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FULL PAPER

Contrast-enhanced ultrasonography used for post-treatment responses evaluation of radiofrequency ablations for hepatocellular carcinoma: a meta-analysis

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Objective: This meta-analysis aims to analyze the usefulness of contrast-enhanced ultrasonography (CEUS) for post-treatment responses evaluation of radiofrequency ablation (RFA) for hepatocellular carcinoma (HCC) management.
Methods: Literature retrieval in three databases PubMed, Embase and Cochrane Library was conducted up to September 2015, with pre-defined criteria. The technical success rate, local tumour recurrence and local tumour progression were the measurement indexes. Cochran's *Q* test and I^2 were used for heterogeneity detection. Subgroup analyses were performed for complete ablation rate stratified by study designs, contrast agents and post-operative testing time points. Statistical analyses were conducted using Stata® 12.0 software (Stata Corporation, College Station, TX).
Results: 12 studies consisting of 772 patients were included in this study. The CEUS-evaluated success rate of RFA for HCCs was 91%. The proportion of ablative margin <5 mm was 53%. The local tumour recurrence rate and local tumour progression rate were 4% and 8%, respectively. Subgroup analysis indicated that the CEUS-assessed technical success rate with Sonazoid™ (Daiichi-Sankyo, Tokyo, Japan) as the contrast agent was higher (95%) than those with other agents [SH U 508A (Schering AG, Berlin, Germany) 86%; SonoVue (Bracco SpA, Milan, Italy) 87%]. The success rate assessed within 24 h (94%) after treatment was higher than longer time (1–3 days 86%; 1 month 91%).
Conclusion: The meta-analysis showed that the CEUS-evaluated success rate of RFA for HCCs was 91%. The local tumour recurrence rate and local tumour progression rate were 4% and 8%, respectively.
Advances in knowledge: Using meta-analysis, the study provided more reliable assessment of usefulness of CEUS, which could provide guidelines for HCC treatment.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common malignant tumour that seriously threatens human healthy and life, causing about 690,000 deaths per year worldwide.[1,2] Surgical resection of HCC is the major treatment for HCC but with a relative high post-surgical recurrent rate.[3] Recently, local ablative therapy represented by radiofrequency ablation (RFA) is gradually used in clinics as the third treatment means following surgery and transhepatic arterial chemotherapy and embolization for HCC because of its effective, minimally invasive and safe properties.[4–6]

Post-operative tumour residue and intrahepatic reoccurrence are the main factors affecting the curative effect of RFA.[7] Scholars[8,9] think that because the residual tumours are not accurately found within short term following RFA treatment, the residues progressed to local recurrence focuses. Early detection and treatment of the residue lesions can substantially raise the complete coagulated rate from 77% to 99.7% by radiofrequency coagulation.[10] Thus, accurate evaluation of the post-treatment curative effect of RFA is critical to improve the complete ablation rate. Imaging methods are commonly used in the evaluation of RFA curative effect, but the conventional ultrasound is limited and contrast-enhanced CT is not suitable for repeated check during short time. Contrast-enhanced ultrasonography (CEUS) due to its advantages of moderate price and ability to repeated assessment and high spatial resolution is not only used in evaluation

of the local response but also in follow-up of patients.[11] A number of studies have investigated the usefulness of CEUS in evaluation of the post-surgical curative effects of RFA for HCC;[11–20] however, inconsistent results have been reported.

This study therefore aimed to summarize the previously published studies on the role of CEUS in evaluation of therapeutic response of RFA for HCC and systematically analyze the effect. The technical success rate, local tumour reoccurrence and local tumour progression rate were investigated by a meta-analysis.

METHODS AND MATERIALS

Search strategy

Three bibliographic databases PubMed, Embase and Cochrane library were searched up to September 2015 for studies on the post-treatment evaluation of RFA for HCCs. The keywords were radiofrequency ablation, hepatocellular carcinoma and contrast-enhanced ultrasonography. The complete search strategy was ((radiofrequency ablation) OR RFA) AND ((liver cancer) OR (hepatocellular carcinoma) OR HCC) AND ((ultrasound contrast) OR (contrast-enhanced ultrasonography) OR CEUS).

Selection of the eligible studies

Studies conforming to the following criteria were eligible for including in the meta-analysis: (1) study on post-treatment response assessment of RFA for HCCs; (2) study with at least one of the following outcome lesion detection rate, tumour resection rate and the tumour recurrence rate; (3) study in English.

Besides, duplicates, reviews, letters, meeting abstracts, and study with data could not be extracted were excluded.

Data extraction and quality assessment of the included studies

The following data were extracted by two independent investigators (authors A and B): the first author, publication year, study design, country, included patient data, tumour type, lesion size, pre-operative imaging detection method, surgical procedure, post-operative follow-up time, post-operative radiographic detection method, contrast agent, number of cases, number of lesions, ultrasonic testing time and the test results. The quality assessment of the included studies was conducted by using Agency for Healthcare Research and Quality criterion for cross-sectional study (http://www.ncbi.nlm.nih.gov/books/NBK35156/).

Statistical analyses

The technical success/complete ablation rate, local tumour recurrence and local tumour progression were pooled. Cochran's Q test and I^2 test[21] were used for assessment of the heterogeneity. $p < 0.05$ or $I^2 > 50\%$ was considered as heterogeneous, and a random effects model was used for data combination; otherwise, studies were homogeneous and a fixed effect model was utilized. Subgroup analyses for the complete ablation rate based on study design (retrospective, prospective), contrast agents [SH U 508A (Schering AG, Berlin, Germany), SonoVue (Bracco SpA, Milan, Italy), Sonazoid™ (Daiichi-Sankyo, Tokyo, Japan)] and post-operative time points of CEUS detection (<1 h, 1–24 h, 1–3 days and 1 month) were performed. Stata® 12.0 (Stata Corporation, College Station, TX) was used to perform sensitivity analysis, and this analysis was conducted by eliminating one study at a time to observe the change of the pooled estimates. Reversing results indicate unstable results, whereas non-reversing indicates robust results. All analyses were conducted using Stata 12.0 software with 0.05 as the cutoff of significant difference.

RESULTS

Study selection

The flowchart of study selection is shown in Figure 1. The initial search obtained 561 studies (PubMed: 219; Embase: 334; Cochrane library: 8). After removing duplicates (144), 417 studies remained. By rejecting 180 obviously irrelevant studies, there were 237 studies for screening of abstracts. By eliminating 87 studies associated with CT, MRI and other methods not CEUS, 37 reviews and 58 irrelevant studies, there were 55 studies for full text reading. 11 studies of which CEUS was not the only detection method, 11 meeting abstracts, 4 non-English studies and 3 without extractable data were excluded. Finally, a total of 12 studies[11–20,22,23] were enrolled in the meta-analysis.

Characteristics of the eligible studies

Table 1 shows the characteristics of the eligible studies. Among the 12 studies, there were 8 prospective studies and 4 retrospective studies. All the subjects were patients with HCC and accepted RFA for lesion resection. There were totally 772 patients with 933 lesions. All the studies reported treatment effects of RFA those were assessed by CEUS. The contrast agents included SH U 508A, SonoVue and Sonazoid. The time points for CEUS evaluation were <1 h, 1–24 h, 1–3 days and 1 month after surgery.

Figure 1. Flowchart of study selection. CEUS, contrast-enhanced ultrasonography; RFA, radiofrequency ablation.

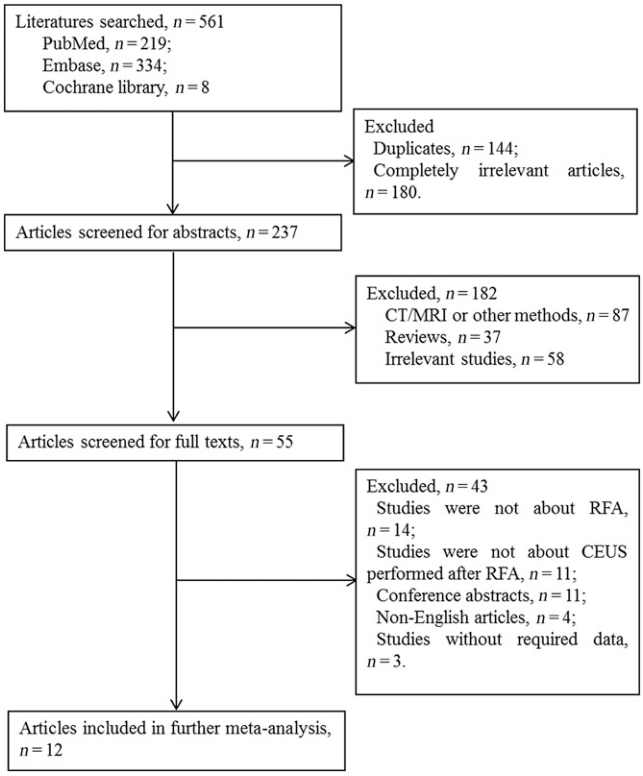


Table 1. Characteristics of the included studies

Study	Study type	Country	Patient selection period	Tumour type	Tumour size	Pre-treatment examinations	RFA technique	Evaluation of post-treatment effect of RFA					Follow-up examination	
								Method	Contrast	Time	Number (patients/ lesions)	Age (years)	Method	Time
Choi et al 2003[12]	R	Republic of Korea	May 2000–December 2001	Nodular HCC	1.3–4.8 cm	Ultrasound	Real-time ultrasound-guided PRFA	CEUS	SH U 508A	<24 h (14–20 h)	75/81	57 (31–72)	CT	12–32 months
Dill-Macky et al 2006[13]	P	Canada	December 2002–February 2004	HCC of hypervascular lesions	1.5–3.7 cm	Multiphasic CT/ dynamic MRI	Sonographic- or CT-guided RFA	CEUS	SH U 508A	<1 h (15–60 min)	19/22	43–83	Multiphasic CT, gadolinium-enhanced dynamic MRI	3–13 months
Du et al 2015[14]	P	China	September 2011–January 2013	HCC	0.8–2.9 cm	Dynamic contrast-enhanced MRI	Conventional greyscale ultrasound, CEUS-guided PRFA	CEUS	SonoVue	<1 h (20–30 min)	63/78	55 ± 7 (41–67)	Contrast-enhanced MRI	11–29 months
Inoue et al 2013[15]	R	Japan	January 2007–December 2011	HCC	16.5 ± 7.1 mm	Dynamic CT	Ultrasound-guided PRFA	CEUS	Sonazoid™	1–3 days	70/86	70.7 ± 7.1	Dynamic CT	873 ± 426 days
Kisaka et al 2006[22]	R	Japan	March 2004–August 2004	HCC	1.0–2.9 cm	Ultrasound	Ultrasound-guided PRFA	CEUS with VUS	SH U 508A	3 days	22/26	68.6 ± 8.08	CECT	–
Kisaka et al 2010[16]	P	Japan	May 2005–August 2006	HCC	17.0 ± 6.5 mm	CT	Ultrasound-guided PRFA	CEUS with VUS	SH U 508A	3 days	25/25	67.0 ± 8.9	Dynamic CT	1 year
Luo et al 2010[17]	P	Japan	February 2007–November 2007	HCC	22 mm (10–30 mm)	3D CECT/CEUS	Real-time ultrasound-guided PRFA	3D CEUS	Sonazoid	1 day	63/63	70 (53–80)	3D CECT	3–21 months
Nishigaki et al 2015[11]	R	Japan	January 2007–June 2010	HCC	16.7 ± 6.1 mm	CT/MRI	CEUS-guided PRFA	CEUS	Sonazoid	3 h	87/87	71 ± 9		1140 days
Numata et al 2012[23]	P	Japan	June 2009–December 2012	HCC	16 mm (10–30 mm)	CECT, fusion imaging combined with CEUS	Real-time ultrasound-guided RFA	Fusion imaging-CEUS	Sonazoid	1 day	67/80	73 (51–84)	CECT	6–24 months
Ricci et al 2009[18]	P	–	January 2001–May 2004	HCC	3.7 ± 1.1 cm (2.6–4.8 cm)	Real time contrast-enhanced examination, helical CT	Real-time sonography-guided RFA	Low-mechanical index CEUS	SonoVue	1 month	100/100	62–76	CT	–
Shimizu et al 2004[19]	P	Japan	October 2000–June 2001	HCC	10–60 mm	ADI, CECT	RFA	CEUS	SH U 508A	1 day	40/64	66.3 (50–85)	Dynamic CT	–
Zheng et al 2013[20]	P	China	May 2007–March 2011	HCC	2.4 cm (0.6–5.7 cm)	CECT, CEUS	Ultrasound-guided PRFA	CEUS	SonoVue	1 month	141/221	53.4 (27–81)	CECT, CEUS	1–31 months

3D, three-dimensional; ADI, agent detection imaging; CECT, contrast-enhanced CT; CEUS, contrast-enhanced ultrasonography; HCC, hepatocellular carcinomas; P, prospective; PRFA, percutaneous radiofrequency ablation; R, retrospective; RFA, radiofrequency ablation; VUS, virtual ultrasonography.

The quality of the included studies was relatively high because most of the studies reported the patient inclusion criteria, consecutive, the reason for exclusion and the blinding methods (Supplementary Table A).

Outcomes measures

Technical success, complete response and complete ablation

A total of 11 studies reported the complete response rate of RFA for HCCs (events: 635, total lesions: 707). Among which, two studies[16,22] reported a response rate of 100%, so they were not included in this analysis. Heterogeneity was found among the remaining 9 studies ($I^2 = 77.3$, $p < 0.01$), thus the random effects model was used. The pooled results indicated that CEUS evaluated success rate of RFA for HCCs was 0.91 [95% confidence interval (CI): 0.87, 0.95] (Figure 2a).

Figure 2b–d shows the results of subgroup analyses. The success rate evaluated by CEUS were 0.92 (95% CI: 0.87, 0.96) and 0.90 (95% CI: 0.84, 0.96) in retrospective studies and prospective studies, respectively (Figure 2b). The success rate assessed by CEUS with Sonazoid as contrast agent (0.95, 95% CI: 0.92, 0.97) was higher than those with other contrast agents (SH U 508A 0.86, 95% CI: 0.76, 0.97; SonoVue 0.87, 95% CI: 0.68, 1.06) (Figure 2c). The success rate assessed within shorter time (<1 h 0.94, 95% CI: 0.90, 0.98; 1–24 h 0.94, 95% CI: 0.89, 0.98) after treatment showed higher success rate than longer time (1–3 days 0.86, 95% CI: 0.67, 1.05; 1 month 0.91, 95% CI: 0.87, 0.95) (Figure 2d).

Ablative margin

Two studies[15,22] in the present study explored the success rate of CEUS for safe margin. The proportion of adequate safety margin >5 mm detected by CEUS was 0.47 (95% CI: 0.30, 0.64) while that of ablative margin <5 mm was 0.53 (95% CI: 0.36, 0.70) (Figure 3).

Local tumour recurrence

5 studies reported the local tumour recurrence of HCCs after RFA (events: 15, total lesions: 307). Significant heterogeneity was

Figure 2. Meta-analysis of the technical success rate. (a) The overall effects; (b) subgroup analysis based on the study design; (c) subgroup analysis based on the contrast agents; (d) subgroup analysis based on the time points of detection. CI, confidence interval.

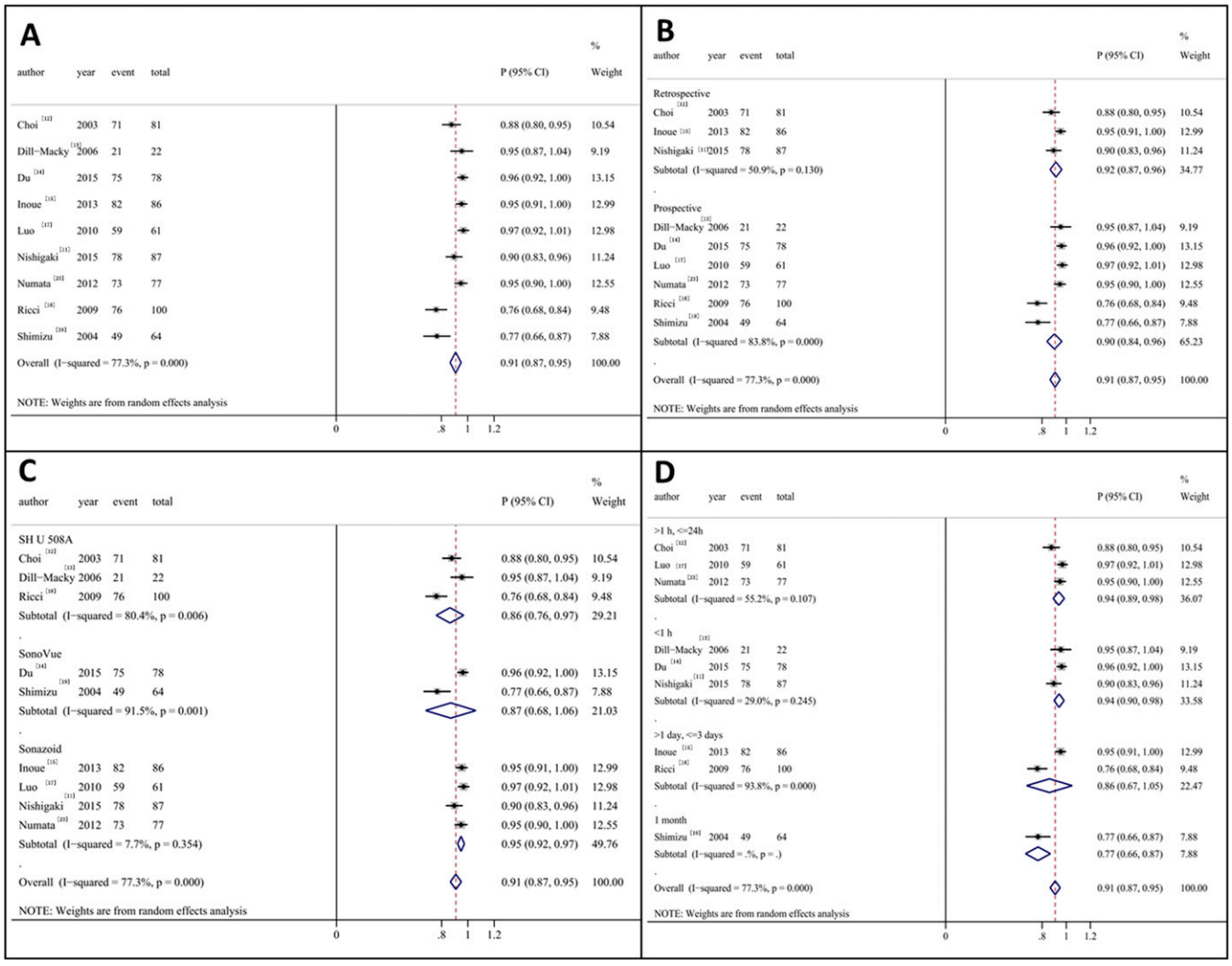
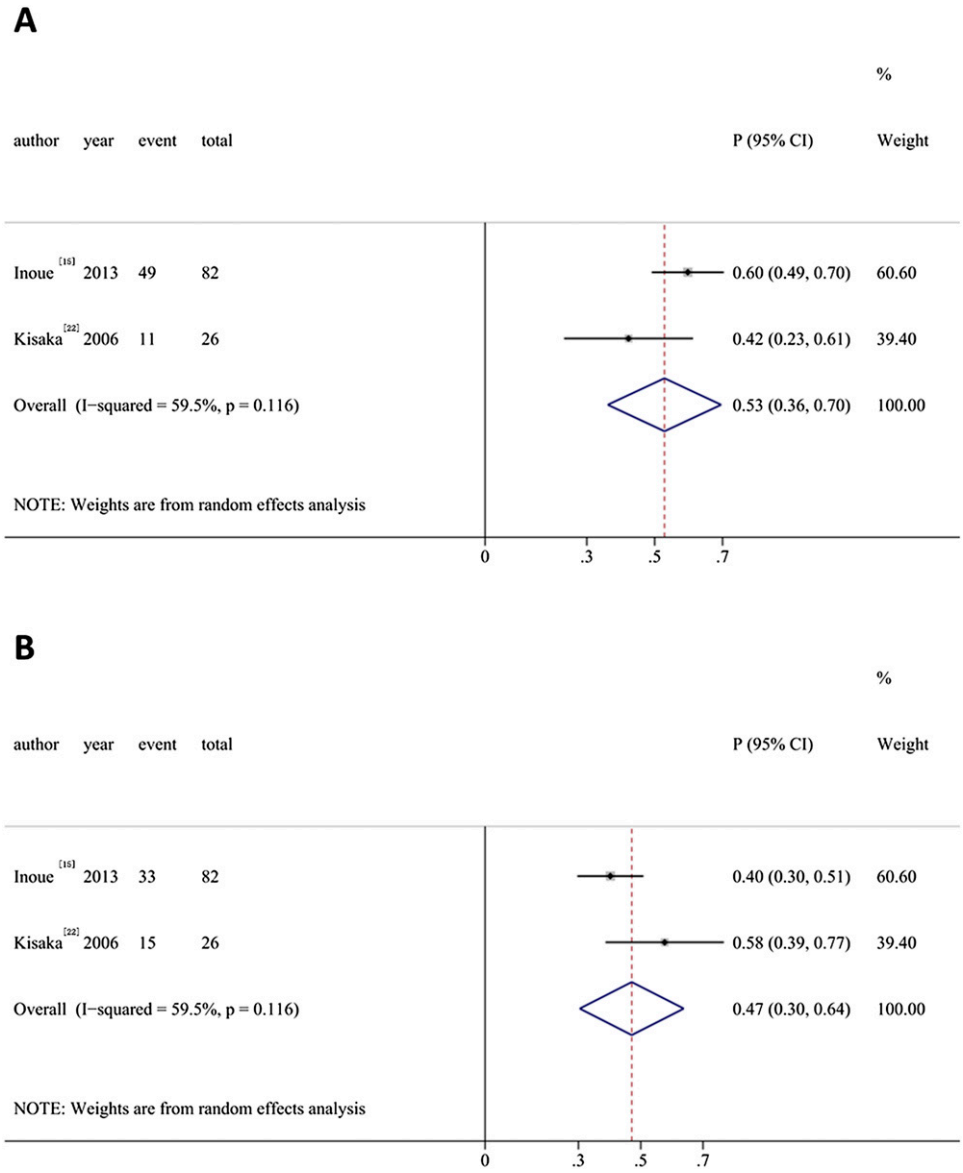


Figure 3. Meta-analysis of the ablative margin. (a) Ablative margin <5 mm; (b) adequate safety margin >5 mm. CI, confidence interval.



found among studies ($I^2 = 62.3\%$, $p = 0.031$), and the random effects model was utilized for combination of the results (Figure 4a). The pooled local tumour recurrence rate was 0.04 (95% CI: 0.00, 0.07).

Local tumour progression

4 studies reported the local tumour progression of HCCs after RFA (events: 45, total lesions: 427). Significant heterogeneity was found among studies ($I^2 = 86.1$, $p < 0.01$), and the random effects model was utilized for combination of the results (Figure 4b). The pooled local tumour recurrence rate was 0.08 (95% CI: 0.01, 0.14).

Sensitivity analysis

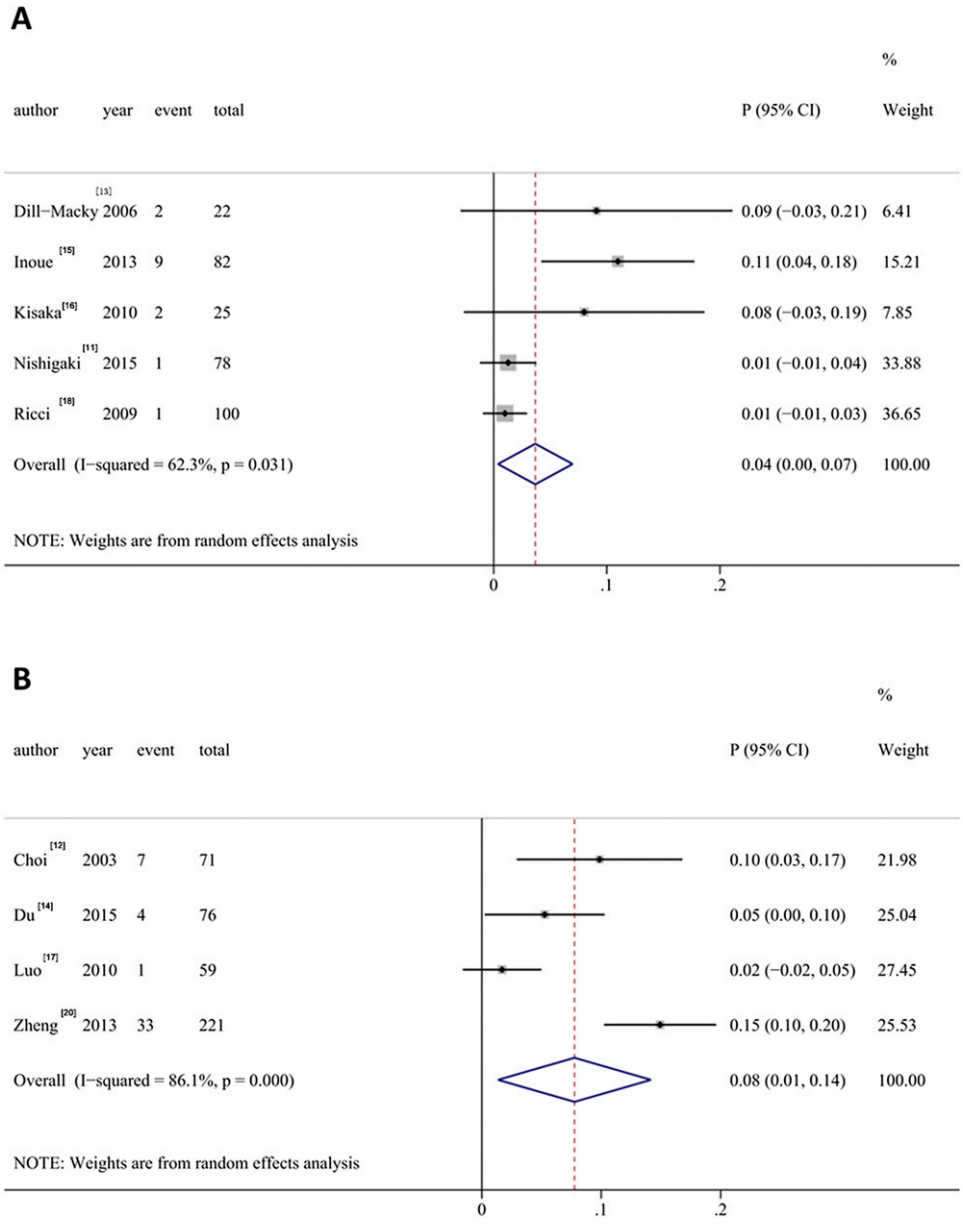
Sensitivity analysis showed that there was no reversing in result by eliminating any of the included studies, indicating the results of the present study was robust (Figure 5).

DISCUSSION

CEUS has been used in assessment of post-treatment effects of RFA for HCC. Many studies have reported the results of CEUS used for the assessment of post-treatment effects of RFA for HCC, but there are inconsistencies in results and slight differences in methods. This study explored the use of CEUS for post-treatment responses evaluation of RFA for HCC by a meta-analysis of 12 studies including 772 patients with 933 lesions. Subgroup analyses based on different contrast agents and time points of test were conducted. The results indicated that the CEUS-evaluated success rate of RFA for HCCs was 91%, and it was higher (95%) with Sonazoid as the contrast agent than with the other agents. We also found that early detection within 24 h following RFA resulted in a higher success rate.

CEUS showed a comparable competence to other imaging methods in assessment of the treatment effect of RFA in HCCs.

Figure 4. Meta-analysis of the local tumour recurrence (a) and local tumour progression (b). CI, confidence interval.

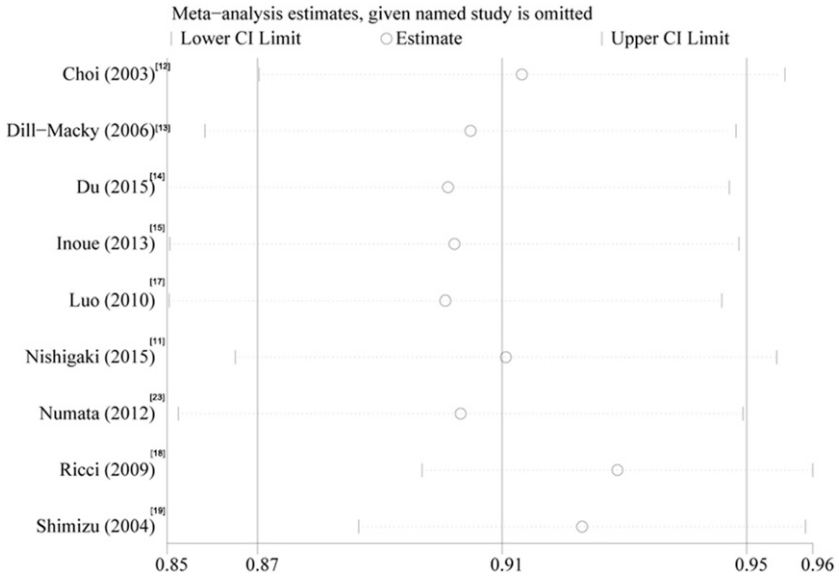


Ricci et al[18] reported a CEUS accuracy of 92.3% compared with four-row spiral CT. Shimizu et al[19] indicated that CEUS with agent detection imaging had similar competence to dynamic CT in assessment of the post-treatment response of RFA for HCC. The sensitivity of CEUS is >90% for detecting adequate ablation when compared with other approaches such as three-dimensional CT scans (97%)[17] and contrast-enhanced CT (91.6%).[22] The combined results from our study showed a 91% complete ablation rate, supporting the opinion that CEUS can be a substitute of CT scans in evaluation of the RFA effectiveness for HCCs.

This study concerned on the assessment of safe margin of RFA by CEUS. Generally, ablative margin width >5 mm after RFA was considered as safe "disease-free margin".[24] A sufficient safe margin can greatly decrease the local tumour recurrence rate, whereas with the ablative margin width of <5 mm, secondary RFA should be performed to ensure complete ablation.[22] Among the included studies, only two studies[15,22] have reported the complete ablated rate based on safe margin width. The results of our study indicated that the proportion of ablative margin width <5 mm was 53%, which might be useful for the judgment of the following treatment effect for patients with HCC. More attention should be drawn on the detection of safe margin in future studies.

The use of different contrast agents affects the evaluation effects of CEUS in RFA for HCC. Among the included studies in this meta-analysis, SH U 508A,[12,13,18] SonoVue[14,19] and Sonazoid[11,15,17,23] were used in as the contrast agents. SH U 508A is the first agent approved by Europe and Canada for radiology application.[25] However, Levovist (SH U 508A) has been withdrawn from the market after having been used for almost 10 years,[26] and thus contrast specific modes were

Figure 5. Sensitivity analysis. CI, confidence interval.



much less effective in Levovist times. SonoVue and Sonazoid (also NC100100) are transpulmonary vascular agents with half-life >5 min after an intravenous bolus injection.[25,27] The results of the present study showed that Sonazoid had higher performance and lower heterogeneity among studies than other agents. From Figure 2c, we can also find that Sonazoid is more frequently used than other agents in recent years. The possible reasons for this situation are that Sonazoid permits real-time and precise observation of the hepatic hemodynamics during a long period,[11] and Sonazoid has more stable Kupffer phase image than SH U 508A.[15] However, as regards comparison between Sonazoid and SH U 508A, a bias may arise from evolution of ultrasonography equipment.

There was inconsistency among studies in the time point for detection, which also influences the evaluation results. We performed subgroup analysis to explore the suitable testing time point. The results showed that early detection by CEUS had a higher complete ablation rate (94%) in evaluation of RFA for HCC, and there was no heterogeneity among studies with time point <1 h and 1–24 h. Thus, we recommended early detection within 24 h after RFA by CEUS with Sonazoid as the contrast agent. However, the comparison of the efficiency of CEUS at different time points by well-designed study is needed to confirm the observation.

There are some concerns which should be taken into account. There was high heterogeneity among studies which might be caused by the different study design, contrast agents and time point of test. Although subgroup analyses for study design, contrast agents and time point of test were conducted, heterogeneities still existed among the analysis of perspective studies, SH U 508A, SonoVue and test point of 1–3 days. Other confounding factors such as population, age and slight modification of RFA or CEUS may also affect the results of the meta-analysis. Another concern is the small sample size. Despite the systematic analysis, there were only 772 patients with 933 lesions included. The small sample size may have influence in the statistical power. Thus, future studies with large sample size, well design by taking in account the agents, detection time and other factors, are necessary to warrant the findings in this study.

CONCLUSION

In conclusion, this meta-analysis showed that the CEUS-evaluated success rate of RFA for HCCs was 91%, and it was higher (95%) with Sonazoid as the contrast agent. The local tumour recurrence rate and local tumour progression rate were 4% and 8%, respectively. Early detection within 24 h after RFA is recommended.

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