

The Predictive Value of Vessels Encapsulating Tumor Clusters in Treatment Optimization for Recurrent Early-Stage Hepatocellular Carcinoma

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Research

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Abstract

Background. The vessels encapsulating tumor clusters (VETC) pattern is an effective predictor of survival in patients with hepatocellular carcinoma (HCC) after resection. The predictive value of VETC in recurrent early-stage HCC remains unclear. Therefore, the aim of the present study was to investigate the prognostic significance of VETC in patients with recurrent early-stage HCC after repeat hepatic resection (RHR) or radiofrequency ablation (RFA).

Methods. From December 2005 to December 2016, 138 patients who underwent RHR and 188 patients who underwent RFA were enrolled. VETC was evaluated by immunohistochemical staining for CD34. The survival outcomes of treatment for patients with or without the VETC pattern was investigated.

Results. Among VETC-positive HCC patients, 50 patients underwent RHR, and 69 patients underwent RFA; among VETC-negative HCC patients, 88 patients underwent RHR, and 119 patients underwent RFA. There was no significant difference between the RHR and RFA groups in disease-free survival (DFS) or overall survival (OS) as determined by univariate analysis of the whole cohort. In the subgroup analysis of the VETC-positive cohort, the patients in the RHR group had a longer median DFS time compared to those in the RFA group (15.0 vs 5.0 months, $P=0.001$). Similarly, the patients in the RHR group had a longer median OS time compared to those in the RFA group (39.5 vs 19 months, $P=0.001$). In the VETC-negative cohort, there was no significant difference in DFS and OS rates between the RHR and RFA groups ($P>0.05$).

Conclusions. The results of our study suggested that RHR was relatively safe and superior to RFA in improving survival outcomes for recurrent early-stage HCC after initial hepatectomy. Furthermore, the VETC pattern may represent a reliable marker for selecting HCC patients who may benefit from RHR.

Background

Hepatocellular carcinoma (HCC) is characterized by high vascularization and rapid tumor progression, as well as an extremely poor prognosis[1–3]. Although surgical resection is commonly chosen as a curative treatment for HCC, long-term outcomes are not yet satisfactory, as approximately 70% of patients experience recurrence within 5 years[4]. Approximately 30% of patients with recurrent HCC are diagnosed in the early stage, which has a relatively favorable prognosis[5, 6]. However, guidelines for the management of recurrent early-stage HCC remain controversial and poorly defined.

Available management options for recurrent early-stage HCC are almost identical to those for primary HCC. Repeat hepatic resection (RHR) continues to be the conventional option for recurrent early-stage HCC with preserved liver function and residual liver volume, and advances in surgical techniques have resulted in improved safety in RHR[4, 7, 8]. In addition, as a minimally invasive option, radiofrequency ablation (RFA) has emerged as another alternative treatment modality for early-stage HCC[9, 10]. Several studies have previously recommended RHR when possible in the treatment of recurrent HCC[11–13]. However, conflicting data have shown that RFA, with relatively few complications, could achieve survival

outcomes comparable to those of RHR for recurrent early-stage HCC[14–16]. Therefore, the optimal strategies for recurrent HCC remain unclear and controversial.

We and other researchers have identified two microvessel types in HCC by their distinct morphologic features: capillary-like with small, scattered capillaries having no or narrow lumen and sinusoid-like that form a cobweb-like pattern and encapsulate tumor clusters, also named vessels encapsulating tumor clusters (VETC)[17, 18]. The VETC pattern was found to be consistently and easily detectable in HCC and to indicate a poorer prognosis in patients after recurrence[19, 20]. Moreover, the VETC pattern may act as predictor of sorafenib benefit in patients with HCC, especially those with VETC. VETC can be an indicator for guiding the treatment of patients with recurrent HCC[21]. However, there are no studies regarding the prognostic value of the VETC pattern in recurrent early-stage HCC.

In this study, we used tissue microarrays containing 326 HCC samples from our center to compare the survival outcomes of recurrent early-stage HCC treated by RHR or RFA and explore whether VETC pattern could guide the identification of appropriate candidates for optimization treatment.

Methods

Patients

From December 2005 to December 2016, a total of 1206 HCC patients developed intrahepatic recurrence after initial R0 resection. Recurrence was diagnosed either by histologic findings or by the noninvasive criteria used by the American Association for the Study of Liver Diseases. Among the patients, 144 (11.9%) were amenable to RHR, and 203 (16.8%) received RFA. The inclusion criteria were as follows: (a) age between 18 and 75 years; (b) first intrahepatic recurrent HCC after curative hepatectomy; (c) single lesion ≤ 3 cm or 2–3 lesions ≤ 3 cm recurrent HCC; (d) no radiologic evidence of macroscopic vascular invasion or extrahepatic metastasis; (e) RHR or RFA performed as the initial treatment for recurrent HCC; and (f) Child-Pugh class A or B. Thus, six patients in the RHR cohort were excluded, namely, four patients who received palliative repeated resection and two patients with other malignancies. Fifteen patients in the RFA cohort were excluded, including five patients older than 75 years, four patients with extrahepatic metastasis, and six patients whose samples were not sufficient for immunohistochemistry staining. Finally, a total 326 patients were enrolled into our study, including 138 patients who underwent RHR and 188 patients who underwent RFA as the first treatment for recurrent early-stage HCC. The study was approved by the Institutional Review Board of our center and conducted according to the ethical guidelines of the Declaration of Helsinki.

Treatment strategy

Treatment for recurrent recurrent early-stage HCC was performed as previously described[22]. RHR was assigned when there was the possibility for the complete removal of all tumors while retaining a sufficient liver remnant, with an expected remnant liver volume of no less than 250 ml/m², as assessed by our multidisciplinary team. Resection was avoided if patients had gross ascites, severe portal

hypertension, or an inadequate liver remnant. Reasons for assigning RFA instead of RHR included psychological resistance to invasive treatment, refusal of general anesthesia, and an insufficient liver remnant.

Immunohistochemistry staining

Immunohistochemical staining for CD34 was carried out using a sensitive streptavidin-biotinylated horseradish peroxidase complex system (Catalyzed Signal Amplification System, DAKO, Carpinteria, CA) according to the manufacturer's instructions. Formalin-fixed, paraffin-embedded sections were deparaffinized in xylene and rehydrated in a graded series of ethanol. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 10 min. Antigen retrieval was performed by microwave pretreatment in 100 W citrate buffers for 5 min and 30 W for 25 min. Then, the sections were incubated at 4 °C overnight with mouse anti-human CD34 monoclonal antibody (mAb; working dilution 1:200, QBEnd10, DAKO). After washes using TBS with 0.1% Tween 20, the sections were incubated at room temperature with biotinylated rabbit anti-mouse secondary antibody for 30 min followed by TBS washes. The sections were then incubated with streptavidin-biotin complex for 15 min. All sections were counterstained with hematoxylin.

Outcome measures

Immunohistochemical staining was independently evaluated using the same diagnostic criteria by two pathologists who were blinded to the patient data. In case of discrepancy, the specimens were referred to a third observer, and the majority decision was considered final. Using a Leica inverted research microscope (Leica Microsystems, Wetzlar, Germany), the slides were examined under 100 × magnification to identify the highest vascular density area within the tumor, and five representative fields were observed at a higher magnification of 200×. Microvascular invasion (MVI) was defined as microscopic tumor invasion identified in the portal or hepatic vein of the surrounding liver tissue contiguous to the tumor[23]. Tumor differentiation was histologically graded according to the Edmondson system[24].

Statistical analysis

Disease-free survival (DFS) and overall survival (OS) rates were calculated with the life table method, and the survival time was calculated from the day of surgery to the day of death or the most recent follow-up visit. The patient survival curves were compared using the Kaplan-Meier method and analyzed using the log-rank test. Chi-square tests and Spearman's rank tests were used to evaluate the univariate correlation between the biological parameters and clinicopathological variables, as well as the recurrence. Multivariate Cox proportional hazards models were performed for the identification of relevant prognostic factors. All statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS version 19.0, IBM, Armonk, NY, USA). The significance level was defined as two-sided ($P < 0.05$).

Results

Subject characteristics

Table 1 summarizes the baseline characteristics of patients with recurrent early-stage HCC. In all, 80 patients were VETC-positive, and 145 patients were VETC-negative. We also found 101 patients with mixed VETC and capillary-like microvessels in the tumor tissue simultaneously (Fig. 1). Lastly, 119 patients were classified as VETC-positive (defined as staining of VETC > 50%), and 207 patients were classified as VETC-negative (defined as staining of capillary-like microvessels > 50%) (Fig. 2).

Table 1
 Patent characteristics at the time of recurrence

Variables	VETC (+)			VETC (-)		
	RHR	RFA	<i>P</i>	RHR	RFA	<i>P</i>
Age, y	49.5 ± 10.9	48.9 ± 10.7	0.822	51.51 ± 10.3	49.4 ± 11.2	0.498
Sex			1.000			0.647
Male	47	64		78	108	
Female	3	5		10	11	
HBsAg			0.427			0.201
Positive	41	61		76	94	
Negative	9	8		12	25	
Background liver			0.708			0.137
Normal	19	29		34	34	
Cirrhosis	31	40		54	85	
Histological grade			0.850			0.776
Well differentiated	21	27		38	48	
Poorly differentiated	29	42		50	71	
Microvascular invasion			0.136			0.472
Present	18	35		14	24	
Absent	32	34		74	95	
ALB at recurrence, g/L	41.9 ± 6.6	42.0 ± 3.3	0.154	42.6 ± 5.8	42.4 ± 7.7	0.488
TBIL at recurrence, umol/L	15.5 ± 4.7	14.7 ± 4.9	0.922	15.5 ± 5.6	15.2 ± 5.9	0.147
HGB at recurrence, g/L	143.6 ± 17.8	143.9 ± 16.8	0.421	144.3 ± 22.4	142.7 ± 24.0	0.777
AFP at recurrence, ng/ml			0.838			0.884
> 20	35	50		56	77	
≤ 20	15	19		32	42	
Child-Pugh score at recurrence			0.488			0.363
5	42	54		73	94	

	VETC (+)		VETC (-)			
6	8	15	13	25		
Tumor size at recurrence, cm	2.4 ± 0.6	2.2 ± 0.5	0.472	2.4 ± 0.5	2.3 ± 0.4	0.630
Tumor multiplicity at recurrence			0.451			0.158
Solitary	44	57		75	91	
Multiple	6	12		13	28	
Time to recurrence			0.192			1.000
< 1 year	23	27		34	54	
≥ 1 year	41	28		46	73	

Among VETC-positive HCC patients, 50 patients underwent RHR, and 69 patients underwent RFA; among VETC-negative HCC patients, 88 patients underwent RHR, and 119 patients underwent RFA. The proportions of VETC cases were similar in cohorts 1 and 2 (42.0% vs 42.5%) and between the RHR and RFA groups. Notably, the VETC pattern was more frequently observed with the occurrence of MVI and early recurrence. In the subgroups of patients with VETC-positive or VETC-negative lesions, no significant difference was observed between the RHR and RFA groups for any of the baseline characteristics.

Survival analysis in the overall cohort

The median duration of follow-up was 39 months (range, 2–118 months). At the time of censoring, 256 (78.5%) of 326 patients had recurred, and 153 (46.9%) had died of tumor progression. The 1-, 3-, and 5-year DFS rates for the VETC-positive group and VETC-negative group were 39.5%, 17.6%, and 8.4% and 50.7%, 29.5%, and 18.8%, respectively. The VETC-negative group had a longer median DFS time compared with the VETC-positive group (6.9 vs 12.0 months, $P=0.011$). Similarly, the 1-, 3-, and 5-year OS rates for the VETC-positive group and VETC-negative group were 74.8%, 44.5%, and 19.3% and 87.4%, 58.9%, and 33.8%, respectively. The VETC-negative group had a longer median OS time compared with the VETC-positive group (27 vs 46 months, $P=0.002$) (Table 2).

Table 2
Comparison of DFS rate and OS rate in entire series (Kaplan-Meier method)

Variables	n	DFS	P	OS	P
HBsAg			0.935		0.932
Positive	272	10.0 ± 0.9		38.5 ± 13.2	
Negative	54	14.1 ± 4.8		39.0 ± 16.2	
Background liver			0.609		0.674
Normal	116	11.0 ± 1.4		39 ± 13.3	
Cirrhosis	210	10.0 ± 1.6		39.0 ± 14.8	
Histological grade			0.028		0.648
Well differentiated	192	10.8 ± 2.2		39.0 ± 13.7	
Poorly differentiated	134	10.0 ± 1.3		38.5 ± 14.4	
Microvascular invasion			< 0.001		0.002
Present	90	13.8 ± 1.8		46.0 ± 14.1	
Absent	236	5.7 ± 0.9		27.0 ± 13.5	
AFP at recurrence, ng/ml			0.204		0.123
> 20	108	11.0 ± 3.4		43.5 ± 14.6	
≤ 20	218	10.0 ± 1.2		36.5 ± 9.4	
Child-Pugh score at recurrence			0.148		0.195
5	146	11.0 ± 1.5		34.5 ± 11.5	
6	180	10.0 ± 1.6		44.0 ± 13.5	
Tumor multiplicity at recurrence			0.094		0.686
Solitary	267	11 ± 1.5		39 ± 17.9	
Multiple	59	7 ± 2.3		39 ± 14.3	
Time to recurrence			< 0.001		< 0.001
< 1 year	144	7.0 ± 0.9		33 ± 8.3	
≥ 1 year	182	18.0 ± 2.7		50.5 ± 15.0	
VETC			0.011		0.002
Positive	119	6.9 ± 1.4		27.0 ± 10.1	
Negative	207	12.0 ± 1.8		46.0 ± 13.5	

Variables	n	DFS	P	OS	P
Treatment allocation			0.114		0.259
RHR	138	11.8 ± 3.3		42.0 ± 12.6	
RFA	188	10.0 ± 1.1		37.5 ± 10.4	

The results of univariate analysis showed that time to recurrence, VETC and microvascular invasion were found to be significant risk factors affecting DFS and OS. There was no significant difference between the RHR and RFA groups for DFS and OS. By multivariate analysis, time to recurrence ($P < 0.001$), tumor multiplicity ($P = 0.008$), Child-Pugh score at recurrence ($P < 0.001$), microvascular invasion ($P < 0.001$) and VETC pattern ($P = 0.015$) were identified as independent predictors of DFS. Time to recurrence ($P = 0.002$), Child-Pugh score at recurrence ($P = 0.004$), microvascular invasion ($P = 0.019$) and VETC pattern ($P = 0.025$) were identified as independent predictors of OS (Table 3).

Table 3
Multivariate analysis of the risk factors related to DFS and OS of recurrent early-stage HCC

	DFS			OS		
	P	HR	95% CI	P	HR	95% CI
HBsAg (positive/negative)	0.912	1.019	0.725–1.433	0.779	1.065	0.685–1.656
Cirrhosis (present/absent)	0.627	0.937	0.719–1.220	0.326	1.186	0.844–1.667
Tumor multiplicity at recurrence (solitary/multiple)	0.008	1.554	1.124–2.148	0.514	1.150	0.755–1.752
AFP at recurrence, ng/mL ($\leq 20 / > 20$)	0.461	1.107	0.845–1.450	0.193	1.262	0.889–1.793
Child-Pugh score at recurrence (5/6)	0.000	0.466	0.338–0.641	0.004	0.541	0.357–0.820
Histological grade (well/poorly differentiated)	0.292	1.147	0.889–1.479	0.354	0.855	0.614–1.191
Time to recurrence (< 1 year/ ≥ 1 year)	0.000	0.556	0.431–0.716	0.002	0.601	0.435–0.832
Microvascular invasion (present/absent)	0.000	2.997	2.103–4.273	0.019	1.739	1.097–2.757
Treatment allocation (RHR/RFA)	0.228	1.170	0.907–1.510	0.459	1.133	0.815–1.575
VETC (positive/negative)	0.015	1.454	0.854–1.948	0.025	1.486	1.050–2.102

First, the survival benefit of treatment for recurrent early-stage HCC in the VETC-positive and VETC-negative groups was investigated. In the VETC-positive cohort, the 1-, 3-, 5-year DFS rates were 58.0%, 30.0%, and 14.0% for the RHR group and 26.1%, 8.7%, and 4.3% for the RFA group, respectively. The RHR group had a longer median DFS time compared to the RFA group (15.0 vs 5.0 months, $P = 0.001$). Similarly, the 1-, 3-, and 5-year OS rates for RHR group and RFA group were 82.0%, 54.0%, and 28.0% and 69.6%, 37.7%, and 13.0%, respectively ($P = 0.008$). The RHR group had a longer median OS time compared to the RFA group (39.5 vs 19 months, $P = 0.001$) (Fig. 3). In contrast, in the VETC-negative cohort (Fig. 4) and the entire cohort (Fig. 5), there was no significant difference in DFS and OS rates between the RHR and RFA groups ($P > 0.05$) (Fig. 4).

Characteristics after treatment

Treatment-related complications are summarized in Table 4. Complications were reported according to the Clavien-Dindo classification. No treatment-related mortality was reported in this study, and the incidence of major complications did not differ significantly between the RHR and RFA groups. However, the delay in the RHR group led to a significantly longer length of hospital stay (6.7 vs 4.1 day, $P < 0.001$) and higher total hospital charges (52,645 vs 27,949 RMB, $P < 0.001$).

Table 4
Major characteristics after treatment

Variables	RHR	RFA	<i>P</i>
Postoperative hemorrhage			0.087
Absent	135	187	
Present	5	1	
Ascites			0.120
Absent	128	182	
Present	10	6	
Bile leakage			0.178
Absent	136	188	
Present	2	0	
Pleural effusion			0.178
Absent	136	188	
Present	2	0	
Liver failure			0.167
Absent	134	187	
Present	4	1	
Length of stay (days)	8.7 ± 1.2	4.3 ± 0.9	< 0.001
Total hospital charges (RMB)	52,645 ± 7055	27,949 ± 3165	< 0.001

Discussion

Because of the high incidence of intrahepatic recurrence, which affects the prognosis of HCC patients, the available treatment options are not that different from those for primary tumors. Many recent articles have reported the feasibility, safety, and efficacy of RHR or RFA for intrahepatic HCC recurrence. However, there is no consensus on the most appropriate choice for HCC recurrence with regard to RHR or RFA. Our study demonstrated that RHR was relatively safe and superior to RFA in improving survival outcomes for recurrence of early-stage HCC for our whole cohort. Moreover, we first explored the VETC pattern to identify the patient subgroup mostly likely to benefit from this treatment. The result showed that HCC patients who were VETC-positive could benefit from RHR, while there was no significant difference between RHR and RFA in the VETC-negative cohort.

Indeed, following surgical resection of the primary tumors, intensive screening was usually applied using AFP levels and CT or MRI, and the recurring tumors were usually detected in the early stage, with tumor sizes smaller than 3 cm[25]. Our study showed that approximately 30% of recurrent HCCs were smaller than 3 cm, which was more similar to the real situation in the recurrence of tumors. As expected, recurrent early-stage HCC with a smaller tumor size was closely related to a higher rate of complete tumor elimination after RFA and a greater safety resection margin with fewer resections of tumor-free liver parenchyma in RHR, which in turn leads to better prognosis. However, different from the prognosis of first treatment in early-stage HCC, several studies have shown that 70–80% of patients developed a second recurrence with either RHR or RFA[26–28]. Our study found that 78.5% of patients had recurred, and 46.9% had died of tumor progression after the second treatment. This may be because recurrent HCC, with a higher degree of malignancy, more easily leads to a second recurrence after treatment.

The debate about whether resection or ablation should be first-line therapy for early-stage HCC has intensified over the years, and there has also been controversy about treatment optimization for recurrent early-stage HCC. Several studies revealed similar survival outcomes between RHR and RFA in recurrent small tumors[22, 29, 30]. Compared with RHR, RFA is a highly selective, targeted thermal treatment technique to conserve tumor-free liver parenchyma and cause a minimum of damage to the small or cirrhotic liver remnant. Unlike surgery, RFA can be performed under conscious sedation and has a shorter hospital day, making it more cost-effective than surgical resection. In our study, the median total hospital stay duration was shorter and median total charges for patients who underwent RFA were significantly lower than those for patients who underwent RHR. Therefore, RFA is usually the first choice for patients with early-stage HCC, whether primary or recurrent. Our study showed that 57.7% of patients underwent RFA and 42.3% of patients underwent RHR. There was no significant difference in survival outcomes between RFA and RHR in the whole cohort.

The VETC pattern has been identified as an effective predictor of survival in patients with HCC after resection, and it can be easily identified and distinguished from capillary vessels by using IHC staining for a single endothelial marker, CD34[17]. In our study, we found that the VETC pattern was correlated not only with recurrence after first resection but also with second recurrence. Furthermore, we found that the VETC-positive patients in the RHR group had longer median DFS and OS rates than those in the RFA group, while among the VETC-negative cohort, there was no significant difference in DFS or OS rates between the RHR and RFA groups. VETC is closely associated with tumor micrometastases and venous thrombus in the tumor margins. We postulate that RFA cannot achieve sufficient ablation in hepatic tumors with potential adjacent venous invasion or microscopic lesion remnants. In contrast, RHR can usually achieve a relatively satisfactory margin of normal liver tissue if possible. With the recent advances in hepatic surgical techniques, anatomic resection is well developed and is performed more actively for hepatic tumors, which can remove the tumor and the entire Couinaud segment potentially containing the adjacent venous invasion or microscopic lesions simultaneously[31].

There were several limitations to our study. First, this was a retrospective study, and selection bias may have influenced the study results. Second, treatment selection for recurrent HCC was decided by our

multidisciplinary team and not randomly assigned. For HCC patients who had a major resection, when recurrence was present and located deep within the liver or in a patient with insufficient liver function reserve, RFA was usually the first choice as it causes less damage, making patients more likely to benefit from treatment.

Conclusions

In conclusion, the results of our study showed that RHR was relatively safe and superior to RFA in improving survival outcomes for recurrent early-stage HCC after initial hepatectomy. Additionally, the VETC pattern may represent a reliable marker for selecting HCC patients who may benefit from RHR; however, large-scale studies are required to confirm these findings.

List Of Abbreviations

VETC
vessels encapsulating tumor clusters; HCC:hepatocellular carcinoma; RHR:repeat hepatic resection; RFA:radiofrequency ablation; DFS:disease-free survival; OS:overall survival.

Declarations

Acknowledgments

None

Author' contributions

ZYC, ZXG, LHL contributed equally to this work; WW and RPG also contributed equally to this work. ZYC, WW and RPG conceived and designed the experiments; ZYC, ZXG, LHL and WPL collected the data; ZXG and LHL analyzed the data; ZYC wrote the paper. All authors have read and approved the final version of the manuscript.

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Available of date and materials

All the supported data is included in the manuscript and where applicable hyperlinks are provided.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors have read and approved the manuscript in all respects for publication.

Competing interests

The authors have no conflicts of interest to declare.

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Figures

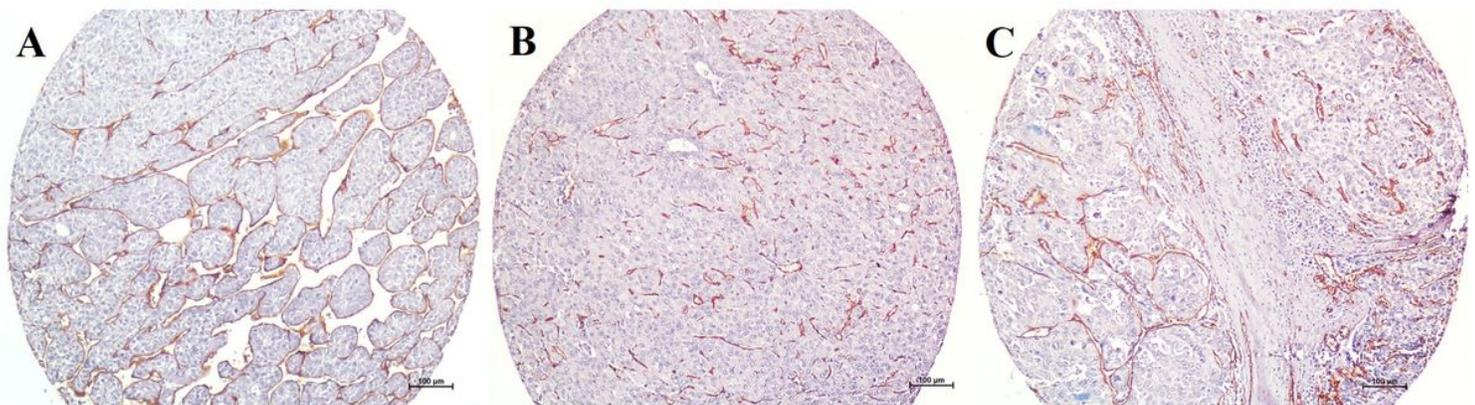


Figure 1

IHC staining for human CD34 was performed to detect vascular patterns in human HCC tissues. (A) VETC-positive: sinusoid-like that form a cobweb-like pattern and encapsulate tumor clusters; (B) VETC-negative: capillary-like microvessel; (C) mixed VETC and capillary-like microvessel. IHC:

immunohistochemical staining, HCC: hepatocellular carcinoma, VETC: vessels encapsulating tumor clusters.

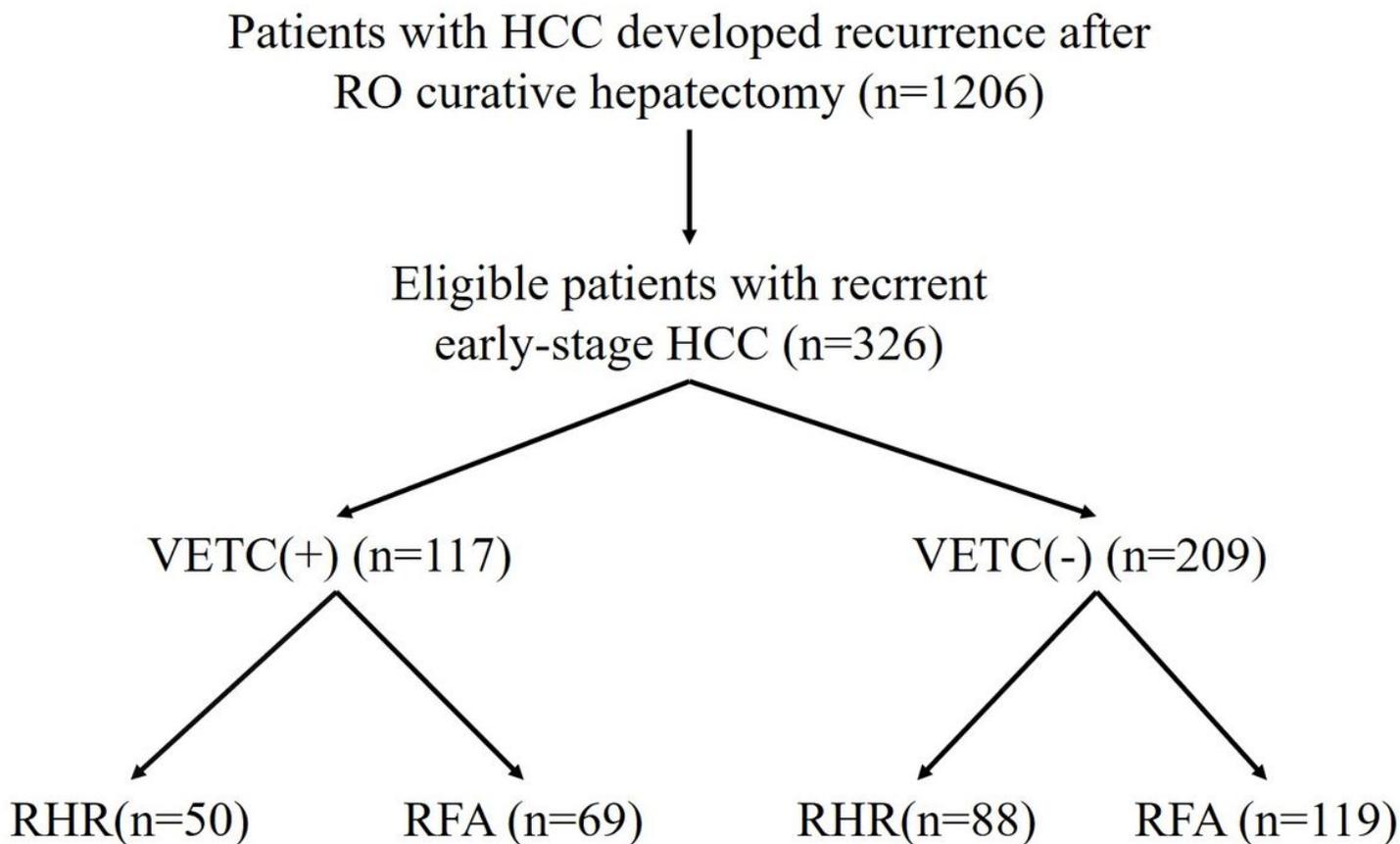


Figure 2

Flow chart of the study protocol. RHR: repeat hepatic resection, RFA: radiofrequency ablation.

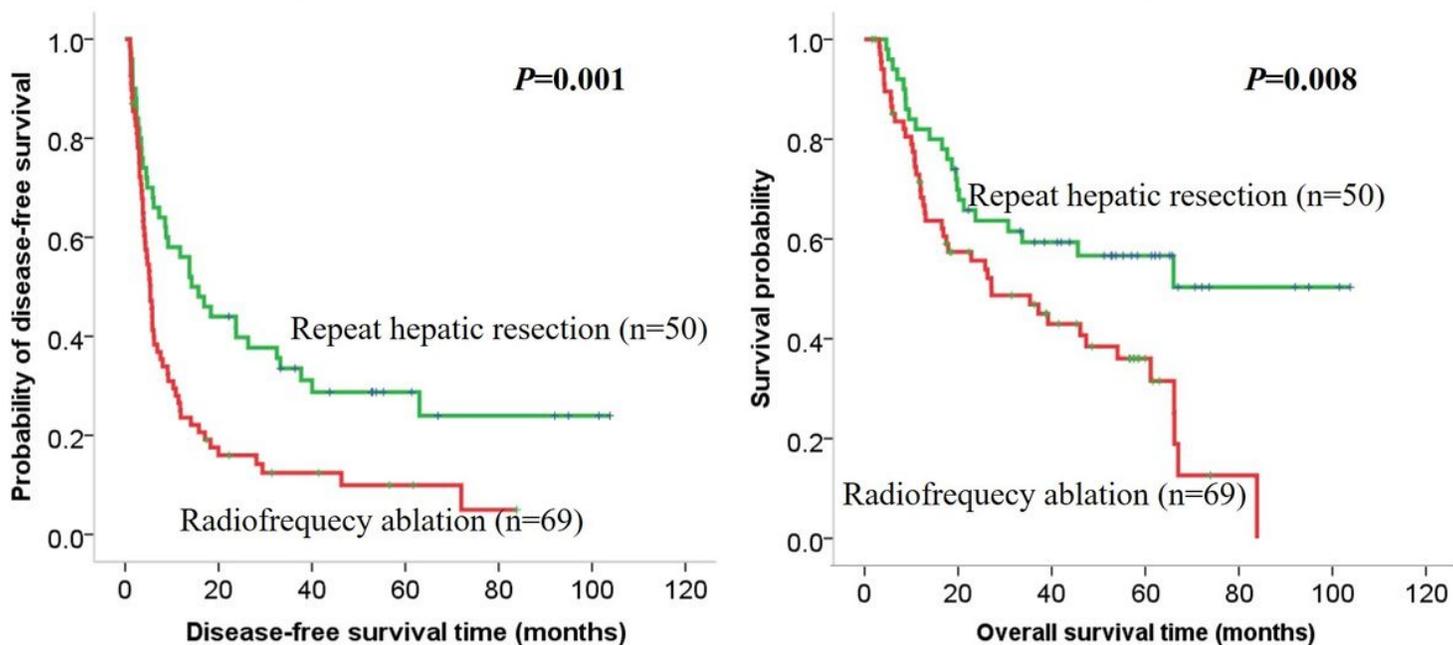


Figure 3

Kaplan-Meier curves of survival outcomes of recurrent early-stage HCC. Kaplan-Meier curves of (A) DFS and (B) OS in the VETC-positive cohort. DFS: disease-free survival, OS: overall survival.

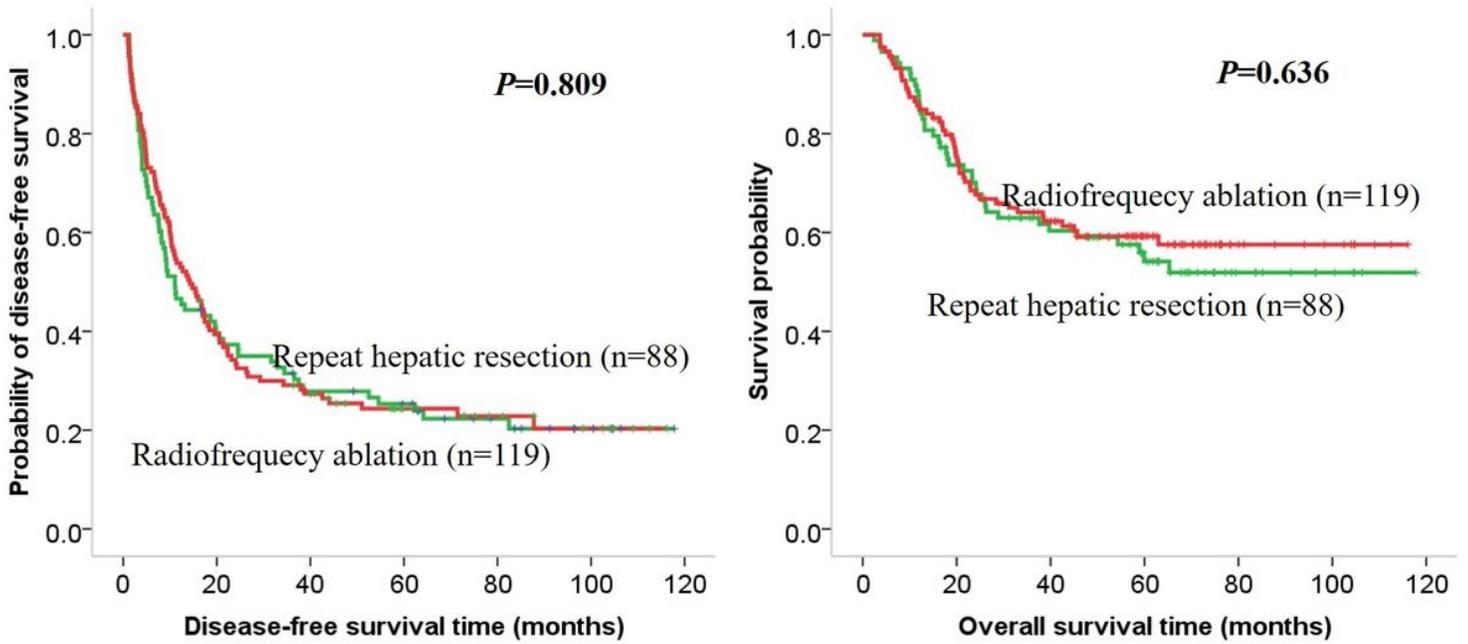


Figure 4

Kaplan-Meier curves of survival outcomes of recurrent early-stage HCC. Kaplan-Meier curves of (A) DFS and (B) OS in the VETC-negative cohort.

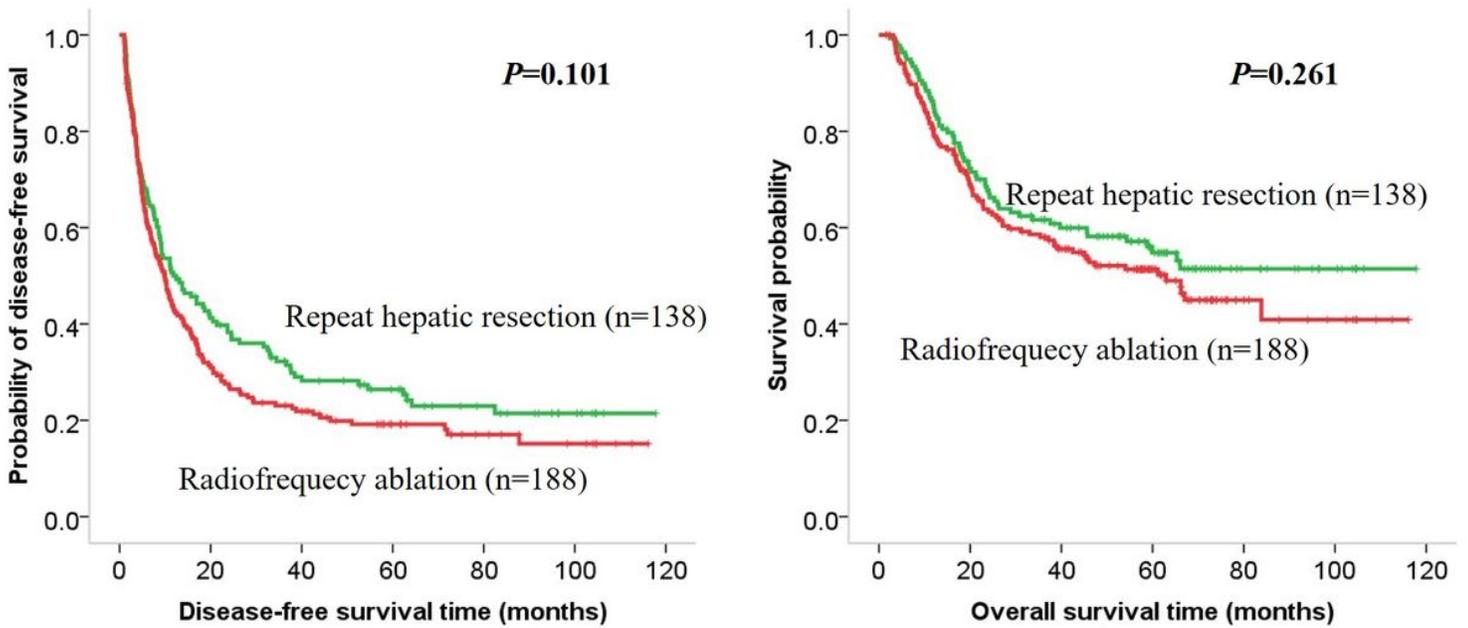


Figure 5

Kaplan-Meier curves of survival outcomes of recurrent early-stage HCC. Kaplan-Meier curves of (A) DFS and (B) OS in the whole cohort.