

# Radiofrequency Ablation of Hepatocellular Carcinomas Adjacent to the Gallbladder Without Isolation Under Contrast-Enhanced Ultrasound Monitoring: A Comparative Study with Long Term Follow-Up

Liping Luo<sup>1,2,\*</sup>, Ronghua Yan<sup>3,4,\*</sup>, Qingjing Zeng<sup>2</sup>, Yinglin Long<sup>2</sup>, Xuqi He<sup>2</sup>, Kai Li<sup>2</sup>, Erjiao Xu<sup>1</sup>

<sup>1</sup>Department of Medical Ultrasonics, The Eighth Affiliated Hospital of Sun Yat-sen University, Shenzhen, People's Republic of China; <sup>2</sup>Department of Medical Ultrasonics, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China; <sup>3</sup>Department of Radiology, Peking University Shenzhen Hospital, Shenzhen, People's Republic of China; <sup>4</sup>Department of Radiology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China

\*These authors contributed equally to this work

Correspondence: Erjiao Xu, Department of Medical Ultrasonics, The Eighth Affiliated Hospital of Sun Yat-sen University, No. 3025, Shennanzhong Road, Shenzhen, 518033, People's Republic of China, Email xuerjiao@mail.sysu.edu.cn; Kai Li, Department of Ultrasound, The Third Affiliated Hospital of Sun Yat-sen University, 600 Tianhe Road, Guangzhou, 510630, People's Republic of China, Email likai@mail.sysu.edu.cn

**Objective:** This study intends to compare the efficacy and safety between patients undergoing invasive isolation or monitoring measures and patients undergoing intra-operative contrast-enhanced ultrasound (CEUS) monitoring who underwent radiofrequency ablation (RFA) of hepatocellular carcinomas (HCC) adjacent to the gallbladder (GB).

**Methods:** We retrospectively assessed patients with HCC adjacent to the GB who underwent ultrasound-guided RFA. They were divided into two groups: group A was monitored under intra-operative CEUS, while group B was assisted by invasive auxiliary means. The efficacy, complications and survival were followed up and compared.

**Results:** Thirty-eight patients with 39 HCCs were enrolled into group A and 31 patients with 35 HCCs were enrolled into group B. The technique efficacy rates were both 100% in the two groups. There were no significant differences of the cumulative 1-, 3-, and 5-year local tumor progression, tumor-free survival and overall survival between the two groups ( $P = 0.851$ ,  $0.081$  and  $0.700$ , respectively). There were no significant differences of major and minor complications rates between the two groups ( $P = 1.000$ ,  $0.994$ , respectively). More importantly, no GB related complications occurred in group A.

**Conclusion:** Intra-operative CEUS monitoring without protective isolation of the GB might be also a potentially safe and effective method for the RFA of HCC adjacent to the GB, when compared with those assisted with invasive auxiliary means.

**Keywords:** hepatocellular carcinoma, gallbladder, contrast-enhanced ultrasound, thermal ablation, monitoring

## Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor in the world and the third cause of cancer-related mortality as estimated by the World Health Organization.<sup>1</sup> Liver transplantation, hepatectomy and radiofrequency ablation (RFA) are considered as three radical modalities for early-stage HCC.<sup>2</sup> As a safe, effective and minimally invasive therapeutic means, RFA has been recommended as a first-line treatment for HCC.<sup>2,3</sup> However, RFA is considered as a relative contraindication for tumors adjacent to extrahepatic critical organs, such as the gallbladder (GB), extrahepatic bile duct and intestines.<sup>3-5</sup> The collateral thermal damage caused by ablation cannot be ignored. For the GB, if thermal damage occurs, it can cause some serious major complications such as

cholecystitis and perforation.<sup>6–10</sup> Meanwhile, to avoid the thermal damage to the GB, the ablation range tends to be moderated. This leads to an increased incidence of residual and local tumor progression (LTP).<sup>11,12</sup>

For the RFA of the HCC adjacent to the GB, various auxiliary measures have been reported in the literature. Some of these studies reported GB isolation methods including artificial ascites or needle decompression.<sup>6,13,14</sup> Some of these studies reported the use of a thermometric needle inserted in the GB fossa or laparoscopy monitoring to predict the potential thermal damage to the GB.<sup>15,16</sup> Even cholecystectomy before or immediately after RFA was also reported to prevent the potential thermal damage.<sup>17</sup> However, all these extra auxiliary measures were invasive which may increase the cost and operative trauma.

Previously, a few articles had reported that the RFA of HCC adjacent to the GB without extra auxiliary means was feasible and safe.<sup>12,18</sup> However, due to lack of an effective monitoring technique, the incidences of LTP were as high as 14%.

Contrast-enhanced ultrasound (CEUS) was recommended for immediate evaluation of the treatment effect after ablation.<sup>19</sup> At the same time, it was regarded as an effective evaluation method for the microvascular perfusion in non-liver organs,<sup>20</sup> including the GB. So that we supposed thermal damage of the GB could be monitored by CEUS evaluation of the perfusion of the GB wall. We have successfully reported RFA of liver cancers adjacent to the GB without extra invasive measures under the monitoring of intra-operative CEUS.<sup>21</sup> The preliminary results showed that the complete ablation rate was 100% (23/23), no LTP and no serious complications of the GB occurred in all patients with a median follow-up time of 17 months. However, this was a single arm and small sample study with a short follow-up period. The results of the previous study confirmed the feasibility of CEUS monitoring, but could not cover the long-term efficacy and late complications, such as long-term biliary complications. In addition, it needs to be compared with invasive gallbladder isolation, which was more frequently reported, to further verify the safety and effectiveness of the CEUS monitoring method for RFA of HCC adjacent to the GB.

Therefore, in this study, we intend to compare the long-term follow-up results between the patients undergoing invasive isolation or monitoring measures and the patients who employed CEUS monitoring who underwent RFA of the HCC adjacent to the GB.

## Materials and Methods

### Study Population

From January 2016 to December 2019, patients with HCC adjacent to the GB underwent ultrasound-guided RFA in the Third Affiliated Hospital of Sun Yat-sen University were consecutively included in this retrospective study. The inclusion criteria of this study were as follows: (1) patients diagnosed with HCC; (2) HCC within Milan criteria (one lesion up to 5 cm, or two to three lesions with the maximum diameter <3 cm); (3) the distance between the lesion and the GB less than 10 mm; (4) all parameters agreed with the indication for the RFA of HCC. The exclusion criteria were: (1) patients lost to follow up; (2) patients treated by RFA combined with ethanol injection; (3) patients allergic to ultrasound contrast agents (UCAs).

The diagnosis of HCC was based on the biopsy pathological result or was consistent with LI-RADS 5 criteria on contrast-enhance computed tomography/magnetic resonance imaging (CECT/CEMR).<sup>2,22</sup>

All the patients were divided into two groups. Patients in group A were ablated without additional auxiliary means under the monitoring of CEUS. Patients in group B were ablated with additional auxiliary means (ie: artificial ascites, or laparoscopy).

This study was in compliance with the Declaration of Helsinki. Informed consent of RFA and CEUS was obtained from each patient. The study was approved by the Medical Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University (the approval number is [2022]02-162-01).

### Equipment

RFA was performed using the Cool-tip RFA system (Covidien, Mansfield, MA, USA) with a 17-gauge, internally cooled-tip electrode with a 3 cm tip. The RF generator was usually set in the impedance mode with maximum output. Four to twelve minutes were used for each insertion under the real-time ultrasound monitoring during RFA procedure.

MyLab Twice ultrasound machine (Esaote, Genoa, Italy) with the convex probe CA541 (frequency: 1–8 MHz) and GE Logiq E9 (General Electric, United States of America) with C1-6 VN (frequency: 1–6 MHz) were used for the ablation procedure.

## Pre-Ablation Assessment of the GB

The clinical signs and imaging findings of the GB were assessed before the RFA procedure. Especially, cholecystitis and cholelithiasis were recorded. The thickness of the GB walls on the liver side and the shortest distance between the lesion and the GB were measured.

## RFA Procedure

All patients underwent ultrasound-guided percutaneous RFA under general anesthesia by one of three experienced interventional physicians (X.E.J., Z.R.Q. and L.K.) with more than ten years of experience in liver ablation procedures. To avoid the difference of technical experience, the RFA procedure for group A was performed by a single experienced interventional physician (X.E.J.). Usually, the direction of the electrode insertion was parallel to the GB wall, and a minimum distance of 5 mm to the GB wall was maintained in group A. While in group B, the minimum distance was not restricted. According to the size of the lesion, single or overlapped multiple punctures were used to encompass the whole lesion and an additional 5–10 mm of the normal liver parenchyma around the tumor to achieve a sufficient ablative margin (AM), whenever possible (because the lesion adjacent to the GB fossa, the ablative margin of position in GB fossa was not always necessary. The ablation zone only needed to cover the lesion completely at this position).

## Invasive Extra Auxiliary Measures in Group B

Artificial ascites were used to separate the HCC lesion adjacent to the GB from the GB by injecting normal saline (about 500–1000 mL) into the GB fossa through the indwelling drainage tube to avoid thermal damage.

For the patients undergoing laparoscopy or open surgery combined RFA, some isolation means were used: 1) the GB could be pulled away by surgical instruments, 2) the gauze was placed in the gallbladder fossa, 3) artificial ascites were produced.

Moreover, for some patients with cholelithiasis or cholecystitis, laparoscopic cholecystectomy (LC) was performed before or after the RFA procedure to avoid the occurrence of GB thermal damage.

## CEUS Monitoring and Evaluation

SonoVue (Bracco, Milan, Italy) was used in this study which was injected as a rapid bolus of 1.0–2.0 mL via the antecubital vein with 5 mL normal saline for flushing. When necessary, SonoVue could be injected repeatedly.

## Monitoring of the GB Wall

The thickness of the GB wall was measured on ultrasound images during and after the RFA procedure. Thickening of the GB wall was defined as an increase in the thickness of the GB wall of more than 2 mm. In group A, CEUS was employed to evaluate the perfusion of the GB wall after each RFA cycle when the electrode insertion came close to the GB wall ( $\leq 10$  mm) during the RFA procedure. When the RFA cycles were away from the GB wall, CEUS was not applied routinely. After the RFA procedure was complete, CEUS was carried out to confirm the intact perfusion of the GB wall. Intact perfusion was considered when both the serosa and the mucosa were perfused continuously and uniformly. In contrast, if a perfusion defect was observed on the GB wall, the perfusion was considered to be not intact, and cholecystectomy or drainage would be suggested in order to avoid GB perforation.

## Immediate Evaluation of Treatment Response

After the RFA completed the ablation plan, CEUS was used to evaluate the treatment response immediately. If no-perfusion zone covered the whole lesion and the surrounding had at least 5 mm normal liver parenchyma (except for the position in GB fossa), the procedure was considered to be technically successful. If not, supplementary ablation was performed immediately.

## Follow-Up and Subsequent Treatment

To exclude early complications and assess the thickness and integrity of the GB wall, ultrasound examination and laboratory examination were carried out within 72 h after the RFA procedure both in group A and group B. Acute cholecystitis and perforation were diagnosed by the clinical manifestations and imaging finding. In group B, with patients who were assisted by artificial ascites, the peritoneal drainage tube was usually removed 1–3 days after the RFA process. CECT/CEMR was the standard reference for the evaluation of technique efficacy which was performed within one to three months after the RFA procedure. If technical efficacy was established, the patients conducted regular follow-up every three months according to the standardization of tumor ablation.<sup>23</sup> When residual, LTP or intrahepatic distant recurrence (IDR) appeared during subsequent follow-up, appropriate treatments were performed and percutaneous ablation was preferred. The follow-up of all patients ended before December 31, 2021 or the date of liver transplantation. The residual, LTP, intrahepatic distant recurrence (IDR), extrahepatic recurrence (ER), overall survival (OS), tumor free survival (TFS), complications and side effects (especially those related to the GB) were monitored and recorded during the follow-up period.

## Data Analysis

All statistical analyses were accomplished by SPSS 22.0.0 (SPSS, Chicago, IL, USA). The measurement data were presented as the average  $\pm$  standard deviation if they displayed a normal distribution (age, follow-up period) or as the median (range) if the data were not normally distributed (the thickness of GB wall, maximum diameter, distance to GB). Mann Whitney *U*-test was used for the comparison of non-parametric data (maximum diameter, distance to GB, and the thickness of GB wall before and after RFA between two groups et al). Comparison of the thickness of the GB wall both in group A and group B before and after RFA were performed by a *t*-test between two paired samples. Chi-square test was used for the comparison of percentages. The cumulative incidences of OS, TFS, LTP and IDR were displayed using a Kaplan-Meier curve and compared by the Log rank test. A *P*-value less than 0.05 indicated significant differences.

## Results

### Enrollment

Finally, 69 patients with 74 HCCs adjacent to the gallbladder were enrolled in this study. Sixty-four patients had one tumor and 5 patients had two tumors adjacent to the gallbladder. Among 69 patients, 38 patients with 39 HCCs who received RFA therapy without additional auxiliary means under the monitoring of CEUS were categorized into group A and other 31 patients with 35 HCCs were categorized into group B. The baseline characteristics of the patients and lesions are shown in Table 1. The baseline characteristics between the two group had no statistical significance ( $P > 0.05$ ).

**Table 1** The Baseline Characteristics of the Two Groups

Characteristics	Group A	Group B	<i>P</i> -value
Patients	38	31	
Age (Year) <sup>a</sup>	58.63 $\pm$ 8.81 (range 30–72)	54.35 $\pm$ 11.71 (range 25–72)	0.088
Gender (Male/Female)	32/6	26/5	1.000
Tumors (Single/Multiple)	19/19	18/13	0.504
Cirrhosis (Yes/No)	35/3	26/5	0.452
Initial treatment/ Recurrence	18/20	20/11	0.154
Cholelithiasis (Yes/No)	9/29	12/19	0.177
The thickness of GB wall (mm)	Median: 4, range 2–8	Median: 4, range 2–7	0.080
Follow-up period (months) <sup>a</sup>	41.29 $\pm$ 15.35 (range 2–64)	37.74 $\pm$ 17.45 (range 2–68)	0.372

(Continued)

**Table I** (Continued).

Characteristics	Group A	Group B	P -value
Auxiliary measures (artificial ascites/monitor during laparoscopy or open surgery/LC)	-	21/2/8	-
Lesions	39	35	
Maximum diameter (mm)	Median: 15, range 8–49	Median: 18; range 6–41	0.119
Tumor location (S4/S5/S8)	12/20/7	7/24/4	0.319
Distance to GB <sup>b</sup> (mm)	Median: 0, range 0–9	Median: 2, range 0–9	0.804
Distance to GB <sup>b</sup> (0/1–5mm/ 6–9mm)	21/8/10	15/16/4	

**Notes:** Data are numbers of cases in parentheses unless specified otherwise; <sup>a</sup>Data are means ± standard deviations (ranges); <sup>b</sup>The shortest distance between the lesion and GB.

**Abbreviations:** GB, gallbladder; LC, laparoscopic cholecystectomy.

## RFA Procedure and Evaluation

In group A, no invasive extra auxiliary measure was used in all patients. All the GBs were preserved after RFA. Under intra-operative CEUS monitoring during the RFA procedures, the perfusion of the GB wall was intact in all 38 patients (Figure 1). No further emergency cholecystectomy or drainage was needed in these patients.

In group B, artificial ascites were utilized in 21 patients to isolate the GB and lesions (Figure 2). All the drainage tubes were successfully removed within 1–3 days after ablation, and no related complications occurred. Two patients underwent RFA combined with hepatectomy or laparoscopic colectomy. During the operation, surgical instruments were used to isolate the GB and lesions. Another eight patients underwent LC after the RFA procedure because of gallbladder polyps, gallstones or due to a history of severe cholecystitis. As a result, only 23 of the 31 patients in group B had their GB preserved.

According to the CEUS evaluation, immediately after the RFA procedure, all lesions in both groups were successfully ablated. The technical success rate for RFA for both groups was 100% (38/38) and 100% (31/31).

However, for one patient in group B with cholelithiasis who was meant to undergo LC after RFA (Figure 3), a perfusion defect of the GB wall was found by accident during the CEUS evaluation after the RFA procedure of the lesion adjacent to the GB (0 mm to the GB). The GB wall was considered to have had thermal damage. During the LC procedure, the GB wall was observed to be blackened by carbonization. The postoperative pathology confirmed the necrosis of the serosa and mucosa of the GB wall. In the other seven patients, a mild serosal edema was found in these GB walls by postoperative pathology.

## Follow Up

### Technique Efficacy Rate

No residual was detected in all lesions of both groups by CECT/MR one month after RFA, and the technical efficacy rate of group A and group B were both 100% ( $P = 1.00$ ).

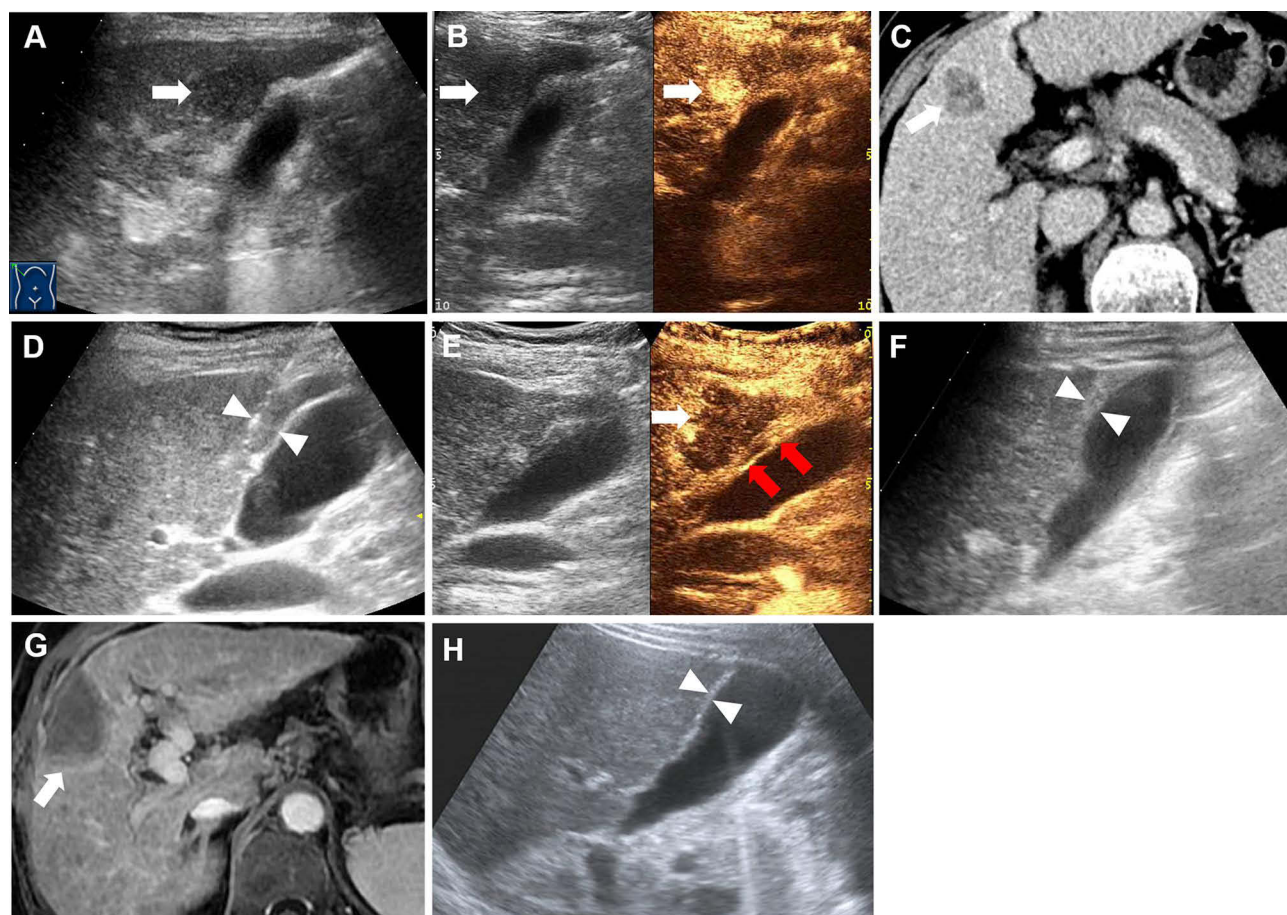
### LTP, IDR and ER

During follow-up, there were two cases with LTP in group A and two cases in group B. And IDR occurred in 15 patients of group A and 18 patients of group B. Their subsequent treatments are shown in Table 2.

The 1-, 3-, and 5-year cumulative LTP rates and cumulative IDR rates in groups A and B are shown in Table 3 and Figure 4. The cumulative LTP rates between group A and group B had no significant differences ( $P = 0.851$ , Figure 4A). While the cumulative IDR rate of group B was higher than that of group A ( $P = 0.039$ , Figure 4B).

Among these patients, a total of 15 patients developed extrahepatic recurrences. There were no significant differences between these two groups ( $P = 0.878$ , Table 2).





**Figure 1** RFA of a 68-year-old female with HCC abutting the gallbladder under intra-operative contrast-enhanced ultrasound (CEUS) monitoring. **(A–C)**. Conventional ultrasound, CEUS and contrast-enhanced CT image showed HCC nodule in segment V abutting to the gallbladder with a maximum diameter of 22 mm (white arrow). Before ablation, the thickness of the gallbladder wall was 4 mm. **(D)** The electrode was inserted parallel to the gallbladder wall with a minimum distance of 6 mm (white triangle). **(E)** CEUS showed that the perfusion of the gallbladder wall was intact (red arrow) and the index tumor had been completely ablated (white arrow). **(F)** The thickness of the gallbladder wall increased to 10 mm one day after ablation (white triangle). **(G)** One month later, the contrast-enhanced MR confirmed the result of complete ablation (white arrow). **(H)** The thickness of the gallbladder wall was restored to 4 mm after 4 months on conventional ultrasound image (white triangle).

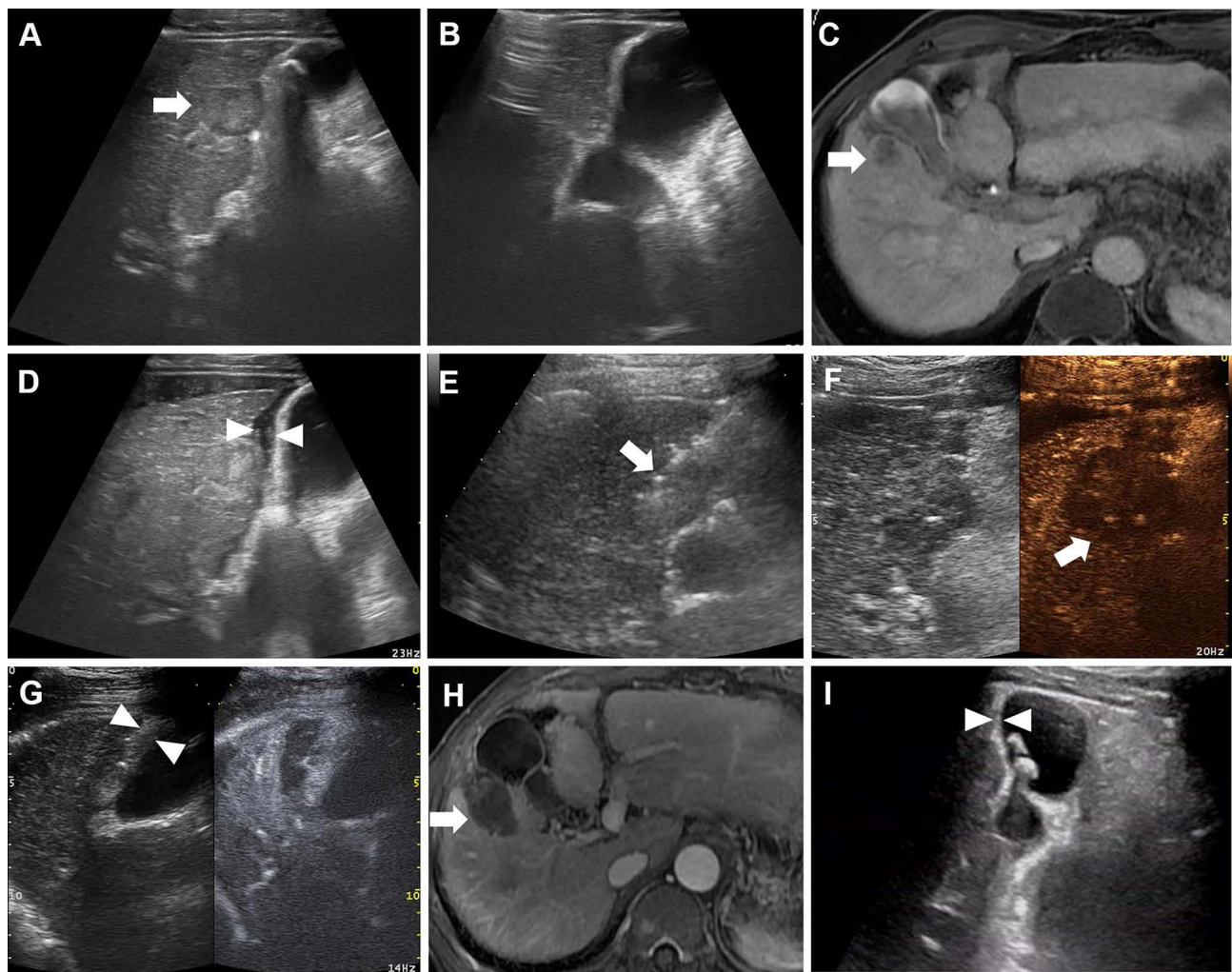
## Complications and Side Effects

The median thickness of the GB wall after RFA was 7 mm (range 2–12 mm) in group A and 4 mm (range 2–7 mm) in group B (Table 4). Thickening of the GB wall was diagnosed in 14 patients in group A. While, thickening of the GB wall was also diagnosed in 4 patients in group B.

The thicknesses of the GB wall, both in group A and group B, after RFA were thicker than those before RFA ( $P < 0.001$  and  $P = 0.004$ ). While the thickness of the GB wall after RFA in group A was thicker than that in group B ( $P = 0.001$ ). However, there was no significant difference in the proportion of patients with thickening of the GB wall (thickening  $>2$  mm) between group A and group B ( $P = 0.106$ ) (Table 4).

No further treatment was required for the thickening of these GB walls. The thickness of the GB wall returned to the pre-ablation level in 7 patients (50.0%, 7/14) of group A and all 4 patients (100%, 4/4) of group B within a follow-up period of 1–5 months (Table 4). No apparent cholecystitis or perforation appeared in these gallbladder-preserving patients of both groups during the follow-up period. CECT/CEMR images one month after RFA confirmed integrity of the GBs in these gallbladder-preserving patients.

Three patients of group A and two patients of group B had major complications with an incidence rate of 7.9% (3/38) and 6.5% (2/31). Drainage was used to treat massive pleural effusions in three patients and a hemothorax in one patient. These thoracic complications were caused by the treatment of lesions adjacent to the diaphragm in patients with multiple



**Figure 2** A 52-year-old male with HCC abutting the gallbladder underwent RFA with auxiliary artificial ascites. (A and C) Conventional ultrasound and contrast-enhanced MR image indicated a HCC lesion located in segment V abutting the gallbladder with a maximum diameter of 15 mm (white arrow). (A and B) Gallbladder stones were found on ultrasound images and the thickness of gallbladder wall was 4 mm. (D) Artificial ascites (white triangle) were employed to isolate the lesion and gallbladder before radiofrequency ablation. (E) The electrode (white arrow) was inserted parallel to the gallbladder wall. (F) After 4 ablation cycles, contrast-enhanced ultrasound showed that the index tumor had been completely ablated (white arrow). (G) One day after ablation, the thickness of the gallbladder wall increased to 7 mm (white triangle). (H) Contrast-enhanced MR one month after the ablation procedure confirmed the complete ablation of the index tumor (white arrow). (I) The thickness of gallbladder wall was restored to 4 mm after 5 months on conventional ultrasound image (white triangle).

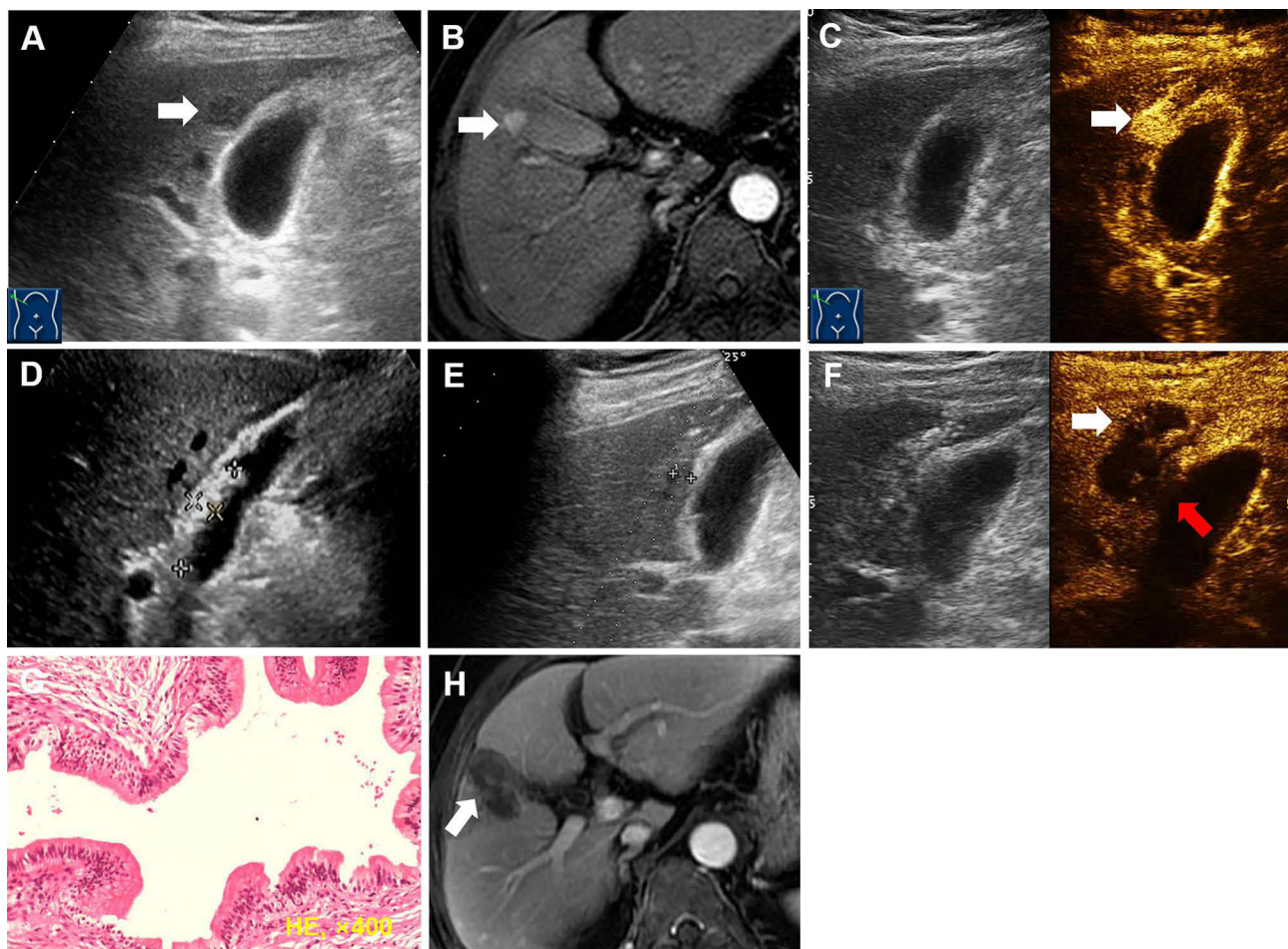
lesions. One patient in Group B occurred peritonitis one week after RFA procedure which was treated by drainage combined with antibiotics. There was no definite manifestation of GB thermal damage in this case.

The minor complication rates were 28.9% (11/38) in group A and 29.0% (9/31) in group B (Table 4). They recovered spontaneously or after conservative treatment. There was no statistically significant difference in the incidence rate of major and minor complications between the two groups ( $P = 1.000$  and  $P = 0.994$ ).

### Cumulative OS and TFS

During the follow-up period, one patient in group A died of liver failure, and one patient in group B died of severe intestinal obstruction. Another three patients in group A and three patients in group B died of HCC progression. The cumulative OS and cumulative TFS in group A and group B are shown in Table 3. There were no significant differences between group A and group B with these two results ( $P = 0.700$ , Figure 4C;  $P = 0.081$ , Figure 4D).





**Figure 3** A 65-year-old male with cholelithiasis who was planned to undergo LC after RFA. (A–C) Conventional ultrasound and contrast-enhanced ultrasound (CEUS), and contrast-enhanced MR image showed a HCC (15×10 mm) in segment V abutting to the gallbladder (white arrow). Before ablation, the thickness of the gallbladder wall was 4 mm. (D) Sediment-like gallstones (white cross) were found on ultrasound images. (E) The electrode was inserted parallel to the gallbladder wall with a distance less than 5 mm (white cross). (F) CEUS showed that the index tumor had been completely ablated (white arrow) while the perfusion defect of the gallbladder wall was found by accident (red arrow). Patient underwent LC soon after RFA procedure, thermal damage of the gallbladder wall was found in postoperative specimens. (G) Postoperative pathology confirmed the thermal damage of the serosa and mucosa of the GB wall. (H) CEMR one month after the RFA procedure confirmed the technical efficacy (white arrow).

## Discussion

HCC adjacent to the GB is considered as a high-risk site for thermal ablation in the literature, including guidelines of HCC management, which affects the efficacy and safety of thermal ablation.<sup>4,5</sup> Several articles had reported a higher incidence of tumor residue and progression because of the limited ablation zone to reduce the thermal damage.<sup>12,24</sup> For

**Table 2** Therapeutic Effect and Re-Treatment

Variable	Group A	Group B	P-value
Residual	0% (0/39)	0% (0/35)	1.000
LTP	5.1% (2/39)	5.7% (2/35)	1.000
IDR	39.5% (15/38)	58.1% (18/31)	0.124
ER	21.1% (8/38)	22.6% (7/31)	0.878
Major complications	7.9% (3/38)	6.5% (2/31)	1.000
	Hemothorax (n=1)	Peritonitis (n=1)	
	Massive pleural effusion (n=2)	Massive pleural effusion (n=1)	

(Continued)



**Table 2** (Continued).

Variable	Group A	Group B	P-value
Minor complications	28.9% (11/38) Fever (n=4) Abdominal pain (n=5) Nausea and vomit (n=2)	29.0% (9/31) Fever (n=4) Abdominal pain (n=4) Vomit (n=1)	0.994
Treatment of recurrences	17	20	
RFA/MWA/PEI	8/2/1	5/7/0	
Resection	0	1	
TACE	3	3	
Radiotherapy	0	1	
Liver transplantation	1	1	
Palliative care	2	2	

**Notes:** Data are numbers of cases or with percentages in parentheses unless specified otherwise.

**Abbreviations:** LTP, local tumor progression; IDR, intrahepatic distant recurrence; ER, extrahepatic recurrence; RFA, radiofrequency ablation; MWA, microwave ablation; PEI, percutaneous ethanol injection. TACE, trans-arterial chemoembolization.

**Table 3** Cumulative LTP, IDR, OS and TFS Rates of Group A and Group B

Cumulative Rates	Group A			Group B			P-value <sup>a</sup>
	1-Year	3-Year	5-Year	1-Year	3-Year	5-Year	
LTP	2.6%	5.7%	5.7%	5.9%	5.9%	5.9%	0.851
IDR	27.2%	30.6%	53.5%	33.8%	56.5%	73.9%	0.030
OS	97.3%	88.8%	88.8%	96.8%	85.8%	85.8%	0.700
TFS	73.1%	55.8%	42.9%	64.2%	39.5%	23.7%	0.081

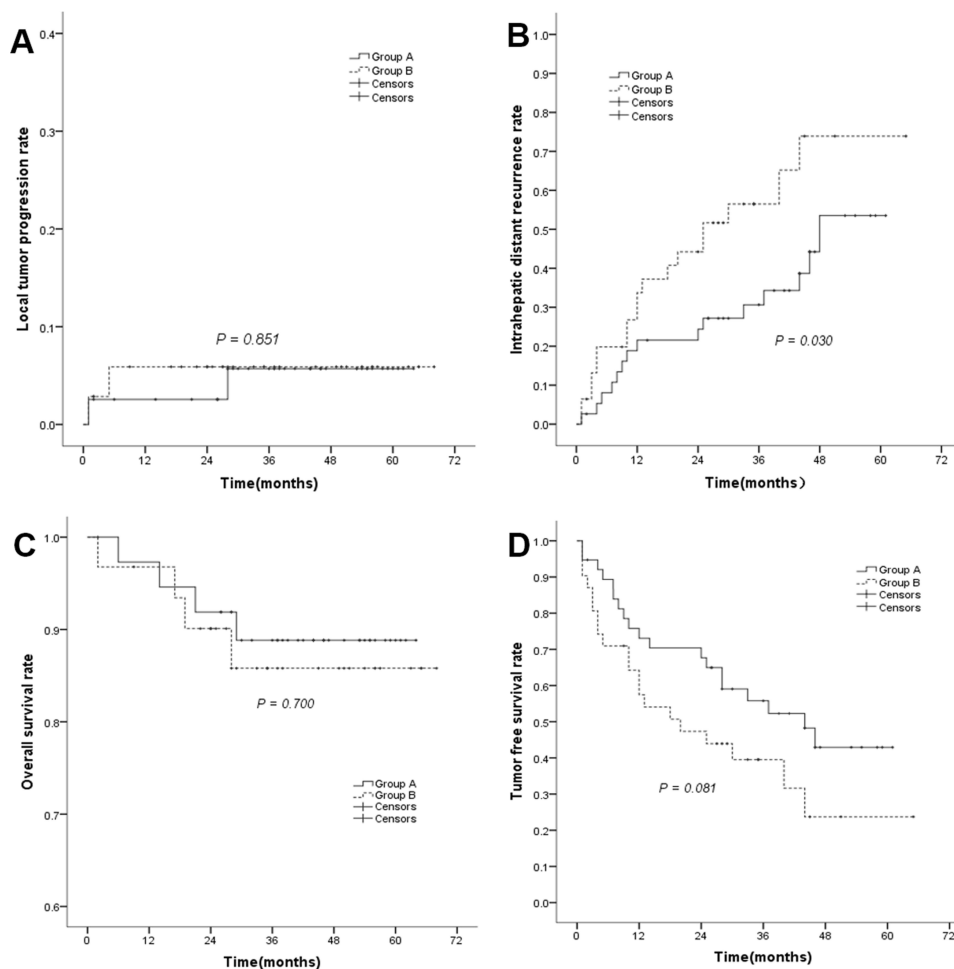
**Note:** <sup>a</sup>Data were compared using the Log rank test.

**Abbreviations:** LTP, local tumor progression; IDR, intrahepatic distant recurrence; OS, overall survival; TFS, tumor free survival;

the thermal ablation of this site, most of the current clinical practices and literatures employ a variety of invasive methods to assist ablation.<sup>6,13,15,17</sup>

Previously, we reported the preliminary results of RFA of liver tumors without GB isolation under the monitoring of CEUS, suggesting that this strategy might be safe, feasible and effective.<sup>21</sup> However, there are a lack of long-term follow-up results and comparative studies.

In this study, we compared the strategies of ultrasound guided RFA of HCC adjacent to the GB with or without extra invasive auxiliary methods in our hospital with long-term follow-up. The results showed that RFA of HCC without GB isolation under the monitoring of CEUS had similar efficacy and safety to RFA with different extra invasive auxiliary means (mainly artificial ascites isolation).<sup>6,13</sup> There were no significant differences between group A and group B in terms of local tumor control rate (technical efficacy and LTP), survival rate (OS and TFS), or the incidence of complications (major and minor complications) ( $P > 0.05$ ). On the basis of the preliminary study, it was further proved that RFA of HCC without GB isolation under the monitoring of CEUS was feasible, safe and effective. According to our practical experience, for the RFA of HCC located at the high-risk site close to the GB, we adopted the strategy of shortening the distance between the electrode and the GB wall (within 5–10 mm) and applying intraoperative CEUS monitoring to evaluate the perfusion of the GB wall and tumor necrosis after RFA, which improved the efficacy and ensured the safety. The results of local tumor control rate (technical efficacy and LTP) in group A (94.9%, 37/39) were better than those reported in the literature on thermal ablation of HCC adjacent to the GB (82.6–92.0%).<sup>12,13,15,25</sup> At the same time, there was no increase in the incidence of major complications and thermal damage of the GB. Although the cumulative IDR rate in group B was higher than that in group A, the IDR was not only related to the ablation effect, but



**Figure 4** Comparison of the cumulative local tumor progression (A), intrahepatic distant recurrence (B), overall survival (C) and tumor free survival (D) curves between group A and group B.

also related to the patient's own conditions and tumor heterogeneity.<sup>3</sup> To some extent, the lesion size in Group B was slightly larger than that in group A (but not statistically significant), which might also lead to the higher recurrence rate. But at least it showed that the RFA of HCC without GB isolation under the monitoring of CEUS had no adverse effect on IDR results.

Our results also showed that although there were no GB related complications in group A, the incidence of GB wall thickening in group A (36.8%) was higher than that in group B (17.4%) and in previous studies (6.1–27.8%),<sup>6,13</sup> which was mainly related to the edema of the GB wall caused by heating due to no isolation of the GB.<sup>7,26</sup> This judgment could

**Table 4** Thickness of Gallbladder Wall

Gallbladder Wall	Group A	Group B	P-value
Thickness before RFA (mm)	Median: 4, range 2–8	Median: 4, range 2–7	0.079 <sup>a</sup>
Thickness after RFA (mm)	Median: 7, range 2–12	Median: 4, range 2–7	0.001 <sup>a</sup>
P-value (Thickness before vs after RFA) <sup>a</sup>	<0.001	0.004	-
Patients with thickening of the GB wall	14 (14/38, 36.8%)	4 (4/23, 17.4%)	0.106 <sup>b</sup>
Thickness recovery	50.0% (7/14)	100% (4/4)	0.119 <sup>b</sup>

**Notes:** <sup>a</sup>Non-parametric data were analyzed using the Mann Whitney U-test; <sup>b</sup>Categorical variable were analyzed using the  $\chi^2$  test.

**Abbreviations:** RFA, radiofrequency ablation; GB, gallbladder.

also be confirmed by the specimen pathology of patients undergoing cholecystectomy in group B. When the intra-operative CEUS images of these patients were reviewed, no incomplete perfusion of gallbladder wall was found. In group A, the GB wall of 14 patients was significantly thicker than that before ablation (>2 mm), of which 50% recovered in the follow-up period. While in another 50% of these 14 patients, the GB wall thickness did not return to the pre-ablation level, but there were no symptoms of cholecystitis during the follow-up period. In group B, there were also four patients with GB wall thickening, but 100% recovered, indicating that GB isolation still had a certain protective effect on the GB wall.

In group B, a patient with HCC adjacent to the GB, complicated with cholelithiasis, planned to undergo LC after RFA. Because the radiofrequency electrode was too close to the GB (<5 mm) during RFA, the perfusion defect of the GB wall was observed during intraoperative CEUS in this patient. The thermal damage of the GB wall was confirmed by the post-operative pathology result, which verified the reliability of intra-operative CEUS monitoring of RFA strategy. Once the GB perfusion defect was detected on CEUS, the interventional therapy should be done in time, such as drainage or cholecystectomy, so as to avoid serious complications.

There were some limitations of this study. Firstly, this is a single center study. Secondly, the sample size of this study is still small. Thirdly, in group A, in order to ensure the consistency of ablation strategy, it was performed by a single experienced interventional doctor. Fourthly, this study is a non-randomized controlled comparative study. Therefore, it is necessary to carry out a multi-center, larger sample study with long-term follow-up with randomized control in the future to verify the effect of RFA without GB isolation under the monitoring of intra-operative CEUS.

In conclusion, intra-operative CEUS monitoring without protective isolation of GB might be a potentially safe and effective method for RFA of HCC adjacent to the GB, when compared with those assisted with invasive auxiliary means such as artificial ascites.

## Abbreviations

HCC, hepatocellular carcinoma; RFA, radiofrequency ablation; GB, gallbladder; LTP, local tumor progression; CEUS, contrast-enhanced ultrasound; UCAs, ultrasound contrast agents; CT, computed tomography; MR, magnetic resonance; AM, ablative margin; LC, laparoscopic cholecystectomy; IDR, intrahepatic distant recurrence; ER, extrahepatic recurrence; OS, overall survival; TFS, tumor free survival; MWA, microwave ablation; PEI, percutaneous ethanol injection; TACE, trans-arterial chemoembolization.

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## Disclosure

No potential conflict of interest was reported by the authors.

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