

Circulating Tumor Cell is a Clinical Indicator of Pretransplant Radiofrequency Ablation for Patients with Hepatocellular Carcinoma

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Research Article

Keywords: circulating tumor cells, radiofrequency ablation, hepatocellular carcinoma, recurrence

Posted Date: April 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-442626/v1>

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Abstract

Introduction:

It is of great significance to confirm reliable indicators for the guidance of pretransplant radiofrequency ablation (RFA) for hepatocellular carcinoma (HCC). In this study, we aim to investigate whether circulating tumor cell (CTC) status is clinical indicator for RFA before liver transplantation (LT) in HCC patients.

Method:

79 HCC patients with pretransplant CTC analysis were enrolled in this retrospective study. Clinical outcomes including recurrence and survival were compared and analyzed between patients with and without pretransplant RFA.

Result

Forty-two patients were detected as CTC- positive and 18 patients received pretransplant RFA. Recurrence was correlated with CTC count ($P= 0.024$), tumor number ($P= 0.035$), liver cirrhosis ($P= 0.001$), Milan criteria ($P= 0.003$) and University of California San Francisco (UCSF) criteria ($P= 0.001$). Kaplan-Meier analysis revealed that patients with CTC-positive had higher recurrence rate ($P= 0.0257$). For CTC-positive patients, the recurrence rate of pretransplant RFA group were significantly lower than non- pretransplant RFA group (0 vs. 46.7%, $P= 0.0236$). For CTC-negative patients, both recurrence rate and OS rate were similar and without significantly differences. In multivariate analysis, pretransplant RFA was the independent factor for recurrence ($P= 0.025$).

Conclusion

Pretransplant CTC status can guide the administration of pretransplant RFA in HCC patients which can reduce recurrence in CTC-positive HCC patients.

Introduction

Hepatocellular carcinoma (HCC) is regarded as the most common malignancy and is a leading cause of cancer-related death in the world as the 6th most common worldwide and 4th leading cancer-related death^{1, 2}. In China, HCC is the fourth most diagnosed cancers and the fourth leading cause of cancer death³. Treatment of HCC should be carefully selected to achieve promising outcomes. Hepatic resection (HR) is considered as the first-line treatment for patients without vasculature invasion in China^{4, 5}. However, the numbers of patients who are suitable for radical resection are limited and the overall 5-year recurrence rate remains high⁶. Liver transplantation (LT) has been accepted as the most effective and

curative treatment for patients with both HCC and decompensated cirrhosis⁷. In patients for whom transplantation is not an option (Tumor size and numbers is beyond Milan criteria), local and systemic treatment are available as bridging therapy for HCC. Thermal ablation, for example, radiofrequency ablation (RFA), is considered as the preferred treatment for local tumor control and used for bridging or downstaging HCC patients before LT⁸. However, the current clinical use of RFA depends on the experiences based on the traditional tumor characteristics, like tumor size, tumor numbers, and alpha-fetoprotein (AFP), and it remains controversial that whether patients will benefit from pretransplant RFA⁹⁻¹². Therefore, it is of great significance to confirm reliable indicators for the guidance of pretransplant RFA for HCC.

Our previous study confirmed that positive circulating tumor cell (CTC) count ($\geq 1/3.2\text{ml}$) was related to the early recurrence of patients with HCC after LT and showed that pretransplant CTC status may be useful to predict recurrence¹³. Whether it also be useful to guide the application of pretransplant RFA remains unclear. In this study, we aim to investigate whether CTC status is clinical indicator for RFA before LT in patients with HCC.

Materials And Methods

Patient enrollment

Between January 2016 and January 2020, 713 patients received LT in our center and 373 patients met inclusion criteria. The inclusion criteria were as follows: 18 to 75 years of age, a diagnosis of HCC confirmed by postoperative pathological examination, and follow-up of more than 1 year. The exclusion criteria were as follows: patients with perioperative or nonrecurrence-related mortality, a diagnosis of other types of tumors, and follow-up of less than 1 year (Figure 1). Afterwards, 79 patients who were tested for CTCs were enrolled in this study. Eighteen patients only received pretransplant -RFA 6 months before LT.

All the procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. The study was approved by the Institutional Ethics Committee for Clinical Research and Animal Trials of the First Affiliated Hospital of Sun Yat-sen University and informed consent waiver was granted by the IEC given the retrospective, minimal risk nature of the study. No organs from executed prisoners were transplanted into any of the patients reported in this study.

Perioperative management and Follow-up

The immunosuppressive regimen after LT was tacrolimus (Tac)+mycophenolate mofetil (MMF). The followed-up period was at least 1 year. Postoperative visits were performed on postoperative day (POD) 1-7, POD14 and each postoperative month (POM). Laboratory tests, imaging examinations and tumor markers were documented. Routine Doppler ultrasound of the liver graft blood flow and biliary tract was

performed once every 2 days for 7 days. Afterward, imaging studies were performed based on patients' clinical status or laboratory findings. HCC recurrence was diagnosed according to Guidelines for the Diagnosis and Treatment of Primary Liver Cancer (2019 edition) in China¹⁴. For deceased patients/ patients with recurrences, the date of death/ recurrence was used as the last follow-up for overall survival (OS) and progression-free survival (PFS), respectively. The follow-up deadline was January 1st, 2021.

CTCs detection

The specific method has been described in the previous study¹³. In brief, the samples (3.2ml peripheral blood collected from median cubital vein) for CTCs analysis were collected within 1 month before LT. Negative enrichment and imFISH methods were introduced to detect CTCs. The identification of enriched CTCs was performed by imFISH, which combined the FISH probes with chromosome 8 (orange) centromere probes (Abbott Molecular Diagnostics, Des Plaines, IL, USA) and anti-CD45 monoclonal antibodies (Red, Cytel). To be considered positive, CTCs needed to be hyperdiploid and have the phenotype CEP8+/DAPI+/CD45-.

RFA procedures

Indications for pretransplant RFA was evaluated by physicians primarily¹⁵. Briefly, artificial ascites was created firstly by injecting with 100ml 5% glucose solution to separate gastrointestinal tract and liver. An 18G biopsy needle was used for biopsy of lesion sent for pathological examination. Afterward, the lesions were ablated with anhydrous alcohol and injected with 3ml anhydrous alcohol by 21G PTC needle. RFA was then performed with Cool-tipTM electrode needle (ACT2020) for 10-30 minutes. The primary endpoint of RFA is to obtain a complete necrosis of liver tumors and create a safety margin of at least 10mm round the external margin of the lesion. Contrast enhanced ultrasound (CEUS) was performed on the second day after ablation to confirm the margin of tumor necrosis.

Statistical analysis

All statistical analyses of the data were performed by SPSS version 26.0. All data are expressed as the number and percentage of patients. For comparison between groups, the chi-square and Fisher's exact tests were performed for frequencies and continuous data, respectively. Cox proportional hazards model was performed for multivariate analysis. Overall and disease-free survival were compared using the Kaplan–Meier method. A P -value<0.05 was considered statistically significant.

Results

Baseline characteristics of patients with HCC in CTC-test group

To eliminate selection bias, we compared baseline data between CTC-test group and no CTC-test group (Supplementary Table 1) and there were no significant differences in age, gender, AFP, diagnosis with cirrhosis and TNM staging between groups ($P \geq 0.05$). Baseline characteristics of 79 patients enrolled in

this study are presented in Table 1. The median follow-up time was 15.7 and 17.3 months for PFS and OS, respectively. Of the 79 patients, 42 patients (53.2%) were detected as CTC-positive ($\geq 1/3.2\text{ml}$) and 18 (22.8%) patients received pretransplant RFA only. Fifteen patients (19.0%) had multinodular tumors and 31 (39.2%) patients had tumor with larger size ($\geq 3\text{cm}$). Most patients were diagnosed with Liver cirrhosis (92.4%) and Hepatic B virus (HBV) infection.

CTC result is related with the early recurrence of patients with HCC after LT

Analysis of the 79 patients revealed that 20 (25.3%) patients had a recurrence after LT (Table 2). Fifteen of 20 (75%) patients with recurrence and 27 of 59 (45.7%) patients without recurrence were positive for CTCs, respectively. The results showed that recurrence was correlated with CTC count ($\chi^2=5.128$, $P=0.024$), tumor number ($\chi^2=4.464$, $P=0.035$), liver cirrhosis ($\chi^2=11.559$, $P=0.001$), Milan criteria ($\chi^2=8.773$, $P=0.003$) and University of California San Francisco (UCSF) criteria ($\chi^2=10.225$, $P=0.001$), while there were no significant differences in other groups like preoperative AFP ($\chi^2=1.328$, $P=0.249$). Kaplan-Meier analysis revealed that patients with CTC-positive had higher recurrence rate compared with patients with CTC-negative ($P=0.0257$; Figure 2A). However, OS rate seemed to be similar and not significantly different between CTC-negative and CTC-positive groups ($P=0.5543$, Figure 2B).

Pretransplant RFA reduces recurrence in CTC-positive patients

Baseline characteristics in HCC patients with or without RFA were shown in Table 3 and no significant differences were found between groups. The association between pretransplant RFA and posttransplant tumor recurrence was analyzed in HCC patients stratified by CTC status. During the follow-up period, recurrence was observed in 15 of 42 CTC-positive patients and 5 of 37 CTC-negative patients, respectively. For CTC-positive patients, the recurrence rate of pretransplant RFA group were significantly lower than non-RFA group (0 vs. 53.3%, $P=0.0236$; Figure 2C), whereas the OS rates between the groups were similar (87.5% vs. 83.3%, $P=0.5543$; Figure 2D). For CTC-negative patients, both recurrence rate and OS rate were similar and without significant differences ($P=0.6636$ and 0.0677 , respectively; Figure 2E-F).

Furthermore, the predictive value of CTC-positive for benefit of pretransplant RFA was evaluated within clinical subgroups (Figure 3). The result showed that the recurrence rates were lower in patients with pretransplant RFA. However, no significant differences were found between patients with/without pretransplant RFA in these subgroups. Kaplan-Meier survival analyses for clinical subgroups are shown in Figure 3A-H. The efficacy of RFA to recurrence rate and OS in CTC-positive HCC patients were also evaluated in multivariate analysis. The result showed that pretransplant RFA was the independent factor for recurrence but not for OS ($P=0.025$ and 0.382 , respectively; Table 4).

Discussion

LT has been regarded as the only curative method for patients with HCC. However, posttransplant tumor recurrence was the major limitation for the survival of these patients^{16,17}. RFA is widely used for bridging or downstaging HCC patients before LT¹⁸. It remains controversial that whether patients will benefit from pretransplant RFA for the lack of reliable indicators¹⁹⁻²¹. In this retrospective study, we aimed to investigate whether CTC result could indicate the application of pretransplant RFA for HCC patients. Overall, our result showed that pretransplant RFA reduces recurrence effectively in CTC-positive patients with HCC. However, For CTC-negative patients, pretransplant RFA cannot reduce both recurrence rate and OS rate. Therefore, pretransplant CTC result may be used as an indicator for RFA before LT for patients with HCC.

Recurrences is the most negative factor affecting survival for LT patients with HCC^{7,22}. The main cause of recurrence is tumor cell dissemination via blood vessel infiltration²³. In our study, recurrence after LT was related with CTC count, Milan criteria and UCSF criteria. The recurrence of LT within the Milan criteria was 13.0%, and it is better than 43.6% for those beyond Milan criteria. The recurrence of LT within the UCSF criteria was 13.7% and it is also better than 46.4% for those beyond UCSF criteria. This indicates that Milan criteria and UCSF criteria are the promising criteria for favorable outcomes²⁴⁻²⁶. However, in China, patients tend to have HR or conservative treatment due to economic or ideological reasons, even if tumors are detected early. LT would be considered only when other treatments were ineffective or if the tumor progressed. Therefore, finding a method to predict the prognosis after LT is of great significance. CTCs were first discovered and described by Ashworth et al. in 1869²⁷. Vona et al. first explored the prognostic value of blood CTCs in patients with HCC and it was related with prognosis and recurrence in patients with HCC²⁸. CTC detection can be applied as a method for early cancer detection and prediction of recurrence or metastasis risk²⁹⁻³¹. Our previous study also revealed that CTC-positive patients had a worse prognosis after LT than those with CTC-negative¹³. In current study, it remains unclear whether patients can benefit from RFA with achieving a high degree of tumor necrosis before LT³². Agopian et al. showed in their study including 3601 recipients of LT that none of significantly differences were identified in survival between patients with/without locoregional treatment (LRT) prior to LT⁸. Our result showed that pretransplant RFA reduces recurrence effectively in CTC-positive patients with HCC. However, in clinical subgroups significantly differences were not found between patients with/without pretransplant RFA. We consider these results in traditional clinical subgroups were not conflict with previous studies^{33,34}, for CTC result is a novel biomarker to evaluate the risk of recurrence and may be a complementary biomarker of traditional clinical indicators to pretransplant RFA in HCC patients. It can be applied to the guidance for the downstaging treatment before LT³⁵. In addition, CTC result can be used to guide the pretransplant management for HCC patients. In our study, CTCs were detected in 42 of 79 (53.2%) patients before LT. The sensitivity and specificity of CTCs detection were 75% and 54.2%, respectively. The results showed that CTCs test had good sensitivity and specificity so it could be helpful to predict recurrence. An additional pretransplant RFA may be not necessarily needed for CTC-negative patients. A further prospective, multicenter, and large population study is needed to investigate the value of CTC result as an indicator in guiding pretransplant treatment.

RFA was first applied and described by Rossi et al. in 1993³⁶. In China, the use of RFA for HCC was quickly developed in recent years. Compared with HR, RFA is minimally invasive and has lower morbidity and mortality rates, especially in cases with impaired liver functions. However, the tumor size and stage are important factors for the outcome of RFA^{37,38}. Yan et al. showed in their study that larger tumor size (≥ 5 cm) would result in less complete necrosis rate and RFA alone for HCC is limited³⁹. The combination of RFA and other methods would have further benefit for patients. RFA can be used for bridging or downstaging HCC patients before LT therefore it may help to prolong time on waiting-list and reduce waiting-list mortality rate⁴⁰. The current clinical use of RFA depends on the experiences based on the traditional tumor characteristics and a novel biomarker to guide pretransplant treatment is of great significance. Our result showed that transplant RFA reduces recurrence rates effectively of patients in the status of CTC-positive. For CTC-negative patients, pretransplant RFA did not decrease the early recurrence rate, and this suggested that RFA may be not necessary for such patients. Furthermore, we made a multivariate analysis and figured that pretransplant RFA was the independent factor for recurrence.

Our study has limitations. First, the sample size is small and from a single-center institution. Larger multicenter studies are needed to determine whether pretransplant RFA can reduce recurrence in patients with HCC. Second, the value of postoperative CTCs in guiding pretransplant RFA should be analyzed in further study. For future studies, the 3-year and 5-year recurrence time and OS values should be calculated to obtain more convincing conclusions.

Conclusion

In conclusion, this study provides evidence that CTC results can be used for guiding pretransplant RFA for patients with HCC. Therefore, CTC result is a potentially promising biomarker and clinical indicator for administration of pretransplant RFA for HCC patients.

Declarations

Acknowledgement

This work was supported by the National Natural Science Foundation of China (81401324 and 81770410), the Science and Technology Planning Project of Guangdong Province (2016A020215048), the Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology (2013A061401007), the Guangdong Basic and Applied Basic Research Foundation (2020A1515011557, 2020A1515010903), and the Guangdong Provincial International Cooperation Base of Science and Technology (Organ Transplantation) (2015B050501002), China.

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Tables

Table 1 Baseline characteristics of HCC patients for the entire study

Variable		N= 79	
		n	%
Gender	Male	74	93.7
	Female	5	6.3
Age (years)	>50	47	59.5
	≤50	32	40.5
CTC count	>1	42	53.2
	≤1	37	46.8
Tumor number	>3	15	19.0
	≤3	64	81.0
Tumor diameter (cm)	>3	31	39.2
	≤3	48	60.8
PVT	Yes	16	20.3
	No	63	79.7
MVI	Yes	15	19.0
	No	64	81.0
Edmonson stage	I-II	48	60.8
	III-IV	31	39.2
Liver cirrhosis	Yes	73	92.4
	No	6	7.6
Milan Criteria	Yes	46	58.2
	No	33	41.7
UCSF Criteria	Yes	51	64.6
	No	28	35.4
HBsAg (+)	Yes	65	82.3
	No	14	17.7
AFP (ng/ml)	>400	20	25.3
	≤400	59	74.7
TNM stage	I	10	12.7
	II	23	29.1
	III-IV	46	58.2
Transplantation treatment	Yes	51	64.6
	No	28	35.4
RFA only	Yes	18	22.8
	No	61	77.2
Recurrence	Yes	20	25.3
	No	59	74.7

Abbreviations: AFP, alpha-fetoprotein; CTC, circulating tumor cells; HBsAg, Hepatitis B surface antigen; HCC, hepatocellular carcinoma; MVI, microvascular invasions; PVT, portal vein thrombosis; RFA, Radiofrequency Ablation; UCSF, University of California San Francisco.

Table 2 Analysis of Relevant factors for Recurrence of HCC in 79 Patients

Variable	N= 79		χ^2	P-value	
	Recurrence (n=20)	Non-recurrence (n=59)			
Gender, n (%)	Male	19(24.1)	55(69.6)	0.08	0.778
	Female	1(1.3)	4(5.1)		
Age (years), n (%)	≤50	11(13.9)	36(45.6)	0.224	0.636
	>50	9(11.4)	23(29.1)		
CTC count, n (%)	≤1	15(19.0)	27(30.4)	5.128	0.024
	>1	5(6.3)	32(40.5)		
Tumor number, n (%)	≤3	7(8.9)	8(10.1)	4.464	0.035
	>3	13(16.5)	51(64.6)		
Tumor diameter (cm), n (%)	≤3	10(12.7)	19(24.1)	2.036	0.154
	>3	10(12.7)	40(50.6)		
PVT, n (%)	Yes	6(7.6)	10(12.7)	1.575	0.209
	No	14(17.7)	49(62.0)		
MVI, n (%)	Yes	7(8.9)	10(12.7)	2.882	0.090
	No	13(16.5)	49(62.0)		
Edmonson stage, n (%)	I-II	9(11.4)	39(49.4)	2.790	0.095
	III-IV	11(13.9)	20(25.3)		
Liver cirrhosis, n (%)	Yes	15(19.0)	58(73.4)	11.559	0.001
	No	5(6.3)	1(1.3)		
Milan Criteria, n (%)	Yes	6(7.6)	40(50.6)	8.773	0.003
	No	14(17.7)	19(24.0)		
UCSF Criteria, n (%)	Yes	7(8.8)	44(55.6)	10.225	0.001
	No	13(16.4)	15(18.9)		
HBsAg (+), n (%)	Yes	16(20.3)	49(62.0)	0.095	0.757
	No	4(5.1)	10(12.7)		
RFA only, n (%)	Yes	2(2.5)	16(20.3)	2.448	0.115
	No	18(22.8)	43(54.4)		
AFP (ng/ml), n (%)	>400	7(8.9)	13(16.5)	1.328	0.249
	≤400	13(16.5)	46(58.2)		
TNM stage, n (%)	I	0	10(12.7)	6.334	0.042
	II	4(5.1)	19(24.1)		
	III-IV	16(20.3)	30(38.0)		

Bold *P*-values indicates statistical significance.

Abbreviations: AFP, alpha-fetoprotein; CTC, circulating tumor cells; HBsAg, Hepatitis B surface antigen; HCC, hepatocellular carcinoma; MVI, microvascular invasions; PVT, portal vein thrombosis; RFA, Radiofrequency Ablation; UCSF, University of California San Francisco.

Table 3 Baseline characteristics in HCC patients with/without RFA

Variable		N= 79		χ^2	P-value
		RFA (n=18)	non-RFA (n=61)		
Gender, n (%)	Male	16(20.3)	58(73.4)	0.899	0.343
	Female	2(2.5)	3(3.8)		
Age (years), n (%)	≤50	8(10.1)	39(49.4)	2.191	0.139
	>50	10(12.7)	22(27.8)		
CTC count, n (%)	≤1	8(10.1)	34(43.0)	0.712	0.399
	>1	10(12.7)	27(34.2)		
Tumor number, n (%)	≤3	1(1.3)	14(17.7)	2.734	0.098
	>3	17(21.5)	47(59.5)		
Tumor diameter (cm), n (%)	≤3	6(7.6)	9(11.4)	3.119	0.077
	>3	12(15.2)	52(65.8)		
PVT, n (%)	Yes	3(3.8)	13(16.5)	0.186	0.667
	No	15(19.0)	48(60.8)		
MVI, n (%)	Yes	3(3.8)	12(15.2)	0.082	0.775
	No	15(19.0)	49(62.0)		
Edmonson stage, n (%)	I-II	11(13.9)	37(46.8)	0.001	0.972
	III-IV	7(8.9)	24(30.4)		
Liver cirrhosis, n (%)	Yes	17(21.5)	56(71.0)	0.138	0.710
	No	1(1.3)	5(6.3)		
Milan Criteria, n (%)	Yes	14(11.4)	32(40.5)	3.663	0.056
	No	4(5.0)	29(36.7)		
UCSF Criteria, n (%)	Yes	15(18.9)	36(45.5)	3.592	0.058
	No	3(3.7)	25(31.6)		
HBsAg (+), n (%)	Yes	14(17.7)	51(64.6)	0.324	0.569
	No	4(5.1)	10(12.7)		
AFP (ng/ml), n (%)	≤400	3(3.8)	17(21.5)	0.922	0.337
	>400	15(19.0)	44(55.7)		
TNM stage, n (%)	I	1(1.3)	9(11.4)	1.723	0.423
	II	7(8.9)	16(20.3)		
	III-IV	10(12.7)	36(45.6)		

Abbreviations: AFP, alpha-fetoprotein; CTC, circulating tumor cells; HBsAg, Hepatitis B surface antigen; HCC, hepatocellular carcinoma; MVI, microvascular invasions; PVT, portal vein thrombosis; RFA, Radiofrequency Ablation; UCSF, University of California San Francisco.

Table 4 Multivariate analysis to identify independent risk factors of progression free survival and overall survival in CTC- positive HCC patients.

Variable	Progression-free survival		Overall survival	
	HR (95%CI)	P-value	HR (95%CI)	P-value
Tumor number (≤3)	3.059 (0.672~13.394)	0.148	0.380 (0.005~30.134)	0.664
Tumor size (≤3cm)	0.348(0.051~2.395)	0.284	2098.606(0~5.316*10 ⁹)	0.934
Edmonson stage (III-IV)	0.693 (0.136~3.523)	0.659	1.297 (0.068~24.657)	0.863
TNM stage (III-IV)	1.394 (0.340~5.721)	0.644	8.755 (0.272~281.503)	0.220
AFP (≤400ng/ml)	2.047 (0.340~12.329)	0.434	1.944 (0.04~91.723)	0.735
HBsAg (+)	0.348 (0.054~2.259)	0.269	0.045 (0.000~61.85)	0.216
Adjuvant RFA (Yes)	0.076 (0.008~0.724)	0.025	0.140(0.002~11.449)	0.382

Bold *P*-values indicates statistical significance.

Abbreviations: AFP, alpha-fetoprotein; CTC, circulating tumor cells; HBsAg, Hepatitis B surface antigen; HCC, hepatocellular carcinoma; MVI, microvascular invasions; PVT, portal vein thrombosis; RFA, Radiofrequency Ablation; UCSF, University of California San Francisco.

Figures

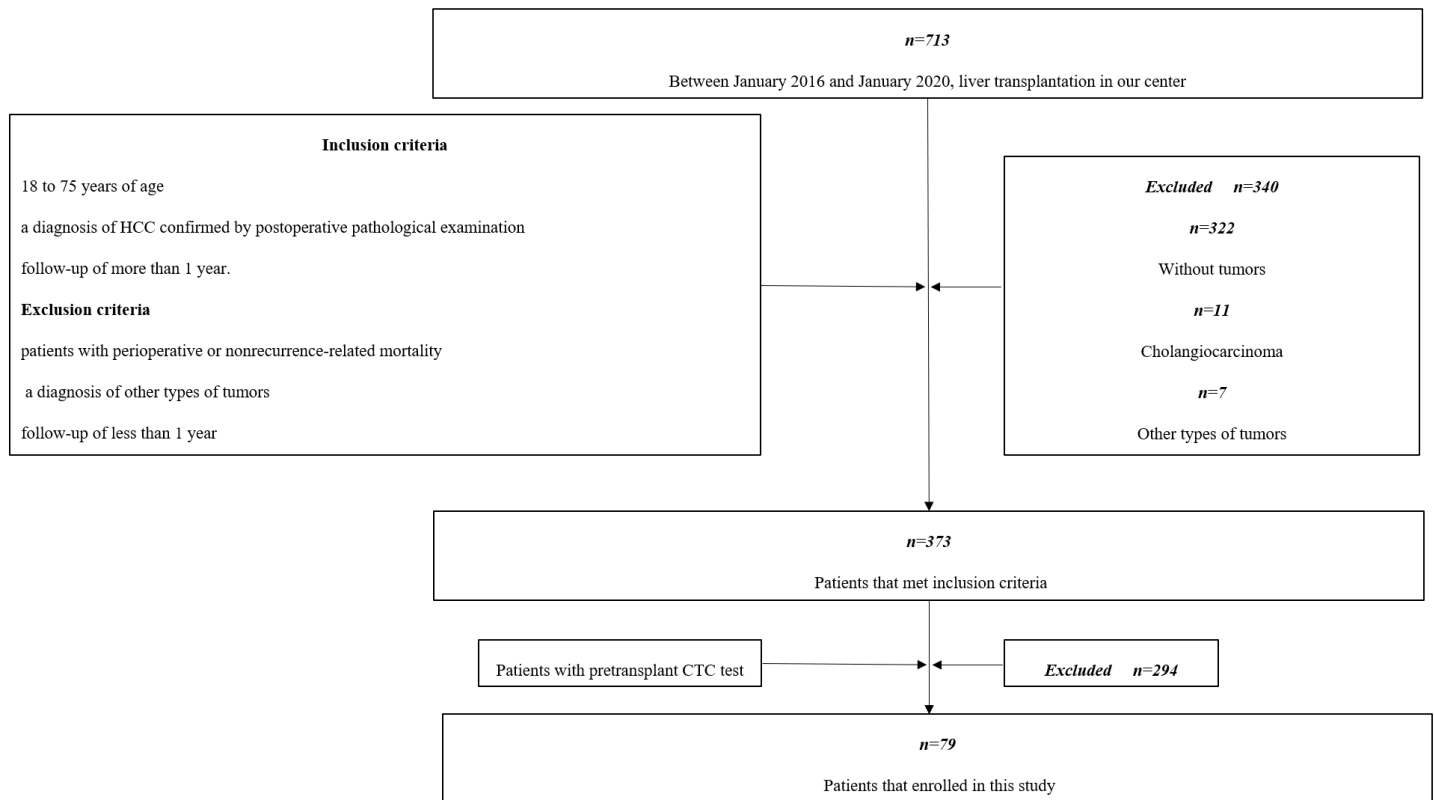


Figure 1

Flow chart for patients' selection in this study

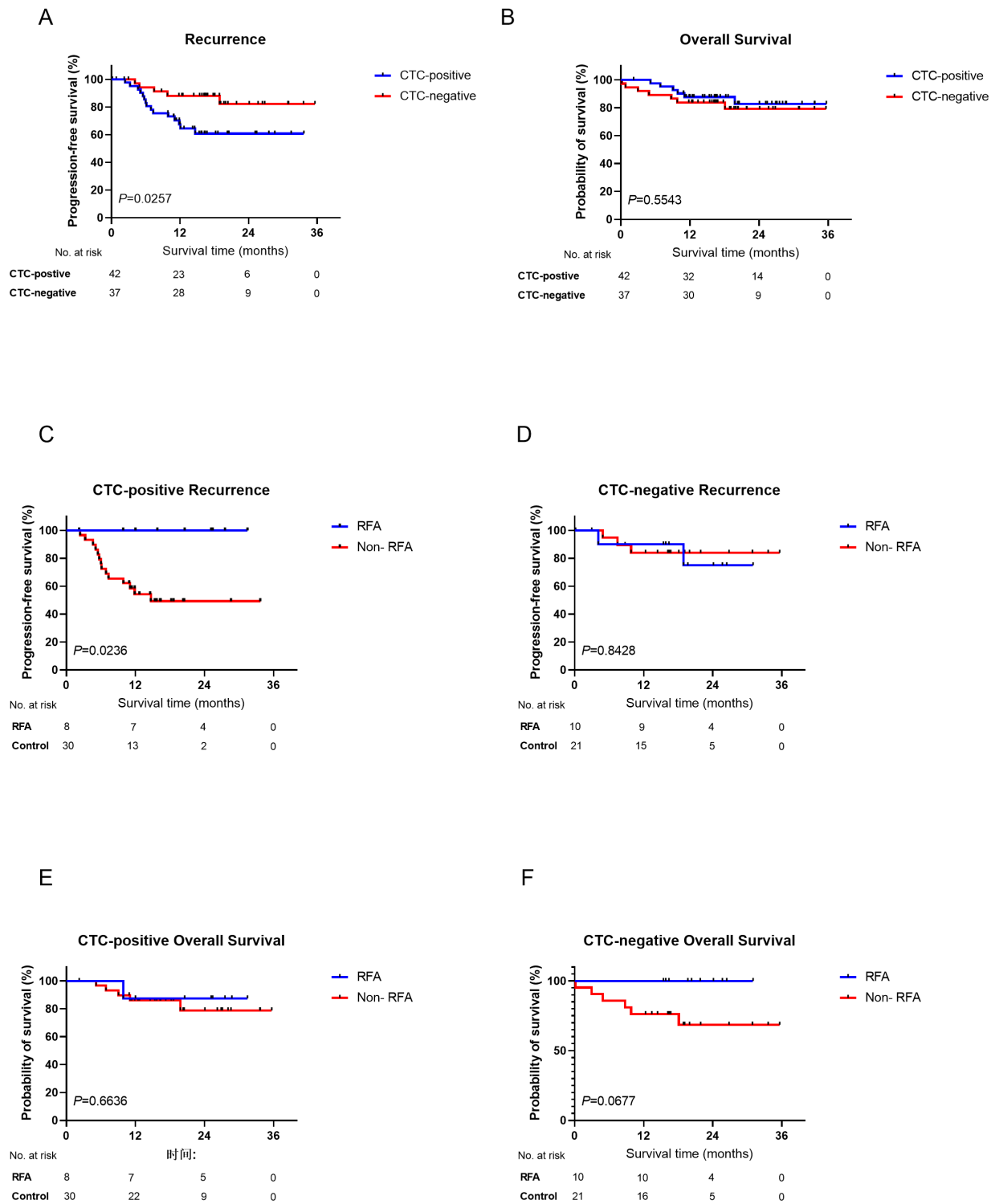


Figure 2

Comparison of PFS and OS between different groups of HCC patients. A: PFS between CTC-positive and CTC-negative groups; B: OS between CTC-positive and CTC-negative groups; C: PFS between RFA and non-RFA groups in CTC-positive HCC patients; D: PFS between RFA and non-RFA groups in CTC-negative HCC patients; E: OS between RFA and non-RFA groups in CTC-positive HCC patients; F: OS between RFA and non-RFA groups in CTC-negative HCC patients.

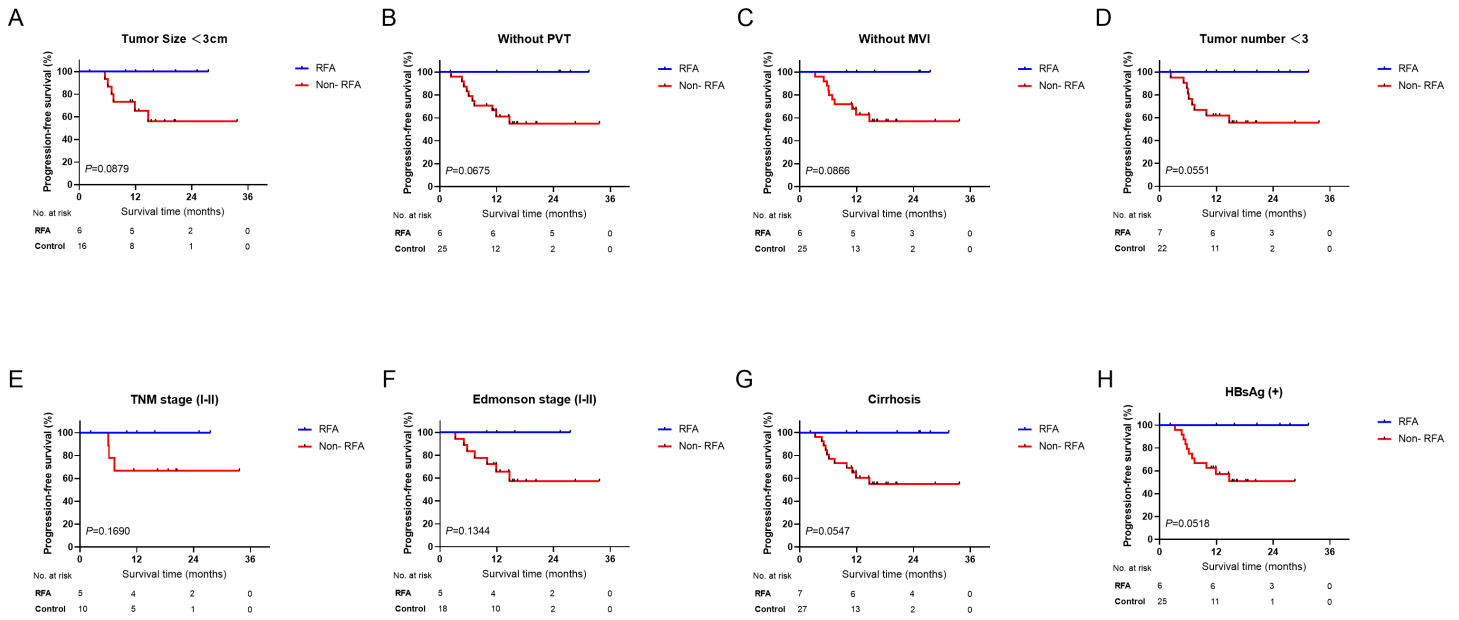


Figure 3

Kaplan-Meier analysis of PFS in subgroups of CTC-positive HCC patients. A Tumor size $\leq 3\text{cm}$; B Without PVT; C Without MVI; D Tumor number ≤ 3 ; E Tumor stage (I-II); F Edmonson stage (I-II); G With cirrhosis; H HBsAg (+). PVT, portal vein thrombosis; MVI, microvascular invasions; HBsAg, Hepatitis B surface antigen.