 2D ultrasound and enhanced CT/MRI, the lesion size before RFA was found to be significantly different from those at 1 month and 3 months after RFA. However, there was no statistically difference after measurements using RTE and enhanced CT/MRI (**Table 2**).

VTQ

The VTQ values of lesions before RFA and at 1-2 days, 1 month, and 3 months after RFA were presented (**Figure 3**; **Table 3**). Differences between values at each time points were statistically significant.

Discussion

The application of RFA for the treatment of liver cancer was first reported in 1995 by Rossi, and it has become an important nonsurgical treatment for liver cancer with the subsequent development of technology. It is most commonly applied in primary or metastatic liver cancer under the circumstances that the tumor cannot be removed by surgery or is not suitable for surgical resection [6]. Objective evaluation on the therapeutic effect of RFA is necessary to determine the location and extent of necrosis after treatment and, most importantly, to assess whether there remain any residual cancer tissue. At present, enhanced CT or MRI have been the modalities which are most commonly used to assess the success rate of tumor ablation [7, 8]. However, ultrasound is considered to have a great number of advantages such as convenience, low cost, and real-time kinematics, and has been commonly applied in the clinic stage.

CEUS is believed to have the ability of effectively evaluating the perfusion of the lesion before RFA and revealing residual cancer or relapse after ablation. RTE is also an effective noninvasive method to evaluate the success rate of RFA, since it can clearly demonstrate

Table 3. VTQ at different time points

VTQ	Before RFA	1-2 days after RFA	1 month after RFA	3 months after RFA
Mean (m/s)	1.57 ± 0.61	2.43 ± 0.58*	3.21 ± 0.44*,▲	3.65 ± 0.38*,▲,#
Maximum (m/s)	2.83	3.30	4.17	4.33
Minimum (m/s)	0.57	1.39	2.30	2.78

Comparison between groups showed that there was difference in VTQ at different time points. (F=115.560, P<0.001). The results were compared between groups multiply by Bonferroni method. VTQ values were increased steadily by time and significant increases were found between each time points. VTQ obtained before RFA vs. VTQ obtained at 1-2 days after RFA, and at 1 month and 3 months after RFA (**P*<0.001); VTQ obtained at 1-2 days after RFA vs. VTQ obtained at 1 month and 3 months after RFA (▲*P*<0.001); and VTQ obtained at 1 month after RFA vs. VTQ obtained at 3 months after RFA (#*P*<0.010).

the morphology and size of the treated lesion, and VTQ for treated lesions increased obviously after RFA. Both of them can contribute to the evaluation of RFA efficacy. In our study, results suggested that EI was inferior to CEUS for the diagnosis of HCC before RFA and was more suitable for the evaluation of survival of ablated lesions. However, CEUS might be prior for the evaluation of lesion's extent when compared with the enhanced CT or MRI. But different from elastography, it cannot assess the hardness of the ablated lesion. The combination of CEUS and EI, by providing information on different aspects of the treated tumor, can be expected to be more effective than either single method.

To the best of our knowledge, CEUS has unique advantages in the evaluation of RFA for liver cancers. First, the contrast used in CEUS is different from that in CT or MRI. As a consequence, it will not be diffused into interstitial spaces and therefore reflect the blood supply of the tumor more accurately. Second, low-speed blood flow in the tumor can be revealed in CEUS, and the whole process of blood perfusion of capillaries within the tumor in real time can also be demonstrated by it [9].

In HCC, a rapid enhancement can be shown through CEUS in the arterial phase and the enhancement will fade rapidly in the portal phase to become equal to or lower than that of the surrounding liver parenchyma, thus revealing an obvious hypoechoic "defect" area that contrasted to be significantly different from the surrounding liver parenchyma [10]. In this study, in all 36 lesions, CEUS before RFA showed rapid enhancement in the arterial phase and low (or equal) enhancement in the portal and delayed phases. Furthermore, typical malignancy could be confirmed by the above-mentioned findings. Thus, CEUS displayed 100% sensitivity in the detection of HCC. After RFA, no enhancement could be found in any of the three phases (arterial phase, portal vein phase, or delayed phase), and lesions were confirmed to be completely inactivated at 3 months after RFA. Two patients were diagnosed with tumor relapse under enhanced CT/MRI findings (local enhancement was found in the ablated lesion edge), while only one case was found to have relapse with suggestive changes indicated by CEUS (i.e., rapid local enhancement in the arterial phase and ablation area edges decline in the portal phase). The concordance between CEUS and enhanced CT/MRI was 97.2% (35/36). The lesion in one case was located near the diaphragm and right upper lobe of the liver, and CEUS failed to show the enhancement at the edge of the lesion.

The good concordance of CEUS and enhanced CT/MRI indicates a clear beneficial effect of CEUS on early detection of liver cancer. However, there are two shortcomings of CEUS. The first one is the blind spot in the examination of the liver. Lesions in some locations may be missed (as demonstrated in one patient in our cohort). The other weakness is that the contrast enhancement time is too short to make an accurate assessment of the entire blood perfusion of the liver.

Ultrasound elasticity imaging is capable of detecting the hardness of different tissues (distinguishing between normal tissues and lesion tissues). Those areas with high elastic coefficient (hard) had less strains while areas with low elastic coefficient (soft) usually had more [11]. On the monitor, degrees of hardness would be represented by different colors, and a 5-point grading scale was applied [12]: one point could be awarded when the lesion and surrounding tissues were completely green; 2 points indicated a mixture of blue and green lesion, and green color was predominated; 3 points indicated predominantly blue lesion, with some green color in the surrounding tissues; 4 points indicated completely blue lesion; 5 points indicated completely blue lesion and a small part of tissues around the lesion was also blue.

The size of the treated liver lesion was another measurement aspect for the efficacy of RFA. Due to the underlying imaging principles, RTE measurement was relatively little impacted by the presence of gas in the intestinal loops and it was, therefore, likely to become a useful method for assessing the scope of treated lesions. Some researchers have also reported that there is no significant difference between the ablation volume measured by ultrasound elasticity imaging and gross specimen examination [13]. In this study, the size of the treated lesion could be accurately assessed with RTE. Under the measurement of RTE and enhanced CT/MRI, there was no significant difference in the maximum cross-sectional area. However, under the measurement of 2D ultrasound and enhanced CT/MRI, the cross-sectional area before RFA was found to be significantly different from those at 1 month and 3 months after RFA, whilst lesion size measured by RTE was not significantly different from the CT/MRI measurements. Moreover, RTE also contributed to the identification of residual tumor. There are some limitations of RTE. False-positive and false-negative results were possibly towing to the overlap of different tissues and artificial subjectivity in a certain degree [14].

VTQ, also known as acoustic radiation force impulse (ARFI), is a noninvasive ultrasound imaging technique for the assessment of tissue elasticity. It can be used to detect the hardness of tissues in a particular region. The VTQ techniques measured the acoustic radiation force impulse and shear wave propagation, and accurately and quickly calculated the elasticity of interested region and surrounding tissues [15]. In traditional ultrasound elasticity imaging, manual pressure exerted by the examiner was utilized to assess the elasticity of tissues, but it might cause subjective errors. During VTQ measurement, however, shear waves were generated in the deep tissues with the use of a focused ultrasound beam [16]. Additionally, this procedure could help to avoid errors due to subjective factors.

At present, VTQ technology is mainly used for the assessment of liver fibrosis [17, 18], but more and more researchers have been interested in exploring its application in the diagnosis of liver tumors [19-21]. In this study, the VTQ for examining the lesion showed a statistically significant increase with time after RFA. Such increase in hardness of the lesion might be related to the coagulative necrosis induced by RFA and therefore shows the efficacy of RFA. VTQ could be expected to become an important indicator for the efficacy of RFA treatment.

Our study had several limitations. For example, the sample size was small and the follow-up period was short. In the future investigations, the difference in the detection efficacy for residual or relapse between combined CEUS and EI and CEUS alone could be evaluated more accurately. In conclusion, our study found that the combination of CEUS and ultrasound EI could save treatment costs and effectively evaluate the efficacy of RFA on liver cancer treatment.

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Disclosure of conflict of interest

None.

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