Impact of Real-Time Contrast-Enhanced Ultrasound-Guided Radiofrequency Ablation on Progression-Free Survival in Patients with Hepatocellular Carcinoma: a Retrospective Case-Control Study

Xiao Shang, Guang Yang, Heng Jun Zhao, Ming Zhang, Guo Zhen Cui, Jiu Wei Cui, Nan Ya Wang[#]

Cancer Center of the First Hospital, Jilin University, Jilin 130021, Changchun, China

Abstract: Objective To compare the value of contrast-enhanced ultrasound (CEUS) and conventional ultrasound (US) during radiofrequency ablation (RFA) for the treatment of hepatocellular carcinoma (HCC) ≥ 3.0 cm in diameter. **Methods** A total of 149 HCC patients treated with RFA guided by either CEUS or conventional US between January 2012 and June 2013 were retrospectively analyzed. Patients were divided into different groups based on the type of ultrasound guidance (CEUS or conventional US) and tumor volume (diameter < 3.0 or ≥ 3.0 cm). The progression-free survival (PFS) and complete ablation rates were compared between groups, and risk factors for the PFS were investigated. **Results** Seventy four patients received CEUS-guided RFA, and conventional US was performed in 75 patients. Among patients with a tumor < 3.0 cm, the PFS and complete ablation rates were similar. However, for patients with a tumor ≥ 3.0 cm, those treated with CEUS had a significantly longer PFS (17.3 vs. 3.1 months, HR = 2.73; 95% CI, 1.28~5.81; $P = 0.007$) and higher complete ablation rates at 6- and 12-month post-treatment (87.5% vs. 57.7%, $P = 0.042$; 75.0% vs. 38.5%, $P = 0.009$, respectively) than those treated with conventional US-guided RFA. The type of treatment ($P = 0.024$) and maximum tumour size ($P = 0.011$) were both found to be independent factors associated with the PFS. **Conclusion** Compared with conventional US, CEUS is more effective for guiding RFA in patients with HCC ≥ 3.0 cm. CEUS-guided RFA could target HCC more accurately, and its ability to immediately detect any residual tumor during RFA might contribute to an increase in complete ablation rates and reduced progression.

Key words: Hepatocellular carcinoma; Radiofrequency ablation; Contrast-enhanced ultrasound; Conventional ultrasound; Progression-free survival; Complete ablation rate

Introduction

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related death worldwide, and the incidence is especially high in China [1,2]. Owing to the development of screening programs for patients at high risk, patients are being increasingly diagnosed with small HCC lesions (maximum diameter of tumor ≤ 3.0 cm) [3]. However, some patients are still diagnosed with intermediate (3.1~5.0 cm in diameter) or large (> 5.0 cm in diameter) lesions at presentation. Radiofrequency ablation (RFA) is one of the most popular curative therapies, and the prevailing method of treatment for small HCC lesions [4-6]. The complete ablation rate for small HCC is reported to be more than 95%, with a local recurrence rate $< 20\%$ [7]. However, for intermediate to large HCC, RFA confers lower complete ablation rates ranging from 29%~70% [8]. To widen the scope of RFA, it has been combined with assistant technologies including percutaneous ethanol injection (PEI) and transcatheter arterial chemoembolization (TACE) [9-12]. Additional innovations could further improve the outcomes of ablation. Currently, conventional US is the

imaging technique most commonly used during RFA. However, it does not provide accurate information about the exact position and vascular flow of the targeted lesions [13]. Contrast-enhanced ultrasound (CEUS) is extremely sensitive to the presence of microbubble agents, thus allowing physicians to evaluate the microvasculature and enhancement patterns of hepatic tumors [14]. Many experimental and clinical studies have indicated that CEUS can be used to evaluate the blood perfusion status of HCC [15-18]. During RFA, CEUS allows the monitoring and multi-angle observation of the targeted lesion, providing objective and accurate information on its position [19]. It is also helpful to detect viable tumor persistence after locoregional therapy, reducing the incidence of local tumor progression and the cost to patients [20,21]. This retrospective case-control study was designed to evaluate the therapeutic efficacy of CEUS in guiding RFA in patients with hepatocellular carcinoma.

Materials and Methods

Patients population and study design

We enrolled 149 patients (116 males and 33 females) who were diagnosed with HCC and underwent either CEUS- or conventional US-guided RFA. HCC was diagnosed based on either enhanced computed tomography (CT) or magnetic resonance imaging (MRI) scans, or

[#]Corresponding author: Nan Ya Wang, MD, PhD, Cancer Center of the First Hospital, Jilin University, Jilin 130021, Changchun, China; Tel: +86 158 0430 2611, +86 0431 8878 3984; Fax: +86 0431 8878 3373; Email: nanyawang@yahoo.com.

pathological evidence. Patients were included in the study if they met the following criteria: ① maximum tumor diameter < 7.0 cm; ② three or fewer lesions; ③ Child-Pugh grade A or B; ④ adequate hematologic function (platelet count > $6 \times 10^9/L$). Patients were subsequently allocated into two groups depending on the type of ultrasound guidance they received. The clinical characteristics of the two groups were compared, including sex, age, Child-Pugh grade, tumor size, and tumor number. To further evaluate the Progression-free survival (PFS) and complete ablation rates among patients with tumour size ≥ 3.0 cm, the patients were divided into four groups based on the tumour size and treatment modality, as follows: group 1, tumor size ≥ 3.0 cm treated with CEUS; group 2, tumor size ≥ 3.0 cm treated with conventional US; group 3, tumour size < 3.0 cm treated with CEUS; group 4, tumour size < 3.0 cm treated with conventional US. Enhanced CT was performed 4 weeks after treatment. Thereafter, the patients were followed up every 3 months. The completely ablated lesions exhibited no enhancement on contrast-enhanced CT. The median follow-up was 10 months (6–21 months).

Contrast agent, CEUS technique and RFA technique

All patients underwent conventional US prior to RFA treatment to determine the location, number, size, and margins of the tumor(s), and the largest diameter was chosen as the most appropriate plane to display the lesions.

The conventional US group then received conventional US-guided RFA. The other group underwent CEUS-guided RFA, and the lesion was observed continuously for 3 min after the injection of contrast agents to further determine the actual lesion region and scope of ablation scope. All ultrasound evaluations were conducted with a GE LOGIQ9 instrument. The US contrast agent used was a second-generation microbubble, sulphur hexafluoride-filled microbubbles (SonoVue, Bracco, Italy), which were supplied as a lyophilized powder and reconstituted with 5 ml of saline to form a homogeneous micro-bubble suspension. Each injection was administered as a 1.2–2.4 ml bolus of contrast agent via the antecubital vein within 2–3 s, followed by a flush with 5 ml of 0.9% normal saline. The arterial (8–30 s), portal venous (31–120 s), and delayed (121–360 s) phases were then monitored in real time. HCC is characteristically hypervascular in the arterial phase, and washed out during the portal venous phase and delayed phase. CEUS was repeated 5 min after the ablation to identify any residual arterial phase enhancement and assess the adequacy of the ablation. Incomplete ablation, usually appearing as an irregular or peripheral nodular enhancement in the ablation zone, required repeated procedures until a complete ablation was achieved.

A LDRF-120S multipole radiofrequency ablation instrument was used. Multiple RFA needle electrodes were used to destroy large tumors (> 5.0 cm in diameter); single RFA needle electrodes were used to ablate exceptional

locations in which multiple needles were difficult to deploy.

Statistical methods

Statistical analyses were conducted using IBM SPSS 22.0. Categorical and continuous variables were analyzed using the χ^2 test and *t*-test, respectively. The PFS was analyzed using the Kaplan-Meier method, and the significance of differences was determined by log-rank analysis. Cox proportional hazards model was used for univariate and multivariate analysis of risk factors related to the PFS. All *P* values were two-sided, and *P* < 0.05 was considered statistically significant.

Results

Patient characteristics at baseline

Among the 149 patients, 74 (49.7%) and 75 (50.3%) were in the CEUS and conventional US groups, respectively. Male patients constituted the majority in both groups. There were 58 male patients in each group, and 16 and 17 female patients in the CEUS and conventional US groups, respectively (*P* = 0.878). The mean tumor sizes were 2.46 ± 0.96 (0.7–5.5) cm and 2.66 ± 1.12 (1–6.1) cm, respectively (*P* = 0.231). There were 59 Child-Pugh grade A cases in the CEUS group and 56 in the conventional US group (*P* = 0.462). The tumor numbers were comparable between the two groups (*P* = 0.624). No significant differences were observed in the baseline characteristics between the two groups (Table 1).

Table 1 Baseline characteristics of patients in the CEUS and conventional US groups.

Variables	CEUS (<i>n</i> = 74)	Conventional US (<i>n</i> = 75)	<i>P</i>
Characteristics			
Sex			
Male	58	58	0.878
Female	16	17	
Age (years)			
≥ 60	35	38	0.681
< 60	39	37	
Child-Pugh grade			
A	59	56	0.462
B	15	19	
Maximum HCC size (cm)			
< 3.0	50	49	0.773
≥ 3.0	24	26	
Tumor number			
1 < <i>n</i> ≤ 3	20	23	0.624
1	54	52	
Mean HCC size (cm)	2.46 ± 0.96 (0.7~5.5)	2.66 ± 1.12 (1~6.1)	0.231

HCC, hepatocellular carcinoma; CEUS, contrast-enhanced ultrasound; US: ultrasound

Figure 1 The progression-free survival (PFS) of patients with tumors < 3.0 cm treated with CEUS- and conventional US-guided RFA (HR = 1.252; 95% CI, 0.674–2.327; $P = 0.474$).

Comparisons of the PFS and complete ablation rates among the different groups

PFS and complete ablation rates in patients with tumors < 3.0 cm group

There were 50 (67.6%) and 49 (65.3%) patients with a maximum tumour size < 3.0 cm in the CEUS and conventional US groups, respectively. The PFS rates are shown in Figure 1. No significant differences in the PFS were noted between the two groups (HR = 1.252; 95% CI, 0.674–2.327; $P = 0.474$), and median PFS were not reached in either groups. The complete ablation rates in the CEUS group were 92.0%, 80.0%, and 70.0% at 6-, 12-, and 18-month post-treatment, respectively; the corresponding figures in the conventional US group were 79.6%, 77.6%, and 71.4%, respectively. No statistically significant differences were observed in the complete ablation rates between the two groups at 6 ($P = 0.138$), 12 ($P = 0.766$), or 18-month ($P = 0.876$) post-treatment (Table 2).

Table 2 Complete ablation rates at different times post-treatment in patients with tumors < 3.0 cm.

Time	CEUS ($n = 50$)	Conventional US ($n = 49$)	P
6 months	46 (92.0%)	39 (79.6%)	0.138
12 months	40 (80.0%)	38 (77.6%)	0.766
18 months	35 (70.0%)	35 (71.4%)	0.876

CEUS, contrast-enhanced ultrasound; US: ultrasound

PFS and complete ablation rates in patients with tumors ≥ 3.0 cm

There were 24 (32.4%) patients in the CEUS group and 26 (34.7%) in the conventional US group with a maximum tumor size ≥ 3.0 cm. The PFS rates are shown in Figure 2. Patients who received CEUS had significantly better PFS compared with those who received conventional US (17.3 m vs 3.1 months, HR = 2.73; 95% CI, 1.28–5.81; $P = 0.007$). The complete ablation rates in the CEUS group at 6-, 12-, and 18-month were 87.5%, 75.0%, and 45.8%, respectively,

whereas those in the conventional US group were 57.7%, 38.5%, and 34.6%, respectively. Significant differences in complete ablation rates were observed between the two groups at 6- ($P = 0.042$) and 12-month ($P = 0.009$) post-treatment (Table 3).

Figure 2 The progression-free survival (PFS) of patients with tumors ≥ 3.0 cm treated with CEUS- and conventional US-guided RFA (17.3 m vs 3.1 months, HR = 2.73; 95% CI, 1.28–5.81; $P = 0.007$).

Table 3 Complete ablation rates at different times post-treatment in patients with tumors ≥ 3.0 cm.

Time	CEUS ($n = 24$)	Conventional US ($n = 26$)	P
6 months	87.5% (21/24)	57.7% (15/26)	0.042
12 months	75.0% (18/24)	38.5% (10/26)	0.009
18 months	45.8% (11/24)	34.6% (9/26)	0.419

CEUS, contrast-enhanced ultrasound; US: ultrasound

Factors that affect the PFS

The univariate analysis identified that the use of conventional US (HR = 1.655, 95% CI, 1.032–2.652, $P = 0.036$) and a maximum tumour size ≥ 3.0 cm in diameter (HR = 1.768, 95% CI, 1.108–2.823, $P = 0.017$) were significant factors associated with a lower PFS. In a multivariate analysis, the use of conventional US (HR = 1.732, 95% CI, 1.077–2.786, $P = 0.024$) and a maximum tumour size ≥ 3.0 cm in diameter (HR = 1.844, 95% CI, 1.152–2.949, $P = 0.011$) were both found to be risk factors for a decreased PFS (Table 4).

Recurrence

Distant recurrence was defined as the emergence of a tumor not adjacent to the ablation zone and local recurrence was defined as a reappearance of tumor progression adjacent to the treated site. During the follow-up, the 1-, 6- and 12-month local recurrence rates for the CEUS and the conventional US groups were 0%, 9.5%, and 21.6% and 6.7%, 28.0%, and 36.0%, respectively. The corresponding distant recurrence rates for the 2 groups were 0%, 12.2%, and 23.0% and 0%, 13.3%, and 20.0%, respectively (Table 5).

Table 4 Results of the univariate and multivariate analyses of the risk factors for the progression free survival (PFS).

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Age (≥ 60 vs < 60 y)	1.150 (0.723~1.828)	0.555		
Type of treatment (US vs CEUS)	1.655 (1.032~2.652)	0.036	1.732 (1.077~2.786)	0.024
Child-Pugh class (B vs A)	0.870 (0.499~1.518)	0.624		
Tumor number (multiple vs single)	1.277 (0.778~2.097)	0.333		
Maximum tumor size (≥ 3.0 vs < 3.0 cm)	1.768 (1.108~2.823)	0.017	1.844 (1.152~2.949)	0.011

CEUS, contrast-enhanced ultrasound; US: ultrasound

Table 5 Recurrence rates at different times post-treatment in the CEUS-guided and conventional US-guided groups

Group	Local recurrence (%)	Distant recurrence (%)
CEUS		
1 month	0	0
6 months	9.5 (7/74)	12.2 (9/74)
12 months	21.6 (16/74)	23.0 (17/74)
Conventional US		
1 month	6.7 (5/75)	0
6 months	28.0 (21/75)	13.3 (10/75)
12 months	36.0 (27/75)	20.0 (15/75)

CEUS, contrast-enhanced ultrasound; US: ultrasound

Discussion

RFA is a safe and effective approach for treating small hepatic tumors, and is associated with a 5-year survival rate higher than 50% [22,23]. Imaging-based guidance has an important role in RFA for HCC by ensuring accurate targeting of the lesion and in assessing the efficacy of the ablation. Despite advances in computed tomography (CT) and magnetic resonance imaging (MRI), US continues to be the most commonly performed imaging technique during the treatment of HCC due to its low cost, availability and lack of radiation exposure. Unfortunately, conventional US may provide insufficient detection and characterization of liver lesions. In particular, the identification of residual disease is often difficult. In most cases, conventional US cannot distinguish viable tumor from necrotic tissue in the treated area [22]. Therefore, at the end of treatment, conventional US cannot precisely assess the size and completeness of coagulation-induced necrosis. Many researchers have noted that the therapeutic effects of RFA decreased as the tumor size increased. For example, Livraghi T et al. [24] reported that the RFA-induced complete tumor necrosis rates was 90%, 71%, and 25% for tumors ≤ 3.0 cm, 3.1~5.0 cm, and > 5.0 cm, respectively. Liang HH et al. [25] demonstrated that the 1-, 2-, and 3-year overall survival rates of patients with tumors ≤ 3.0 cm after RFA were higher than those with tumors 3.1~5.0 cm and > 5.0 cm. The results of our present study also revealed

that the complete ablation rates decreased as tumor size increased. These drawbacks have prevented the widespread use of RFA in treating intermediate- to large-sized hepatic tumors. In our study, there were no significant differences in the complete ablation rates between the conventional US and CEUS in patients with tumors < 3.0 cm. This is likely because small HCC can often be successfully treated by conventional US-guided RFA, with efficacy almost the same as that of surgical resection. Thus, it is difficult to further improve the results of RFA for small tumors [26-28]. In contrast, for tumors ≥ 3.0 cm, the complete ablation rates were higher in the CEUS group than in the conventional US group at 6 and 12 months' post-treatment. This suggests that CEUS-guided RFA may be more effective in treating HCC ≥ 3.0 cm compared with conventional US-guided RFA.

Using CEUS with a second-generation contrast agent provides an excellent visualization of both microvascularization and the overall perfusion patterns of liver lesions. It is easily performed and can be repeated as needed because microbubbles are associated with a low risk of adverse events, lower than the risks of contrast agents used in CT or MRI scans [29]. Several studies have shown that CEUS has the ability to characterize HCC lesions, in addition to guiding RFA treatment [30]. It plays a pivotal role both before and during RFA treatment by permitting real-time vascular imaging with high spatial and temporal resolution. Before RFA, real-time targeting under the guidance of CEUS has been used to facilitate accurate needle positioning and precisely target the areas that require treatment [31]. During ablation treatments, CEUS can be used to identify residual areas of enhancement, with the possibility of immediately performing re-treatment within the same session. This application has been shown to reduce the number of failed radiofrequency procedures and decrease the rate of incomplete ablation from 16% to 6% [32, 33].

In our study, the PFS of patients who received CEUS were longer than those of patients who received conventional US (17.3 vs 3.1 months, $P = 0.007$) among those with tumors ≥ 3.0 cm. The complete ablation rates were significantly higher in patients with tumors ≥ 3.0 cm treated with CEUS-guided RFA than in those treated with conventional US-guided RFA at 6- and 12- month post-

treatment (87.5% vs 57.7%, $P = 0.042$; 75.0% vs 38.5%, $P = 0.009$, respectively). And another study has revealed that the complete ablation rates of the group treated with CEUS-guided RFA were significantly higher than those of the conventional US-guided group at the 1-month follow-up (90.5% vs 52.4%, $P = 0.017$) [34]. The univariate and multivariate analyses in our study suggested that the type of treatment and maximum tumor size were significant factors affecting the PFS. HCC ≥ 3.0 cm was a risk factor for a shorter PFS (HR = 1.844, $P = 0.011$). Compared with conventional US, CEUS-guided RFA could improve the PFS (HR = 1.732, $P = 0.024$), especially for patients with tumors ≥ 3.0 cm. We believe that this is because CEUS can more easily detect HCC lesions by confirming the area of enhancement, making it possible to ensure that there is a sufficient ablative margin during RFA. One study found that the enhanced area of HCC detected using CEUS was larger than that measured by conventional US in 40%~60% patients, which indicates that CEUS can more better reflect the actual condition of the tumor and more accurately determine the appropriate range of RFA [35]. In brief, RFA guided by CEUS can help to confirm that there is a sufficient ablation margin, which is critical for preventing the local recurrence of HCC ≥ 3.0 cm.

Conclusion

This study indicated that the therapeutic efficacy of RFA guided by CEUS was better than that of RFA guided by conventional US in patients with hepatocellular carcinoma ≥ 3.0 cm.

Conflict of Interest

The authors declare that there is no conflicts of interest.

Funding

This work was supported by the Training Plan for Outstanding Young Teachers of Jilin University (No. 419080500356).

References

1. Bray F, Ferlay J, Soerjomataram I, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68(6):394-424.
2. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016;66(2):115-32.
3. Renzulli M, Tovoli F, Clemente A, Ierardi AM, Pettinari I, Peta G, Marasco G, Festi D, Piscaglia F, Cappabianca S, Carrafiello G, Golfieri R. Ablation for hepatocellular carcinoma: beyond the standard indications. *Med Oncol* 2020;37(4):23.
4. Zhang YJ, Liang HH, Chen MS, Guo RP, Li JQ, Zheng Y, Zhang YQ, Lau WY. Hepatocellular carcinoma treated with radiofrequency ablation with or without ethanol injection: a prospective randomized

trial. *Radiology* 2007;244(2):599-607.

5. Yu SCH, Hui JWY, Chong CCN, Chu CM, Cheung S, Wong J, Lee KF. Transarterial ethanol ablation for small hepatocellular carcinoma (≤ 3 cm): a comparative study versus radiofrequency ablation. *Cardiovasc Intervent Radiol* 2020;43(5):732-9.
6. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, Tateishi R, Han KH, Chawla Y K, Shiina S, Jafri W, Payawal DA, Ohki T, Ogasawara S, Chen PJ, Lesmana CRA, Lesmana LA, Gani RA, Obi S, Dokmeci AK, Sarin SK. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017;11(4):317-70.
7. Vigano L, Laurenzi A, Solbiati L, Procopio F, Cherqui D, Torzilli G. Open liver resection, laparoscopic liver resection, and percutaneous thermal ablation for patients with solitary small hepatocellular carcinoma (≤ 30 mm): review of the literature and proposal for a therapeutic strategy. *Dig Surg* 2018;35(4):359-71.
8. Morimoto M, Numata K, Kondou M, Nozaki A, Morita S, Tanaka K. Midterm outcomes in patients with intermediate-sized hepatocellular carcinoma: a randomized controlled trial for determining the efficacy of radiofrequency ablation combined with transcatheter arterial chemoembolization. *Cancer* 2010;116(23):5452-60.
9. Lencioni RA, Allgaier HP, Cioni D, Olschewski M, Deibert P, Crocetti L, Frings H, Laubenberger J, Zuber I, Blum HE, Bartolozzi C. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. *Radiology* 2003;228(1):235-40.
10. Min JH, Lee MW, Rhim H, Choi D, Kim YS, Kim YJ, Cha DI, Lim HK. Recurrent hepatocellular carcinoma after transcatheter arterial chemoembolization: planning sonography for radio frequency ablation. *J Ultrasound Med* 2011;30(5):617-24.
11. Kurokohchi K, Masaki T, Himoto T, Deguchi A, Nakai S, Yoneyama H, Yoshida S, Kimura Y, Inoue H, Kinekawa F, Yoshitake A, Izuishi K, Watanabe S, Kuriyama S. Successful laparoscopic radiofrequency ablation of hepatocellular carcinoma adhered to the mesentery after transcatheter arterial embolization. *Oncol Rep* 2005;13(1):65-8.
12. Yan S, Xu D, Sun B. Combination of radiofrequency ablation with transarterial chemoembolization for hepatocellular carcinoma: a meta-analysis. *Dig Dis Sci* 2013;58(7):2107-13.
13. Dong Y, Wang WP, Gan YH, Huang BJ, Ding H. Radiofrequency ablation guided by contrast-enhanced ultrasound for hepatic malignancies: preliminary results. *Clin Radiol* 2014;69(11):1129-35.
14. Kim AY, Lee MW, Rhim H, Cha DI, Choi D, Kim YS, Lim HK, Cho SW. Pretreatment evaluation with contrast-enhanced ultrasonography for percutaneous radiofrequency ablation of hepatocellular carcinomas with poor conspicuity on conventional ultrasonography. *Korean J Radiol* 2013;14(5):754-63.
15. Doury M, Dizeux A, de Cesare A, Lucidarme O, Pellot-Barakat C, Bridal SL, Frouin F. Quantification of tumor perfusion using dynamic contrast-enhanced ultrasound: Impact of mathematical modeling. *Phys Med Biol* 2017;62(3):1113-25.
16. Zhang P, Chen Y, Liu J, Yang Y, Lv Q, Wang J, Zhang L, Xie M. Quantitative evaluation of combretastatin A4 phosphate early efficacy in a tumor model with dynamic contrast-enhanced ultrasound. *Ultrasound Med Biol* 2018;44(4):840-52.

17. Delorme S, Krix M. Contrast-enhanced ultrasound for examining tumor biology. *Cancer Imaging* 2006;6(1):148-52.
18. Lo GM, Al Zahrani H, Jang HJ, Menezes R, Hudson J, Burns P, McNamara MG, Kandel S, Khalili K, Knox J, Rogalla P, Kim TK. Detection of early tumor response to axitinib in advanced hepatocellular carcinoma by dynamic contrast enhanced ultrasound. *Ultrasound Med Biol* 2016;42(6):1303-11.
19. Wu H, Wilkins LR, Ziats NP, Haaga JR, Exner AA. Real-time monitoring of radiofrequency ablation and postablation assessment: accuracy of contrast-enhanced US in experimental rat liver model. *Radiology* 2014;270(1):107-16.
20. Meloni MF, Andreano A, Franza E, Passamonti M, Lazzaroni S. Contrast enhanced ultrasound: Should it play a role in immediate evaluation of liver tumors following thermal ablation? *Eur J Radiol* 2012;81(8):e897-902.
21. Mauri G, Porazzi E, Cova L, Restelli U, Tondolo T, Bonfanti M, Cerri A, Ierace T, Croce D, Solbiati L. Intraprocedural contrast-enhanced ultrasound (CEUS) in liver percutaneous radiofrequency ablation: clinical impact and health technology assessment. *Insights Imaging* 2014;5(2):209-16.
22. Dohmen T, Kataoka E, Yamada I, Miura K, Ohshima S, Shibuya T, Segawa D, Sato W, Anezaki, Y, Ishii H, Kamada K, Goto T, Ohnishi H. Efficacy of contrast-enhanced ultrasonography in radiofrequency ablation for hepatocellular carcinoma. *Intern Med* 2012;51(1):1-7.
23. Takai Takamatsu R, Okano A, Yamakawa G, Mizukoshi K, Obayashi H, Ohana M. Impact of an ultrasound-guided radiofrequency ablation training program on the outcomes in patients with hepatocellular carcinoma. *Diagn Interv Imaging* 2019;100(12):771-80.
24. Livraghi T, Lazzaroni S, Meloni F. Radiofrequency thermal ablation of hepatocellular carcinoma. *Eur J Ultrasound* 2001;13(2):159-66.
25. Liang HH, Chen MS, Wang XD, Zhang YQ, Zheng Y, Li JQ. Analysis of the therapeutic efficiency of percutaneous radiofrequency ablation on hepatocellular carcinomas. *Zhonghua Gan Zang Bing Za Zhi* 2004;12(12):756-7.
26. Chen MS, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ, Lau WY. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006;243(3):321-8.
27. Peng ZW, Chen MS, Liang HH, Gao HJ, Zhang YJ, Li JQ, Zhang YQ, Lau WY. A case-control study comparing percutaneous radiofrequency ablation alone or combined with transcatheter arterial chemoembolization for hepatocellular carcinoma. *Eur J Surg Oncol* 2010;36(3):257-63.
28. Zhu ZX, Huang JW, Liao MH, Zeng Y. Treatment strategy for hepatocellular carcinoma in China: radiofrequency ablation versus liver resection. *Jpn J Clin Oncol* 2016;46(12):1075-80.
29. Cantisani V, Wilson SR. CEUS: where are we in 2015? *Eur J Radiol* 2015;84(9):1621-2.
30. Chan AK, Hegarty C, Klass D, Yoshida E, Chung S, Liu DM, Ho SG, Harris AC. The Role of contrast-enhanced ultrasound in guiding radiofrequency ablation of hepatocellular carcinoma: a retrospective study. *Can Assoc Radiol J* 2015;66(2):171-8.
31. Solbiati L, Ierace T, Tonolini M, Cova L. Guidance and monitoring of radiofrequency liver tumor ablation with contrast-enhanced ultrasound. *Eur J Radiol* 2004;51 Suppl:S19-23.
32. Kim TK, Khalili K, Jang HJ. Local ablation therapy with contrast-enhanced ultrasonography for hepatocellular carcinoma: a practical review. *Ultrasonography* 2015;34(4):235-45.
33. Durot I, Wilson SR, Willmann JK. Contrast-enhanced ultrasound of malignant liver lesions. *Abdom Radiol (NY)* 2018;43(4):819-47.
34. Lu SC, Ren QH, Huang Q, Lu HM. Therapeutic effect comparison of radiofrequency ablation guided by conventional ultrasound and contrast-enhanced ultrasound in patient with advanced primary liver cancer following transcatheter arterial chemoembolization. *J. Chinese Journal of Bases and Clinics in General Surgery* 2018;25(11):1318-22.
35. Yan K, Chen MH, Zhang XM, Gao W, Yang W, Zhang H, Fu Y, Wu W. Radiofrequency ablation of small hepatic malignancies guided by contrast enhanced ultrasonography. *J. Chinese Journal of Ultrasonography* 2011;20(1):18-21.