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Stereotactic Radiofrequency Ablation of Hepatocellular Carcinoma: a Histopathological Study in Explanted Livers

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This retrospective study was performed to evaluate the efficacy of three-dimensional (3D)-navigated multiprobe radiofrequency ablation (RFA) with intraprocedural image fusion for treatment of hepatocellular carcinoma (HCC) by histopathological examination. From 2009 to 2018, 97 patients (84 men, 13 women; median age, 60 years; range, 1-71) were transplanted after bridging therapy of 195 HCCs by stereotactic RFA (SRFA). The median interval between the first SRFA and transplantation was 6.8 months (range, 0-71). The rate of residual vital tissue (RVT) could be assessed in 188 of 195 lesions in 96 of 97 patients by histological examination of the explanted livers using hematoxylin and eosin (H&E) and Tdt-mediated UTP nick-end labeling (TUNEL) stains. Histopathological results were compared with the findings of the last computed tomography (CT) imaging before liver transplantation (LT). Median number and size of treated tumors were 1 (range, 1-8) and 2.5 cm (range, 1-8). Complete radiological response was achieved in 186 of 188 nodules (98.9%) and 94 of 96 patients (97.9%) and complete pathological response in the explanted liver specimen in 183 of 188 nodules (97.3%) and 91 of 96 patients (94.8%), respectively. In lesions ≥3 cm, complete tumor cell death was achieved in 50 of 52 nodules (96.2%). Residual tumor did not correlate with tumor size (P = 0.5). *Conclusion:* Multiprobe SRFA with intraprocedural image fusion represents an efficient, minimally invasive therapy for HCC, even with tumor sizes larger than 3 cm, and without the need of a combination with additional treatments. The results seem to justify the additional efforts related to the stereotactic approach. (Hepatology 2019;70:840-850).

Liver transplantation (LT) represents the only curative option for both hepatocellular carcinoma (HCC) and underlying chronic carcinogenic liver disease. Tumor progression beyond conventional transplant criteria during the prolonged waiting period for transplantation may result in a substantial patient dropout rate from the waiting list.[1] Neoadjuvant locoregional therapy (LRT) is an established treatment option in HCC patients considered for LT to both decrease the waiting list dropout rate[2,3] and downstage tumors to meet transplant eligibility.[3-6] Presence of partial necrosis after LRT

Abbreviations: 3D, three-dimensional; BCLC, Barcelona Clinic Liver Cancer; CT, computed tomography; HCC, hepatocellular carcinoma; H&E, hematoxylin and eosin; IQR, interquartile range; LRT, locoregional therapy; LT, liver transplantation; RFA, radiofrequency ablation; RVT, residual vital tissue; SRFA, stereotactic radiofrequency ablation; TACE, transarterial chemoembolization; TCD, tumor cell death; TUNEL, Tdt-mediated UTP nick-end labeling; US, ultrasound.

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has been identified as a major risk factor for tumor recurrence after LT.[7-12]

A large, monocenter study[13] reported a very low HCC recurrence rate (<3% within 5 years) in patients with complete histological response after LRT compared to 10%-15% of tumor recurrence observed in patients meeting Milan criteria at explant pathology. These results were confirmed by a recent multicenter study, where only 5% of patients with no evidence of residual vital tumor (RVT) in explant histopathological exams showed a 5-year HCC recurrence compared to 40% of recurrences observed in patients having an RVT ≥2 cm.[12] Thus, the ultimate goal of neoadjuvant LRT before LT is induction of complete response.

Because of its great potential for local curative tumor control,[14-17] radiofrequency ablation (RFA) is the first choice of treatment of HCCs in this condition in many centers. In the very early tumor stage (<2 cm), therapeutic response is comparable to surgical resection.[18] However, larger tumor size bears a greater risk of recurrence in conventional ultrasound (US)- and computed tomography (CT)- guided single-probe RFA.[19,20] To overcome this limitation,[21,22] three-dimensional (3D) navigation systems have been introduced to allow for a more sophisticated 3D planning of multiple overlapping ablation zones, precise probe placement, and intraoperative assessment of the result by means of image fusion.[23] Long-term survivals after stereotactic RFA (SRFA) of intrahepatic cholangiocellular carcinomas,[24] colorectal liver metastases,[25] breast cancer liver metastases,[26] and melanoma liver metastases[27] were comparable to resection. On follow-up imaging, SRFA showed excellent local tumor control rates in primary and secondary liver tumors.[28] The question arises whether the implementation of high-end stereotactic techniques, including 3D planning, 3D guidance, and image fusion, can improve the results of thermal ablation.

The purpose of this study has been to evaluate the efficacy of SRFA for treatment of HCC by determining the tumor cell death (TCD) rate on histopathological examinations of whole-liver specimens upon subsequent transplantation. TCD was determined by hematoxylin and eosin (H&E) staining and the Tdt-mediated UTP nick-end labeling (TUNEL) assay.[29-31]

Patients and Methods

PATIENT COHORT AND INCLUSION CRITERIA

This is a single-institution, retrospective study of prospectively collected data for consecutive HCC patients who underwent LT from January 2009 to July 2018 after previous bridging therapy by SRFA. The study protocol was in conformity with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by our institutional review board. No donor organs were obtained from executed prisoners or other institutionalized persons. In all patients, the treatment plan was established by a multidisciplinary tumor board consisting of hepatologists, oncologists, transplant surgeons, and interventional radiologists. Decisions for therapy were based on tumor characteristics, Child-Pugh classification, anatomical considerations, and the general patient condition. Written informed consent for the ablation, transplantation, and prospective patient data collection for study purposes was obtained

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from all patients or their legal representatives before treatment. The patient registry for prospective data collection was approved by the institutional ethical committee. A total of 97 patients with 195 HCCs underwent LT from January 2009 to July 2018 after a previous bridging therapy by SRFA.

Inclusion criteria for SRFA as bridging to LT were as follows:

1. Tumors showed the typical imaging characteristics (with arterial hypervascularity and washout) on contrast-enhanced CT and were accessible by a percutaneous approach.
2. Prothrombin time ratio greater than 50% (prothrombin time with international normalized ratio, G1.7); and platelet count greater than 60,000 cells/mm^3 (60 cells × 109/L).
3. Absence of portal vein thrombosis and extrahepatic metastases.

RFA

A rapid-switching, multiple-electrode RFA system (Covidien, Burlington, MA) was used. It includes a 200-W generator, three internally cooled monopolar electrodes, and a rapid-switching multielectrode control; the current flows between the needle-like probe tips and two grounding pads.

SRFA

The technique of SRFA has been described in detail.[25] The entire procedure was performed during general anesthesia and muscle relaxation. Intravenous broadband antibiotics (1,500-mg cefuroxime) were administered as a single shot approximately 30 minutes before needle placement. A contrast-enhanced (100-150 mL of Iopromide [Ultravist 370; Schering AG, Berlin, Germany], 370 mg I/mL, 3 mL/s) helical CT (Somatom Open; Siemens, Erlangen, Germany) with a slice thickness of 3 mm was acquired. Images were obtained 35-40 and 70-80 seconds after initiation of contrast material injection, representing the late arterial and late portal phase. Images were obtained during breath hold by temporary disconnection of the tracheal tube from the respirator.

Using the planning software of the Treon optical frameless stereotactic navigation system (Medtronic Inc., Louisville, KY), multiple electrode probe positions were planned on the 3D-CT data set in order to cover the entire tumor volume with overlapping necrosis. After skin fiducial-based registration, an accuracy check, and scrubbing and draping, the Atlas aiming device (Elekta Inc., Schwabmünchen, Germany) was manually adjusted using the guidance software of the navigation system. Coaxial needles (15-G; Bard Inc., Covington, GA) were sequentially advanced through the targeting device to the preplanned depth during repeated temporary breath holds. To verify correct needle placements, one unenhanced CT with all coaxial needles in place was obtained and superpositioned to the contrast-enhanced planning CT (with the corresponding plans of the needle trajectories) by means of image fusion.

Thereafter, three RFA probes with a 3-cm active tip were inserted through the shorter coaxial needles, the latter being retracted to uncover the active probe exposure. At each position, ablations were performed using the switching control mode for up to three probes during the 16-minute ablation per cycle.

After hot withdrawal of all probes, a contrast-enhanced control CT in the late arterial and portal phase was obtained to exclude treatment-related complications (e.g., bleeding, pneumothorax) and confirm sufficient coverage of the tumor by the ablation zone.

Within 24 hours of treatment, a US examination was performed to exclude delayed treatment-related complications.

IMAGING FOLLOW-UP STUDIES

After SRFA, contrast-enhanced follow-up CTs (including native, late arterial, late portal, and delayed phase) were performed in 3- to 6-month intervals until LT.

IMAGE ANALYSIS

In addition to the original report, completeness of treatment was retrospectively determined in consensus and blinded by two experienced abdominal radiologists (D.P., G.E.). On follow-up images, any new foci of abnormally enhanced tissue in the late arterial phase and washout in the delayed phase that were located within or along the margin of the coagulation zone were considered tumor recurrence.

Contrast-enhanced CT images after thermal ablation and before LT were available in all patients.

HISTOPATHOLOGICAL EXAMINATION

Explanted livers were processed immediately after retrieval according to routine protocol. After formalin fixation, livers were dissected in approximately 1-cm-thick slices to identify focal lesions. All tissue areas suspicious of HCC nodules, treated or untreated, were collected, macroscopically described, and, finally, microscopically investigated.

Treated tumor areas were examined histologically by two experienced surgical pathologists (G.O., P.M.), blinded to pre-LT imaging on efficacy of RFA but working in consensus. Whenever viability of the treated HCC could not be determined in conventional H&E sections, a TUNEL assay (ApopTag; EMD Millipore Corporation, Burlington, MA) was performed. Furthermore, areas that were considered viable on H&E-stained sections were also retrospectively examined by a TUNEL assay.

The TUNEL assay was performed on formalin-fixed, paraffin-embedded tissue sections according to the instruction manual. In short, the histological slides were deparaffinized and treated with Proteinase K (20 μg/mL). Endogenous peroxidase was quenched in 3% hydrogen peroxide in phosphate-buffered saline (PBS) for 5 minutes at room temperature. After applying the equilibration buffer for at least 10 seconds at working strength, TdT enzyme was incubated in a humidified chamber at 37°C for an hour. After applying a stop/wash buffer and washing the specimens in PBS, the antidigoxigenin conjugate was incubated for 30 minutes. After washing the specimens, the brown color was developed with a peroxidase substrate and finally cells were visualized with a counterstain. Sections were dehydrated by xylene and, after having been covered with a coverslip, examined by light microscopy.

COMPARISON BETWEEN FOLLOW-UP IMAGING AND HISTOPATHOLOGICAL FINDINGS

The comparison was based on the presence or absence of any viable tumor at the ablation site by imaging and histopathological examination.

STATISTICAL ANALYSIS

The influence of several variables (tumor size, tumor stage, location, sex, and age) on treatment outcome was tested by using the Pearson correlation or Fisher's exact test for nominal variables and the *t* test for continuous variables. A threshold *P* value of 0.05 was chosen to indicate a statistically significant difference. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the last imaging follow-up before LT were calculated and compared with histological findings from explanted livers.

The statistical software program, SPSS (Version 20; IBM Inc., Armonk, NY), was used.

Results

PATIENT CHARACTERISTICS

Patient characteristics are listed in Table 1. One patient with seven lesions was excluded, because a correlation of radiological imaging and histopathological findings was not possible attributable to inadequate sampling of treated lesions from the explanted liver.

Median patient age in the final study population (n = 96) was 60 years, with a range of 1-71 (male:female = 13:83). Some 75 patients fulfilled the Milan criteria[32] and 20 patients the "up-to-seven" criteria.[33] One patient whose tumor size was exceeding the up-to-seven criteria received urgent transplantation because of RFA-induced liver failure.

Seventy-six patients with 131 HCCs underwent RFA only, whereas 51 tumors in 16 patients received RFA after unsuccessful transcatheter arterial chemoembolization (TACE). Four patients had successful resection of six nodules in total before ablation. In follow-up imaging, two lesions in 2 patients showed local recurrences after initial SRFA and were retreated.

Ninety-four of 96 patients suffered from liver cirrhosis, 2 from advanced liver fibrosis. According to Barcelona Clinic Liver Cancer (BCLC), the majority of the patient cohort (n = 58; 59.1%) was classified as early stage A, whereas 11 patients (11.2%) were staged very early stage 0, 25 patients (25.6%) intermediate stage B, 1 patient advanced stage C (1%), and 2 patients stage D (2.0%), respectively.

The etiology of liver disease was hepatitis B virus (HBV; n = 6), hepatitis C virus (HCV; n = 28),

TABLE 1. Patient Characteristics

	Patients n = 96
Age, years	60 (1-71)
Sex (female/male), n (%)	13/83 (13.5/86.5)
Cirrhosis, n (%)	94 (97.9)
– Child A, n (%)	65 (69.1)
– Child B, n (%)	27 (28.7)
– Child C, n (%)	2 (2.1)
BCLC stage	
– Very early stage (0), n (%)	11 (11.5)
– Early stage (A), n (%)	57 (59.4)
– Intermediate stage (B), n (%)	25 (26)
– Advanced stage (C), n (%)	1 (1.0)
– Terminal stage (D), n (%)	2 (2.1)
Etiology	
– FLC, n (%)	56 (60)
– HBV, n (%)	6 (6.3)
– HCV, n (%)	28 (28.7)
– Hemochromatosis, n (%)	1 (1.3)
– Cryptogenic, n (%)	3 (3.8)
Ablations, n (median/range)	120 (1/1-4)
Tumors, n	188
– No. per patient, median/range	1/1-8
– Size, cm median/range	2.5/1.0-8.0
– Location:	
– seg I, n (%)	2 (1.1)
– seg II, n (%)	14 (7.4)
– seg III, n (%)	20 (10.6)
– seg IVa, n (%)	19 (10.1)
– seg IVb, n (%)	8 (4.3)
– seg V, n (%)	18 (9.6)
– seg VI, n (%)	40 (21.3)
– seg VII, n (%)	44 (23.4)
– seg VIII, n (%)	42 (22.3)

Abbreviation: FLC, fatty liver cirrhosis.

alcoholic steatohepatitis and nonalcoholic steatohepatitis (n = 56), hemochromatosis (n = 1), and cryptogenic (n = 3).

The median number of treated tumors per patient was 1 (range, 1-8 lesions/patient), and the median tumor size was 2.5 cm (range, 1-8), including 52 lesions ≥3 cm in diameter. In total, 124 (median, 1; range, 1-3) ablation sessions were performed. Eighty patients required one, 13 patients two, and the remaining 3 patients three sessions, respectively.

Locations of tumors included all liver segments. The median number of coaxial needles per lesion was three (range, 1-17), and in lesions ≥3 cm five.

All patients were transplanted in a median time between initial SRFA and LT of 6.8 months (range, 0-71) and between last SRFA and LT of 5.7 months (range, 0-66). Median imaging follow-up time after the first and last SRFA was 7.2 months (range, 0.0-36.4) and 6 months (range, 0.0-36.4), respectively.

COMPLICATIONS AND SURVIVAL

In 125 sessions, five (4%) major complications, including liver failure (n = 1), pulmonary embolism (n = 1), diaphragmatic injury (n = 1), bleeding (n = 1), and pleural effusion (n = 1), were observed.

At the study endpoint, 20 patients had deceased. Causes of death were mycotic infections with subsequent multiorgan failure (n = 7), liver failure attributed to viral recurrence or rejection (n = 5), posttransplant neoplastic disease (n = 6), cardiac arrest (n = 1), and progressive amyotrophic lateral sclerosis (n = 1), respectively. Mean 1-, 3-, 5-, and 10-year overall survival rates dating back to the first SRFA and LT were 114.3 months, 92%, 83%, 79%, and 64%, and 83.9 months, and 84%, 76%, 73%, and 64%, respectively.

PATHOLOGICAL EXAMINATIONS OF TREATED LESIONS

Gross examination clearly revealed zones of necrosis. Whereas in cases with a recently performed ablation the HCC was still grossly recognizable, this was not true for older cases. In the latter, the necrotic zone appeared amorphic and was bordered by a fibrous capsule; histology revealed an amorphous necrotic zone as well, with debris showing no remaining cellular nor nuclear elements. In other specimens, tumor cells were still clearly recognizable, and in a few cases, they were indiscernible from living tumor cells, despite appearing necrotic during gross examination. In this situation, apoptotic cell death was assessed by a TUNEL assay (Fig. 1). Freshly apoptotic cell areas showed a distinct nuclear staining, whereas in more-advanced lesions the cytoplasm of tumor cells depicted a positive reaction as well.

Viable carcinoma was never found in the tumor center, but only in the area of the tumor margin. Residual tumor or recurrence was diagnosed when the viable tumor was in contact with the necrotic zone. Cases with a tumor-free area in between the ablation zone and the viable tumor were considered new

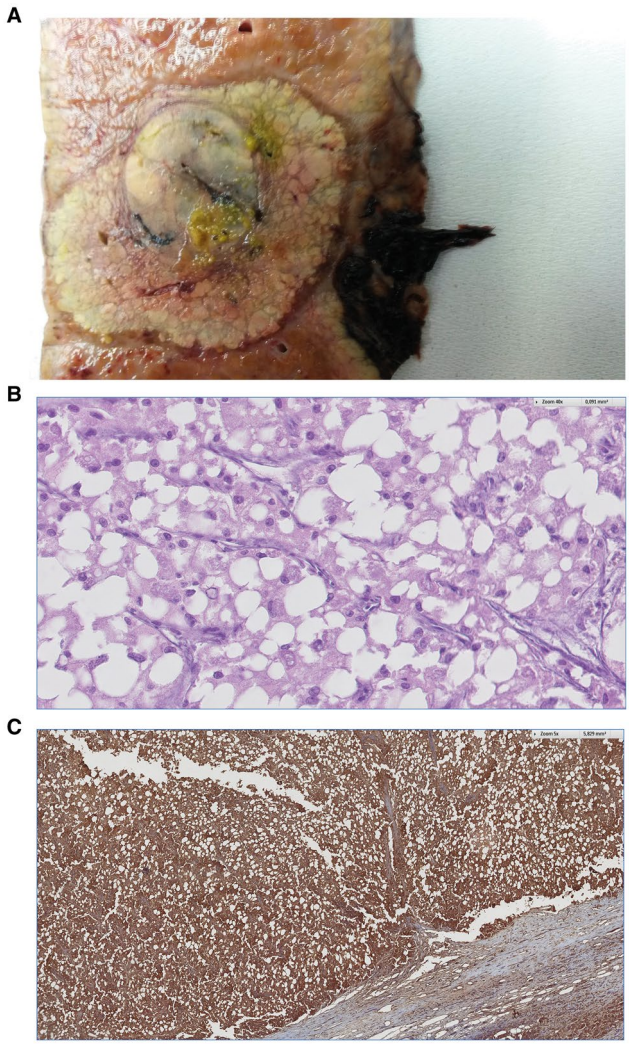

FIG. 1. Macroscopic and histopathological correlation after SRFA of one HCC in segment VI with a diameter of 35 mm in March 2018 and LT in May 2018. Macroscopic examination suggested full coverage of the lesion by the ablation zone. However, viability of the treated HCC was suspected in conventional H&E sections attributed to preserved appearing nucleoli and cytoplasm. Therefore, an additional TUNEL assay was performed. (A) Gross examination revealed complete coverage of the lesion by the ablation zone. (B) However, in conventional H&E sections viability of the treated HCC could not be excluded. (C) The additional TUNEL stain shows the typical pattern of cell death in all tumor cells, without any evidence of RVT.

manifestations of the disease. This was observed in a total of 4 patients. In 5 of 188 treated lesions, RVT was found; thus, ablative treatment was considered successful in the remaining 183 of 188 (97.3%) lesions.

CORRELATION OF IMAGING WITH HISTOPATHOLOGICAL FINDINGS

With two in five RVTs, an insufficient ablation margin had already been described in the original SRFA report (Table 2). However, LT was performed before reintervention. According to the original pretransplant imaging reports, the three remaining RVTs were missing. None of the RVTs were detected false positively, with a negative predictive value of 97.33%, specificity of 100%, sensitivity of 28.6%, and a diagnostic accuracy of 97.35%. After awareness of the results of the histopathological assessment, only one of the three remaining lesions could be identified retrospectively.

The lesions showing incomplete overall TCD were characterized by a mean size of 2.9 cm (interquartile range [IQR], 2.1-3.8). Nodules that had been successfully treated had a mean diameter of 2.4 cm (IQR, 1.4-3.0). Statistical analysis revealed that recurrence did not correlate with tumor size (P = 0.500), tumor stage (BCLC, P = 0.810), location (P = 0.609), sex (P = 0.323), and age (P = 0.801).

ANATOMICAL LOCATION OF RVT

Three in five treated tumors that showed RVT were located in a subcapsular position, two of them being adjacent to the bowel and the other two adjacent to the diaphragm/lung, respectively. One lesion was located close (0.6 cm) to the central bile ducts.

TABLE 2. Comparison of Post-SRFA Imaging Findings With Histopathological Findings

Imaging	
Complete radiological response	186/188 lesions (98.9%)
	94/96 patients (97.9%)
False-negative radiological response	3/188 (1.6%)
False-positive radiological response	0/188 (0%)
Histopathology	
Complete pathological response	
Total	183/188 lesions (97.3%)
	91/96 patients (94.8%)
Lesions ≥3 cm	50/52 (96.2%)
Mean tumor size with incomplete TCD	2.9 cm (IQR, 2.1-3.8)
Mean tumor size with complete TCD	2.4 cm (IQR, 1.4-3.0)

DETAILED DESCRIPTION OF CASES WITH RVT

In two lesions of two different patients, an incomplete ablation margin had been described in the original SRFA report and the RVT found in histopathological examination corresponded well to the residual lesions described in the interventional radiological report (Table 3). As mentioned above, in both patients LT was performed before reintervention. In the first 56-year-old male patient, RVT was found after SRFA of a singular HCC in segment IVa with 2 cm in diameter. Because of the close proximity to the central bile ducts (0.6 cm) and in order to prevent biliary stricture or biliary leakage, only one straight probe had been applied and therefore only insufficient coverage of the lesion had been achieved.

The second RVT was observed in a 60-year-old male patient after SRFA of a subcapsular lesion in segment VI with a diameter of 2.8 cm and with close vicinity to the large bowel. The remaining five treated HCC nodules of 1, 1.5, 1.5, 2, and 2.3 cm in the same patient showed complete response in the radiological as well as in the histopathological examinations.

Another 65-year-old male patient had undergone SRFA of one subcapsular subphrenic lesion in segment VIII with 3 cm in diameter and one subcapsular lesion in segment VII in two different sessions. Whereas one lesion was completely necrotic, RVT was found in the other lesion in segment VII in the histopathological exam. Even retrospectively, the lesion is not detectable on the last pre-LT imaging. The most likely reason for this lies in the five-month gap between the last pre-LT imaging to LT. A clear differentiation between RVT after SRFA and a new lesion is not possible.

The fourth RVT (Fig. 2) was described in the explanted liver of a 60-year-old male patient who had undergone SRFA of two ill-defined HCC nodules with 5.2 and 4.0 cm after unsuccessful TACE. LT was performed 4 months after SRFA. A histopathological exam of the explanted liver specimen revealed a tiny RVT (0.4 cm) in segment IVa/IVb, which had not been described in the original pre-LT magnetic resonance imaging (MRI) and which could not be identified in retrospective review either. The most likely reason for this is the inadequate image quality caused by respiration artefacts. In addition, this patient developed post-LT HCC recurrence in a hilar lymph node,

TABLE 3. Description and Clinical Correlation of Pathological Tumor Recurrences

Patient No.	Sex	Age, Years	Child-Pugh Class	Month of First SRFA	Month of Last SRFA	Month of LT	Tumor Size, cm	Tumor Location	Special Risk Attributed to Proximity to	No. of Needles	Previous Treatment of Respective Lesion	Residual Tumor Described in Original SRFA Report
28	Male	65	A	11/2010	07/2012	12/2012	2.5	VII	None	5	None	No
31	Male	56	B	11/2012	11/2012	02/2013	2.0	IVa	Bile ducts	1	None	Yes
48	Male	60	A	01/2015	02/2015	05/2015	2.8	VI	Large bowel	3	None	Yes
74	Male	60	A	08/2016	08/2016	12/2016	4.0	IVa, IVb	Diaphragm/lung	4	TACE	No
78	Male	61	B	03/2017	03/2017	03/2017	1.8	VI	Large bowel	3	None	No

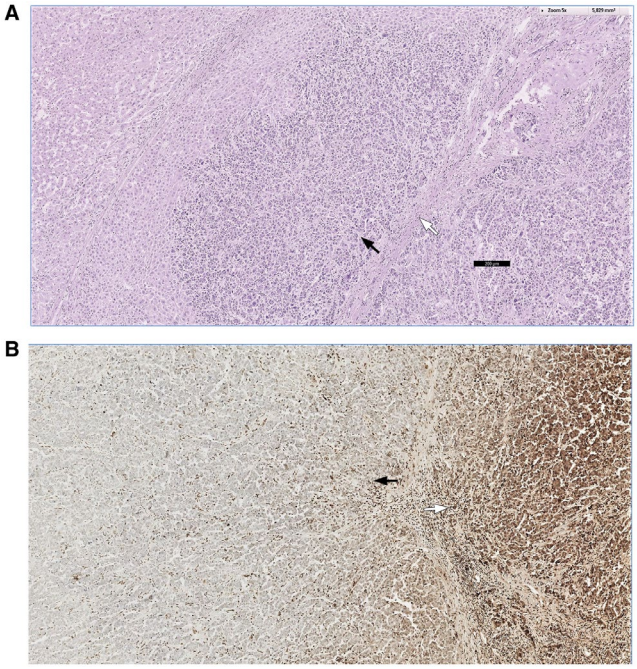

FIG. 2. Histopathologic assessment in an explanted liver of a 60-year-old male patient who had undergone SRFA of two ill-defined HCC nodules with 5.2 cm and 4.0 cm after unsuccessful TACE. Histopathological exam of the explanted liver specimen revealed a tiny RVT (0.4 cm) in segment IVa/IVb, which was not described in the original pre-LT MRI and which could also not be identified in retrospective review. (A) The main tumor on the right is surrounded by a thin fibrous capsule (white arrowhead), with an area of vital HCC on the left (black arrowhead). In the conventional H&E-stained sections differentiation between vital and necrotic tumor is hardly feasible. (B) The TUNEL assay clearly shows the large TUNEL-positive avital tumor (white arrowhead). Only a small tumor part transgressing the capsule is vital (black arrowhead) as is the adjacent normal liver tissue.

which was also not identifiable in the pre-LT radiological examinations.

The fifth RVT was diagnosed in a 61-year-old male patient who received SRFA of a single subcapsular nodule in segment VI with a diameter of 1.8 cm in 2016. Because of the close vicinity of the large bowel, three probes were positioned more centrally in order to cut the blood vessel supply on the one hand and to preserve the large bowel on the other hand. A thin crescent-shaped RVT with a size of 0.2-1.0 cm was observed in the histopathological exam at the periphery of the lesion. This residual tumor had not been reported initially, but could be identified retrospectively on the last pre-LT CT.

Discussion

According to the European Association for the Study of the Liver guidelines, locoregional treatments, including RFA, are considered a bridge to transplantation, if the waiting list exceeds 6 months.[34] The ultimate goal of thermal ablation is induction of irreversible tumor cell destruction. In small tumors (<2 cm, BCLC-0), conventional US-/CT-guided RFA achieved complete response rates in >90% of the cases, as confirmed by multiphase contrast-enhanced imaging, with an excellent long-term outcome.[34,35] However, because of a high risk of tumor recurrence, a combination of RFA and TACE is recommended for tumors exceeding 3 cm.[36]

Most clinical studies have relied on imaging characteristics for assessment of treatment response after RFA. However, a few studies used the histopathological analysis of the explanted liver after LT as their endpoint. In an early study comprising 14 patients, complete necrosis was observed in 8 of 24 nodules (33%).[2] In a multicenter study, median necrosis was only 70%. With 102 in 276 (36.9%) patients, extent of TCD ranged between 50% and 90%, with a median size of the RVT nodule being 0.7 cm. In the case of multiple nodules (n = 152), only 26 in 276 (9.4%) patients had complete TCD and 33 in 276 (21.7%) reached 50%-90% of TCD.[12] In further studies, a histological evaluation of the explanted liver revealed complete tumor necrosis in only 47%-75% of cases, with apparent discrepancies between histopathological and radiological tumor response to RFA.[20,22,32,37-40] These disagreements can be attributed to several histological features, such as thermal fixation or apoptosis, which were not included in the conventional histopathological diagnostic criteria.[41] It has been demonstrated in animals[42] and in humans[29,42-44] that H&E stain under-represents the amount of tumor necrosis on ablation sites because thermal fixation of necrotic areas may demonstrate a striking preservation of the sinusoidal HCC architectural pattern.

Martin et al.[29] performed a histological examination of explanted livers with H&E and TUNEL staining in 35 patients with 38 HCCs after RFA. The percentage of tumor cell death analyzed by H&E and TUNEL staining was 19.6% higher than that assessed by H&E staining only. In a similar study[30] including 35 patients, addition of TUNEL findings increased the ≥90% TCD from 22 of 36 (61.1%) to 26 of 36 (72.2%) and the complete (100%) TCD from 12 of 36 HCC (33.3%) to 14 of 36 HCC (38.8%), respectively.

Therefore, whenever viability of the treated HCC could not be ruled out in conventional H&E sections, an additional TUNEL assay was performed in this study. In histopathological examination, SRFA resulted in complete necrosis in 183 of 188 nodules (97.3%) and 91 of 96 patients (94.8%), respectively. In contrast to previous studies,[20,32] and most likely attributed to the additional use of the TUNEL assay, histopathological examination correlated well with radiological imaging before LT. To the best of our knowledge, these results are superior to all results published so far on conventional thermal ablation, including similar histopathological studies with additional TUNEL staining.

In addition, in most reports dealing with conventional US-/CT-guided thermal ablation tumor size has been a major prognostic factor for tumor persistence, the rate of complete necrosis ranging between 50% and 78% in small HCCs (up to 3 cm) and decreasing to rates between 13% and 43% in larger lesions.[20,22,32] Lu et al.[20] treated 47 HCCs in 24 patients by conventional CT-guided RFA. In the nodules smaller than or equal to 2.5 cm, complete ablation was achieved in 87%. In contrast, 47% of tumors >2.5 cm showed residual tumor at histopathological examination. Univariate analysis confirmed increasing size to be an indirect predictor of incomplete response to treatment (P = 0.017, Fisher's exact test). These findings reflect a well-known limitation of conventional US-/CT-guided single-probe RFA.[2]

Using the stereotactic approach with intraoperative image fusion, even in lesions ≥3 cm, complete TCD was achieved in 50 of 52 (96.2%). In contrast to previous reports about conventional RFA, no correlation between residual tumor and tumor size was found (P = 0.5).

The results of our study need to be seen in the light of limiting factors of the trial. First, viable nodules may have been missed because of the thickness of slices for histopathological evaluation. Second, evaluation of the microscopic necrosis rate with semiquantitative methods of visual estimation by two pathologists (G.O., P.M.) might result in inaccurate assessment. Nevertheless, histological findings provide a more accurate method of assessing the efficacy of LRT as compared to imaging.

Despite these limitations, our data represent the largest patient cohort in the literature who the outcome after SRFA have been evaluated for by histopathology, including TUNEL staining.

In our opinion, the results justify specialized training in stereotactic techniques as well as additional costs related to infrastructure. We therefore encourage other centers to adapt 3D planning, 3D image guidance, and image fusion to improve the outcome of ablation techniques.

In conclusion, SRFA is an effective—histologically proven—method for the treatment of HCC. Because of the creation of multiple overlapping ablation zones, even tumors exceeding >3 cm can be effectively treated without the need of a combined additional treatment. The results after SRFA are superior to conventional RFA in small and large HCCs and seem to justify the implementation of sophisticated guidance technology into clinical routine.

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