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Prognostic Factors after Percutaneous Radiofrequency Ablation in the Treatment of Hepatocellular Carcinoma. Impact of Incomplete Ablation on Recurrence and Overall Survival Rates

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ABSTRACT

Aims: To report on the long-term impact of tumor and non-tumor related parameters on local recurrence, distant recurrence and survival in patients with naïve or recurrent type hepatocellular carcinoma (HCC) treated by radiofrequency ablation (RFA).
Methods: We performed 240 RFA sessions on 133 patients with 156 HCC nodules developed on a background of liver cirrhosis and analyzed the outcomes.
Results: Contrast-enhanced ultrasound performed one month after RFA showed complete ablation in 119 out of 133 (89.65%) patients. With a median follow-up of 46 months, 3-, 5- and 7-year survival rates were 61.7%, 35.7%, and 22.6%, respectively. Previous ethanol injection and histological grade were significantly related to local tumor progression. Child-Pugh class, incomplete ablation, histological grade, previous ethanol injection, alpha-fetoprotein level before the treatment, and local recurrence were all significantly related to distant recurrence. Multivariate analysis demonstrated that age, Child-Pugh class, distant recurrence and multiple incomplete ablations were significantly related to survival.
Conclusion: Radiofrequency ablation could be locally curative for HCC, resulting in a survival longer than 7 years. Previous ethanol injection and incomplete ablations were strongly associated with poor outcomes.

Key words: hepatocellular carcinoma – radiofrequency ablation – recurrences – incomplete ablation.

Abbreviations: AFP: alpha fetoprotein; APF: arterio-portal fistula; BCLC: Barcelona clinic liver cancer; CEUS: contrast enhanced ultrasound; CT: computer tomography; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; LTP: local tumor progression; MRI: magnetic resonance imaging; MWA: microwave ablation; OLT: orthotopic liver transplantation, PAT: percutaneous ablation therapy; PEI: percutaneous ethanol injection; RFA: radiofrequency ablation; TACE: transarterial chemoembolization; US: ultrasound.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common malignancy worldwide, with its incidence increasing due to the dissemination of hepatitis B and C virus infection [1]. Cirrhotic patients in particular, have the highest risk of developing HCC. According to the European Association for the Study of Liver diseases (EASL), they should be monitored every 6 months with ultrasound. Surveillance can lead to the diagnosis of HCC at early stages, when the tumor may be treated with a curative intent. Among the curative treatment modalities, surgical resection, liver transplantation, or percutaneous ablation therapies (PATs) have all proven their efficacy [2].

Unfortunately, only 20% of HCC patients are candidates for surgical resection and the reported rate of recurrence is high even after curative resection [3]. By far, the best treatment modality is liver transplantation. However, the waiting time for orthotopic liver transplantation (OLT) is more than 1 year in Europe [4]. Among various PATs, radiofrequency ablation (RFA) is currently the most used [5, 6]. However, in developing countries percutaneous ethanol injection (PEI) was extensively used even after RFA was widely available, especially in lesions smaller than 2 cm [7, 8]. Until now, several studies that have evaluated tumor or non-tumor related parameters as prognostic factors for local or distant recurrence are controversial [9–11]. Anyway, there is scarce data about tumor or non-tumor related parameters as prognostic factors for long-term survival [12]. After gaining more and more experience

with PATs or surgical resection for HCC, we now have to deal with recurrent type HCC. The best treatment modality for recurrent type HCC is not established. There is some data about a more aggressive phenotype in case of recurrence after PATs [13]. However, there is no data regarding the impact of prior ethanol injection, before RFA, on patient outcome. The purpose of this retrospective study was to evaluate the impact of tumor and non-tumor related parameters on local recurrence, distant recurrence and survival in 133 consecutive cirrhotic patients with very early and early-stage HCC treated by RFA in a single institution. The impact of prior treatment (ethanol injection or surgical resection) on RFA outcome was also evaluated.

METHODS

We conducted a retrospective cohort study, on a consecutive series of patients with HCC on a background of liver cirrhosis in a single tertiary hospital. Informed consent was not required because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent. The Institutional Review Board of our hospital does not require approval for a retrospective study.

The criteria of patient selection were the following: tumor detectable by ultrasound (US), single nodular HCC <5 cm in maximum diameter or multinodular (up to three in number) HCCs ≤ 3 cm in maximum diameter each, tumors accessible via the percutaneous approach at US, the absence of portal vein thrombosis and known extrahepatic metastases, well compensated Child-Pugh A or B cirrhosis without ascites, prothrombin time ratio >50%, and a platelet count > 70,000 cells/mm3 (70 cells x 109/L).

The study flowchart is depicted in Fig. 1.

Diagnosis and staging of HCC

Prior to the treatment, all patients underwent imaging studies including US; contrast enhanced computed tomography (CT) and/or magnetic resonance imaging (MRI), a physical examination, and laboratory tests. The diagnosis of HCC was histologically proven in 49 patients. In the remaining 71 patients, the diagnosis was established according to non-invasive criteria as defined by EASL [12]. All biopsies were performed with a 1.2 mm (18G) Bard needle coupled on an automatic biopsy device (Biopty, Bard Radiology Division, Covington, GA).

Radiofrequency ablation procedure

All procedures were performed percutaneously in the surgical theater under local anesthesia and intravenous deep sedation using US guidance (Aloka Prosound SSD 3500SX system with a 3.5 MHz transducer) by the same operator, who had 10 year-experience in interventional ultrasound by the time the first patients were recruited. All patients underwent RFA with a 200 W generator (model 1500X; Rita Medical Systems, AngioDynamics) that was coupled to an expandable array with nine electrode tines. The needle was placed into the target and deployed to 3.0 cm, 4 cm or 5 cm (full deployment), depending on tumor size and proximity with surrounding structures. When reaching the target temperature (105^{0}C), the needle tip was continuously perfused with sodium chloride by an internal channel inside the needle throughout the ablation to keep the temperature of the tip low, thus preventing charring around. At the end of the procedure, the generator was reactivated and the needle track was ablated. Each ablation cycle lasted between 6 and 24 minutes depending on tumor size and number. For sub-capsular tumors, we avoided direct perpendicular puncture and in case of medium sized tumors (3-5 cm), multiple overlapping ablations were performed. All ablations aimed at reaching at least 0.5-mm margin of non-tumorous liver parenchyma. All the RFA procedures were performed in the surgical department using a local US machine with no possibility to perform CEUS. In our US department we had at our disposal a US machine with CEUS software. After RFA, the patients were rescanned by US 60 minutes later to detect any bleeding in the liver or the peritoneal cavity. In accordance with the Society of Interventional Radiology guidelines, major complications were defined as an unexpected event increasing the level of care, and/or prolonged hospital stay and/or permanent adverse sequelae. All the others were considered minor.

Follow-up

The short-term effects of ablation were assessed using CEUS with second generation contrast agents (SonoVue, Bracco, Milano, Italy) 1 or 2 days after ablation. In case of incomplete ablation, additional percutaneous ethanol injection was performed in the residual tumoral tissue. The efficacy of RFA was assessed one month after each procedure, using CEUS. According to CEUS results, a response to RFA

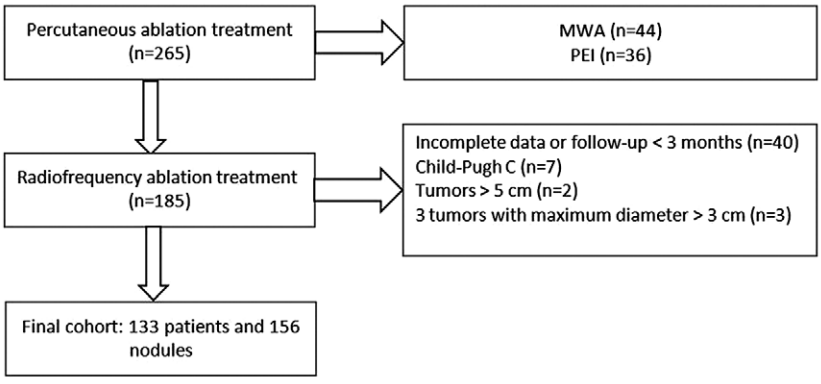

Fig. 1. Flow chart of the patients in this study. MWA, microwave ablation, RFA, radiofrequency ablation, PEI, percutaneous ethanol injection, n = number.

was classified as complete ablation (indicated by the absence of enhancing tissue at the tumor site) or incomplete ablation (when enhancing tissue was still observed at the tumor site). Patients with more than one incomplete ablation either consecutively or during the follow-up period were classified as patients with multiple incomplete ablations. Dynamic CT scans were performed 3 months after RFA in all patients to assess the treatment outcome. Thereafter, patients were followed-up with US, CEUS and/or CT and/or MRI every 3 months. After one year of follow-up, patients with no signs of recurrences were evaluated with the same imaging tools every 6 months. Local recurrence was defined as a reappearance of tumor progression at or adjacent to the treated site, and distant as the emergence of one or several tumor(s) not adjacent to the ablation zone.

Statistical analysis

Categorical variables were compared with the χ^2 test. Overall survival was defined as the interval between treatment initiation and death. Survival curves were generated by the Kaplan-Meier method and compared with the log-rank test. The observation period for tumor recurrence was defined as the interval between the first RFA and either the detection of tumor recurrence, death, or the last visit before December 31, 2017, whichever came first. We defined a session and a treatment according to the working party report on image guided tumor ablation [14]. Technical success rate was defined as the percentage of successfully eradicated macroscopic tumors, as evidenced by CEUS 1-2 days after the procedure [8]. Incomplete ablation rate was evaluated with CEUS 1 month after RFA. In cases of incomplete ablation, the local tumor progression rate was calculated from the second RFA session. A history of previous treatment was defined if the patients had been treated with a curative intent in the past and subsequently presented local or distant recurrence.

The prognostic relevance of baseline characteristics (Table I) was analyzed by univariate and multivariate Cox proportional hazards regression models. All variables taken into account in the univariate analysis were assessed in multivariate analysis. Each variable in the multivariate analysis was adjusted for different cofounders found by other studies to be relevant (age, anti-hepatitis C virus (HCV) positive status, Child-Pugh class, tumor size, tumor number, and the appearance of distant recurrence). For all regressions, we checked the proportional hazard assumption, and for multivariate analyses we checked the presence of multicollinearity. The results of multivariate analysis were presented as a hazard ratio with corresponding 95% confidence interval (CI), with p values based on the Wald statistic. All significance tests were two-tailed, and tests with a p value < 0.05 were considered statistically significant. All tests were performed using the compress package in R version 3.2.3 software.

RESULTS

Characteristics of the patients

The baseline characteristics of the patients are reported in Table I. Out of 133 patients, 112 had one nodule, 19 had two nodules and 2 had three nodules. Out of 156 nodules, 49 (36.8%) tumors were ≤ 2 cm in diameter, 73 (54.9%) tumors were between 2 and 3 cm, and 34 (25.6) were larger than 3 cm. Patients with previous treatment (PEI in 33 patients, 7 with distant recurrence and 26 with local tumor progression, LTP), surgery in 9 patients (4 with distant recurrence and 5 with LTP) were categorized as recurrent type HCC. In patients with recurrent type HCC, the initial PEI was performed with a curative intent. The median time between previous treatment and subsequent RFA treatment was 5 months, range 1-54. One day after the initial RFA session 6 patients had hyperenhancing areas within the treated lesion. In 2 out of 6 the hyperenhancing area was inside the necrotic area (<1 cm) (Fig. 2) and additional PEI was performed. The decision to perform PEI in such cases had several reasons: a) PEI was demonstrated to be equivalent to RFA in small HCC (<2cm); b) in our US department PEI is performed without deep sedation and can be very quickly scheduled; b) the possibility to discharge the patient on the same day and c) PEI is less time consuming. In the remaining 4 cases, the contrast-enhanced ultrasound (CEUS) performed 1 or 2 days after RFA depicted hyperenhancing rim like peripheral areas in which the differential diagnosis between true incomplete ablation and hyperemia or small arterio-portal shunts was challenging. In the end, 2

Table I. Baseline characteristics of the 133 patients undergoing radiofrequency ablation for hepatocellular carcinoma

Variable	
Age (years)	64.2 ±8.9
Males, n (%)	99 (74.43)
Viral infection	
HBs-Ag-positive, n (%)	27 (26.61)
Anti-HCV-positive, n (%)	74 (56.49)
Both positive, n (%)	2 (1.53)
Both negative, n (%)	28 (21.37)
Alcohol consumption > 80g/d	33 (24.81)
Child-Pugh class, n (%)	
A	122 (91.73)
B	11 (8.27)
Tumor size (cm)	2.47 ± 0.84
Tumor number/patient	1.18 ± 0.43
Serum AFP (ng/dl), n (%)	
≤ 20	49 (61.25)
20-100	23 (28.75)
>100	8 (10)
Histological Grade	
ES I, n (%)	10 (20.83)
ES II, n (%)	22 (45.83)
ES III, n (%)	16 (33.33)
Previous treatment, n (%)	
Total	42 (31.5)
Surgery	9 (6.76)
PEI	33 (24.8)

AFP: alpha fetoprotein; ES: Edmonson-Steiner, PEI: percutaneous ethanol injection; HCV: hepatitis C virus, n: number of patients; ng: nanograms. Data are expressed as means ± SD. Serum AFP level was not available in 53 patients. Histological grade was not available in 85 patients.

were true incomplete ablations and in 2 there were no signs of residual tissue and were considered as false positive (Fig. 3). Fourteen patients presented incomplete ablation one month after RFA and subsequent RFA was performed.

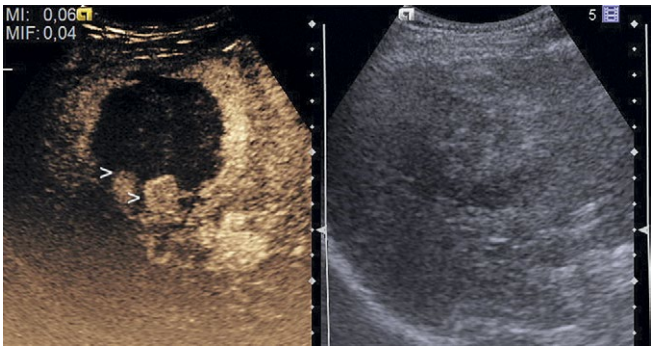

Fig. 2. Hyperenhancing areas inside a treated HCC nodule consistent with incomplete ablation. A CEUS guided PEI session was subsequently performed in the tumoral areas.

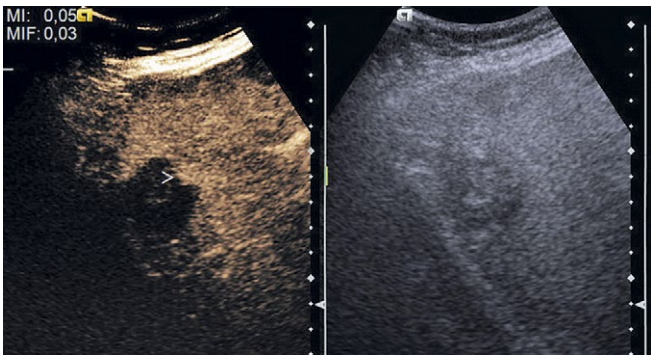

Fig. 3. Hyperenhancing area at tumor border after RFA ablation consistent with perilesional perfusion disturbance.

Radiofrequency ablation procedures and complications

For the treatment of initial tumors, 240 overall RFA sessions were performed: 1 in 94; 2 in 45; 3 in 12 and 4 in 5. When considering CEUS performed at 1 month as a gold standard, CEUS performed one or two days after RFA had an accuracy of 91.66%. CEUS performed one month after RFA showed incomplete ablation in 14 (10.5%) out of 133 patients, resulting in a technique effectiveness rate of 89.65%. All patients with residual viable tumor were retreated with RFA. Both tumor size and a history of previous treatment had no impact on technique effectiveness rate (p=0.181 and 0.11, respectively). Complete ablation rate was not significantly different in patients with solitary nodules compared to patients with multiple nodules (88.51% vs. 91.3%, p=0.77), or in patients stratified according to tumor size (93.55% vs. 91.18% vs. 81.82% in tumors ≤ 2 cm vs. 2-3 cm vs. > 3 cm, respectively, p=0.31). Further on, when stratifying the cohort in patients with tumor size ≤ 3 cm or > 3 cm, the complete ablation rate was 81.81% versus 91.30% (p=0.09).

In the recurrent type HCC, the complete ablation rate was lower as opposed to naïve HCC (83.33% vs. 91.49%). However, the difference was not statistically significant (p=0.23). In patients treated with PEI before RFA the median size of the tumors at the time of PEI was significantly lower (22 mm; 18-22.5 IQR vs. 25; 21.72-11.25 IQR) compared to those not treated with PEI (p=0.0017). After a median follow-up of 46 months (25-80 IQR), one patient was lost to follow-up after 3 months and was censored at this time point. No procedure related death was observed. Major complications were observed in 5 (3.75%) of 133 patients, 2 intraperitoneal bleeding treated conservatively, 1 needle track seeding and 2 hepatic abscesses that were drained percutaneously. Minor complications were observed in 21 (15.78%) of 133 patients and included asymptomatic arterio-portal fistula (APF) (n =18, 13.5%), skin burn (n=1, 0.8%) and asymptomatic biloma (n=1, 0.8%).

Survival

Of the 133 patients, only 1 patient was transplanted and was censored at the time of liver transplantation. As of December 2016, the number of 5-year survivors was 30 (22.55%). The median overall survival was 40 months. Survival rates of different subgroups are detailed in Table II, Fig.4 and 5.

In the univariate analysis, age, anti-HCV positive status, Child-Pugh B class, histological grade, previous PEI treatment, history of multiple incomplete ablations and appearance of distant recurrence all had a statistically significant impact on survival. In the multivariate analysis only age, Child-Pugh class B, distant recurrence, and a history of multiple incomplete ablations had a statistically significant impact on survival (Table III).

Recurrence

Recurrence developed in 96 patients (72.18%). Local tumor progression alone was found in 11 (8.3%) patients, local tumor progression with distant recurrence was found in 40 (30.1%) and distant recurrence alone was found in 45 (33.8%) patients. In case of recurrence, the retreatment strategy was based on Barcelona Clinic Liver Cancer (BCLC) classification.

The 1-, 2-, 3-, and 4-year rates of local tumor progression with or without distant recurrence were 20.4%; 31.1%; 39.7% and 44.3%, respectively. Both univariate and multivariate

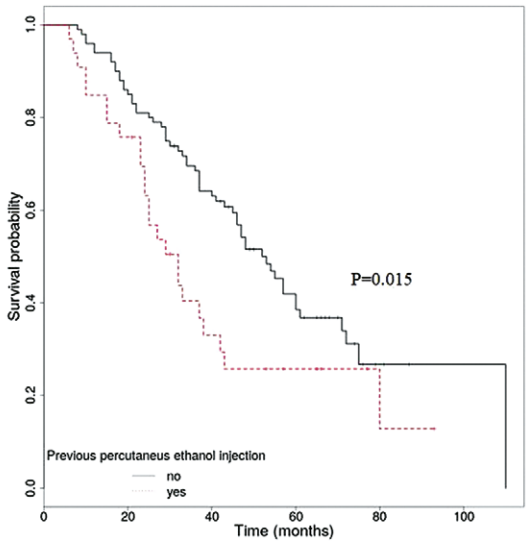

Fig. 4. Overall cumulative survival curves of patients with previous ethanol injection (n=33) and of those with no previous treatment (n=91).

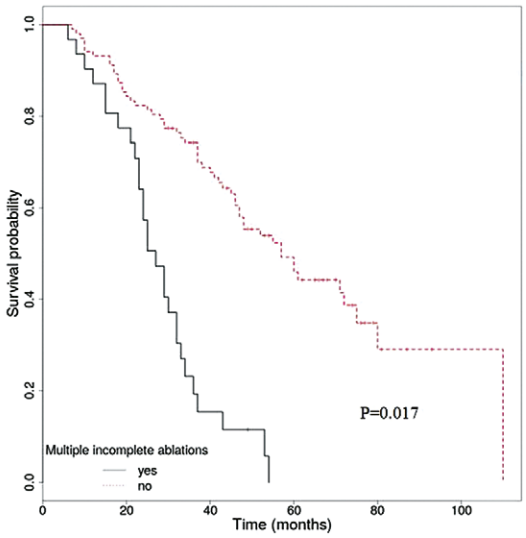

Fig. 5. Overall cumulative survival curves of patients with a history of more than one incomplete ablation (n=31) and of those with one or no incomplete ablation (n=102).

analysis demonstrated that histological grade and previous PEI therapy were associated with local tumor progression.

The 1-,2-,3-, 4- and 5-year rates of distant recurrence were 22.5%; 44.6%; 57.9%; 68.3% and 75.2%, respectively. Univariate analysis showed that Child-Pugh class, histological grade, local tumor progression and previous PEI therapy were significantly related to distant recurrence. The multivariate analysis showed that Child-Pugh class, histological grade, incomplete ablation, previous PEI therapy, AFP serum levels, and local tumor progression were significantly related to distant recurrence.

DISCUSSION

This study describes our clinical experience with RFA in the treatment of HCC at a single institution. Tumors were considered to have been completely ablated by CEUS performed 1 month after treatment in 89.65 % of the cases. The complete response rate may be lower in our study than others [10]. There are several reasons that may explain the suboptimal response rate. First, we did not have the possibility

Table II. Survival of patients undergoing radiofrequency ablation, based on tumor number, tumor size, Child-Pugh, AFP, history of incomplete ablation, type of previous treatment and Edmonson Steiner

Grading	n	Survival (%)					P value
		1 -year	2-year	3 year	5-year	7-year	
Overall Survival	133	91.7	76.6	61.7	35.7	22.6	
Tumor number							
Solitary	102	94.25	80.41	64.58	38.34	18.93	0.485
Multiple	21	86.96	69.57	56.52	32.02	25.16	
Tumor size							
≤3 cm	122	91.92	75.68	64	39.75	25.77	0.276
>3 cm	34	93.94	81.82	57.58	26.98	20.24	
Child-Pugh class							
A	122	94.26	82.76	67.36	39.02	24.66	<0.001
B	11	63.64	9.09	0	0	0	
AFP							
< 20 ng/ml	49	91.84	75.35	68.85	31.42	15.71	0.777
20-100 ng/ml	23	82.61	69.57	46.35	29.97	28.97	
>100 ng/ml	8	87.5	75	60	NA	NA	
Edmonson-Steiner							
ES I	10	90	90	80	70	35	0.063
ES II	22	82.82	59.09	45.45	21.92	21.92	
ES III	16	NA	62.5	49.92	21.09	21.09	
Incomplete ablations							
Yes	31	87.1	57.33	19.27	0	0	0.001
No	102	93.14	82.35	74.23	45.92	29.2	
Previous Treatment							
No treatment	91	93.41	81.32	67.64	38.08	26.24	0.046
PEI	33	84.85	63.13	40.4	25.71	12.86	
Surgery	9	NA	77.78	77.78	41.48	NA	
Distant recurrence							
Yes	85	94.12	74.03	52.77	28.1	18.26	0.013
No	48	87.5	81.25	77.08	49.19	28.69	

AFP: alpha fetoprotein; PEI: percutaneous ethanol injection; NA: not available.

Table III. Cox Survival Analysis of predictors for survival in patients with hepatocellular carcinoma after radiofrequency ablation

	Univariate			Multivariate		
	HR	95% CI	p	HR	95% CI	p
Age (years)	1.03	1-1.05	0.029	1.03	1-1.06	0.036
Anti-HCV positive	2.31	1.22-4.36	0.01	1.58	0.99-258	0.053
Child-Pugh Class (B/A)	8.95	4.41-18.18	<0.001	8.08	3.55-18.41	<0.001
Tumor size (>3/ ≤3 cm)	1.31	0.81-2.11	0.277	0.98	0.59-161	0.936
Tumor number (single/multiple)	0.85	0.55-1.33	0.485	1.19	0.72-1.96	0.505
Edmonson-Steiner grade (II/I)	3.82	1.11-13.18	0.034	3.09	0.8-11.98	0.103
Edmonson-Steiner grade (III/I)	3.7	1.03-13.28	0.045	3.4	0.83-13.92	0.089
Incomplete ablation	0.92	0.47-1.79	0.81	1.06	0.52-2.15	0.877
Local recurrence	1.46	0.94-2.26	0.092	1.48	0.93-2.36	0.1
Distant recurrence	1.85	1.13-3.03	0.014	1.84	1.1-3.1	0.021
Previous surgery	0.75	0.27-2.08	0.58	1.15	0.41-3.23	0.787
Previous PEI	1.76	1.08-2.86	0.023	1.44	0.85-2.45	0.171
Multiple incomplete ablations	4.14	2.52-6.8	<0.001	3.78	2.23-6.4	<0.001
AFP (≤20/20-100ng/ml)	0.84	0.44-1.59	0.588	1	0.46-2.16	0.998
AFP (>100/20-100ng/ml)	0.68	0.19-2.36	0.541	0.67	0.17-2.58	0.56

HR: hazard ratio; AFP: alpha fetoprotein. All variables in the multivariate analysis were adjusted for: age, Anti-HCV positive status, Child-Pugh class, tumor size, tumor number, and the appearance of distant recurrence.

to use intraprocedural CEUS to optimize the ablation. We used instead CEUS 1 or 2 days after ablation to confirm the complete ablation. The role of CEUS in the first or second day after the RFA is controversial, but generally it is aimed to detect incomplete ablations or possible complications. Compared to CEUS performed immediately after RFA, the 24-48 hours evaluations might be more accurate due to less periablation hyperemia. However, the results of the available studies failed to demonstrate an increased sensitivity in detecting residual tumoral tissue (27% vs. 20%) [15]. Based on the actual knowledge, the 24-hour evaluation is indicated if the periprocedural CEUS is not available. Interestingly, we obtained very high 24-48 hour sensitivity (91.4%) in detecting residual tumors. Possible explanation is a delayed evaluation (48 hours in some cases), and a high end US machine with very good CEUS performances. If further studies will confirm our findings, the 24-48 hours evaluation should be part of the CEUS follow up schema.

Table IV. Cox Survival Analysis of predictors for local recurrence in patients with hepatocellular carcinoma after radiofrequency ablation

	Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P
Age (years)	1	0.97-1.03	0.916	1.01	0.97-1.04	0.711
Anti-HCV positive	1.19	0.68-2.09	0.534	1.16	0.65-2.08	0.613
Alcohol (>80 g/day /<80 g/ day)	0.89	0.46-1.7	0.719	0.9	0.46-1.78	0.763
Child-Pugh Class (B/A)	0.82	0.2-3.4	0.781	1.02	0.23-4.44	0.983
Tumor size (>3/ ≤3 cm)	1.03	0.55-1.93	0.93	1.01	0.53-1.89	0.987
Tumor number (single/ multiple)	1.51	0.82—2.81	0.189	1.55	0.81-2.96	0.183
Edmonson-Steiner grade (II/I)	6.39	0.8-50.71	0.079	8.36	0.97-72.11	0.054
Edmonson-Steiner grade (III/I)	8.37	1.03-67.96	0.047	11.5	1.28-103.04	0.029
Incomplete ablation	1.59	0.75-3.4	0.229	1.73	0.77-3.88	0.184
Previous surgery	0.56	0.13-2.32	0.421	0.52	0.12-2.24	0.381
Previous PEI	2.06	1.13-3.78	0.019	1.97	1.01-3.71	0.037
AFP (≤20/20-100 ng/ml)	0.59	0.26-1.32	0.2	0.6	0.26-1.4	0.24
AFP (>100/20-100 ng/ml)	0.63	0.14-2.86	0.546	0.52	0.11-2.5	0.416

HR: hazard ratio; CI: confidence interval; HCV: hepatitis C virus; AFP: alpha fetoprotein. All variables in the multivariate analysis were adjusted for age, Child-Pugh class, tumor size and tumor number.

Table V. Cox Survival Analysis of predictors for distant recurrence in patients with hepatocellular carcinoma after radiofrequency ablation

	Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P
Age (years)	1.01	0.98-1.03	0.639	1.01	0.98-1.03	0.58
Anti-HCV positive	1.36	0.88-2.1	0.164	1.27	0.81-1.98	0.292
Alcohol (>80 g/day /<80 g/day)	0.81	0.49-1.35	0.422	1.07	0.56-2.05	0.828
Child-Pugh Class (B/A)	3.56	1.6-7.92	0.002	4.16	1.75-9.87	0.001
Tumor size (>3/ ≤3 cm)	1.22	0.76-1.97	0.417	1.04	0.64-1.71	0.861
Tumor number (single/multiple)	1.4	0.87-2.24	0.165	1.59	0.97-2.63	0.068
Edmonson-Steiner grade (II/I)	3.87	1.12-13.44	0.033	4.22	1.1-16.09	0.035
Edmonson-Steiner grade (III/I)	5.8	1.61-20.86	0.007	5.89	1.5-23.1	0.011
Incomplete ablation*	1.28	0.67-2.43	0.453	3.08	1.14-8.34	0.027
Previous surgery	0.85	0.34-2.12	0.725	1.12	0.43-2.89	0.816
Previous PEI	1.76	1.09-2.86	0.021	1.7	1.03-2.83	0.04
AFP (≤20/20-100 ng/ml)	0.68	0.36-1.28	0.228	0.83	0.41-1.71	0.614
AFP (>100/20-100 ng/ml) *	1.28	0.49-3.31	0.612	38.44	2.56-576.38	0.008
Local recurrence	1.85	1.2-2.84	0.005	1.87	1.21-2.89	0.005

HR: hazard ratio; AFP: alpha fetoprotein. All variables in the multivariate analysis were adjusted for: age, anti-HCV status, Child-Pugh class, tumor size, and tumor number; * adjusted for age, Anti-HCV status, Child-Pugh class, tumor size, tumor number and Edmonson-Steiner grade.

With no intraprocedural CEUS, the efficacy of RFA after one RFA session varied between 65% [16] and 83.9% [17]. Secondly, we defined incomplete ablation after the first RFA treatment while others [12, 18] defined it after up to 3 RFA procedures. Thirdly, we used CEUS rather than CT at one month to depict an incomplete ablation. However, both procedures were found to have similar accuracy to detect a residual tumor [19].

We found no host or tumor factors associated with incomplete ablation. One study [20] found that in tumors larger than 3 cm the incomplete ablation rate was significantly higher. In our study, probably due to a low number of medium size HCCs (n=34, 21.7%), the difference did not reach statistical significance.

We have showed that RFA could be associated with long-term survival for as long as 7 years. Age, Child-Pugh class, distant recurrence and multiple incomplete ablations were found to be associated with survival. Contrary to other studies [12, 21], we did not find the tumor number, tumor size and AFP serum levels as being related to survival. This is probably because of the small number of patients with more than one tumor (n=21, 15.7%) and with tumors larger than 3 cm (n=34, 21.7%). Moreover, only 8 patients from our study had an AFP level higher than 100 ng/dl. We also found age as a prognostic factor, probably because 12.78% of the patients were older than 75 years and might have died because of co-morbidities rather than advanced cirrhosis or HCC. An important observation is the fact that patients with recurrent type HCC had a worse overall survival compared to patients with naïve HCC. The largest study that compared the outcomes of RFA in naïve and recurrent type HCC found no such difference [18]. However, they only included recurrent type HCC after surgical resection, while most of our patients were previously treated by PEI. It is worth mentioning that in our study, local recurrence itself was not associated with overall survival. Interestingly, a similar result was reported by others [10]. The main explanation of this apparently paradoxical result is that the majority of these local recurrences were small enough to be completely ablated by additional RFA, highlighting the benefits of a careful post-treatment follow-up thus allowing additional procedure as soon as possible. HCC frequently recurred after RFA; most recurrences were, however, not local tumor progression, but distant recurrence. Frequent recurrences are also specific to surgical resection, where the tumor recurrence rate exceeds 70% at 5 years [22]. Due to the periodic follow-up in this study, we were able to detect most recurrences at a limited stage, which allowed us to perform another PAT in almost 62% of cases. On the other hand, repeat resection rate for first recurrence has been reported to range between 10.4 to 30.6% [22, 23]. Due to its safer profile, RFA can be performed for recurrence more easily than surgery. LTP was found more frequently in this study than in other studies (around 10% at 3 years) [10, 12, 18]. Several factors may explain this difference. First of all, we used middle range US machine for RFA guidance with medium image resolution (clearly affecting not only the visibility of the tumors, but also the result of the treatment) and no possibility to perform CEUS intraprocedurally to detect incomplete ablations. Secondly, we included patients with more advanced liver disease, only 14 of them being candidates for surgical resection. Not least, we had no data regarding the safety margin of 5 mm or proximity with large vessels in our patients. Furthermore, different from previous reports [24, 25], tumor size was not significantly related to local tumor progression in this study. One report on the 10-year outcome of RFA [12] showed similar findings. The frequency of distant recurrence in this study was similar to that reported in other studies [10, 12, 18]. The fact that Child-Pugh class has an impact on distant recurrence suggests that the severity of underlying liver disease, which is a risk factor for HCC occurrence, may also be a risk

of HCC recurrence, reinforcing the important role of the liver status in hepatocarcinogenesis.

The novelty of this study stands in the fact that both incomplete ablation and previous PEI treatment were associated with distant recurrence. We found no other study supporting this evidence. However, one experimental study showed that sublethal heat treatment transforms HCC cells to a progenitor-like, highly proliferative cellular phenotype *in vitro* and *in vivo* and suboptimal RFA accelerates HCC growth and spread [26]. It is possible that the previous PEI treatment only induced sublethal treatment in our patients and thus, these patients were more prone to develop local and distant recurrences. In a similar way, incomplete ablation also increased the likelihood of distant recurrence. This is the first report showing that sublethal treatment increases the likelihood of developing recurrences, supporting the evidence coming from experimental models of HCC [26].

In our study, RFA was a safe procedure, major complications occurred in only 3.75% of cases. Other investigators have also reported low complication rates of 2% [27]. For hepatic resection, morbidity rates of 38-47% have been reported [28, 29]. An unexpectedly high rate of asymptomatic APF was found in our study compared to others [10, 27]. A possible explanation could be the "umbrella type" of the electrode used in this study. Different from other studies [10, 27], all patients were treated with the same type of RFA electrode. Therefore, it is possible that, this type of electrode might create more APFs than others.

The retrospective analysis is the main limitation of the present study. We had no data regarding the proximity of the tumors to large vessels, the number of sub-capsular tumors, or the number of patients that might have been treated by surgery. Furthermore, not all patients had available data about AFP serum levels or ES grade. The wide range of follow-up is another limitation. Therefore, survival outcomes should be regarded carefully because they were determined in a relatively small number of patients. One further limitation is the fact that this was a single center study.

CONCLUSIONS

According to our study, RFA ablation could be currently considered as the first-line treatment of choice for patients with early stage HCC who were excluded from surgery. The overall survival was similar for both naïve and recurrent type HCC after surgical resection. Whenever possible, RFA should be performed as an initial treatment rather than PEI, since previous unsuccessful PEI treatment before RFA strongly impacts the outcome. When treating patients with HCC, all efforts should be made to completely ablate the entire tumor. One or more incomplete ablations might convert the residual HCC tissue into a more aggressive tumor.

Conflicts of interest: The authors declare no conflicts of interest.

Authors' contributions: Z.S.: substantial contribution to the concept of the study and interpretation of data; T.M. analyzed the data and drafted the manuscript; T.M, P.R., L.P.M, and M.S.: data acquisition; D.C.L.: statistical analysis; N.A.H. supervised the study and revised the manuscript for important intellectual content. All authors critically revised the manuscript and approved the final version to be published.

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