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Laparoscopic Contrast-Enhanced Ultrasonography for Real Time Monitoring of Laparoscopic Radiofrequency Ablation for Hepatocellular Carcinoma: an Observational Pilot Study

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ABSTRACT

Background & Aims: Laparoscopic radio-frequency ablation (L-RFA) for hepatocellular carcinoma (HCC) is used for unresectable tumors, with difficult location, unfitted for a percutaneous ablation technique. L-RFA has a high incidence of local recurrence. Even if intraoperative-ultrasound is standardized for staging and RFA probe guidance, the role of laparoscopic contrast-enhanced ultrasound (L-CEUS) for the real time monitoring of L-RFA efficacy has not been previously reported. We evaluated in a pilot observational study the efficacy of L-CEUS to assess the necrotic post-ablative area in difficult to treat HCC.

Methods: Eight consecutive patients diagnosed with HCC (peripherally located) on liver cirrhosis were referred for L-RFA between May 2016 and December 2018. For L-RFA a SturBurst XL (AngioDinamics®) internally cooled electrode was used, being placed under ultrasound guidance. L-CEUS was used to assess the necrotic post-ablative area. The median follow up period was 18 months.

Results: L-CEUS real time monitoring of the L-RFA efficacy indicated residual neoplastic tissue in 4 cases (50%). The procedure was repeated by reinserting the needle in the suspected areas indicated by L-CEUS. Complete tumor ablation was achieved in all treated patients. After a median follow-up of 18 months no recurrence of HCC was observed in 7 patients (87.5%).

Conclusions: L-CEUS was a reliable procedure for the immediate assessment of L-RFA efficacy; half of the ablated HCC nodules required a second ablation session. This approach might decrease the local recurrences, but its role must be further investigated in larger cohorts.

Key words: laparoscopic radiofrequency ablation – hepatocellular carcinoma – contrast-enhanced intraoperative ultrasound – laparoscopic contrast-enhanced ultrasound

Abbreviations: CE-CT: contrast enhanced computed tomography; CEUS: contrast-enhanced ultrasound; CE-IOUS: contrast-enhanced intraoperative ultrasound; HCC: hepatocellular carcinoma; L-CEUS: laparoscopic contrast-enhanced ultrasound; L-US: laparoscopic ultrasound; RFA: radiofrequency ablation; L-RFA: laparoscopic radiofrequency ablation; P-RFA: percutaneous radiofrequency ablation.

INTRODUCTION

Hepatocellular carcinoma (HCC) treatment represents a challenge [1], a personalized care being frequently required [2] as the underlying liver disease might limit the therapeutic options. Radiofrequency ablation (RFA) is indicated in patients unfit for surgery due to limited liver function, for unresectable HCC or as a bridge to transplantation. Multiple RFA approaches are available: percutaneous, through laparotomy or laparoscopy [3]. Wong et al. [4] demonstrated that short- and long-term outcomes of patients treated by percutaneous RFA (P-RFA) are similar to those treated through surgical approach. RFA through the classic surgical approach is indicated when is associated with liver resection. Laparoscopic-RFA (L-RFA) represents an alternative when P-RFA is not feasible and laparotomy is contraindicated or is too invasive. The specific indications for L-RFA are represented by: severe impairment of the coagulation tests, large tumors (but < 3.5 cm) or multiple lesions requiring repeated punctures, superficial lesions adjacent to visceral structures, deep-situated lesions with a very difficult or impossible percutaneous approach, short-term recurrence of HCC following percutaneous loco-regional therapies [5-7]. For HCC patients unfit for surgery, the efficacy of L-RFA appeared to be superior to P-RFA in terms of survival in the study conducted by Eun et al. [6].

As L-RFA is indicated for difficult to treat HCC, it was combined with laparoscopic intraoperative ultrasound (L-IOUS) for correct staging and RFA probe guidance [8], following the concept of "radical, but conservative surgery" [9].

The reported overall survival after HCC treatment by L-RFA ranged between 77 and 98% [10-13] at 12 months. The drawback of RFA is the high recurrence rate: 15-25.5% [14, 15]. One predictor factor for recurrence after RFA is microvascular invasion [16]. The assessment of neoangiogenesis can be achieved with the contrast-enhanced ultrasound (CEUS) using sulfur hexafluoride microbubbles; the contrast agent, SonoVue (Bracco Imaging), reaches the vessels as small as 40 microns [17] and can depict the vascular pattern of the HCC. Using contrast-enhanced intraoperative ultrasonography (CE-IOUS) just before open surgery for HCC complements the accuracy of IOUS for staging and probe guidance [9, 18, 19]. Compared to preoperative imaging and to conventional IOUS, more than 50% additional lesions were found using high resolution linear probes for CE-IOUS, leading to therapeutic consequences for patients with HCC [20].

The role of L-IOUS was documented for L-RFA, but the potential benefits of L-CEUS for the real time monitoring of L-RFA efficacy has not been previously reported in clinical settings. In a living swine liver model, Liu et al. [20] documented that CEUS provided a real time imaging foundation for evaluating RFA efficiency compared to histology assessment. The use of ultrasound contrast agents immediately after computer tomography (CT) guided P-RFA or after combined open liver tumor surgery and RFA allowed a reliable assessment of ablative status [21, 22].

Monitoring L-RFA for difficult to treat HCC, in real time, using L-CEUS, requires increased experience in laparoscopic and hepatic surgery, but also in diagnostic and interventional ultrasound.

The aim of this pilot observational study was to evaluate the efficacy of L-CEUS to assess the necrotic post-ablative area in difficult to treat HCC.

METHODS

We prospectively enrolled the patients diagnosed with peripheral HCC that were referred for L-RFA with L-CEUS real time monitoring, between May 2016 and December 2018, at the Regional Institute for Gastroenterology and Hepatology, Cluj-Napoca, Romania. The local Ethics Committee approved this study. Data of all patients were prospectively collected and retrospectively analyzed. The procedures were performed with the patient hospitalized and all patients gave written informed consent before the procedure.

Indications for laparoscopic ablation technique were: subcapsular HCC on cirrhotic liver (< 3.5 cm), dome location and/or in contact with viscera (gallbladder, stomach), with contraindication for open surgery resection due to comorbidities or altered liver function. The contraindications of the procedure were: severe coagulation disorder (thrombocytes < 50,000) and no informed consent given. Previous surgery of the abdomen represented a relative contraindication.

Laparoscopic RFA technique

Laparoscopic RFA was performed under general anesthesia, with the patient positioned on the operating table in dorsal decubitus, in the standard position used for liver resections through laparoscopic approach. In all patients, we used one 10 mm supraumbilical trocar for 30° optics, one 12 mm trocar for the laparoscopic transducer, and 1-2 x 5 mm trocars for the use of working instruments (retractors, electrocautery). Depending on the patient's physiognomy and the location of the targeted tumor, the scope trocar (for optics) was positioned more cranially in the epigastric or the right subcostal area. For intraoperative ultrasound we used a dedicated transducer for laparoscopic surgery (HitachiAloca®).

The RFA was achieved with the RITA device, with a long StarBurst XL electrode (AngioDinamics®). The electrode was internally cooled with iced saline solution. In all cases, L-IOUS was required to confirm the presence of the tumor and to establish the relationships with the intrahepatic structures. The rest of the hepatic parenchyma was also scanned, in order to find possible undetected lesions by preoperative imaging investigations. All ablation procedures were performed with a preset radiofrequency power of 100-150 W, the application time being 10 minutes.

For subcapsular tumors, where complete necrosis area could not be achieved with StarBurst ablation, the laparoscopic Habib 4X device was used to complete the area of superficial ablation. For deep tumors, hardly to visualize in the same ultrasound plane as both the RFA probe and the tumor, we obtained an approximation of the probe insertion area after tattooing the tumor projection area with the electrocautery on the liver capsule. This area was approximated by the use of L-IOUS in transverse and sagittal sequences. L-CEUS with SonoVue 4.8 ml was used in all cases to evaluate the post-ablation necrotic area, at 20 minutes after the RFA (gas bubble induced during RFA can affect the detection zone, being necessary a period of at least 20 minutes after finishing RFA for L-CEUS). Focal areas with irregular peripheral enhancement within the ablation zones were considered as residual tumor foci and the L-RFA procedure was repeated by reinserting the needle into another part of the lesion until obtaining the complete ablation of the tumor (overlapping technique).

Post-procedural follow-up

Postoperatively, the patients were clinically monitored in the surgery unit. Laboratory tests (complete blood count, liver function tests) were performed at 6, 24 and 48 h after the procedure. An ultrasound examination of the abdomen was performed at day 1 after surgery, to assess the abdominal cavity for free liquid or hematomas. Contrast-enhanced CT (CE-CT) was performed one month after the procedure to check the treatment outcome and to exclude any adverse events (portal thrombosis, hematoma, abscess).

Technical success was defined by achieving HCC ablation (i.e., the presence of a markedly hypodense area inside the tumor that was depicted by L-CEUS 20 minutes after L-RFA and confirmed at one month follow up by CE-CT scan).

The median follow up period was 18 months. The monitoring visits at 3 months interval consisted of clinical, biological and imaging assessment (CE-CT).

RESULTS

Eight consecutive patients (5 men and 3 women, median age 59.5 years) diagnosed with HCC on liver cirrhosis were enrolled for L-RFA with real time monitoring by L-CEUS. The demographic and clinical data are listed in Table I. All HCC nodules were visible on the surface of the liver or abutting the liver surface on L-IOUS.

Laparoscopic RFA was feasible in all 8 cases. One single case had prior surgery (hysterectomy by laparotomy). Preoperative mean MELD score was 12 (range 10-17). Two patients had moderate ascites at admission. The average of International Normalized Ratio (INR) was 1.48 with range between 1.22-2.09. Average platelet count at admission was 80,125/μL (range 53,000-114,000 /μL). Due to low platelet count, 4 patients required preoperative platelet transfusion. Tumors were located in hepatic segments II, III, IVB, V and VIII, 4 (50%) on the diaphragmatic surface, 3 (37.5%) on the visceral surface and 1 (12.5%) on both sides of the liver. Mean tumor size (longest axis) was 26 mm (range 15-33 mm) (Table I). Cholecystectomy was associated in 2 cases.

For 7 patients (87.5%), no technical difficulty in inserting the RFA probe by ultrasound guidance was recorded (Fig. 1A-B). In one patient, with the tumor located in segment IVB, due to the small liver and constitutional anatomy of the thoracic anterior wall, the L-IOUS guided insertion of the RFA needle was not possible. The needle was inserted after approximation of the future ablation area by the L-IOUS measurements (depth of the tumor, cranial-caudal and right-left angles) and tattooing with the monopolar cautery the insertion site. The time of a single L-RFA application was 10 minutes in 7 (87.5%) patients and 6 minutes in 1 patient (12.5%), with a mean number of applications of 3 (range 2-5).

Laparoscopic CEUS assessed the necrotic area at 20 minutes after L-RFA. Complete response was considered as the absence of contrast enhancement during all phases, reflecting coagulative and vascular necrosis. Treatment failure was defined as persistence of areas of focal enhancement inside the ablated region, reflecting the presence of well-perfused residual tumor (Fig 2A). We considered a peripheral residual disease if there was hyperenhancing pattern in the arterial phase (sometimes weak and transient) and if the pattern was focal, irregular, heterogeneous, larger than 7-8 mm. In four cases (50%) we detected incomplete ablation based on the peripheral enhancement aspect. In all these cases, the remnant neoplastic tissue was located near the surface of the liver (Fig. 2A). Due to this suboptimal necrotic post-ablative area depicted by L-CEUS in these four cases, the Habib 4x laparoscopic RFA device was used to complete the ablation of superficial tumoral tissue (Fig 2B). An ablated area in the tumor was obtained in all patients, confirmed by the L-CEUS (Fig. 3) and postoperative CE-CT (100%) at one month.

No major postoperative adverse events were recorded. The mean hospitalization time was 4 days (range 2 – 8). The longest

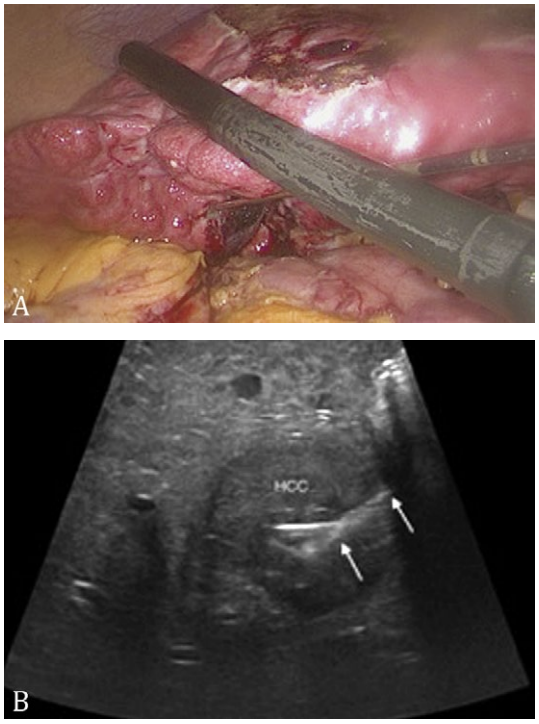

Fig. 1. Laparoscopic IOUS, to confirm the topography of the tumor, vascular rapports and to guide the insertion of RFA probe (arrows). (A) Intraoperative aspect; (B) Ultrasound aspect.

Table I. Clinical characteristics of the patients.

Case	Gender, age (years)	Etiology	Child score	PLT count/μL	MELD score	Tumor location (liver segment/ liver surface)	Tumor size: (longest axis)
1	M, 61	HCV + Alcohol	B	59 x 10^3	15	IVB/diaphragmatic	20 mm
2	M, 62	HCV	A	96 x 10^3	10	V/ visceral	31 mm
3	M, 55	HBV	A	114 x10^3	11	V/ diaphragmatic and visceral	30 mm
4	M, 61	Alcohol	A	53 x 10^3	10	II/III diaphragmatic	33 mm
5	F, 57	Alcohol	B	68 x 10^3	17	III/visceral	30 mm
6	M, 64	Alcohol	A	101 x 10^3	10	VIII/visceral	23 mm
7	F, 58	Alcohol	B	81 x 10^3	11	VI/diaphragmatic	15 mm
8	F, 56	HCV	B	72 x 10^3	12	III/diaphragmatic	26 mm

HCV: hepatitis C virus

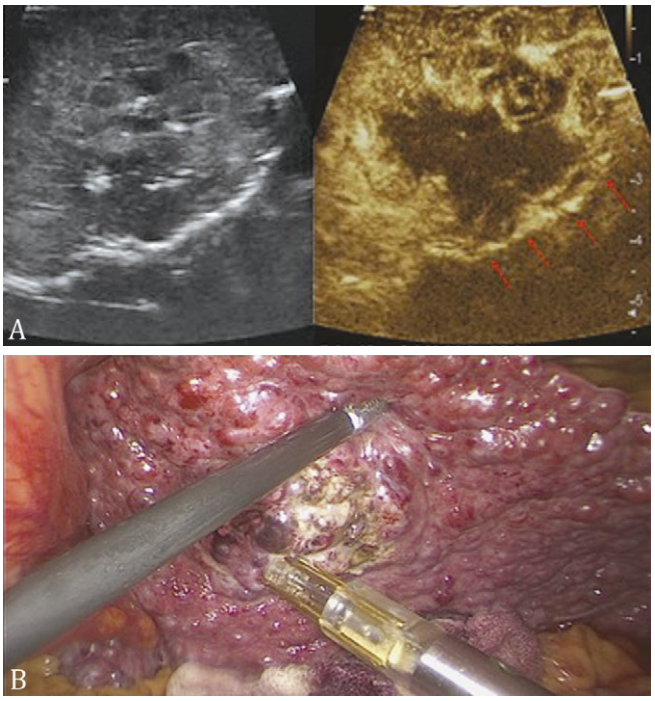

Fig. 2. (A) L-CEUS of a HCC nodule situated on segment III, visceral surface of the liver: a superficial unablated zone (red arrows); (B) Completing the ablation on liver surface with the RFA Habib 4x device.

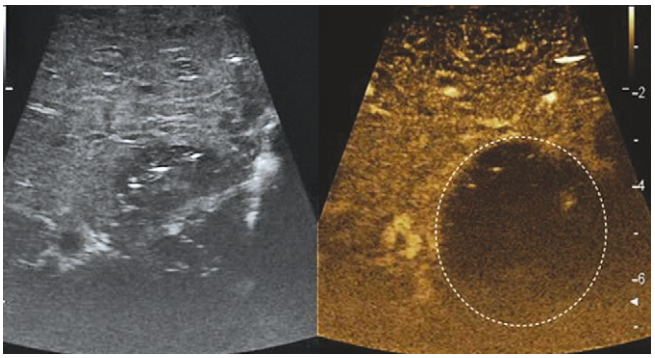

Fig. 3. L-CEUS with confirmation of complete necrotic area after repeating the ablative technique (dotted white line).

hospitalization was required for postoperative platelet count and INR monitoring.

At 18-month median of follow up, no local HCC recurrence was recorded in 7 (87.5%) patients. One patient had HCC recurrence, associated with 5 newly appeared nodules at 6 months after the L-RFA.

DISCUSSION

The treatment of HCC, even standardized by international guidelines, is facing nowadays a need for personalized care, especially in some difficult to treat patients with advanced liver disease or comorbidities [2]. Among loco-regional treatments for HCC, RFA has been accepted as the most popular alternative to curative transplantation or resection as it shows an excellent local tumor control rate and acceptable morbidity. Also, the ablative procedure by RFA is continuously evolving, being guided by different imaging procedure and being used for other digestive malignancies [23, 24]. The techniques for L-RFA for HCC have been previously reported [14, 25]. This minimally invasive technique might represent the proper approach for patients with HCC on liver cirrhosis with coagulopathy and difficult locations for percutaneous approach. The risk of bleeding is better controlled directly during L-RFA. Another important aspect is the possibility to reduce the cooling effect exerted during RFA by the main glissonian pedicle flow on the tumors in close contact with them ("heat-sink" effect), by performing the Pringle maneuver (intermittent clamping of the hepatic pedicle). General anesthesia is not absolutely mandatory. On selected cases, a thoracic peridural anesthesia is possible, which along with a loco-regional block would allow L-RFA to be performed safely.

The L-RFA benefits from L-IOUS, which is able to locate, characterize and guide the procedure [9]. The introduction of ultrasound contrast agents, which are easy to use, increased the accuracy of IOUS and affected the radicalness of the surgical procedure for HCC [18]. Torzilli et al. [18] classified the vascular pattern of HCC nodules assessed during open surgery into four categories; resection was recommended for three vascular patterns and the operative decision was modified in 78% of cases after CE-IOUS. The authors concluded that the specificity of CE-IOUS for HCC assessment was not so high, as there were intrinsic drawbacks in the diagnostic criteria of HCC vascularity. Different vascular patterns correspond to different histological grades [26].

The standard algorithm for RFA ablations requires at least 1 cm of margins to be included in the ablative area, unless limited by adjacent structures, including major biliary or vascular structures, diaphragm and viscera [14, 25]. For peripheral HCC nodules, the intended ablation margins cannot be achieved, and this is one of the major causes of local recurrences [25, 27]. Incomplete margin ablation argues for the role of microvascular invasion for tumor recurrence [16]. As ultrasound agents are able to detect vessels as small as 40 microns [17], they may detect residual neoplastic tissue after RFA technique. Beside its role in detecting new lesions and guiding percutaneous treatments, L-CEUS can also be used to evaluate in real time the response to treatment. The efficiency of CEUS was proved for real-time monitoring RFA guided by CT [21] and immediately after open surgical RFA [22].

We explored for the first time the utility of ultrasound contrast agents for real-time assessment of L-RFA in difficult locations for HCC, not allowing complete ablations margins.

Residual viable tumor foci were suspected in the presence of enhancement areas in an ablated zone, allowing re-treatment during the same session. An immediate assessment of the deeper portion of a lesion is limited by gas formation or cavitation in cases of RFA, being necessary a period of time for the majority of artifacts to disappear [28]. We expected 20 minutes after the procedure to evaluate the residual vascular tumor with ultrasound contrast agents.

Initial studies suggested a time span of 24 hours between the treatment and CEUS for optimal visualization, but it would prevent immediate re-treatments [28]. This might explain the limited number of studies related to the efficiency of CE-IOUS for real time monitoring of RFA. Nowadays, CEUS is routinely performed 10 minutes after RFA, when the majority of artifacts

have disappeared [29]. Peripheral hyperemia is a common, transient finding after percutaneous ablation reflecting peritumoral inflammation secondary to thermal damage and might persist 1-2 months. The peripheral enhancement at the borders of the ablation zones may be misjudged as a peripheral residual tumor. There are some characteristics of the enhancement, which may help in the differentiation [30]. For the peritumoral inflammation, the pattern of enhancement is diffuse, homogeneous, uniform, ring like, 4-5 mm thick. In the arterial phase, there is hyperenhancement, which persists during the portal and venous phase. Hyperenhancing area lies inside the border of the initial tumor. The CEUS aspect for remnant peripheral neoplastic tissue is focal, irregular, heterogeneous, larger than 7-8 mm, hyperenhanced in the arterial phase (sometimes weak and transient) and then hypoenhanced (wash out). The hyperenhancing area lies outside the border of the initial tumor [30].

The real-time monitoring of L-RFA with L-CEUS detected the incomplete margin ablations, allowing immediately a second ablation session, thus limiting one of the predictors of local recurrence. Our new approach for peripheral HCC nodules difficult to ablate improved the immediate results in 50% of the HCC nodules and reduced the local recurrence in one patient (12.5%).

Another proposed technique to improve the ablative margin after RFA is the use of intraoperative CEUS-CT or magnetic resonance imaging (MRI) fusions technique [31], which combines the advantages of ultrasound of real-time guidance, accessibility and non-invasiveness with CT or MRI. However, the equipment is expensive, requiring expertise in CT or MRI.

At one-month follow-up, contrast-enhanced CT represents the standard of care. High accuracy (91%) in the assessment of the residual tumor after ablative techniques was also demonstrated for CEUS in a meta-analysis [32].

The small number of included patients represents a limitation of our study. Further studies from centers with expertise in both laparoscopic liver surgery and L-CEUS might validate our results. Another limitation is represented by the lack of preoperative CEUS. In order to correctly evaluate the treatment efficacy, a review of pre-ablative and post-ablative images is mandatory to compare the diameters of the ablation zone with those of the tumor before treatment [33].

CONCLUSIONS

Fifty percent of our patients benefitted from using L-CEUS immediately after L-RFA, which detected residual malignant vascular tissue. L-CEUS is a simple, non-expensive procedure that accurately assesses the ablative area after L-RFA, allowing complete treatment of HCC nodules in one surgical procedure for difficult to treat HCC nodules. This approach might reduce the local recurrence, but its role must be further investigated in larger cohorts.

Conflicts of interest: None to declare.

Authors' contributions: A.B. and D.B. drafted the manuscript. I.I. collected the data. A.B. performed the L-CEUS. I.I., L.C., C.B. managed the patients. Z.S., L.C. and C.I. critically revised the paper. All the authors approved the final version of the manuscript.

REFERENCES

1. Dimitroulis D, Damaskos C, Valsami S, et al. From diagnosis to treatment of hepatocellular carcinoma: An epidemic problem for both developed and developing world. World J Gastroenterol 2017;23:5282–5294. doi:10.3748/wjg.v23.i29.5282
2. Bruix J, Han KH, Gores G, Llovet JM, Mazzaferro V. Liver cancer: Approaching a personalized care. J Hepatol 2015;62:S144–S156. doi:10.1016/j.jhep.2015.02.007
3. Eisele RM. Advances in local ablation of malignant liver lesions. World J Gastroenterol 2016;22:3885-3891. doi:10.3748/wjg.v22.i15.3885
4. Wong J, Lee KF, Yu SC, et al. Percutaneous radiofrequency ablation versus surgical radiofrequency ablation for malignant liver tumours: the long-term results. HPB (Oxford) 2013;15:595-601. doi:10.1111/hpb.12014
5. Yu B, Ding Y, Liao X, Wang C, Wang B, Chen X. Radiofrequency ablation versus surgical resection in elderly patients with early-stage hepatocellular carcinoma in the era of organ shortage. Saudi J Gastroenterol 2018;24:317-325. doi:10.4103/sjg.SJG_261_18
6. Eun HS, Lee BS, Kwon IS, et al. Advantages of Laparoscopic Radiofrequency Ablation Over Percutaneous Radiofrequency Ablation in Hepatocellular Carcinoma. Dig Dis Sci 2017;62:2586-2600. doi:10.1007/s10620-017-4688-6
7. Santambrogio R, Opocher E, Costa M, Cappellani A, Montorsi M. Survival and intra-hepatic recurrences after laparoscopic radiofrequency of hepatocellular carcinoma in patients with liver cirrhosis. J Surg Oncol 2005;89:218-225. doi:10.1002/jso.20204
8. Casaccia M, Andorno E, Nardi I, et al. Laparoscopic US-guided radiofrequency ablation of unresectable hepatocellular carcinoma in liver cirrhosis: feasibility and clinical outcome. J Laparoendosc Adv Surg Tech A 2008;18:797-801. doi:10.1089/lap.2008.0039
9. Donadon M, Costa G, Torzilli G. State of the art of intraoperative ultrasound in liver surgery: current use for staging and resection guidance. Ultrashall Med 2014;35:500-511. doi:10.1055/s-0034-1385515
10. Montorsi M, Santambrogio R, Bianchi P, et al. Survival and recurrences after hepatic resection or radiofrequency for hepatocellular carcinoma in cirrhotic patients: a multivariate analysis. J Gastrointest Surg 2005;9:62-67. doi:10.1016/j.gassur.2004.10.003
11. Hsieh CB, Chang HM, Chen TW, et al. Comparison of transcatheter arterial chemoembolization, laparoscopic radiofrequency ablation, and conservative treatment for decompensated cirrhotic patients with hepatocellular carcinoma. World J Gastroenterol 2004;10:505-508. doi:10.3748/wjg.v10.i4.505
12. Tanaka S, Shimada M, Shirabe K, et al. Surgical radiofrequency ablation for treatment of hepatocellular carcinoma: an endoscopic or open approach. Hepatogastroenterology 2009;56:1169-1173.
13. Sakaguchi H, Seki S, Tsuji K, et al; Japan Society for Laparoscopic Therapy Research. Endoscopic thermal ablation therapies for hepatocellular carcinoma: a multi-center study. Hepatol Res 2009;39:47-52. doi:10.1111/j.1872-034X.2008.00410.x
14. Herbold T, Wahba R, Bangard C, Demir M, Drebber U, Stippel DL. The laparoscopic approach for radiofrequency ablation of hepatocellular carcinoma--indication, technique and results. Langenbecks Arch Surg 2013;398:47-53. doi:10.1007/s00423-012-1018-5
15. Takahashi H, Akyuz M, Aksoy E, Karabulut K, Berber E. Local recurrence after laparoscopic radiofrequency ablation of malignant liver tumors: Results of a contemporary series. J Surg Oncol 2017;115:830-834. doi:10.1002/jso.24599

16. Lee S, Kang TW, Song KD, et al. Effect of microvascular invasion risk on early recurrence of hepatocellular carcinoma after surgery and radiofrequency ablation. Ann Surg 2019 Apr 30. doi:10.1097/SLA.0000000000003268
17. Badea R, Ciobanu L. Contrast enhanced and Doppler ultrasonography in the characterization of the microcirculation. Expectancies and performances. Med Ultrason 2012;14:307-317.
18. Torzilli G, Palmisano A, Del Fabbro D, et al. Contrast-enhanced intraoperative ultrasonography during surgery for hepatocellular carcinoma in liver cirrhosis: is it useful or useless? A prospective cohort study of our experience. Ann Surg Oncol 2007;14:1347-1355. doi:10.1245/s10434-006-9278-3
19. Loss M, Schneider J, Uller W, et al. Intraoperative high resolution linear contrast enhanced ultrasound (IOUS) for detection of microvascularization of malignant liver lesions before surgery or radiofrequeny ablation. Clin Hemorheol Microcirc 2012;50:65-77. doi:10.3233/CH-2011-1444
20. Liu J, Liu Y, Huang J, Huang L, Zhao P. Real-time Monitoring of Contrast-enhanced Ultrasound for Radio Frequency Ablation. Open Med (Wars) 2017;12:474-480. doi:10.1515/med-2017-0066
21. Wiggermann P, Zuber-Jerger I, Zausig Y, et al. Contrast-enhanced ultrasound improves real-time imaging of ablation region during radiofrequency ablation: preliminary results. Clin Hemorheol Microcirc 2011;49:43-54. doi:10.3233/CH-2011-1456
22. da Silva NPB, Beyer LP, Hottenrott MC, et al. Efficiency of contrast-enhanced ultrasound for immediate assessment of ablation status after intraoperative radiofrequency ablation of hepatic malignancies. Clin Hemorheol Microcirc 2017;66:357-368. doi:10.3233/CH-179112
23. Crinò SF, D'Onofrio M, Bernardoni L, et al. EUS-guided Radiofrequency Ablation (EUS-RFA) of Solid Pancreatic Neoplasm Using an 18-gauge Needle Electrode: Feasibility, Safety, and Technical Success. J Gastrointestin Liver Dis 2018;27:67-72. doi:10.15403/jgld.2014.1121.271.eus
24. Bartos A, Breazu C, Sparchez Z, Tantau M, Iancu C, Bartos D. Radiofrequency ablation of locally advanced pancreatic tumors. The surgical approach. J Gastrointestin Liver Dis 2018;27:102-104. doi:10.15403/jgld.2014.1121.271.spz
25. Siperstein A, Garland A, Engle K, et al. Laparoscopic radiofrequency ablation of primary and metastatic liver tumors. Technical considerations. Surg Endosc 2000;14:400–405. doi:10.1007/s004640000067
26. Iavarone M, Sangiovanni A, Forzenigo LV, et al. Diagnosis of hepatocellular carcinoma in cirrhosis by dynamic contrast imaging: the importance of tumor cell differentiation. Hepatology 2010;52:1723-1730. doi:10.1002/hep.23903
27. Berber E, Siperstein AE. Local recurrence after laparoscopic radiofrequency ablation of liver tumors: an analysis of 1032 tumors. Ann Surg Oncol 2008;10:2757–2764. doi:10.1245/s10434-008-0043-7
28. Salvatore V, Gianstefani A, Negrini G, Allegretti G, Galassi M, Piscaglia F. Imaging Diagnosis of Hepatocellular Carcinoma: Recent Advances of Contrast-Enhanced Ultrasonography with SonoVue®. Liver Cancer 2016;5:55-66. doi:10.1159/000367748
29. Salvatore V, Borghi A, Piscaglia F: Contrast-enhanced ultrasound for liver imaging: recent advances. Curr Pharm Des 2012;18:2236-2252. doi:10.2174/138161212800099892
30. Sparchez Z, Radu P, Anton O, Socaciu M, Badea R. Contrast enhanced ultrasound in assessing therapeutic response in ablative treatments of hepatocellular carcinoma. J Gastrointestin Liver Dis 2009;18:243-248.
31. Li K, Su ZZ, Xu EJ, Ju JX, Meng XC, Zheng RQ. Improvement of ablative margins by the intraoperative use of CEUS-CT/MR image fusion in hepatocellular carcinoma. BMC Cancer 2016;16:277. doi:10.1186/s12885-016-2306-1
32. Shi W, He Y, Ding W, et al. Contrast-enhanced ultrasonography used for post-treatment responses evaluation of radiofrequency ablations for hepatocellular carcinoma: a meta-analysis. Br J Radiol 2016;89:20150973. doi:10.1259/bjr.20150973
33. Nicolau C, Vilana R, Bianchi L, Brú C. Early-stage hepatocellular carcinoma: the high accuracy of real-time contrast-enhanced ultrasonography in the assessment of response to percutaneous treatment. Eur Radiol 2007;17(Suppl 6):F80-F88.