Journal of Hepatocellular Carcinoma

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Long-Term Analysis of Recurrence Beyond Milan Criteria Following Ablation of Solitary Early-Stage Hepatocellular Carcinoma ≤3 cm in Potentially Transplantable Patients: A Over 10-Year Survival Study

Shuanggang Chen[1,2,*], Han Qi[1,2,*], Hongtong Tan[1,2,*], Fei Cao[1,2], Lin Xie[1,2], Tao Huang[1,2], Ying Wu[3], Chunyong Wen[1,2], Yujia Wang[1,2], Lujun Shen[1,2], Weijun Fan[1,2]

[1]Department of Minimally Invasive Interventional Therapy, Sun Yat-Sen University Cancer Center, Guangzhou, 510060, People's Republic of China; [2]State Key Laboratory of Oncology in South China, Guangdong Provincial Clinical Research Center for cancer, Sun Yat-Sen University Cancer Center, Guangzhou, 510060, People's Republic of China; [3]Department of Interventional Therapy, Shenzhen second People's Hospital, The First Affiliated Hospital of Shenzhen University, Shenzhen, 518035, People's Republic of China

*These authors contributed equally to this work

Correspondence: Weijun Fan, Email fanwj@sysucc.org.cn; Lujun Shen, Email shenlj@sysucc.org.cn

Background: Salvage liver transplantation is promising for hepatocellular carcinoma(HCC) recurrence post-ablation but is significantly affected by recurrence beyond Milan Criteria (RBM).

Materials and Methods: A retrospective cohort study of potentially transplantable HCC patients undergoing ablation between 2007 and 2017 assessed median time to recurrence beyond Milan Criteria(TRBM) via Kaplan-Meier curves and predictive capacity of recurrence and RBM for overall survival(OS) via Receiver Operating Characteristic Curves, and identified independent risk factors for TRBM and RBM via Cox and binary logistic regression models.

Results: We enrolled 191 potentially transplantable patients with early-stage HBV-related HCC ≤3 cm who underwent ablation. During a median follow-up of 7.64 years, HCC recurrence occurred in 126 patients(65.9%), with RBM 86 patients(45.0%). The median TRBM was 10.54 years. Cumulative survival rates without RBM at 3, 5, 8, 10, and 13 years were 77.3%, 65.9%, 56.5%, 51.0%, and 37.6%, respectively. Multivariable analysis identified older age, C-reactive protein(CRP)≥1.81 mg/L, and platelet(PLT)≤80×10^9/L as independent risk factors for TRBM. Also, cirrhosis, CRP≥1.81 mg/L and PLT≤80×10^9/L were identified as independent risk factors of the occurrence of RBM. Elevated Platelet-CRP Score(PCS), integrating CRP and PLT, correlated significantly with an increased incidence of RBM and a more aggressive phenotype, characterized by vascular invasion or metastatic dissemination ($P<0.05$). Notably, RBM was a superior predictive indicator for OS compared to recurrence (P<0.05).

Conclusion: When using ablation as a bridge to liver transplantation for solitary HBV-related early HCC (≤3 cm), it is crucial first to identify key preoperative features, including high CRP, low PLT, cirrhosis, and older age.

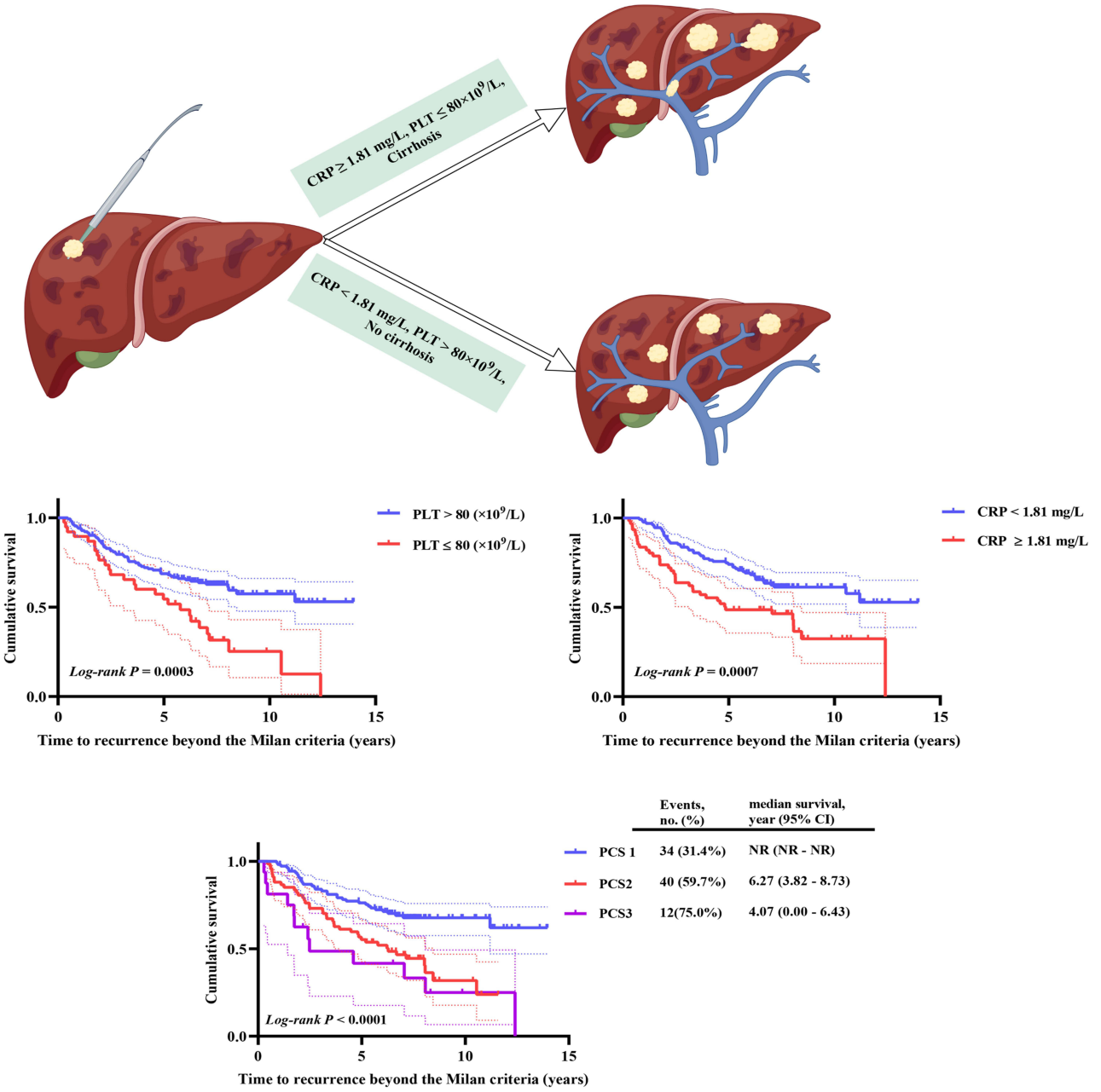
Keywords: hepatocellular carcinoma, hepatitis B virus, ablation techniques, transplantable liver transplantation, recurrence beyond the Milan criteria

Introduction

About 50~80% of hepatocellular carcinoma (HCC) is attributed to hepatitis B virus (HBV).[1] Liver transplantation is the preferred recommended first-line treatment option for patients with early-stage HCC.[2] However, due to a variety of limitations, such as shortage of donors and financial pressure, many patients with early-stage HCC are unable to receive first-line liver transplantation. Ablation can serve as a bridge for liver transplantation in patients with early-stage HCC,

Received: 12 November 2024
Accepted: 27 January 2025
Published: 3 February 2025

Graphical Abstract



but disease recurrence is a concern.[2–5] Encouragingly, compared with re-resection or re-ablation, salvage liver transplantation is also a more promising treatment option for HCC patients with recurrence after resection or ablation.[6–8] For example, previous studies have shown that HCC patients who undergo salvage liver transplantation have a higher 5-year disease-free survival rate, which is significantly higher than that of patients who undergo salvage hepatectomy or re-ablation. However, some studies have demonstrated that HCC recurrence beyond Milan criteria (RBM) following radical resection or ablation is an independent risk factor for prognosis after salvage liver transplantation.[9,10] Therefore, Investigating RBM patterns and predictive factors after radical ablation in potentially transplantable early-stage HCC patients is crucial.

In our study, we summarized over 10 years of experience at our cancer center, with the primary aim of further exploring the RBM patterns post-ablation in potentially transplantable patients with HBV-related early-stage HCC. Additionally, we aimed to identify predictive factors for time to recurrence beyond Milan Criteria (TRBM) over a 10-year period. The secondary objectives were to predict overall survival (OS) and recurrence-free survival (RFS) following radical ablation over a 10-year period, and determine associated risk factors.

Patients and Methods

Patients

We retrospectively analyzed 246 patients with solitary early-stage HCC ≤3 cm treated by ablation at the Sun Yat-sen University Cancer Center (SYSUCC) between September 2007 and December 2017. Inclusion criteria comprised: (1) histological or radiological confirmation of HCC; (2) receipt of curative ablation therapy as the initial intervention, with no evidence of local HCC recurrence at the first follow-up assessment; (3) Hepatitis B viral infection; (4) preserved hepatic function (Child-Pugh class A or B; albumin-bilirubin grade 1 or 2); (5) age bracket between 18 and 70 years; (6) absence of prior HCC management strategies; (7) lack of extrahepatic disease spread; (8) absence of bile duct involvement and vascular invasion; (9) no clinical manifestations of acute infection within one week preceding ablation. Conversely, exclusion criteria encompassed: (1) presence of a secondary primary malignancy; (2) significantly impaired liver function (Child-Pugh class C or albumin-bilirubin grade 3); (3) severe coagulopathy. A total of 191 potentially transplantable patients with solitary early-stage HBV-associated hepatocellular carcinoma less than 3 cm were included (Figure 1). We defined potentially transplantable patients as those aged under 70 years without any comorbidities that would contraindicate transplant surgery. Informed consent was waived due to the retrospective nature of the study, in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of SYSUCC.

Ablation Protocols

Radiofrequency ablation (RFA) and microwave ablation (MWA), guided by real-time ultrasound or computed tomography (CT), were performed by experienced doctors under anesthesia. These procedures, conducted under sterile conditions, involved five stages: assessment, puncture site localization, guided insertion, ablation, and post-procedure evaluation. The treatment parameters adjusted to the HCC's location and size for thorough elimination, typically using a single electrode or antenna for tumors ≤3 cm. The post-ablation endpoint was defined as a safety margin of at least 5–10 mm beyond the HCC tissue boundary.[11,12] The 5–10 mm ablation margin was evaluated by measuring the minimum

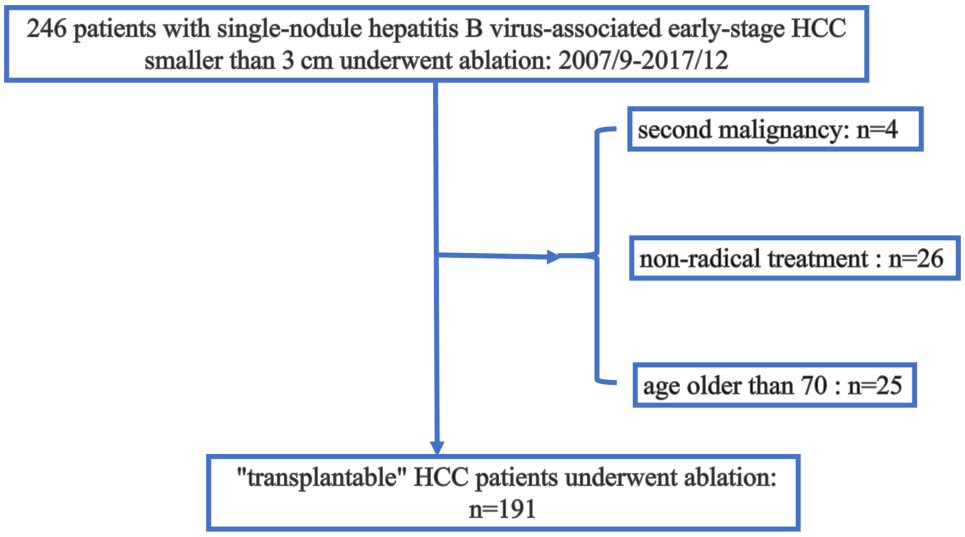


Figure 1 The flowchart of the selection process.
Abbreviation: HCC, hepatocellular carcinoma.

https://doi.org/10.2147/JHC.S505979

distance between the boundary of the ablated area and the tumor margin immediately after ablation. If the minimum distance exceeded 5–10 mm, it was considered that a safe margin had been achieved.

Follow-up and the Follow-up Endpoint

Patients underwent follow-up imaging (ultrasound, CT, or MRI) about one month post-ablation, then every 2–3 months for two years if no recurrence. Afterward, imaging is at 3–6 month intervals for 2–5 years, followed by 6–12 month intervals until recurrence signs.

The primary endpoint was time to recurrence beyond Milan criteria (TRBM), defined as the interval between initial ablation therapy and HCC recurrence beyond Milan criteria (RBM) or death due to RBM. RBM was characterized by one lesion > 5 cm, more than three lesions, at least one of the three lesions > 3 cm, vascular invasion, or extrahepatic metastases visible on imaging examination during the follow-up period. Secondary endpoints included recurrence-free survival (RFS) and overall survival (OS). RFS measured from initial ablation to HCC recurrence or death due to HCC, while OS measured from ablation to death from any cause. The median follow-up interval for the entire cohort was 7.64 years (quartile ranges 6.74–9.85 years).

Statistical Analysis

Mean and standard deviation were typically used to describe normally distributed continuous variables, while median and interquartile range (IQR) were more appropriate for non-normally distributed continuous variables. For comparing continuous variables, *t*-tests or Mann–Whitney *U*-tests were employed depending on the normality of the data. Categorical variables, on the other hand, were compared using chi-square tests or Fisher's exact tests. Kaplan-Meier method estimated median RFS, OS, and TRBM, with Log rank test for comparisons. Univariate and multivariate Cox proportional hazards models identified independent risk factors. The Cox proportional hazards model for identifying independent prognostic factors was internally validated through 1000 bootstrap resampling. This approach allowed us to assess the stability and reliability of our findings by drawing multiple samples with replacement from the original dataset, simulating the sampling distribution, and providing an empirical estimate of variability and confidence intervals for our results, as well as calculating the C-index after each bootstrap sample and the confidence interval after 1000 resamples. Binary logistic regression (forward: likelihood ratio) was used to determine independent predictors of the occurrence of RBM. The predictive abilities of RBM and recurrence for overall survival (OS) were compared using time-dependent receiver operating characteristic (ROC) curves, with the corresponding area under the curve (AUC) estimates providing a measure of their discriminatory power.[13] The optimal cut-off values for platelet (PLT) counts (80×10^9/L) and C-reactive protein (CRP) levels (1.81 mg/L) were determined based on our previously published study, which utilized survival ROC curves to calculate the ideal thresholds.[14] Analyses were bilateral; statistical significance was $P < 0.05$, conducted in R version 4.4.1 (https://www.r-project.org/), SPSS version 25.0 (IBM, United States), and GraphPad Prism 8.0.1(244) (USA, GraphPad Software).

Results

Study Population

The baseline characteristics of the potentially transplantable patients with solitary early-stage HBV-related hepatocellular carcinoma 3 cm or smaller in diameter, who underwent radical ablation, are presented in Table 1. A total of 191 patients (161 men, 30 women) were included in our study. Among these potentially transplantable patients, the median age and tumor size were 55 years (interquartile range 46–62 years) and 2.1 cm (interquartile range 1.6–2.5 cm), respectively. Of the enrolled patients, 30 (15.7%) patients had Albumin-bilirubin (ALBI) grade 2, and no patients had ALBI grade 3. Cirrhosis was present in 108 (56.5%) patients. PLT counts $\leq 80\times10^9$/L were observed in 38 (19.9%) patients, and CRP level ≥ 1.81 mg/L was observed in 61 (31.9%) patients. The remaining baseline characteristics are detailed in Table 1.

Table 1 Demographic and Clinical Characteristics of the Enrolled HCC Participants

Variables	N=191 or Median (n % or Interquartile Q_1-Q_3)
Gender (male vs female)	161 vs 30 (84.3 vs 15.7)
Age (y)	55 (46, 62)
ALB (g/L)	42.40 (39.00, 45.60)
Cirrhosis (Absent vs Present)*	83 vs 108 (43.5 vs 56.5)
TBIL (μmol/L)	15.10 (11.30, 21.20)
Prothrombin Time (s)	12.20 (11.50, 13.10)
PLT ($\times10^9$/L)	132.40 (87.00, 179.00)
PLT ($\leq80\times10^9$/L vs $>80\times10^9$/L)	38 vs 153 (19.9 vs 80.1)
CRP (mg/L)	1.32 (0.66, 2.27)
CRP (≥1.81 mg/L vs <1.81 mg/L)	61 vs 130 (31.9 vs 68.1)
AFP (ng/mL)	44.02 (5.03, 297.60)
AFP	
0–100	117(61.3)
101–1000	53 (27.7)
>1000	21 (11.0)
Tumor Size (cm)	2.1 (1.6, 2.5)
Tumor Size (>2 cm vs ≤2 cm)	107 vs 84 (56.0 vs 44.0)
ALBI (Grade 2 vs Grade 1)	30 vs 161 (15.7 vs 84.3)

Note: *The diagnosis of cirrhosis is based on imaging tests.
Abbreviations: ALB, Albumin; TBIL, Total bilirubin; PLT, Platelet; CRP, C-reactive protein; ALBI, Albumin-bilirubin; AFP, alpha-fetoprotein.

Time to Recurrence Beyond Milan Criteria (TRBM), Overall Survival (OS) and Recurrence-Free Survival (RFS) in the Entire Cohort

With regard to TRBM, 45.0% (86/191) potentially transplantable HCC patients experienced final recurrence beyond the Milan criteria (RBM) during follow-up. The median TRBM was 10.54 years (95% CI: 8.26–12.83). The cumulative rates of survival without RBM at 3, 5, 8, 10 and 13 years were 77.3%, 65.9%, 56.5%, 51.0% and 37.6%, respectively. In terms of OS, 34.0% (65/191) potentially transplantable HCC patients died during follow-up. The median OS was 12.61 years (95% CI: 8.50–16.73). The cumulative OS rates at 5, 8, 10 and 14 years were 76.4%, 66.8%, 60.1% and 44.5%, respectively. Concerning the RFS, 65.9% (126/191) potentially transplantable HCC patients experienced a final recurrence during follow-up. The median RFS was 4.07 years (95% CI: 2.91–5.23). The cumulative RFS rates at 3, 5, 8 and 10 years were 60.4%, 44.8%, 32.6% and 28.1%, respectively (shown in Figure 2).

Analysis of Risk Factors of TRBM, OS and RFS

Analysis of Risk Factors of TRBM

To identify potential predictors of TRBM, we conducted a stepwise univariate analysis using the Cox proportional hazards model with all baseline variables. This initial assessment indicated that age, albumin concentration, presence of cirrhosis, prothrombin time, PLT counts, CRP level, tumor size, and ALBI grade may be predictors of TRBM. To mitigate issues related to multicollinearity, these factors were selected for inclusion in a subsequent time-dependent multivariate Cox regression analysis, specifically focusing on age, cirrhosis, prothrombin time, PLT counts ($\leq80\times10^9$/L vs $>80\times10^9$/L), CRP level (≥1.81 mg/L vs <1.81 mg/L), tumor size (>2 cm vs ≤2 cm) and ALBI grade. The multivariate analysis revealed that advanced age, elevated CRP levels (≥1.81 mg/L), and reduced PLT counts ($\leq80\times10^9$/L) emerged as independent predictors of TRBM (Table 2). The robustness and reliability of these findings were internally validated through 1000 bootstrap resampling (Supplementary Figure 1A and B). Building upon these findings, we devised a novel scoring system, the Platelet-CRP Score (PCS), by integrating PLT and CRP values. The median TRBM between the different groups of PLT, CRP and

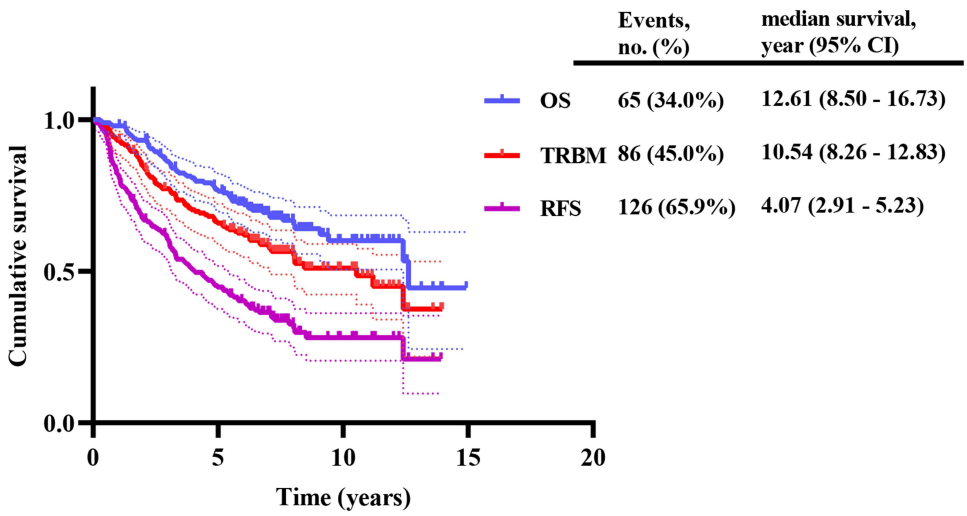


Figure 2 Kaplan-Meier curves for time to recurrence beyond Milan criteria (TRBM), overall survival (OS), and recurrence-free survival (RFS) in the entire cohort.The median TRBM was 10.54 years (95% CI: 8.26–12.83 years); the median OS was 12.61 years (95% CI: 8.50–16.73 years); and the median RFS was 4.07 years (95% CI: 2.91–5.23 years).
Abbreviations: TRBM, time to recurrence beyond Milan criteria; OS, overall survival; RFS, recurrence-free survival; CI, confidence interval.

PCS was compared by Log rank test (shown in Figure 3A-3C). Specifically, PCS 1 encompassed potentially transplantable HCC patients, characterized by PLT >80×10^9/L and CRP<1.81 mg/L, exhibiting an RBM incidence rate of 31.4% (34 out of 108 patients) and a median TRBM period that was not reached (NR, 95% confidence interval: NR - NR). Conversely, PCS 2

Table 2 Univariate and Multivariate Analyses of TRBM in the Whole Cohort

Variable	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Gender (female vs male)	1.283 (0.735–2.241)	0.381	–	–
Age (y)	1.027 (1.005–1.050)	0.016	1.030 (1.007–1.053)	0.011
ALB (g/L)	0.932 (0.890–0.977)	0.004	–	–
Cirrhosis (Present vs Absent)	1.938 (1.228–3.059)	0.004	–	0.114
TBIL (μmol/L)	1.012 (0.982–1.043)	0.428	–	–
Prothrombin Time (s)	1.342 (1.120–1.608)	0.001	–	0.301
PLT (×10^9/L)	0.993 (0.989–0.997)	0.001	–	–
PLT (≤80×10^9/L vs >80×10^9/L)	2.255 (1.428–3.562)	<0.001	2.269 (1.424–3.615)	0.001
CRP (mg/L)	1.029 (1.002–1.056)	0.034	–	–
CRP (≥1.81 mg/L vs <1.81 mg/L)	2.057 (1.341–3.156)	0.001	1.832 (1.190–2.820)	0.006
AFP (ng/mL)	1.000 (1.000–1.000)	0.509	–	–
AFP		0.696	–	–
0–100	Reference			
101–1000	1.095 (0.677–1.769)	0.712	–	–
>1000	0.759 (0.345–1.668)	0.493	–	–
Tumor Size (cm)	1.040 (1.000–1.082)	0.052	–	–
Tumor Size (>2 cm vs ≤2 cm)	1.046 (0.939–2.271)	0.093	–	0.148
ALBI (Grade 2 vs Grade 1)	2.069 (1.345–3.183)	0.001	–	0.278

Notes: Data were analyzed using Cox regression, with variables showing a P-value <0.1 in univariate analysis included in the multivariate model. The significance level was 0.05.
Abbreviations: TRBM, time to recurrence beyond the Milan criteria; ALB, Albumin; TBIL, Total bilirubin; PLT, Platelet; CRP, C-reactive protein; ALBI, Albumin-bilirubin; HR, hazard ratio; AFP, Alpha-fetoprotein; CI, Confidence Interval.

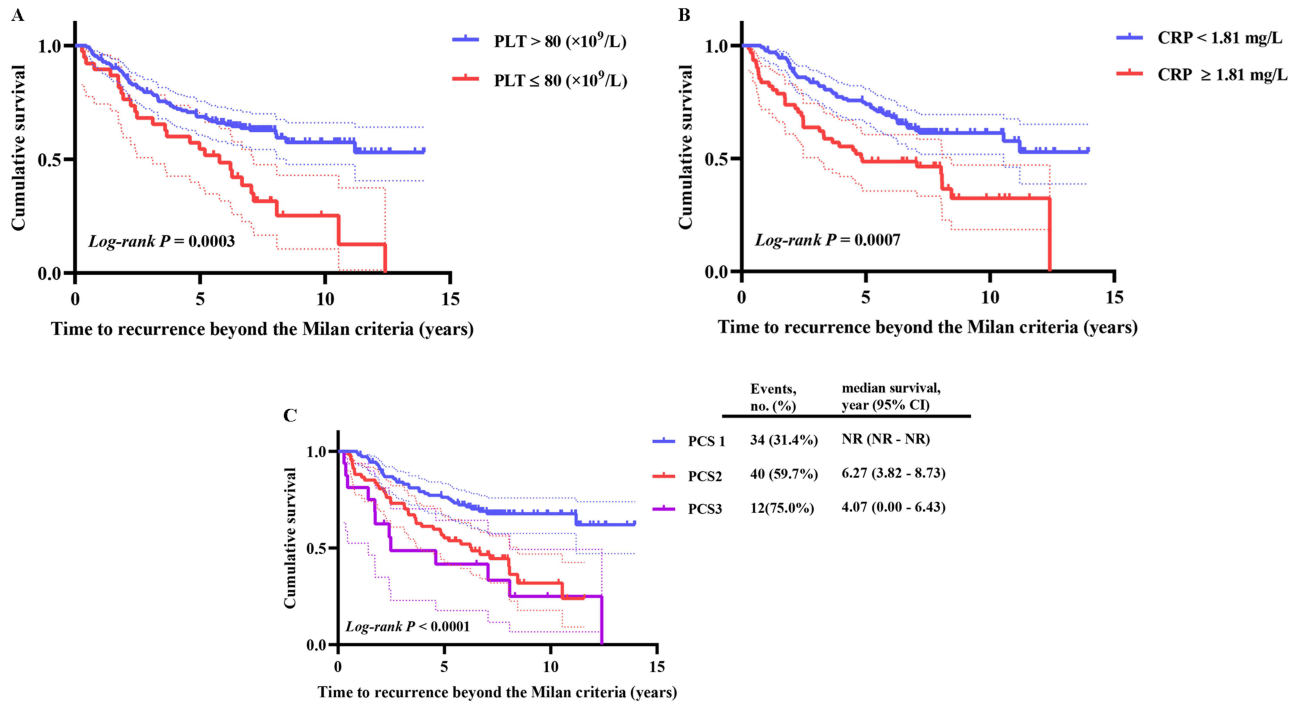


Figure 3 Kaplan-Meier curves for independent prognostic factors of time to recurrence beyond Milan criteria (TRBM).(**A** and **B**) Patients with elevated serum C-reactive protein (CRP) levels or reduced platelet (PLT) counts exhibited a lower TRBM rate compared to those with lower serum CRP levels and higher PLT counts. (**C**) PLT and CRP levels were integrated to devise a novel prognostic score, termed the PLT-CRP Score (PCS). The PCS classification is as follows: PCS 1 includes potentially transplantable HCC patients characterized by PLT >80×10^9/L and CRP <1.81 mg/L, demonstrating an RBM incidence rate of 31.4% (34 out of 108 patients) with a median TRBM not yet reached (NR, 95% confidence interval: NR - NR). In contrast, PCS 2 comprises patients meeting either criterion of PLT≤80×10^9/L and CRP <1.81 mg/dL or PLT>80×10^9/L coupled with CRP≥1.81 mg/dL, exhibiting a heightened RBM incidence of 59.7% (40 out of 67 patients) and a median TRBM of 6.27 years (95% CI: 3.82–8.73 years). Finally, PCS 3 is designated for patients with both PLT≤80×10^9/L and CRP≥1.81 mg/dL, revealing the highest RBM incidence at 75.0% (12 out of 16 patients) alongside a median TRBM of 4.07 years (95% CI: 0.00–6.43 years).

Abbreviations: TRBM, time to recurrence beyond Milan criteria; CRP, C-reactive protein; PLT, platelet; PCS, PLT-CRP score; RBM, recurrence beyond Milan criteria; NR, not reached.

grouped patients with either PLT≤80×10^9/L or CRP<1.81 mg/L but not both, demonstrating an RBM occurrence rate of 59.7% (40 out of 67 patients) and a median TRBM of 6.27 years (95% CI: 3.82–8.73 years). Lastly, PCS 3 represented individuals with both PLT≤80×10^9/L and CRP≥1.81 mg/L, who experienced the highest RBM incidence at 75.0% (12 out of 16 patients) and a median TRBM of 4.07 years (95% CI: 0.00–6.43 years)(shown in Figure 3C). Additionally, we compared the ability of PCS, CRP level, and PLT counts to predict TRBM and found that the predictive ability of PCS was significantly superior to that of CRP level and PLT counts (P<0.05)(Supplementary Figure 2 and Supplementary Table 1).

Analysis of Risk Factors of OS

The initial stepwise univariate analysis employing the Cox proportional hazards model for OS identified several baseline characteristics as potential predictors: age, albumin concentration, total bilirubin level, prothrombin time, PLT counts, CRP level, tumor size, and ALBI grade. To address potential collinearity issues among these variables, we selectively incorporated age, prothrombin time, dichotomized PLT counts (≤80×10^9/L versus >80×10^9/L), CRP thresholds (≥1.81 mg/L versus <1.81 mg/L), tumor size, and ALBI grade into a subsequent time-dependent multivariate Cox regression analysis. This rigorous statistical approach confirmed that advanced age, larger tumors, and reduced PLT counts (≤80×10^9/L) stood independently as significant risk predictors for worse OS outcomes (Supplementary Table 2).

Analysis of Risk Factors of RFS

Stepwise univariate analysis suggests that age, albumin concentration, cirrhosis, PLT, CRP, Alpha-fetoprotein (AFP) and tumor size may be predictors of RFS. Moreover, we included these variables into a time-dependent multivariate Cox

https://doi.org/10.2147/JHC.S505979

Table 3 Patterns of HCC RBM Following Ablation as First-Line Therapy

Patterns of RBM	n=191 (%)	PCS			*P*-value
		PCS1 (n=108)	PCS2 (n=67)	PCS3 (n=16)	
Number of RBM†	86 (45.0)	34 (31.4%)[a]	40 (59.7%)[b]	12 (75.0%)[b]	<0.001
RBM at first recurrence†	25 (13.1)	9 (8.3%)[a]	13 (19.4%)[a]	3 (18.8%)[a]	0.056*
Phenotypes of RBM†	-	-	-	-	-
Tumor size or/and number	29 (15.2)	11 (10.2%)[a]	17 (25.0%)[b]	1 (6.2%)[a,b]	0.019*
Vascular invasion or/and metastatic disease	57 (29.8)	23 (21.3%)[a]	23 (34.3%)[a]	11 (68.8%)[b]	<0.001

Notes: † Among the HCC patients in the whole cohort or in each group;*Fisher's exact test; Superscripts "a" and "b" are used to describe statistical differences between different groups. Different superscripts indicate significant differences between the corresponding data (eg, "a" vs "b"), while the same superscript indicates no significant difference between the corresponding data (eg, "a" vs "a", "b" vs "b", "a,b" vs "a", or "a,b" vs "b").
Abbreviations: HCC, hepatocellular carcinoma; RBM, recurrence beyond Milan criteria; PCS, PLT - CRP score; PLT, platelet; CRP, C-reactive protein.

proportional hazard regression model, and found that cirrhosis, larger tumors, high CRP concentrations (CRP≥1.81 mg/L) and higher AFP were independent risk predictors of RFS (Supplementary Table 3).

Patterns of Recurrence Beyond Milan Criteria (RBM) After Radical Ablation

The comparison of patterns of RBM across different Platelet-CRP Score (PCS) groups revealed statistically significant variations in RBM incidence among patients stratified by PCS (P<0.001) (Table 3). Notably, despite rigorous post-therapeutic radiographic monitoring, patients classified as PCS2 (19.4%) and PCS3 (18.8%) exhibited elevated RBM rates at the time of first recurrence compared to those in PCS1 (8.3%) (P=0.056) (Table 3). Additionally, we compared the baseline characteristics between patients with HCC recurrence within the Milan criteria (40 cases) and those with HCC recurrence beyond the Milan criteria (86 cases). We found that advanced age, thrombocytopenia, low albumin concentration, prolonged prothrombin time, and poor liver function were significantly associated with HCC recurrence beyond the Milan criteria. However, we did not find a significant association between elevated CRP and HCC recurrence beyond the Milan criteria (Supplementary Table 4). This may be due to the fact that we only analyzed the recurrent population and the sample size was reduced, leading to insufficient representativeness. Therefore, we further included the entire population for binary logistic regression analysis which identified cirrhosis, high CRP levels (≥1.81 mg/L), and diminished PLT counts (PLT≤80×10^9/L) as independent risk predictors of the occurrence of RBM (Table 4). In the

Table 4 Univariate and Multivariate Analysis of the Occurrence of HCC RBM in the Whole Cohort

Variable	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Gender (female vs male)	1.268 (0.581–2.766)	0.551	–	–
Age (y)	1.029 (1.001–1.058)	0.046	–	0.103
ALB (g/L)	0.906 (0.848–0.968)	0.003	–	–
Cirrhosis (Present vs Absent)	2.279 (1.261–4.117)	0.006	1.976 (1.059–3.687)	0.031
TBIL (μmol/L)	1.020 (0.979–1.063)	0.350	–	–
Prothrombin Time (s)	1.629 (1.219–2.176)	0.001	–	0.166
PLT ($\times10^9$/L)	0.990 (0.985–0.995)	<0.001	–	–
PLT (≤80×10^9/L vs >80×10^9/L)	3.911 (1.805–8.471)	0.001	3.260 (1.471–7.228)	0.004
CRP (mg/L)	1.031 (0.975–1.091)	0.285	–	–
CRP (≥1.81 mg/L vs <1.81 mg/L)	2.548 (1.365–4.758)	0.003	2.369 (1.236–4.542)	0.009
AFP (ng/mL)	1.000 (1.000–1.000)	0.415	–	–

(*Continued*)

Table 4 (Continued).

Variable	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
AFP		0.516	–	–
0–100	Reference			
101–1000	0.933 (0.486–1.790)	0.834	–	–
>1000	0.564 (0.212–1.498)	0.250	–	–
Tumor Size (cm)	1.054 (1.001–1.111)	0.048	–	–
Tumor Size (>2 cm vs ≤2 cm)	1.808 (1.009–3.240)	0.046	–	0.067
ALBI (Grade 2 vs Grade 1)	2.692 (1.435–5.051)	0.002	–	0.218

Notes: Data were analyzed using binary logistic regression, with variables showing a P-value <0.1 in univariate analysis included in the multivariate model. The significance level was 0.05.

Abbreviations: HCC, hepatocellular carcinoma; RBM, Recurrence beyond the Milan criteria; ALB, Albumin; TBIL, Total bilirubin; PLT, Platelet; CRP, C-reactive protein; ALBI, Albumin-bilirubin; HR, hazard ratio; AFP, Alpha-fetoprotein; CI, Confidence Interval.

phenotype of RBM related to tumor size or/and number, a marked disparity was observed between PCS groups, with PCS2 patients (25%) demonstrating a significantly heightened RBM incidence relative to PCS1 (10.2%) (P=0.019) (Table 3). In the phenotype of RBM associated with vascular invasion or/and metastatic disease, PCS3 individuals (68.8%) manifested a strikingly higher RBM rate than both PCS1 (21.3%) and PCS2 (34.3%) cohorts (P<0.001), underscoring the prognostic significance of the PCS classification in predicting diverse RBM presentations (Table 3).

RBM Versus Recurrence in Predicting OS

We conducted a comparative analysis of overall survival (OS) between patients with RBM and those without RBM using Kaplan-Meier survival curves. The Log rank test was employed to assess the statistical significance of the differences in survival rates between the two groups. The findings demonstrated a significantly prolonged OS in the non-RBM cohort compared to patients experiencing RBM (P<0.001)(Figure 4A). A similar trend was observed when comparing OS between patients without recurrence and those with recurrence, where the former group exhibited significantly longer survival (P<0.001) (Figure 4B). To further evaluate the predictive efficacy of RBM and recurrence for long-term OS, we generated time-dependent ROC curves and computed the AUC at both 8 and 10 years post-ablation. Our analysis revealed that RBM was a significantly superior predictor of 8-year OS in potentially transplantable patients with solitary early-stage HCC following radical ablation, with an AUC of 0.84 (95% CI: 0.77–0.90), compared to recurrence, which had an AUC of 0.68 (95% CI: 0.60–0.75) (P < 0.001 for comparison) (Figure 4C). This advantage persisted at the 10-year mark, where RBM maintained a high predictive value with an AUC of 0.87 (95% CI: 0.78–0.95), whereas recurrence yielded an AUC of 0.68 (95% CI: 0.58–0.78) (P < 0.001 for comparison) (Figure 4D). The differential predictive performance of RBM and recurrence across various time points is illustrated comprehensively in Supplementary Figure 3, highlighting the consistent superiority of RBM in forecasting OS outcomes. Additionally, our investigation into the timing of recurrence and RBM revealed that early recurrences, defined as those occurring within 2 years post-ablation, were correlated with notably shorter OS compared to late recurrences (P = 0.0004) (Supplementary Figure 4A).[15] Similarly, early RBM (occurring within 2 years post-ablation) was linked to significantly shorter OS compared to late RBM (time interval between ablation and RBM > 2 years) (P< 0.0001)(Supplementary Figure 4B). Those findings underscores the prognostic relevance of both the occurrence and timing of recurrence and RBM in the context of HCC management following radical ablation procedures.

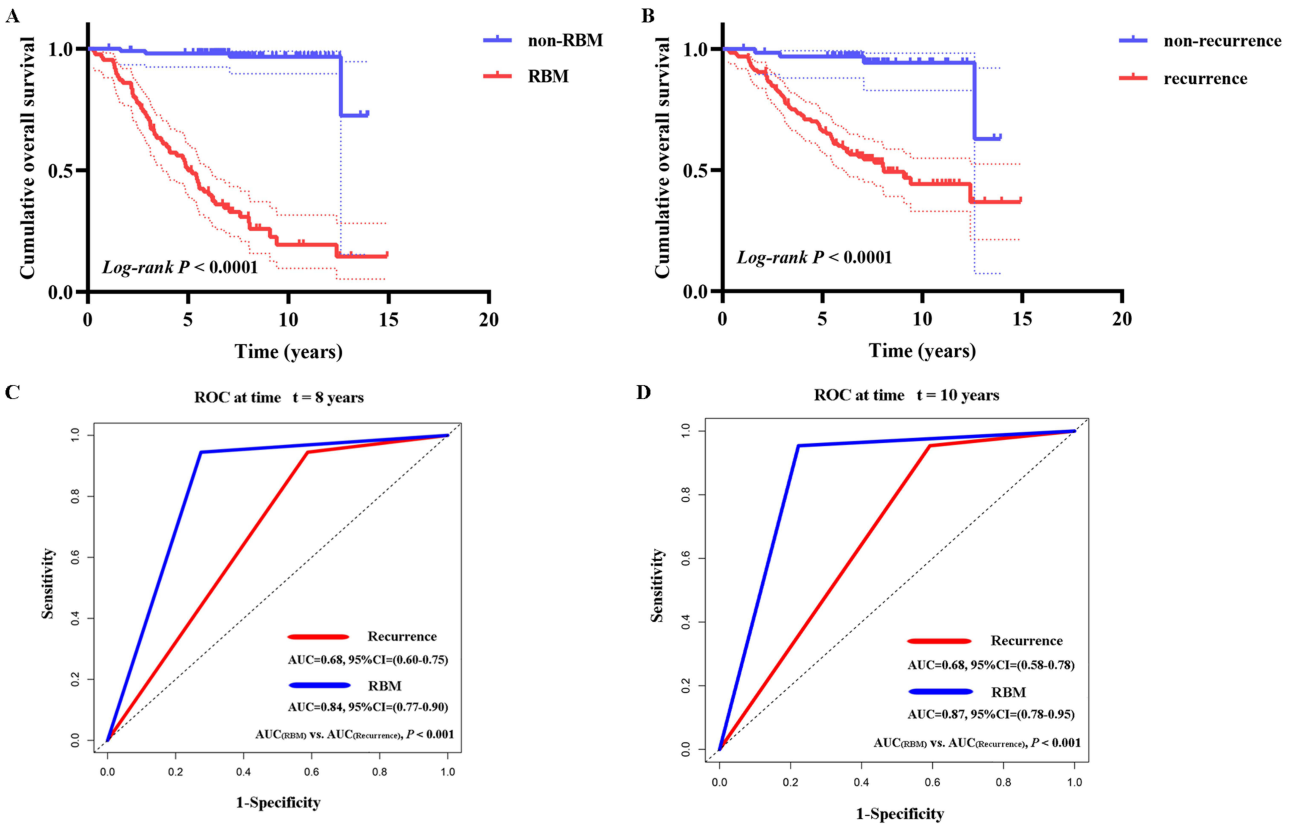


Figure 4 Kaplan-Meier curves for RBM (**A**) and recurrence (**B**). Comparison of the predictive ability of RBM and recurrence for OS using time-dependent ROC and the estimated AUC at 8 years (**C**) and 10 years (**D**).
Abbreviations: RBM, recurrence beyond Milan criteria; ROC, receiver operating characteristic; AUC, area under the curve.

Discussion

In our study, after over 10 years of follow-up, 65.9% (126/191) of potentially transplantable patients with solitary HBV-related early-stage HCC recurred post-ablation, with 68.3% (86/126) exceeding Milan Criteria. The median recurrence-free survival (RFS), time to recurrence beyond Milan Criteria (TRBM) and overall survival (OS) were 4.07, 10.54 and 12.61 years, respectively. The serum CRP ≥1.81 mg/L and peripheral PLT ≤ 80×10^9 /L were independent risk factors for TRBM and RBM incidence. Additionally, these factors formed a PLT-CRP score (PCS), with higher scores correlating to more frequent RBM and severe RBM patterns (vascular invasion or/and metastatic disease).

Due to donor scarcity, ablation serves as a crucial interim therapy for early-stage HCC patients awaiting liver transplantation. However, recurrence, particularly beyond the Milan Criteria (RBM), poses a significant concern, with 68.3% of relapsed patients in our study experiencing RBM, closely aligning with the 71.5% reported by Tsuchiya et al.[5] These findings suggest a substantial decrease in the effectiveness of salvage liver transplantation for most patients with recurrence during follow-up. However, Adam Doyle et al observed that in potentially transplantable patients with solitary HCC ≤3 cm treated with radiofrequency ablation as initial therapy, only 41.7% of those who relapsed progressed to RBM post-ablation,[16] which might be due to the shorter median follow-up of 3.6 years in their study, whereas ours had a 7.64-year follow-up. Although the median follow-up interval reported by Kaoru Tsuchiya et al was only 4.0 years, they included HCC patients with tumors > 3 cm in size and multiple tumors, resulting in a higher proportion of patients with RBM.[5] Ju-Yeon Cho et al reported a median follow-up of 5.7 years, slightly shorter than to ours, but found that only 15.6% of patients with recurrence developed RBM after radiofrequency ablation for single small HCC, lower than in our and other studies.[5,16,17] This underestimation is because they limited RBM to the first recurrence. Interestingly, their results were close to the RBM at first recurrence in our (19.8%, 25/126) and Adam Doyle et al findings (19.1%, 38/199).[16,17]

The association between larger tumor size and higher AFP levels with shorter RFS after ablation is consistent with our findings (Supplementary Table 3). However, the results of these three studies suggest that tumor size ≥2 cm is an independent risk factor for predicting TRBM, inconsistent with our findings (Table 2).[5,16,17] Similarly, higher AFP levels did not show significant predictive power for TRBM in our findings,[5,16] which is consistent with the findings of Ju-Yeon Cho et al.[17] The inconsistency in results may stem from factors like HCC heterogeneity, varying biological backgrounds, and differing endpoint definitions. Notably, Kaoru Tsuchiya et al and Adam Doyle et al had high percentages of early-stage non-HBV-related HCC patients receiving radiofrequency ablation (89.5% and 64.8%, respectively), whereas our study focused exclusively on HBV-related HCC.[5,16] Although Ju-Yeon Cho et al also included a large number of patients with HBV-associated HCC, their definition of RBM differed significantly from other studies, including ours.[5,16,17] In fact, they identified independent risk factors for RBM at the time of the first recurrence, but our identification of RBM continued throughout follow-up.[5,16,17] Results from a previous study showed that 29.8% (37/124) of patients with HBV-associated early-stage HCC with 2–3 cm had RBM after ablation at a median follow-up of 3.14 years.[18] However, our current study updated the median follow-up to 7.64 years and included patients with HBV-associated early HCC less than 2 cm receiving ablation, resulting in an increase in the incidence of RBM to 45.0% (86/191). However, RBM can still significantly improve the ability to predict OS compared to recurrence at any point in time, which largely indicates the stability of RBM in predicting OS[18] (Supplementary Figure 3).

Identification of other risk factors for RBM and TRBM was also acquired in our study. Cirrhosis, serum C-reactive protein (CRP) ≥ 1.81 mg/L, and peripheral platelet (PLT) counts ≤ 80×10^9/L were reported in our study as independent risk factors for predicting the incidence of RBM. In addition, serum CRP ≥ 1.81 mg/L and peripheral PLT ≤ 80×10^9/L were also independent risk factors for TRBM. These results are consistent with the results of our previous study regardless of the updated median follow-up interval and the inclusion of patients with HBV-related early-stage HCC less than 2 cm who underwent ablation.[18] However, these independent risk factors were not reflected in the results of the other three previous studies. The main reason for this was that none of the three studies included serum CRP levels in the baseline characteristics, nor did they explore whether serum CRP levels were an independent predictor of TRBM and the occurrence of BRM.[5,16,17] In addition, two of the studies also did not include platelet count in baseline data, nor did they explore whether peripheral platelet counts were independent predictors of TRBM and the occurrence of BRM.[5,17] Adam Doyle et al, who included peripheral platelet counts in their study and performed independent factor predictions of TRBM, found that PLT counts did not significantly predict TRBM, but they included mainly non-HBV-related HCC patients (only 35.2% of HBV-related HCC patients).[16] In fact, there have been many studies to confirm the association between low preoperative peripheral platelet count and poor prognosis in HCC patients who have undergone ablation or resection, such as intrahepatic distant recurrence, shorter OS and RFS, which has also been further confirmed by Meta-Analysis.[19,20] The mechanism by which platelets and C-reactive protein cause poor prognosis has been elaborated in our previous studies.[18,21] Briefly, in the absence of acute infection, preoperative low peripheral platelet counts and high C-reactive protein may reflect a more obvious background of chronic hepatitis in HCC patients. Inflammation is closely related to the occurrence and development of HCC, and a high inflammatory response indicates more malignant tumor characteristics and resistance to treatment.[21] This is also confirmed by the correlation between higher PCS (consists of high PLT and/or high CRP) and significantly higher incidence of RBM and significantly more malignant RBM phenotype (vascular invasion or/and metastatic disease) (Table 3).

Our study indicates that RBM is a more effective predictor of OS than RFS in early-stage HCC, underscoring the importance of using RBM as the primary endpoint in clinical trials, such as those on postoperative adjuvant therapy for early-stage HCC.[22] Our study highlights that early RBM is significantly linked to shorter median overall survival, similar to early recurrence. This finding emphasizes the need for vigilant monitoring and intervention against both early RBM and recurrence. Notably, as the follow-up period extended, the predominant pattern of RBM shifted from tumor size/number issues to vascular invasion or metastatic disease, accounting for 66.3% of cases. This underscores the criticality of timely and effective strategies to prevent RBM in high-risk patients, irrespective of post-ablation recurrence status.[18]

Our study's main limitations are its single-center, retrospective nature. However, the lengthy median follow-up interval is a significant advantage, allowing more accurate statistical analysis of RBM incidence and reliable prediction of independent risk factors for TRBM. Our reported median follow-up is among the longest in studies on early HCC RBM

post-ablation. Additionally, our focus on potentially transplantable HBV-related early-stage HCC patients suggests further exploration is needed to determine if these conclusions apply to non-HBV-related cases as well.

In conclusion, our study indicates that for transplantable candidates with solitary early-stage HBV-HCC≤3 cm, those with preoperative CRP ≥1.81 mg/L, PLT ≤80×10^9/L, older age, and cirrhosis require closer monitoring and adjunct interventions post-ablation to facilitate timely salvage transplantation if recurrence occurs within Milan criteria. In donor-scarce regions, resection may serve as a bridge to salvage transplantation in the hope of obtaining a longer wait for a donor. Given its strong OS predictive power, recurrence beyond Milan Criteria could be a viable primary endpoint in early-stage HCC trials.

Highlights

- The majority of potentially transplantable patients with solitary HBV-associated early-stage HCC ≤3 cm will experience recurrence beyond Milan Criteria after 10 years following radical ablation. Encouragingly, these outcomes can be effectively predicted by identifying preoperative features including elevated serum C-reactive protein(CRP), low peripheral platelet (PLT) counts, presence of cirrhosis, and older age.
- Furthermore, an elevated PCS, integrating CRP and PLT, is significantly associated with a more aggressive phenotype of RBM, characterized by vascular invasion or metastatic dissemination.
- Compared to recurrence, recurrence beyond the Milan criteria may be more appropriate as an alternative primary endpoint for early HCC-related clinical trials.

Implications for Patients Care

This study will provide important reference significance for selecting the most suitable candidates for thermal ablation therapy as a bridging treatment to liver transplantation and offer valuable insights into intervention and follow-up strategies for high-risk populations of recurrent beyond Milan criteria (RBM). Additionally, RBM serves as a viable primary endpoint in early-stage HCC trials, which may change the existing tumor efficacy evaluation system.

Abbreviations

HCC, hepatocellular carcinoma; RBM, recurrence beyond Milan Criteria; TRBM, time to recurrence beyond the Milan Criteria; OS, overall survival; CRP, C-reactive protein; PLT, platelet; PCS, PLT - CRP score; HBV, Hepatitis-B virus; RFS, recurrence-free survival; ALB, Albumin; ALBI, Albumin-bilirubin; RFA, Radiofrequency ablation; MWA, microwave ablation; CT, Computed tomography; MRI, magnetic resonance imaging; IQR: interquartile range; CI, confidence interval; ROC, receiver operating characteristic; VI, Vascular invasion; AUC, area under the curve; AFP, alpha-fetoprotein; PT, prothrombin time; NR, not reached; TBIL, Total bilirubin; HR, hazard ratio.

Data Sharing Statement

The data that support the findings of this study are available on request from the corresponding author.

Statement of Ethics

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Sun Yat-sen University Cancer Center. The informed consents were waived because of the retrospective nature of this study. We confirm that the data has been anonymized or maintained confidentially.

Acknowledgment

We would like to thank Ms Binyan Shen for her support of Shuanggang Chen. Graphical abstract is drawn by Figdraw.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was funded by the National Key Research and Development Program of China (Grant number: 2023YFC2414000) and the National Natural Science Foundation of China (Grant number: 82372060).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Chen Y, Tian Z. HBV-induced immune imbalance in the development of HCC. *Front Immunol*. 2019;10:2048. doi:10.3389/fimmu.2019.02048
2. Benson AB, D'Angelica MI, Abbott DE, et al. Guidelines insights: hepatobiliary cancers, version 2.2019. *J Natl Compr Canc Netw*. 2019;17:302–310. doi:10.6004/jnccn.2019.0019
3. Boros C, Sutter O, Cauchy F, et al. Upfront multi-bipolar radiofrequency ablation for HCC in transplant-eligible cirrhotic patients with salvage transplantation in case of recurrence. *Liver Int*. 2024;44:1464–1473. doi:10.1111/liv.15900
4. Anselmo A, Siragusa L, Brigato P, et al. Primary versus salvage liver transplantation after curative-intent resection or radiofrequency ablation for hepatocellular carcinoma: long-term oncological outcomes. *Cancers*. 2023;16:15. doi:10.3390/cancers15205030
5. Tsuchiya K, Asahina Y, Tamaki N, et al. Risk factors for exceeding the Milan criteria after successful radiofrequency ablation in patients with early-stage hepatocellular carcinoma. *Liver Transpl*. 2014;20:291–297. doi:10.1002/lt.23798
6. Lim C, Shinkawa H, Hasegawa K, et al. Salvage liver transplantation or repeat hepatectomy for recurrent hepatocellular carcinoma: an intent-to-treat analysis. *Liver Transpl*. 2017;23:1553–1563. doi:10.1002/lt.24952
7. Yoon YI, Song GW, Lee S, et al. Salvage living donor liver transplantation versus repeat liver resection for patients with recurrent hepatocellular carcinoma and Child-Pugh class A liver cirrhosis: a propensity score-matched comparison. *Am J Transplant*. 2022;22:165–176. doi:10.1111/ajt.16790
8. Chan AC, Chan SC, Chok KS, et al. Treatment strategy for recurrent hepatocellular carcinoma: salvage transplantation, repeated resection, or radiofrequency ablation? *Liver Transpl*. 2013;19:411–419. doi:10.1002/lt.23605
9. Hwang S, Song GW, Ahn CS, et al. Quantitative prognostic prediction using ADV score for hepatocellular carcinoma following living donor liver transplantation. *J Gastrointestinal Surg*. 2021;25:2503–2515. doi:10.1007/s11605-021-04939-w
10. Yang Y, Sun JH, Tan XY, et al. MTM-HCC at previous liver resection as a predictor of overall survival in salvage liver transplantation. *Dig Dis Sci*. 2023;68:2768–2777. doi:10.1007/s10620-023-07857-w
11. Liu S, Wu J, Ding W, et al. The tumor ghost on MRI after microwave ablation for hepatocellular carcinoma: a new modality to assess the ablative margin. *Eur J Radiol*. 2023;158:110617. doi:10.1016/j.ejrad.2022.110617
12. Li FY, Li JG, Wu SS, et al. An optimal ablative margin of small single hepatocellular carcinoma treated with image-guided percutaneous thermal ablation and local recurrence prediction base on the ablative margin: a multicenter study. *J Hepatocell Carcinoma*. 2021;8:1375–1388. doi:10.2147/JHC.S330746
13. Rodriguez-Alvarez MX, Meira-Machado L, Abu-Assi E, Raposeiras-Roubin S. Nonparametric estimation of time-dependent ROC curves conditional on a continuous covariate. *Stat Med*. 2016;35:1090–1102. doi:10.1002/sim.6769
14. Chen S, Ma W, Cao F, et al. Hepatocellular carcinoma within the Milan criteria: a novel inflammation-based nomogram system to assess the outcomes of ablation. *Front Oncol*. 2020;10:1764. doi:10.3389/fonc.2020.01764
15. He Y, Luo L, Shan R, et al. Development and validation of a nomogram for predicting postoperative early relapse and survival in hepatocellular carcinoma. *J Natl Compr Canc Netw*. 2023;22:e237069. doi:10.6004/jnccn.2023.7069
16. Doyle A, Gorgen A, Muaddi H, et al. Outcomes of radiofrequency ablation as first-line therapy for hepatocellular carcinoma less than 3cm in potentially transplantable patients. *J Hepatol*. 2019;70:866–873. doi:10.1016/j.jhep.2018.12.027
17. Cho JY, Choi MS, Lee GS, et al. Clinical significance and predictive factors of early massive recurrence after radiofrequency ablation in patients with a single small hepatocellular carcinoma. *Clin Mol Hepatol*. 2016;22:477–486. doi:10.3350/cmh.2016.0048
18. Chen S, Ma W, Shen L, et al. Recurrence beyond the Milan criteria of HBV-related single hepatocellular carcinoma of 2-3 cm: comparison of resection and ablation. *Front Oncol*. 2021;11:757149. doi:10.3389/fonc.2021.757149
19. Pang Q, Qu K, Bi JB, et al. Thrombocytopenia for prediction of hepatocellular carcinoma recurrence: systematic review and meta-analysis. *World J Gastroenterol*. 2015;21:7895–7906. doi:10.3748/wjg.v21.i25.7895
20. Pang Q, Qu K, Zhang JY, et al. The prognostic value of platelet count in patients with hepatocellular carcinoma: a systematic review and meta-analysis. *Medicine*. 2015;94:e1431. doi:10.1097/MD.0000000000001431
21. Chen S, Shen B, Wu Y, et al. The relationship between the efficacy of thermal ablation and inflammatory response and immune status in early hepatocellular carcinoma and the progress of postoperative adjuvant therapy. *Int Immunopharmacol*. 2023;119:110228. doi:10.1016/j.intimp.2023.110228
22. Qin S, Chen M, Cheng AL, et al. Atezolizumab plus bevacizumab versus active surveillance in patients with resected or ablated high-risk hepatocellular carcinoma (IMbrave050): a randomised, open-label, multicentre, Phase 3 trial. *Lancet*. 2023;402:1835–1847. doi:10.1016/S0140-6736(23)01796-8