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Risk factors for recurrence beyond Milan criteria after radiofrequency ablation in transplantable small hepatocellular carcinoma

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Abstract

This study aims to determine the risk factors of recurrence beyond Milan criteria in patients with transplantable early hepatocellular carcinoma (HCC) after the first Radiofrequency ablation (RFA). 95 patients with newly diagnosed transplantable small HCC with single ≤ 3 cm were analyzed retrospectively. During the 39-month median follow-up period, 12 (21.8%) patients with HCC < 2 cm and 22 (56.4%) patients with HCC ≥ 2 cm relapsed beyond Milan criteria (p = 0.001). The 1- and 3-year recurrence rates beyond Milan criteria were 6.3% and 14.7% in HCC < 2 cm group, compared with 24.1% and 55.6% in HCC ≥ 2 cm group(p < 0.0001).HCC ≥ 2 cm, red blood cell distribution width-to-lymphocyte ratio (RLR) ≥ 18.3, alpha-fetoprotein (AFP) > 15 ng/ml and early recurrence after RFA were independent predictors of recurrence exceeding Milan criteria. For patients with transplantable early single small HCC whose tumor diameter ≥ 2cm and have higher RLR and AFP levels before first RFA and early recurrence after RFA (recurrence within 2 years), close follow-up and early liver transplantation should be initiated to obtain the best survival benefit.

Keywords

Hepatocellular carcinoma. Radiofrequency ablation. Recurrence. Milan criteria.

Introduction

HCC is the sixth most common tumor and the fourth leading cause of cancer

death in the world. For HCC with a diameter of less than 3 cm, RFA is recommended as an effective alternative therapy to hepatic resection,and is more cost-effective [1, 2].

Although RFA and hepatic resection can provide excellent outcomes for early HCC, the recurrence rate 5 years after radical treatment is still as high as 75% [3]. Meanwhile, liver transplantation (LT) has become a better choice for HCC. Studies have shown that for HCC patients with a single tumor diameter ≤ 5cm, or at most three tumors with diameter ≤ 3 cm, the long-term survival after LT is comparable to that of non-malignant cirrhosis patients receiving LT, which is the recognized Milan criteria and has become the benchmark for selecting LT for HCC patients[4, 5].

Due to the shortage of donor liver, LT can only be used as a remedial treatment after recurrence of early HCC[6-8]. However, once the tumor relapses, HCC patients after salvage LT are at increased risk of recurrence. Consequently, HCC patients will have to drop out of the transplant list once recurrence exceeds the Milan criteria[9]. However, relevant research on the risk factors of recurrence of early single small HCC exceeding Milan criteria are controversial yet[10].

The aim of this study was to determine risk factors of recurrence beyond Milan criteria in patients with transplantable single HCC ≤ 3 cm after the first RFA, and to provide evidence for clinical decision-making of transplantable small HCC.

Methods

Study design and patients

This is a single-center retrospective study. This study has been approved by the Ethics Committee of Tianjin second people's Hospital and complied with the Code of Ethics of the Helsinki Declaration. This study included 95 cases of HCC patients with transplantable single HCC ≤ 3 cm who received RFA as their first treatment in Tianjin second people's Hospital from November 2011 to October 2020 retrospectively. The detailed filtering process is shown in Fig. 1. The diagnosis of HCC is based on specific imaging findings[11] (Intense contrast uptake during the arterial phase followed by contrast washout during the venous phases in contrast-enhanced CT or MRI). After multidisciplinary team (MDT) decision-making, RFA was determined as the first-line treatment of HCC. All patients met the criteria for LT and had no transplant-related contraindications[12]. Inclusion criteria: (1) Singular HCC with diameter ≤3 cm was first

diagnosed; (2) Hepatic resection is not suitable or patients refuse to perform hepatic resection; (3) No vascular invasion was found in preoperative imaging examination. Exclusion criteria: (1) Any other HCC-related treatment prior to the first RFA; (2) Child-Pugh C patients; (3) Complicated with other malignant tumors.

RFA method and Evaluation of Therapeutic effect

Ultrasound-guided percutaneous RFA was performed. The procedures are strictly in accordance with the 2011 CSLC/CSCO/CMA Expert Consensus on radiofrequency ablation therapy for Liver Cancer. Complete ablation is defined as low echo absence of enhancement throughout the tumor region. The procedure would be repeated until there was no unablated rumor.

Data collection and follow-up

Baseline data of HCC patients were collected at the first RFA, including general demographic data, blood biochemical indexes, systemic inflammatory markers (Neutrophil-to-lymphocyte ratio (NLR), RLR, Lymphocyte-to-monocyte ratio (LMR) and Platelet-to-lymphocyte ratio (PLR), etc.). liver reserve function (Child-Pugh grade), tumor markers (such as AFP), imaging data (CT, MRI，color ultrasound, contrast-enhanced ultrasound, etc.).

The primary end-point events in this study were the incidence of recurrence beyond Milan criteria, the other events of interest were recurrence-free survival (RFS). After complete ablation, AFP and Dynamic CT were reexamined every 3 months in the first 2 years, and then monitored every half a year. If Dynamic CT showed abnormal enhancement in arterial phase around or in the liver, and decreased density in portal phase and equilibrium phase, with or without elevated AFP, it was considered as tumor recurrence. Early recurrence was defined as the first recurrence occurring within 2 years after RFA. Recurrence beyond Milan criteria was defined as the first occurrence of a single tumor > 5 cm, multiple tumors with more than 3 lesions or ≤ 3 lesions but a single tumor diameter > 3 cm, or tumor invasion of portal vessels or extrahepatic metastasis.

Statistical analysis

Statistical descriptions were utilized to summarize the baseline patient

characteristics. Among them, the counting data was expressed by frequency and constituent ratio. Based on the different distribution characteristics of the data, the measurement data that accord with the normal distribution was expressed by mean ± standard deviation (SD), did not conform to normal distribution in median (IQR: P25-P75). X-tile was used to determine the optimal cut-off of NLR, RLR, LMR, PLR and AFP in patients with HCC. Measurement data was tested by normality test firstly, then independent sample *t* test was employed for comparison between groups of normal distribution data, Mann-Whitney U or Kruskal-Wallis H test was adopted for non-parametric test if the data did not conform to normal distribution; the χ^2-test or Fisher's exact test was applied for comparison of counting data between groups. The recurrence-free survival rate and cumulative recurrence-free survival rate beyond Milan criteria were analyzed by Kaplan-Meier method. Log-Rank method was used to compare the differences of recurrence and survival between groups. The risk factors of recurrence beyond Milan criteria were determined by univariate and multivariate Cox regression. The results of multivariate analysis were expressed as hazard ratios with a 95% confidence interval (CI). SPSS 22.0 (SPSS, Inc, an IBM Company, Chicago, IL, USA), X-tile (Copyright Yale University 2003-05) and R software version 4.0.3. (R Foundation for Statistical Computing, Beijing, China) were employed for statistical analysis, and the difference of $p < 0.05$ was considered to be statistically significant.

RESULTS

Baseline patient characteristics

As shown in Table 1, according to the median tumor diameter (1.8cm), the patients were divided into two groups: HCC < 2 cm and HCC ≥ 2cm. There was no significant difference in age, gender and Child-Pugh grade between the two groups. The optimal cut-off value of NLR, RLR, LMR, PLR and AFP were 4.1, 18.3, 5.8, 65.8 and 15 determined by X-tile, and difference between these groups were not statistically significant ($p > 0.05$).

Recurrence patterns

First recurrence

All 95 patients were followed up for a median of 39 months(5-103). During the

follow-up period, a total of 60/95 (64.5%) patients relapsed, which was showed in Table 2. The overall 1-, 3- and 5-year recurrence-free survival rates were 64.4%, 38.2% and 22.9%, respectively. The 1-, 3- and 5-year recurrence-free survival rates of patients with HCC < 2 cm and were 68.6%, 53.9%, 17.6 %, while those of patients with HCC ≥ 2 cm were 55.7%, 10.3%, 6.9% respectively (p = 0.001), as shown in Fig. 2a. The median recurrence-free survival was 41 months (95% CI 27.3-54.7) for HCC < 2 cm and 21 months (95% CI 9.4-32.6) for HCC ≥ 2 cm (p = 0.001).

Recurrence beyond Milan criteria

During the follow-up period, 34/95 (35.8%) of HCC relapsed beyond Milan criteria, as detailed in Table 2. The total recurrence rates beyond Milan criteria after RFA were 12.3% and 29.9% in 1 and 3 years, respectively. For patients with HCC < 2 cm and HCC ≥ 2 cm, the 1-year and 3-year recurrence rates beyond Milan criteria were 6.3%, 14.7% and 24.1%, 55.6%, respectively (p < 0.0001), as shown in Fig. 2b. In addition, patients recurrence beyond Milan criteria were classified according to the recurrence pattern, but the differences between groups were not statistically significant (p = 0.555).

Predictors of recurrence beyond Milan criteria

Univariate Cox regression analysis showed that Child-Pugh B grade, AFP > 15 ng/ml, HCC ≥ 2 cm, RLR ≥ 18.3, LMR ≥ 5.8, PLR < 65.8 and recurrence within 2 years after RFA were significantly correlated with recurrence beyond Milan criteria. Ultimately, the multivariate analysis showed that HCC ≥ 2 cm (HR 4.52, 95%CI 1.86-10.94, p = 0.001), AFP > 15 ng/ml (HR 3.87, 95%CI 1.53-9.77, p = 0.004), RLR ≥ 18.3 (HR 10.69, 95%CI 2.7-42.37, p = 0.001) and early recurrence after RFA (HR 6.22, 95%CI 2.51-15.43, p＜0.0001) were independent predictors of recurrence exceeding Milan criteria(Table 3). Subgroup analysis of patients with HCC < 2cm showed that only 3 (10.71%) of 28 patients with AFP < 15 and RLR < 18.3 relapse exceeded milan criteria (p < 0.0001), compared with AFP > 15 with or without RLR≥18.3. Fig. 3 shows the probability of recurrence-free survival beyond Milan criteria in patients with RLR<18.3 and RLR ≥ 18.3.

Discussion

Patients with single HCC ≤ 3 cm who were not suitable for surgical treatment, but were candidates for LT, were selected for RFA as the first treatment. The incidence of complications after RFA is very small, which confirms the safety of RFA treatment[13]. Among all recurrent patients, 56.7% exceeded the Milan criteria. Obviously, this result could seriously affect the survival prognosis of patients. We found that HCC ≥ 2 cm, AFP > 15 ng/ml and RLR ≥ 18.3 at baseline and early recurrence (recurrence within 2 years after RFA) were independently associated with recurrence that exceeded Milan criteria, which is similar to previous study[10, 14].

The significant correlation between tumor diameter and recurrence and prognosis of HCC has been confirmed in many studies[15, 16]. With the increase of tumor diameter, the incidence of microinvasion is higher. For HCC with diameter ≤ 3 cm, micrometastasis and microvascular invasion are more obvious in HCC ≥ 2cm[17]. The expression level of AFP is related to the proliferation, angiogenesis and apoptosis of HCC cancer cells[18]. AFP has not only clinical diagnostic value, but also prognostic value for HCC[19]. In recent years, it has been found that de-γ-carboxyl prothrombin (DCP) and lentil agglutinin reactive AFP (AFP-L3) have better performance in the diagnosis and prognosis of HCC, but these indexes have not been widely carried out. AFP is still the most widely used diagnostic and prognostic marker of HCC at present[20, 21].

Inflammatory responses play critical roles in tumorigenesis, promotion, and metastasis[22]. Several previous studies have demonstrated that systemic inflammatory response markers such as NLR, RLR, LMR, PLR, etc. have a certain prognostic value for HCC and other malignant tumors[23-26]. Neutrophils can directly release inflammatory factors to promote tumor angiogenesis and accelerate tumor growth and metastasis[27]. On the other hand, in tumor development, reduced number of lymphocytes indicates a suppressed immune state, and reduced number of T cells and functional inhibition may weaken tumor-specific response, which will affect the survival of patients[28, 29]. Red blood cell distribution width (RDW) imply variation in the distribution of RBC volume in circulation, In patients with HCC,

elevated RDW is associated with lower RFS and poor OS[30]. As the ratio of RDW to lymphocyte, RLR has been shown to predict the prognosis of liver failure and colorectal cancer, and is related to the severity of liver cirrhosis[25, 31, 32]. Similarly, we found that RLR was an excellent predictor of relapse beyond Milan criteria.

In addition, our results suggest that recurrence within 2 years after the first RFA is a strong predictor of recurrence beyond Milan criteria, suggesting that early recurrence after RFA indicates poor prognosis even if the lesion was completely ablated. For these patients, close monitoring after RFA is essential, and LT as soon as possible is the best treatment.

There are still some shortcomings in our study, such as the relatively small number of patients included in the study. Based on clinical follow-up observations, results are less controllable than randomized controlled trials. However, this study is based on real-world data, and its conclusions are authentic and reliable.

In conclusion, for patients with transplantable early single small HCC whose tumor diameter ≥ 2cm and have higher RLR and AFP levels, there is a higher chance of recurrence beyond Milan criteria after RFA. For those patients and early recurrence after RFA (recurrence within 2 years), close follow-up and active treatment should be strengthened to obtain better outcomes.

Acknowledgments

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Table 1. Baseline characteristics of patients.

Variables	**Overall**	**Tumor size**		
	n=95	**HCC < 2cm (n=55)**	**HCC≥2cm (n=40)**	***p*-value**
Male （%）	68 (71.6)	36 (65.5)	32 (80)	0.12
Age （years）	58.9 ± 9.4	58 ± 9.7	60.1 ± 9	0.28
Child-Pugh Class (%)				0.13
A	82 (86.3)	50 (90.9)	32 (80)	
B	13 (13.7)	5 (9.1)	8 (20)	
AFP categories, ng/ml (%)				0.63
0-15	55 (57.9)	33 (60)	22 (55)	
＞15	40 (42.1)	22 (40)	18 (45)	
BMI, median （IQR）	24.01 (22.31 - 26.23)	24.39 (22.04 - 25.95)	24.02 (22.58 - 26.43)	0.85

Total bilirubin (μmol/L), median (IQR)	17.8 (13.1-24)	17.7 (12.7-22.9)	18.8 (13.13-27.65)	0.51
Albumin (g/L)	40.9 ± 6.3	42.3 ± 5.6	39.1 ± 6.8	0.02
Platelet count (´109/L), median (IQR)	109 (73-178)	130 (87-184)	99 (55-163)	0.045
Creatinine (mmol/L), median (IQR)	67 (57.5-76)	67 (56-74)	66 (58-77)	0.64
NLR (%)				0.127
< 4.1	82(86.3)	50(90.9)	32(80)	
≥4.1	13(13.7)	5(9.1)	8(20)	
RLR (%)				0.119
＜18.3	76(80)	47(85.5)	29(72.5)	
≥18.3	19(20)	8(14.5)	11(27.5)	
LMR (%)				1
＜5.8	86(90.5)	50(90.9)	36(90)	
≥5.8	9(9.5)	5(9.1)	4(10)	
PLR (%)				0.191
≥65.8	64(67.4)	40(72.7)	24(60)	
< 65.8	31(32.6)	15(27.3)	16(40)	
Hepatitis B with suppression (%)	50 (70.4)	33 (82.5)	17 (54.8)	0.01
Hepatitis C with SVR, (%)	11 (55)	10 (71.4)	1 (16.7)	0.05

Table 2. Recurrent patterns in patients with single HCC ≤ 3cm after RFA.

Recurrence patterns	**Overall**	**Tumor size**		
	n=93	**HCC < 2cm (n=54)**	**HCC≥2cm (n=39)**	***p*-value**
Recurrence (%)	60 (64.5)	28 (51.9)	32 (82.1)	0.003
Recurrence within 2 years after RFA	39 (65)	19 (65.5)	20 (62.5)	0.806
Beyond Milan criteria (%)	34 (56.7)	12 (35.3)	22 (56.4)	0.001

Reason to being classified as beyond Milan criteria（%）				0.555
Tumor size and/or number	25 (73.5)	10 (83.3)	15 (68.2)	
Macrovascular invasion	3 (8.8)	0	3 (13.6)	
Metastatic disease	6(17.6)	2 (16.7)	4 (18.2)	

Table 3. Univariate and multivariate regression predicts the recurrence rate exceeding Milan criteria in patients with single HCC ≤ 3cm after RFA.

Variables	**Univariable**		**Multivariable**	
	HR (95%CI)	***p*-value**	**HR (95%CI)**	***p*-value**
Sex (male)	2.23 (0.92-5.42)	0.076		
Age	1 (0.96-1.04)	0.95		
Child-Pugh score (ref: A)				
B	4.16 (1.81-9.58)	0.001	0.45 (0.1-2.07)	0.303
AFP (ref: ≤15 ng/ml)				
＞15ng/ml	3.78 (1.8-7.93)	＜0.0001	3.87(1.53-9.77)	0.004
HCC size (ref:＜2cm)				
≥2cm	3.35 (1.66-6.79)	0.001	4.52(1.86-10.94)	0.001
NLR (ref:＜4.1)				
≥4.1	2.26 (0.93-5.51)	0.073		
RLR (ref:＜18.3)				
≥18.3	2.74 (1.3-5.77)	0.008	10.69 (2.7-42.37)	0.001
LMR (ref:＜5.8)				
≥5.8	2.9 (1.18-7.1)	0.02	1.57(0.49-5.04)	0.451
PLR (ref: ≥65.8)				
＜65.8	0.46 (0.24-0.91)	0.026	0.56 (0.23-1.4)	0.217
Recurrence within 2 years	2.69 (1.24-5.83)	0.012	6.22 (2.51-15.43)	＜0.0001

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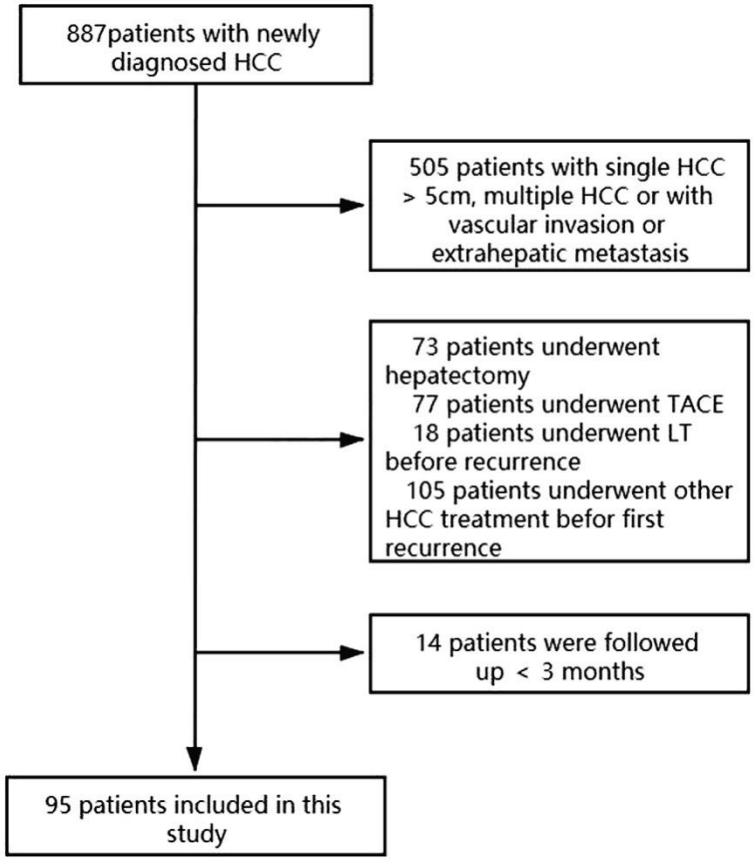

Figure 1. Inclusion and exclusion standard flow chart.

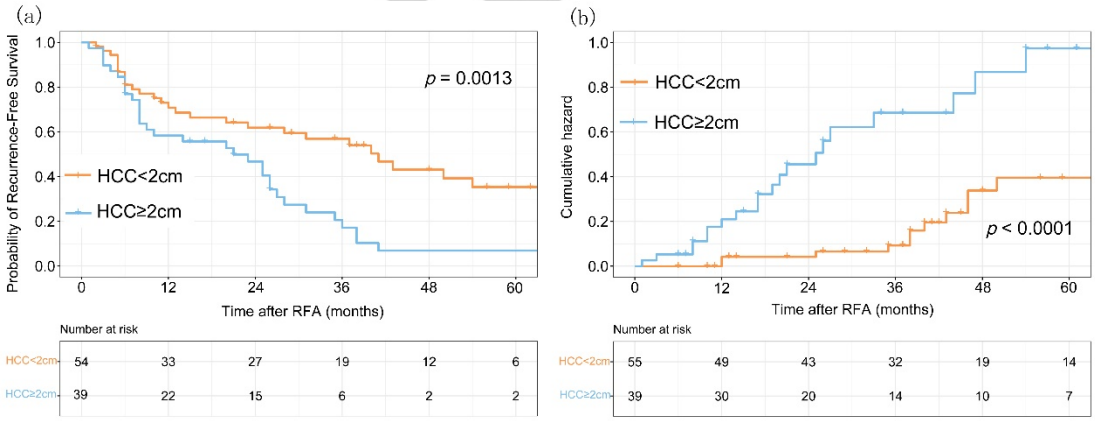

Figure 2. Recurrence-free survival probabilities and cumulative hazards of recurrence beyond Milan criteria.

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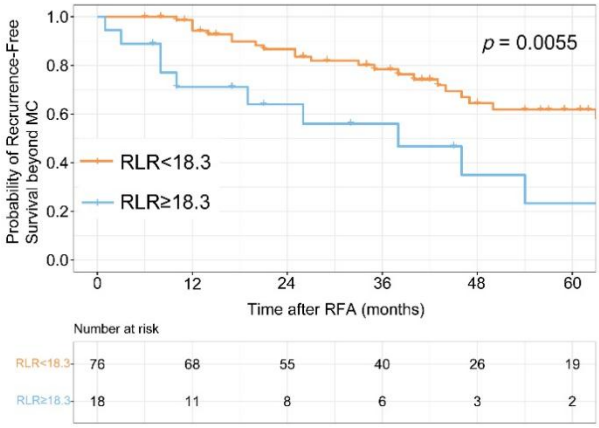

Figure 3. Recurrence-free survival beyond Milan criteria in patients with RLR<18.3 and RLR ≥ 18.3.RLR, red blood cell distribution width-to-lymphocyte ratio.