INTERVENTIONAL THERAPY OF HEPATOCELLULAR CARCINOMA

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INTERVENTIONAL THERAPY OF HEPATOCELLULAR CARCINOMA

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Editorial: Interventional therapy of hepatocellular carcinoma

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Editorial on the Research Topic
Interventional therapy of hepatocellular carcinoma

Globally, liver cancer is the most frequent fatal malignancy. In the United States, it ranks fifth (Sung et al., 2021). Patients are often diagnosed with liver cancer in advanced stages, contributing to its poor prognosis. Of all liver cancer cases, >90% are hepatocellular carcinomas (HCCs) (Sung et al., 2021). Currently, the incidence and mortality are increasing worldwide. Liver cancer is an extraordinarily heterogeneous malignant disease among the tumors that have so far been identified. HCC arises most frequently in the setting of chronic liver inflammation and fibrosis and takes a variety of course in individual patients to process to tumor. Because of the complex anatomy of the liver and association with underlying liver disease, management of these patients has been a challenge, considering both tumor and patient factors.

HCC classically is diagnosed at an advanced stage in a symptomatic patient, with very little therapeutic options. There is a rising incidence of HCC globally, the etiology of which includes viral hepatitis (B and C), alcohol, obesity, and dietary carcinogens, as the most common causes contributing to this high burden of HCC (Sung et al., 2021). Over the past 10 years, there has been considerable progress in the diagnosis and surgical treatment of HCC. Surgical resection is the best treatment option for early HCC. Unfortunately, majority of HCC patients already have advanced disease at the time of presentation. Therefore, liver cancer represents a major therapeutic challenge, and interventional therapy is the most used therapeutic approach today.

In this special issue, we present a summary on the most recent treatment modalities of HCC, using transcatheter arterial chemoembolization (TACE), radiofrequency ablation (RFA) and microwave ablation (MWA), while historical perspectives and the latest key findings are discussed. Comparing the efficacy and safety of TACE combining with apatinib (TACE-apatinib) and TACE-alone for patients with advanced HCC with hepatic arterioportal shunts (APS), it was concluded that TACE-apatinib was an efficacious and safe treatment for patients with advanced HCC with APS, and apatinib improved the efficacy of TACE in the treatment of these patients (Sun et al.). Retrospective study, investigating the efficacy and safety of sorafenib combined TACE (TACE + Sor) vs TACE combined with sorafenib plus

immune checkpoint inhibitors (TACE + Sor + ICIs) in treating intermediate and advanced TACE-refractory HCC, showed that the therapeutic schedule of TACE + Sor + ICIs demonstrated efficacy and safety in intermediate and advanced TACE-refractory HCC (Zheng et al.). In a study to identify the independent risk factors for TACE refractoriness and to develop a novel TACE refractoriness score and nomogram for predicting TACE refractoriness in patients with HCC, the conclusion was that TACE refractoriness impaired the overall survival (OS) of HCC patients, that the number of tumors and bilobular invasion status were independent risk factors for TACE refractoriness, and that TACE refractoriness score could be an effective tool and easy approach to predict the risk of TACE refractoriness status (Chen et al.). A multicenter retrospective study was conducted with a purpose to use baseline variables to predict 1-year disease control for patients with HCC treated with TACE combined with sorafenib as initial treatment by applying a machine learning approach based on the random survival forest (RF) model (Zhong et al.). Because the RF model achieved a higher concordance index of 0.724 compared to that for the logistic regression model (0.709), it was concluded that the RF model was a simple and accurate approach for prediction of 1-year disease control for patients with HCC treated with TACE combined with sorafenib (Zhong et al.). The selection criteria for hepatic resection (HR) in intermediate-stage (IM) hepatocellular carcinoma (HCC) are still controversial. In a study conducted using the real-world data to evaluate the OS in treatment with HR or TACE, the conclusion was that HR was superior to TACE for intermediate-stage HCC in patients with LDH levels >192 U/L (Lu et al.). Investigating the predictive value of inflammatory biomarkers in patients with unresectable HCC for outcomes following the combination treatment of TACE plus sorafenib, the study indicated the prognostic value of quantitative inflammatory biomarkers in correlation with OS and progression-free survival (PFS) in unresectable HCC patients undergoing TACE plus sorafenib treatment (Zhang et al.). Trying to develop and validate a predictive model for early refractoriness of TACE in patients with HCC, an interesting multicenter retrospective study was conducted (Wang et al.), where a predictive model was established using forward stepwise logistic regression and nomogram (Wang et al.). Based on factors selected by logistic regression, a one-to-one propensity score matching (PSM) was conducted to compare PFS between patients who were present or absent of early TACE refractoriness. After PSM, the result showed that patients who were absent of early TACE refractoriness had a significantly higher PFS rate than those of patients who were present. This study presents a predictive model with moderate accuracy to identify patients with high risk of early TACE refractoriness, and patients with early TACE refractoriness may have a poor prognosis (Wang et al.). Trying to evaluate the safety and efficacy of TACE in elderly patients diagnosed as advanced HCC accompanied with different types of portal vein tumor thrombosis (PVTT), it was shown that palliative TACE treatment could be an accessible effective measure to improve the OS and PFS for both type I and type II PVTT patients (Tang et al.). Important study was conducted to establish a magnetic resonance imaging radiomics signature-based nomogram for predicting the progression-free survival of intermediate and advanced HCC patients treated with TACE plus RFA, showing that the radiomics signature was a prognostic risk factor, and a nomogram combined radiomics and clinical factors acted as a new strategy for predicted the PFS of intermediate and advanced HCC treated with TACE plus RFA (Fang et al.). Cases of HCC arising or involving the caudate lobe (HCC-CL) are relatively rare. It was shown that TACE treatment might be associated with better survival benefits in unresectable or "ablation unsuitable" HCC in the CL without macroscopic vascular invasion (MVI) and adequate liver function, compared with the non-selective TACE group, and should be considered as an important reliable therapy for surgeons and interventional radiologists (Yan et al.). To improve treatment of HCC, further research focused on the molecular mechanisms of HCC tumorigenesis is essential. The role of PTEN-Long, a translational variant of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a tumor suppressor frequently lost or mutated in several human tumors was evaluated in the development of liver cancer (Tan et al.). The study identified the antitumor function of PTEN-Long and suggested its potential role and utility for liver cancer treatment (Tan et al.).

In conclusion, with the understanding of the molecular mechanism of HCC and developing of new techniques, the theranostics of HCC have experienced innovations over the last few decades. The best treatment options which is interventional-based minimally invasive therapy have been discussed in this special issue. For example, Transarterial chemoembolization, Radiofrequency ablation, Microwave ablation. In recent years, new emerging interventional therapy has been developing towards non-invasive and intellectual development, playing an increasingly important role in the treatment of liver cancer. Additionally, multidisciplinary strategies for HCC treatment have been highly recommended by the clinical guidelines to further improve the survival and reduce the side-effect for HCC patients.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Advanced Hepatocellular Carcinoma With Hepatic Arterioportal Shunts: Combination Treatment of Transarterial Chemoembolization With Apatinib

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Object: This study aimed to compare the efficacy and safety of transarterial chemoembolization (TACE) combining with apatinib (TACE-apatinib) and TACE-alone for patients with advanced hepatocellular carcinoma (HCC) with hepatic arterioportal shunts (APS).

Materials and Methods: This retrospective study evaluated the medical records of patients with advanced HCC with APS who underwent TACE-apatinib or TACE-alone from June 2015 to January 2019. The occlusion of the shunt was performed during the TACE procedure. The time to tumor progression (TTP) and overall survival (OS) of study patients were evaluated. The modified Response Evaluation Criteria in solid tumors (mRECIST) was used to evaluate the treatment response. The apatinib-related adverse events were recorded.

Results: Fifty-eight patients were included in this study. Twenty-seven patients underwent the treatment of TACE-apatinib, and 31 received TACE-alone treatment. The median overall survival (OS) and median time of tumor progression (TTP) in the TACE-apatinib group were significantly longer than those of the TACE-alone group (OS: 12.0 vs. 9.0 months, $P = 0.000$; TTP: 9.0 vs. 5.0 months, $P = 0.041$). Multivariate analysis revealed that TACE-apatinib was a protective factor for OS, and there was no independent risk factor for TTP. In the TACE-apatinib group, the grade 3 apatinib-related adverse events occurred in four patients.

Conclusion: TACE-apatinib was an efficacious and safe treatment for patients with advanced HCC with APS, and apatinib improved the efficacy of TACE in the treatment of these patients.

Keywords: transarterial chemoembolization, apatinib, advanced hepatocellular carcinoma, hepatic arterioportal shunts, efficacy, safety

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and is one of the most prevalent causes of tumor-related death (Bray et al., 2018). About 35–40% of all HCC patients are diagnosed when the disease has reached an advanced stage (Barcelona Clinic Liver Cancer [BCLC] stage C) owing to an absence of routine screening protocols and to the fact that the disease is often asymptomatic in its early stages, limiting patient treatment options (Forner et al., 2010). These patients must thus rely on palliative therapies, such as sorafenib treatment to prolong their survival (Forner et al., 2010). Recently, many studies have shown that apatinib, which is a newly developed inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2) (Ding et al., 2013), exhibits encouraging antitumor activities and tolerable toxicities when used to treat advanced HCC (Wang and Tang, 2018; Xue et al., 2018; Yang and Qin, 2018).

Hepatic arterioportal shunts (APS) is common in patients with HCC (Wu et al., 2018), affecting up to 60% of these patients (Okuda et al., 1977). The presence of APS has an adverse effect on patient prognosis and increases the incidence of complications, such as esophagus varicose rupture, refractory ascites, and hepatic encephalopathy (Velazquez et al., 2003; Lencioni et al., 2016). APS can not only damage the liver function and aggravate portal hypertension in HCC patients, but can also easily lead to the spread and metastasis of HCC (Murata et al., 2009). Additionally, APS can affect the safety of transcatheter arterial chemoembolization (TACE), as lipiodol can flow through the fistula and thereby access normal hepatic and pulmonary tissues (Ziessman et al., 1984). The standard treatment of APS relies upon the blocking of these shunts using an appropriate embolic material, which can be carried out via the TACE procedure (Kim et al., 2007).

TACE is a standard treatment for BCLC stage B HCC patients based on current BCLC guidelines European Association for the Study of the Liver, 2018). However, some previous studies reported that TACE is beneficial for patients with advanced HCC (Bai et al., 2013; Choi et al., 2013; Zhao et al., 2013). Recently, an increasing number of studies have demonstrated that a combination of TACE with apatinib treatment (TACE-apatinib) may prolong the survival of selected patients with BCLC stage-C HCC (Lu et al., 2017; Yang et al., 2019; Zhao et al., 2020). To our best knowledge, there have been few reports regarding the use of TACE-apatinib as a treatment modality in advanced HCC patients with APS. As such, we conducted a retrospective study to evaluate the efficacy and safety of TACE-apatinib for the treatment of advanced HCC with APS.

Abbreviations: HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer; OS, overall survival; TACE, transarterial chemoembolization; TACE-apatinib, TACE combining with apatinib; ECOG, Eastern Cooperative Group Performance Status; CT, computed tomography; MR, magnetic resonance; AFP, a-Fetoprotein; mRECIST, modified Response Evaluation Criteria in solid tumors; PR, Partial response; PD, Progressive disease; SD, Stable disease; DCR, disease control rate; TTP, time to tumor progression; APS, hepatic Arterioportal shunts; CI, Confidence interval.

MATERIALS AND METHODS

Patient Selections

Approval for this retrospective study was obtained from the institutional review board of our hospital. Between June 2015 and January 2019, 27 advanced HCC patients with APS who underwent TACE-apatinib were included in this study. Prior to initial TACE procedure, patients had been diagnosed with advanced HCC with APS via abdominal contrast-enhanced computed tomography (CT) or magnetic resonance (MRI). Digital subtraction angiography (DSA) was used to confirm APS during the TACE procedure. A written informed consent was obtained from all patients prior to treatment. The inclusion criteria for this study were as follows: (1) patients were diagnosed with HCC based on the guidelines of the European Association for the Study of Liver or the American Association for the Study of Liver Disease; (2) patients were staged at BCLC-C in accordance with the BCLC system; (3) patients were diagnosed with APS via medical imaging; (4) patients with liver function graded at Child-Pugh A or B; (5) patients had the Eastern Cooperative Group Performance Status (ECOG) score of patients were 0–2. The exclusion criteria of this study were as follows: (1) patients who had main portal vein obstruction; (2) patients with a poor performance status (ECOG $>$ 2); (3) patients with significant extra-hepatic disease; (4) patients who had serious medical comorbidities, such as severe dysfunction of the liver, kidney, lung, or heart; (5) patients with massive ascites.

TACE Procedure

The TACE procedure was performed by operators with a minimum of 5 years of experience. First, angiography of the hepatic common artery was used to identify the location, severity and direction of the vessels of APS. Then, a 5-F catheter (Cook, Bloomington, Indian, USA) or a 3-F microcatheter (Progreat, Terumo, Tokyo, Japan) was advanced into the feeding artery of APS. Polyvinyl alcohol particles (500–1,000 um, Cook, USA) that were mixed with contrast media (Hengrui Pharmaceutical Co. Ltd, Jiangsu, China) were then injected to block the APS. An arteriography was then performed to confirm the occlusion of APS. Depending on tumor size and liver function, 2–20 mL of lipiodol (Lipiodol Ultrafluido, Guerbet, France) was mixed with 20–40 mg doxorubicin hydrochloride (Hisun Pharmaceutical Co. LTD, Zhejiang, China) to create an emulsion that was subsequently injected into the tumor feeding arteries. Gelatin sponge particles (300–700 um, Cook, USA) were used to supplement embolization until the stagnation of artery flow appeared.

Apatinib Administration

In the TACE-apatinib group, apatinib (500 mg/day) (Hengrui Pharmaceutical Co. Ltd, Jiangsu, China) was orally administrated 3–5 days after each TACE procedure. Apatinib dose adjustment was based on the tolerance of patients to the drug. The grading of adverse events associated with apatinib was conducted according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0). If apatinib-related adverse events were equal to or greater than grade 3, then the dose of

apatinib was reduced to 250 mg/day to alleviate or eliminate the adverse events. If these events (≥grade 3) didn't disappear after the dose adjustment, the administration of the drug was temporarily interrupted. The dose was resumed at 250 mg/day for patients who have experienced drug interruption when the adverse events had been alleviated or disappeared.

Follow-Up

Follow-up of all patients was conducted through until January 2020. Follow-up contents included imaging examinations, such as abdominal contrast-enhanced CT or MRI, and laboratory tests, such as urine and blood routine test, liver function tests, and renal function analyses. The first follow-up was carried out at the end of the fourth week after the first TACE operation. A repeated TACE procedure was performed when the recurrent tumors or residual lesions were found by medical imaging. The next follow-up interval was extended to every 2 months starting at 4 weeks after the first TACE procedure.

Assessments

Tumor response, overall survival (OS), and time to progression (TTP) were assessed. The medical records including CT, MRI, and follow-up data were reviewed. Treatment response was

TABLE 1 | Baseline characteristics of advanced HCC patients with APS.

Characteristics	TACE-apatinib (*N* = 27) (No, %; Mean ± SD)	TACE alone (*N* = 31) (No, %; Mean ± SD)	*P*-value
Gender			0.107[a]
Male	24 (88.9%)	21 (67.7%)	
Female	3 (11.1%)	10 (32.3%)	
Age (years)	55.56 ± 5.2	58.65 ± 6.6	0.56[b]
Bilirubin (μmol/L)	17.2 ± 7.8	19.5 ± 13.8	0.45[b]
Albumin (g/L)	37.4 ± 4.9	38.1 ± 5.6	0.60[b]
PT (s)	13.9 ± 0.7	14.4 ± 2.0	0.20[b]
ECOG			0.79[a]
1	21 (77.8%)	25 (80.6%)	
2	6 (22.2%)	6 (19.4%)	
Ascites			0.99[a]
Yes	7 (25.9%)	8 (25.8%)	
No	20 (74.1%)	23 (74.2%)	
Portal vein invasion			0.83[a]
Yes	19 (70.4%)	21 (67.7%)	
No	8 (29.6%)	10 (32.3%)	
Extrahepatic metastasis			0.61[a]
Yes	14 (51.9%)	14 (45.2%)	
No	13 (48.1%)	17 (54.8%	
Hepatitis			0.76[a]
Hepatitis B	25 (92.6%)	28 (90.3%)	
Other	2 (7.4%)	3 (9.7%)	
α-Fetoprotein level			0.450[a]
>400 ng/mL	13 (48.1%)	18 (58.1%)	
≤400 ng/ml	14 (51.9%)	13 (41.9%)	
Child-Pugh score			0.75[a]
A	21 (77.8%)	23 (74.2%)	
B	6 (22.2%)	8 (25.8%)	
TACE sessions			0.85[a]
1	12 (44.4%)	13 (41.9%)	
2 or more	15 (55.6%)	18 (58.1%)	

SD, Standard deviation; PT, Prothrombin time; ECOG, Eastern Cooperative Oncology Group; TACE, Transcatheter arterial chemoembolization.
[a]*Chi-square test.*
[b]*Student's t-test.*

TABLE 2 | Univariate and multivariate analysis of prognostic factors for DCR.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Gender				
Male	1			
Female	0.465 (0.125, 1.730)	0.253		
Age (years)	0.956 (0.908, 1.007)	0.092	0.983 (0.926, 1.044)	0.581
ECOG				
2	1			
1	0.769 (0.215, 2.747)	0.686		
Ascites				
Yes	1			
No	1.304 (0.395, 4.306)	0.663		
Portal vein invasion				
Yes	1			
No	0.703 (0.227, 2.183)	0.543		
Extrahepatic metastasis				
Yes	1			
No	1.167 (0.414, 3.290)	0.771		
Bilirubin	1.008 (0.963, 1.056)	0.721		
Albumin	0.955 (0.863, 1.056)	0.371		
PT	1.001 (0.710, 1.412)	0.995		
Hepatitis				
Hepatitis B	1			
Other	0.280 (0.029, 2.674)	0.269		
α-fetoprotein level				
>400 ng/mL	1			
≤400 ng/ml	0.440 (0.153, 1.266)	0.128		
Child-Pugh score				
B	1			
A	0.519 (0.154, 1.754)	0.291		
TACE sessions				
1	1			
2 or more	0.600 (0.210, 1.715)	0.340		
Treatment method				
TACE-apatinib	1			
TACE alone	4.156 (1.382, 12.493)	**0.011**	3.517 (1.019, 12.147)	**0.047**

DCR, Disease control rate; HR, Hazard ratio; CI, Confidence interval; ECOG, Eastern Cooperative Oncology Group; PT, Prothrombin time; TACE, Transcatheter arterial chemoembolization.
Statistical analysis: Logistic regression.
The bold values means that the value of P < 0.05.

evaluated based on the Modified Response Evaluation Criteria in Solid Tumors (mRECIST) criteria (Lencioni and Llovet, 2010). All treatment response evaluations were assessed by a diagnostic radiologist (F.Y., with more than 15 years of experience) and an interventional radiologist (B.L., with more than 10 years of experience). Meanwhile, the treatment information and survival data of patients were blinded to them. Disease control rate (DCR) was defined as the portion of patients who achieved complete response (CR), partial response (PR), and stable disease (SD) (CR or PR or SD). OS was defined as the time from the first TACE to the last follow up or any reason death. TTP was defined as the time from the first TACE to the time that tumor progression.

Statistical Analysis

All statistical analyses were performed by SPSS 24.0 software (IBM, Armonk, New York). Normally distributed data, non-normally distributed data and categorical variables were expressed as mean ± standard deviation, median (quartile range), and frequency (percentage), respectively. Kaplan-Meier method was used to describe the OS and TTP of the study cohort. Univariate analyses were implemented with a log-rank test (OS, TTP) and logistic regression (DCR). Variables with a value of $P < 0.10$ were entered into a multivariate analysis, which was performed by Cox proportional hazard regression model (OS, TTP) and logistic regression model (DCR). P-value <0.05 (two-tailed) was statistically significant.

RESULTS

Study Population

A total of 58 advanced HCC patients with APS were included in this study from June 2015 to January 2019. Twenty-seven patients underwent the treatment of TACE-apatinib, and 31 patients received TACE-alone treatment. The detailed baseline characteristics of the study population (gender, age, bilirubin, albumin, PT, ECOG, ascites, portal vein invasion, extrahepatic metastasis, AFP level, HBV infection, Child-Pugh class, TACE sessions) are listed in **Table 1**. The median follow-up period was 11 months (range, 3–30 months).

The Efficacy of TACE-Apatinib for Advanced HCC With APS

In the TACE-apatinib group, there were no case with CR, 10(37.0%) cases with PR, 7 (25.9%) cases with SD, and 10 (37.0%) cases with progressive disease (PD). In the TACE-alone group, there were no case with CR, 5 (16.1%) cases with PR, 4 (12.9%) cases with SD, and 22 (71.0%) cases with PD. The DCR of tumor response was 62.9% in the TACE-apatinib group, which was significantly higher than 29.0% in the TACE-alone group ($P = 0.01$). Multivariate analysis showed that treatment method (TACE-apatinib) was an independent prognostic factor for DCR (**Table 2**).

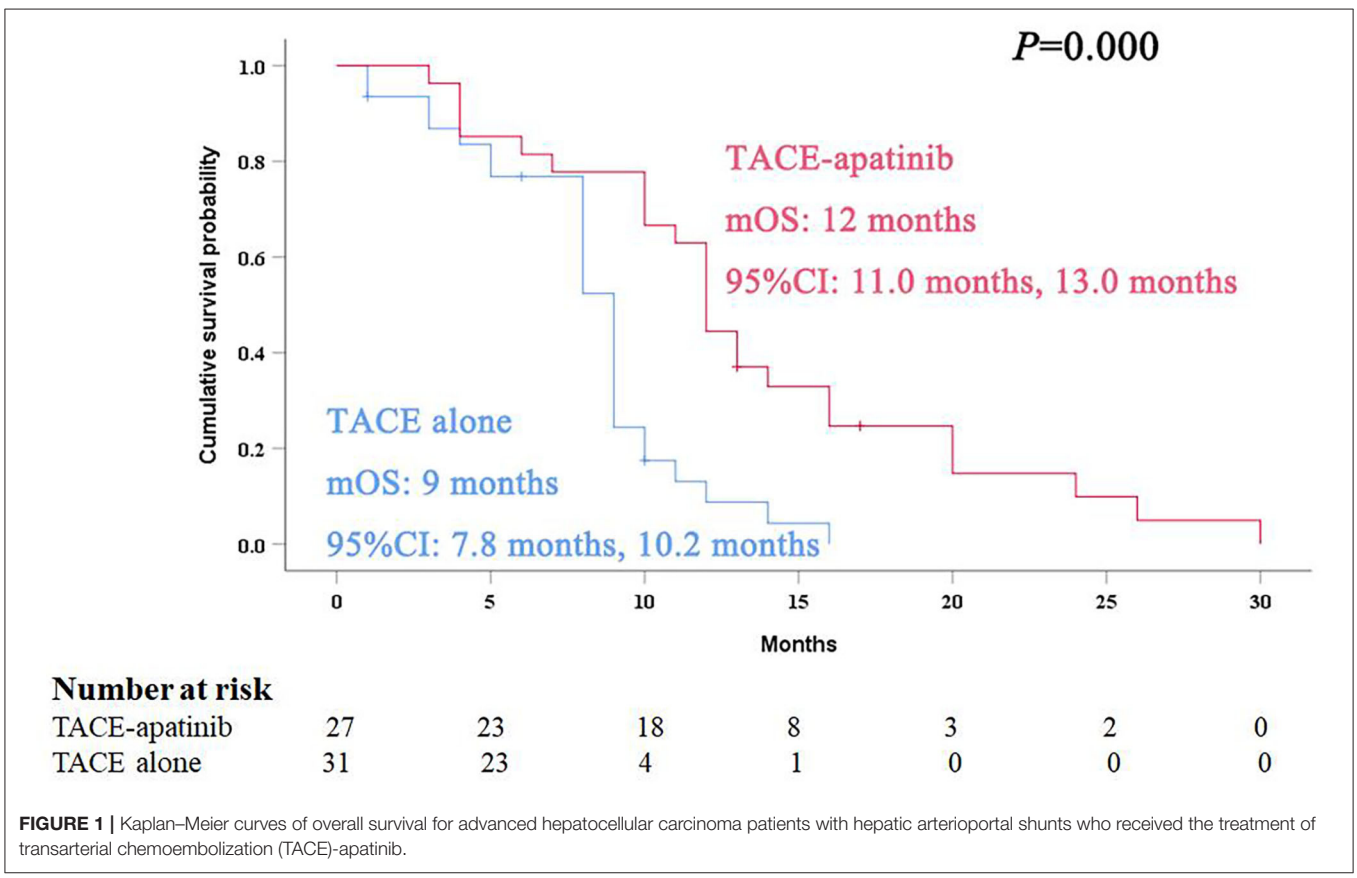

FIGURE 1 | Kaplan–Meier curves of overall survival for advanced hepatocellular carcinoma patients with hepatic arterioportal shunts who received the treatment of transarterial chemoembolization (TACE)-apatinib.

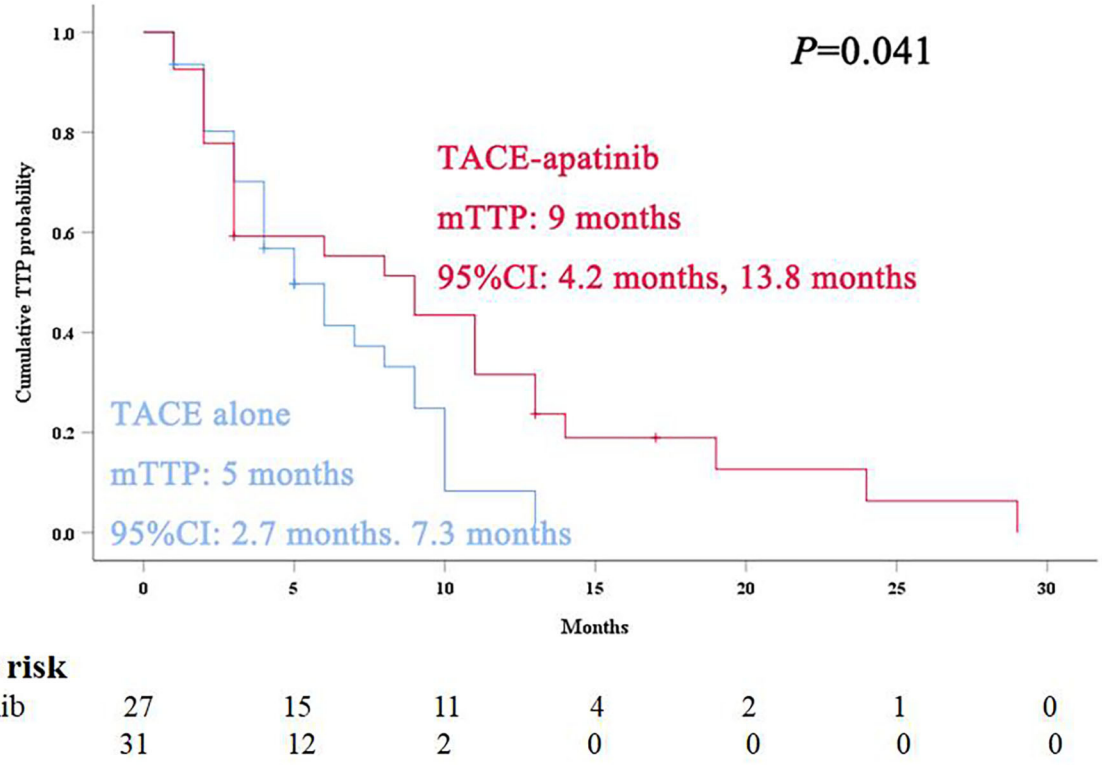

FIGURE 2 | Kaplan–Meier curves of time to progression for advanced hepatocellular carcinoma patients with hepatic arterioportal shunts who received the treatment of transarterial chemoembolization (TACE)-apatinib.

During the follow-up, 24 patients (88.9%) died in the TACE-apatinib group, and 27 patients (87.1%) died in the TACE-alone group. The main causes of death were liver failure and gastrointestinal bleeding. The median overall survival (OS) in the TACE-apatinib group was 12.0 months (95% CI: 11.0, 13.0 months), and in the TACE alone group was 9.0 months (95% CI: 7.8, 10.2 months) (**Figure 1**). The median OS between the two groups was significantly different. The median TTP in the TACE-apatinib group was 9.0 months (95% CI: 4.2, 13.8 months), which was significantly longer than 5.0 months (95% CI: 2.7, 7.3 months) in the TACE-alone group ($P = 0.041$) (**Figure 2**).

Prognostic Factors Affecting OS and TTP

Univariate analyses showed that portal vein invasion, TACE sessions, and treatment method were correlated with OS (**Table 3**). Then, through multivariable analyses, we found that TACE-apatinib was a protective factor for OS (**Table 4**). Univariate analyses showed that albumin and PT were significantly associated with TTP (**Table 3**), but there was no independent risk factor in multivariate analyses for TTP (**Table 5**).

Adverse Events Related to TACE or Apatinib

No treatment-related deaths were observed in this study. Three representative indicators of liver function (bilirubin, albumin, and PT) were observed at 4 weeks after the first TACE-apatinib treatment, and did not differ significantly from the baseline values at this time point (**Figures 3A–C**). The detailed adverse events that related to TACE were listed in **Table 6**. There were 2 (7.4%) patients who had adverse events of hepatorenal syndrome or hepatic arterial dissection after TACE in the TACE-apatinib group. In the TACE-alone group, only 1 patient experienced adverse event of hepatic arterial dissection. There was no significant difference in the adverse events that related to TACE between the two groups. The adverse events that related to apatinib were listed in the **Table 7**. The apatinib-related adverse events in the TACE-apatinib group occurred in 24 (88.9%) out of the 27 patients. Four (14.8%) patients developed grade 3 adverse events, and its duration was 1–2 weeks. The symptoms that related to these adverse events in the patients were alleviated or eliminated after drug reduction or interruption and symptomatic treatments. There was no occurrence of grade 4 and grade 5 adverse events. These symptoms were alleviated within 7 days after treatment and no further complications occurred.

DISCUSSION

TACE is the most widely used and an effective conservative treatment for unresectable HCC (European Association for the Study of the Liver, 2018). Emerging studies have revealed that the combination treatment of TACE with sorafenib

TABLE 3 | Univariate analysis of prognostic factors for overall survival and time to progression.

Variables	OS		TTP	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Gender				
Male	1		1	
Female	0.681 (0.199, 2.328)	0.540	0.636 (0.183, 2.205)	0.475
Age (years)	0.979 (0.937, 1.023)	0.350	0.993 (0.952, 1.036)	0.741
ECOG				
2	1		1	
1	0.904 (0.332, 2.457)	0.843	0.717 (0.264, 1.952)	0.516
Ascites				
Yes	1		1	
No	0.861 (0.334, 2.215)	0.756	1.183 (0.457, 3.065)	0.729
Portal vein invasion				
Yes	1		1	
No	2.794 (1.065, 7.331)	**0.037**	2.027 (0.780, 5.270)	0.147
Extrahepatic metastasis				
Yes	1		1	
No	0.938 (0.411, 2.142)	0.880	1.118 (0.482, 2.597)	0.794
Bilirubin	1.032 (0.972, 1.096)	0.300	1.014 (0.956, 1.076)	0.641
Albumin	1.022 (0.944, 1.107)	0.592	1.086 (0.996, 1.185)	**0.032**
PT	0.763 (0.441, 1.320)	0.333	0.508 (0.255, 1.012)	**0.044**
Hepatitis				
Hepatitis B	1		1	
Other	1.427 (0.329, 6.200)	0.635	0.459 (0.100, 2.100)	0.316
α-fetoprotein level				
>400 ng/mL	1		1	
≤400 ng/ml	1.199 (0.535, 2.688)	0.659	1.301 (0.568, 2.977)	0.534
Child-Pugh score				
B	1		1	
A	0.996 (0.361, 2.749)	0.994	1.086 (0.397, 2.971)	0.873
TACE sessions				
1	1		1	
2 or more	2.031 (0.880, 4.688)	**0.047**	1.820 (0.754, 4.394)	0.183
Treatment method				
TACE-apatinib	1			
TACE alone	2.918 (1.573, 5.415)	**0.001**	1.787 (0.978, 3.266)	0.059

OS, Overall survival; TTP, Time to progression; HR, Hazard ratio; CI, Confidence interval; ECOG, Eastern Cooperative Oncology Group; PT, Prothrombin time; TACE, Transcatheter arterial chemoembolization.
Statistical analysis: log-rank test.
The bold values means that the value of P < 0.05.

TABLE 4 | Multivariate analysis of prognostic factors for overall survival.

Variables	HR (95% CI)	*P*-value
Portal vein invasion		
Yes	1	
No	0.835 (0.437, 1.596)	0.585
TACE sessions		
1	1	
2 or more	0.716 (0.405, 1.266)	0.251
Treatment method		
TACE-apatinib	1	
TACE alone	2.683 (1.441, 4.994)	**0.002**

HR, Hazard ratio; CI, Confidence interval; TACE, Transcatheter arterial chemoembolization.
Statistical analysis: Cox proportional hazards regression model.

TABLE 5 | Multivariate analysis of prognostic factors for time to progression.

Variables	HR (95% CI)	*P*-value
Albumin (g/L)	1.036 (0.969, 1.106)	0.301
PT (s)	0.946 (0.770, 1.163)	0.599
Treatment method		
TACE-apatinib	1	
TACE alone	1.736 (0.909, 3.314)	0.095

HR, Hazard ratio; CI, Confidence interval; PT, Prothrombin time; TACE, Transcatheter arterial chemoembolization.
Statistical analysis: Cox proportional hazards regression model.
The bold values means that the value of P < 0.05.

can benefit advanced HCC patients (Bai et al., 2013; Choi et al., 2013; Zhao et al., 2013), and apatinib functions via similar mechanisms to facilitate HCC treatment (Fontanella et al., 2014). APS is frequently observed in patients with HCC (Wu et al., 2018). The presence of APS affects the survival of HCC patients and the safety of TACE procedure (Murata et al., 2005, 2009). Therefore, timely and complete embolization of shunts before the TACE procedure may represent a suitable treatment approach for advanced HCC with APS.

Few studies to date have reported on use of TACE-apatinib for the treatment of advanced HCC patients with APS. We found that for advanced HCC patients with APS who underwent TACE-apatinib, the median OS was 12.0 months, the median TTP was 9.0 months, and the DCR was 62.9%, with these values being significantly greater than those of the TACE-alone group. Some previous studies have focused on the combined treatment of TACE with apatinib as a means of improving treatment efficacy of advanced HCC (Qiu et al., 2019; Zhu et al., 2019). Kan et al. (2020) reported 90 advanced HCC patients were treated with TACE-apatinib, and the results showed that the median OS was 14.0 months and the median TTP was 7.0 months. Although the results of our study were slightly inferior to Kan et al.'s study, it is acceptable considering that all the patients in our study were with APS. Based on the results of our study, it is believed that TACE-apatinib treatment may benefit advanced HCC patients with APS.

APS may affect the safety of TACE as lipiodol can easily pass through the shunts, potentially resulting in the embolization of normal liver tissue or pulmonary embolism (Ziessman et al., 1984). However, transcatheter arterial occlusion of APS is technically feasible (Chen et al., 2014). We found that performing the TACE procedure after complete shunt embolization was

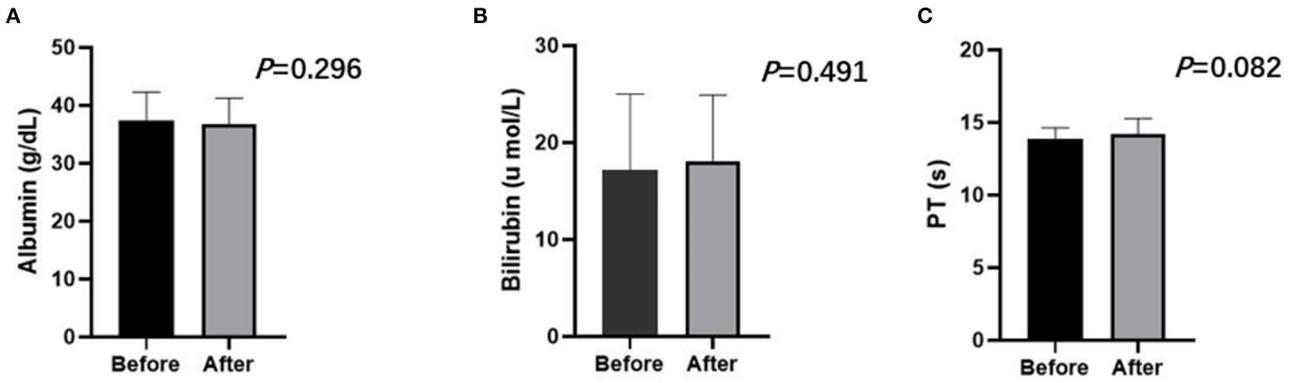

FIGURE 3 | Liver function including albumin **(A)**, bilirubin **(B)**, PT **(C)** of the TACE-apatinib group before and after treatment at 4 weeks. Paired sample *T*-test showed no statistic difference ($P > 0.05$).

TABLE 6 | Adverse events related to TACE.

Adverse events	TACE-apatinib ($n = 27$)	TACE alone ($n = 31$)	*P*-value
Hepatorenal syndrome	1 (3.7%)	0 (0%)	0.944
Inguinal hematoma	0 (0%)	0 (0%)	–
Hepatic arterial dissection	1 (3.7%)	1 (3.2%)	1
Pulmonary oil embolization	0 (0%)	0 (0%)	–

TACE, Transcatheter arterial chemoembolization.
Statistical analysis: Chi-square test.

TABLE 7 | Adverse events related to apatinib in the TACE–apatinib group.

Adverse events	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	All events
Hand foot skin reactions	9 (33.3%)	8 (29.6%)	3 (11.1%)	0 (0%)	0 (0%)	20 (74.1%)
Hypertension	6 (22.2%)	7 (25.9%)	1 (3.7%)	0 (0%)	0 (0%)	14 (51.8%)
Diarrhea	6 (22.2%)	4 (14.8%)	0 (0%)	0 (0%)	0 (0%)	10 (37.0%)
Fatigue	5 (18.5%)	3 (11.1%)	0 (0%)	0 (0%)	0 (0%)	8 (29.6%)
Oral ulcer	2 (7.4%)	1 (3.7%)	0 (0%)	0 (0%)	0 (0%)	3 (11.1%)
Voice change	2 (7.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (7.4%)
Proteinuria	2 (7.4%)	7 (25.9%)	0 (0%)	0 (0%)	0 (0%)	9 (33.3%)
Gastrointestinal hemorrhage	3 (11.1%)	1 (3.7%)	0 (0%)	0 (0%)	0 (0%)	4 (14.8%)

TACE, Transcatheter arterial chemoembolization.

well-tolerated for advanced HCC with APS in both groups, with no patients having experienced pulmonary embolization as a consequence of treatment. Common complications of TACE in this study were fever, abdominal pain, and elevated total bilirubin, consistent with prior reports (Choi et al., 2013; Kan et al., 2020). There were no significant differences in patient liver function when comparing pre- and post-treatment levels at 4 weeks after initial TACE treatment in the TACE-apatinib group, indicating that injury to normal liver tissue was limited. In line with other studies, our study revealed that apatinib was well-tolerated by advanced HCC patients with APS (Yang et al., 2019; Kan et al., 2020). The observed apatinib-related adverse events in our study were predominantly grade 1 or 2, and the symptoms that were related to these adverse events in the patients were alleviated or eliminated after drug reduction or interruption and symptomatic treatments.

There were some limitations to our study. First, this was a retrospective study with a small sample size. Second, our study data came from a single center. It is therefore necessary that prospective multicenter clinical trials be conducted in the future to validate our findings.

In conclusion, our study revealed that TACE-apatinib was safe and effective for advanced HCC patients with APS, and apatinib improved the efficacy of TACE in the treatment of these patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Wuhan Union Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

TS, YR, XK, FY, and CZ: conceptualization. YR and LC: methodology. TS, YR, and WZ: validation. TS and CZ: formal

analysis. TS: writing—original draft preparation. YR, XK, FY, and CZ: writing—review and editing. TS, YR, and XK: visualization. FY and CZ: supervision. All authors have read and agreed to the published version of the manuscript.

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Efficacy and Safety of TACE Combined With Sorafenib Plus Immune Checkpoint Inhibitors for the Treatment of Intermediate and Advanced TACE-Refractory Hepatocellular Carcinoma: A Retrospective Study

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Purpose: The study aims to retrospectively investigate the efficacy and safety of sorafenib combined with transarterial chemoembolization (TACE) (TACE+Sor) vs. TACE combined with sorafenib plus immune checkpoint inhibitors (TACE+Sor+ICIs) in treating intermediate and advanced TACE-refractory hepatocellular carcinoma (HCC).

Materials and Methods: This study was approved by the ethics committee of Lisui Hospital, Zhejiang University, China. From January 2016 to June 2020, 51 eligible patients with intermediate or advanced TACE-refractory HCC received TACE+Sor ($n = 29$) or TACE+Sor+ICIs ($n = 22$). The differences in tumor response, adverse events (AEs), progression-free survival (PFS), and overall survival (OS) were compared between the two groups. Factors affecting PFS and OS were determined by Cox regression.

Results: The disease control rate was higher in the TACE+Sor+ICIs group than in the TACE+Sor group (81.82 vs. 55.17%, $P = 0.046$). Compared with the TACE+Sor group, PFS and OS were prolonged in the TACE+Sor+ICIs group (median PFS: 16.26 vs. 7.30 months, $P < 0.001$; median OS: 23.3 vs. 13.8 months, $P = 0.012$). Multivariate analysis showed that BCLC stage, alpha-fetoprotein and treatment were independent factors of PFS; BCLC, Child-Pugh class, ablation after disease progression and treatment were independent predictive factors of OS. Four patients in the TACE+Sor+ICIs group and three patients in the TACE+Sor group suffered from dose reduction or interruption (18.18 vs. 10.34%, $P = 0.421$). The incidence of ICI-related AEs in the TACE+Sor+ICIs group was well-controlled.

Conclusion: The therapeutic schedule of TACE+Sor+ICIs demonstrated efficacy and safety in intermediate and advanced TACE-refractory HCC.

Keywords: TACE-refractory, immune checkpoint inhibitors, sorafenib, transarterial chemoembolization, progression-free survival, overall survival, adverse events, hepatocellular carcinoma

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INTRODUCTION

Primary liver cancer (PLC) is a common malignant tumor. Its incidence ranks fifth, with 854,000 new cases per year, making PLC the third leading cause of cancer-related death (Bray et al., 2018; Singal et al., 2020). China is the worst-hit region with a heavy burden of liver cancer. Approximately 364,000 new cases were diagnosed, accounting for half of the new cases of PLC worldwide (Zheng et al., 2018). Hepatocellular carcinoma (HCC) is the most common histological type, accounting for ∼75–85% of PLCs. However, ∼70% of new patients are diagnosed with intermediate or advanced HCC, missing the opportunity for curative resection (Morise et al., 2014). Moreover, even when patients undergo curative resection, 70% of patients still suffer from recurrence 5 years later (Lacaze and Scotte, 2015; Xiao et al., 2018).

Transarterial chemoembolization (TACE) is currently recommended for intermediate stage of liver cancer and improves the clinical efficacy both before and after curative resection (Sieghart et al., 2015; Wang et al., 2018). According to the Barcelona Clinic Liver Cancer (BCLC) clinical staging system, HCCs in BCLC stage B are recommended for TACE (Han and Kim, 2015; Raoul et al., 2019). In particular, the application of TACE was expanded from BCLC stage A to stage C with Child-Pugh class A or B liver function according to the Chinese guidelines for the diagnosis and treatment of primary liver cancer (2017 edition) (Zhou et al., 2018). Overall, TACE is recommended as the basic therapy for unresectable HCC. However, the efficacy of TACE declines significantly with the number of TACE procedures, with progressive disease (PD) rates of 18, 21, 25, and 27% for the first, second, third, and fourth TACE procedures, respectively (Peck-Radosavljevic et al., 2018). This phenomenon is defined as "TACE failure or refractory," as proposed by the Japan Society of Hepatology (JSH) and the Liver Cancer Study Group of Japan (LCSGJ) (Kudo et al., 2011, 2014).

The management of advanced TACE-refractory HCC has attracted increasing attention since the concept was proposed by the JSH and LCSGJ in 2014. Efforts have been made to improve the efficacy of TACE by combining with other therapies, including ablation (such as radiofrequency or microwave ablation), radiotherapy (such as stereotactic radiotherapy or radioactive particle seeding), multi-kinase inhibitors (such as sorafenib or apatinib), and immunotherapy (Galle et al., 2017; Guo et al., 2018; Palmer et al., 2020). Sorafenib inhibits tumor angiogenesis by targeting the RAF-MEK-ERK signaling pathway or blocking the expression of vascular endothelial growth factor receptor (VEGFR) and serves as the first line of systemic therapy for HCC (Keating, 2017). Sorafenib can also work as an adjuvant drug for patients diagnosed with advanced HCC (Keating, 2017; Pinyol et al., 2019). Evidence has shown that TACE combined with sorafenib significantly prolongs the recurrence of intermediate or advanced HCC (Kudo et al., 2020). However, data from two phase II/phase III randomized controlled trials (RCTs), the SPACE trial (Lencioni et al., 2016), and the TACE 2 trial (Meyer et al., 2017), failed to demonstrate any clinical benefit from TACE combined with sorafenib. Thus, alternative systemic therapies are urgently needed to improve the patient outcomes of TACE.

Recently, the application of immune checkpoint inhibitors (ICIs) targeting programmed death-1 (PD-1), programmed death-ligand 1 (PD-L1), or cytotoxic T lymphocyte antigen-4 (CTLA-4) has led to a clinical breakthrough for solid tumors (Zhou et al., 2017; Liu and Qin, 2019). Based on a phase I/II clinical trial, CheckMate 040 (El-Khoueiry et al., 2017), the United States Food and Drug Administration (FDA) approved nivolumab for HCC treatment, bringing great promise in restricting tumor recurrence. Due to the need for combinatorial protocols with other antitumor approaches to stimulate the immune system or kill tumor cells directly (Zhou et al., 2017), synergistic combinations with conventional therapies such as radiation, chemotherapy, and targeted therapy have been proposed. Phase II phase III clinical trials of therapies combining ICIs with sorafenib as well as other angiogenesis inhibitors are currently in progress (El Dika et al., 2019).

The hypoxic response induced by TACE boosts the release of proangiogenic cytokines as well as immunogenic cell death (Lencioni, 2012; Huang et al., 2016). These factors further promote tumor angiogenesis and regulate immune function in the tumor microenvironment (Huang et al., 2016; El Dika et al., 2019). Thus, therapy combining TACE with sorafenib plus ICIs may have promising outcomes in intermediate and advanced TACE-refractory HCC. Thus, the aim of this study was to retrospectively compare the efficacy and safety of TACE+sorafenib+ICI treatment with TACE+sorafenib treatment alone in patients with TACE-refractory intermediate and advanced HCC.

MATERIALS AND METHODS

Study Design and Patient Population

Patients diagnosed with intermediate and advanced HCC according to the National Comprehensive Cancer Network (NCCN) Guidelines for Hepatobiliary Cancers were eligible for enrollment (Benson et al., 2019). Candidates from Lishui Hospital of Zhejiang University were enrolled in this retrospective analysis from January 2016 to June 2020. TACE-refractory advanced HCC was determined by the previously published JSH criteria (Kudo et al., 2014). The exclusion criteria were as follows: (1) patients with BCLC stage A or D; (2) patients with Child-Pugh class C liver function; (3) patients with Eastern Cooperative Oncology Group performance status (ECOG-PS) scores was equal to or greater than 3 points; (4) patients with a tumor size <3 cm; (5) patients with grade II-IV myelosuppression; (6) patients with coagulation disorders; (7) patients with other primary malignancies; (8) patients who had received systemic therapy except for TACE and sorafenib before ICI treatment; and (9) patients who previously received immunotherapy except for ICIs (**Figure 1**).

Based on the above exclusion criteria, 51 eligible patients with TACE-refractory intermediate and advanced HCC with an average age of 56 ± 12.0 years were included in this study, including 46 (90.19%) males and 5 (9.81%) females.

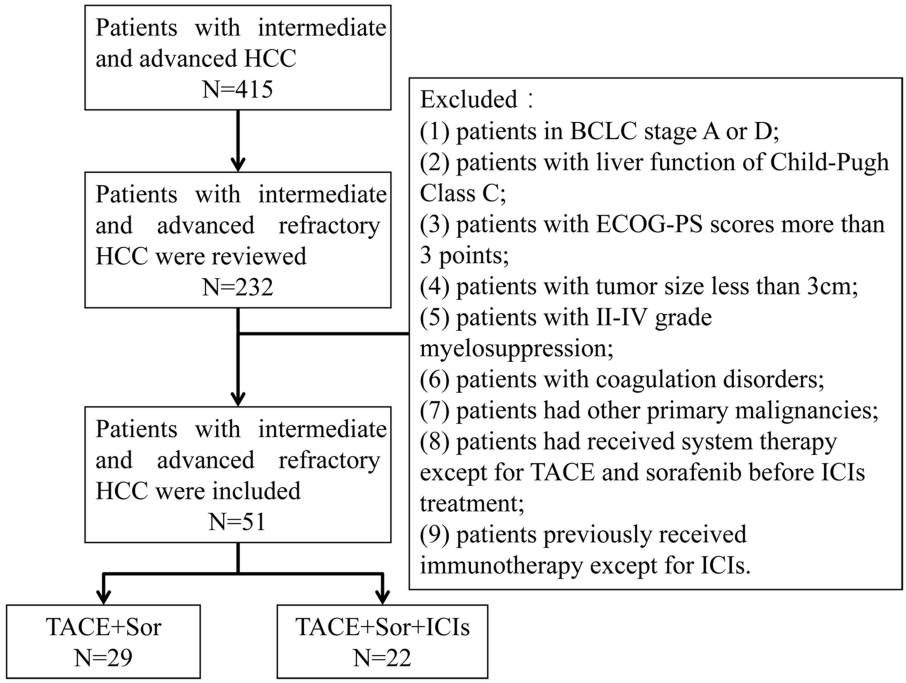

FIGURE 1 | Flowchart shows patient selection. HCC, hepatocellular; BCLC, Barcelona Clinic Liver Cancer; ECOG-PS, Eastern Cooperative Oncology Group performance status; TACE, transarterial chemoembolization; Sor, sorafenib; ICIs, immune checkpoint inhibitors.

The average tumor size was 6.1 ± 2.5 cm, ranging from 3.0 to 12.8 cm. Twenty-three (45.09%) patients with BCLC stage B and 28 (54.91%) patients with BCLC stage B were included. All patients received TACE and sorafenib treatment. Repeated TACE procedures were performed if required after identifying viable lesions or intrahepatic recurrence by MRI imaging. Twenty-nine patients who received TACE plus sorafenib treatment were belong to TACE+Sor group and 22 patients who received TACE plus sorafenib combined with ICIs treatment were served as TACE+Sor+ICIs group. Twelve patients received nivolumab treatment and the other 10 patients selected pembrolizumab treatment in TACE+Sor+ICIs group. This study was approved by the ethics committee of Lishui Hospital, Zhejiang University, China.

TACE Procedure

TACE was conducted by specialists with more than 10 years of experience in the procedure. In brief, after local anesthesia using 1% lidocaine, the patient was punctured, and an arterial sheath was intubated at the root of the femoral artery by the Seldinger method. Under the guidance of digital subtraction angiography (DSA), a catheter was inserted into the hepatic artery, and a superselective microcatheter was inserted into the feeding artery of the tumors. Oxaliplatin (100–150 mg) and 5-fluorouracil (500–750 mg) were infused though the microcatheter; therefore, the mixed emulsion included 10–30 ml of hyper-liquefying iodide oil, and epirubicin (10–20 mg) was injected into the tumor after hepatic angiography. The exact dose administered to each patient was based on their embolization condition. Absorbable gelatin sponge particles were used to completely embolize the feeding arteries. Finally, iodine tablets were obtained to confirm the complete embolism of the feeding arteries. Repeated TACE would be recommended once the lipiodol deposition shrank and residual lesions occurred, indicating viable lesions or intrahepatic recurrence by contrast-enhanced MRI within 6 weeks after TACE therapy.

Sorafenib and ICI Administration

The administration of sorafenib and ICIs was initiated within 2 weeks post-TACE therapy based on the proper condition of liver function (aspartate aminotransferase (AST) <40 U/L. Sorafenib at a dose of 400 mg was orally administered twice a day, and intravenous administration of nivolumab or pembrolizumab at a dose of 3 mg/kg was injected every 3 weeks. If patients could not tolerate side effects, dose reduction was determined and recommended by oncologists with more than 10 years of experience. Once serious adverse events (AEs) occur, drug administration cannot be continued.

Follow-Up and Therapeutic Effect Evaluation

All patients were regularly followed-up and reexamined. The first reexamination of abdominal MRI as well as hematology was conducted within 6 weeks after TACE therapy, and the following reexaminations were recommended every 1–3 months during the treatment. For stable lesions, the time of reexamination was prolonged for 3–6 months. Progression-free survival (PFS) was set as the primary endpoint of this study and was defined

as the time interval from TACE refractoriness to the time of disease progression from any cause. The secondary endpoint was overall survival (OS), defined as the period from the time of TACE refractoriness to the time of death. The results of each patient's imaging examinations were evaluated by two diagnostic radiologists with more than 10 years of experience. The efficacy of each therapy was analyzed according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) as follows. Complete response (CR) was defined as the disappearance of the enhanced tumor area during the arterial phase, meaning complete tissue necrosis. Partial response (PR) was defined as a decrease in the tumor area by at least 30% over a month. PD was defined as an increase of at least 20% in the enhanced tumor area. Stable disease (SD) was defined as neither a sufficient decrease (<30% of the tumor area) nor a sufficient increase in the tumor area (no more than 20% of the tumor area). The overall response rate (ORR) was calculated as (CR+PR)/total number of cases *100%. The formula for calculating the disease control rate (DCR) was (CR+PR+SD)/total number of cases *100%.

Safety Assessment

AEs were recorded and assessed on the basis of the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. According to these criteria, positive AEs were defined as cases with AEs ranked as more than grade 2.

Statistical Analysis

The analysis of this study was conducted with the statistical software SPSS 24.0(NY Armonk, New York, USA). Continuous variables are expressed as medians and ranges, and categorical variables are expressed as numbers or frequencies. Categorical variables were analyzed using the chi-square test. Continuous variables were analyzed using Student's *t*-test. The survival curves of PFS and OS were analyzed based on the Kaplan–Meier method using the log rank test. Factors with $P < 0.10$ in univariate analysis were further combined into a Cox proportional hazards regression model to identify factors independently associated with PFS and OS. $P < 0.05$ was considered statistically significant.

RESULTS

Patient Demographics and Clinical Characteristics

The baseline characteristics, including sex, age, ECOG-PS, HBV infection, cirrhosis, Child-Pugh class, BCLC stage, alpha-fetoprotein (AFP) level, AST level, tumor size, number of tumor nodes, extrahepatic metastasis, portal vein tumor thrombus (PVTT), history of previous surgery, and procedures of TACE, were not significantly different between the two groups (**Supplementary Table 1**). Herein, the last TACE before refactory is considered to be therapeutic schedule of TACE+systemic therapy (sorafenib with or without presence of ICIs). So the last TACE did not calculated into the previous TACE procedures. A total of 115 previous TACE procedures were performed before TACE refractoriness, with a mean of 2.25 times per patient, including 48 TACE procedures in the TACE+Sor+ICIs group (mean of 2.23 procedures per patient; range 1–4) and 67 TACE procedures in the TACE+Sor group (mean of 1.87 procedures per patient; range 1–4). There was no significant difference between the TACE+Sor+ICIs group and the TACE+Sor group in the number of previous TACE procedures ($F = 0.049$, $P = 0.82$).

Tumor Response Evaluation

Two patients (9.1%) showed CR, 10 patients (45.5%) showed PR, six patients (27.3%) showed SD, and four patients (18.2%) showed PD in the TACE+Sor+ICIs group. Ten patients (34.5%) showed PR, six patients (20.7%) showed SD, and 13 patients (44.8%) showed PD in the TACE+Sor group. There was a significant difference between the two treatment groups according to mRECIST ($Z = -2.04$, $P = 0.042$) by Mann-Whitney U test. The ORR in the TACE+Sor+ICIs group was significantly higher than that in the TACE+Sor group (54.6 vs. 34.5%, $\chi^2 = 2.05$, $P = 0.152$). The DCR was significantly different between the two treatment groups (81.8 vs. 55.2%, $\chi^2 = 3.99$, $P = 0.046$).

Safety Assessment

Severe AEs (more than grade 4) did not occur among all patients. Common AEs, including decreased appetite, fatigue, and postembolization syndrome (such as nausea or vomiting, fever, and abdominal pain), were found in the early stage of therapy. However, there was no difference between the TACE+Sor+ICIs group and the TACE+Sor group in embolization-related syndrome. AEs related to sorafenib administration, such as hand-foot syndrome, hypertension, alopecia, and diarrhea, occurred, but there was no significant difference between the two groups. The incidences of pruritus and myalgia were higher in the TACE+Sor+ICIs group than in the TACE+Sor group, but there was no significant difference between the two groups. Grade 3–4 rashes occurred in patients who received TACE combined with sorafenib plus ICI therapy, and the incidence was higher than that in patients who received TACE combined with sorafenib alone (3 (13.63%) vs. 0 (0.00%), $\chi^2 = 4.20$, $P = 0.040$). Fortunately, after receiving glucocorticoid and dose interruptions, the patients recovered within 2 weeks. Even though the incidence rate of hypothyroidism in patients who received TACE combined with sorafenib plus ICI therapy was higher than that in patients who received TACE combined with sorafenib alone [2 (9.09%) vs. 0 (0.00%), $\chi^2 = 2.74$, $P = 0.098$], the difference was not significant. Proteinuria, hypokalemia, increased AST, granulocytopenia, decreased neutrophil count, and hyperbilirubinemia occurred in both groups, but there were no significant differences between the two groups.

Follow-Up Treatment After Tumor Progression

As shown in **Supplementary Table 3**, follow-up treatments, including ablation, radiotherapy, and second-line anti-angiogenesis agents, were applied in both groups. No significant difference was found in the follow-up treatment after disease progression between the two groups.

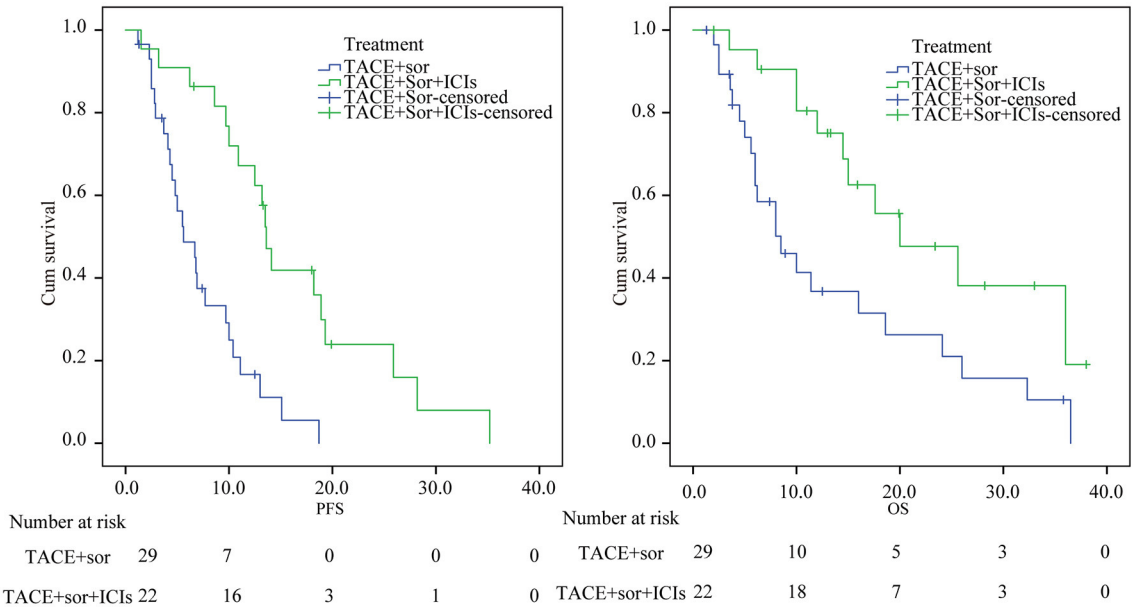

FIGURE 2 | Kaplan–Meier analysis of PFS and OS. TACE, transarterial chemoembolization; Sor, sorafenib; ICIs, immune checkpoint inhibitors; PFS, Progression-free survival; OS, overall survival.

Comparison of PFS and OS Between the Two Groups

The median PFS was 16.26 (95% confidence interval [CI] 12.1–20.37) months in the TACE+Sor+ICIs group, while the median PFS was 7.30 (95% CI 5.49–9.12) months in the TACE+Sor group. Compared with the TACE+Sor group, the OS of the TACE+Sor+ICIs group was significantly longer (log rank test, $z = 15.5$, $P < 0.001$, **Figure 2**). The half-year, 1-year, and 2-year PFS rates were 90.09, 67.59, and 42.56% in the TACE+Sor+ICIs group and 50.0, 18.0, and 7.7 in the TACE+Sor group. The median OS was 13.8 (95% CI 9.11–18.50) months in the TACE+Sor group and 23.3 (95% CI 17.56–29.07) months in the TACE+Sor+ICIs group. Compared with the TACE+Sor group, the OS of the TACE+Sor+ICIs group was significantly longer (log rank test, $z = 6.31$, $P = 0.012$, **Figure 2**). The 1-year, 2-year, and 3-year survival rates were 80, 48.2, and 36.2% in the TACE+Sor+ICIs group and 40.0, 29.1, and 9.7% in the TACE+Sor group. In addition, the median PFS of patients receiving nivolumab was comparable to those receiving pembrolizumab [13.6 months (95% C12.02–15.18) vs. 13.2 months (95% CI 6.85–19.55, $z = 0.32$, $P = 0.859$] in the TACE+Sor+ICIs group. And the median OS of patients selecting nivolumab treatment was similar to those receiving pembrolizumab [20.0 months (95% C12.92–27.08) vs. 25.6 months (95% CI 13.53–37.67, $z = 0.05$, $P = 0.820$] in the TACE+Sor+ICIs group (seen in **Supplementary Figure 1**).

Prognostic Factors Affecting OS and PFS

The univariate analysis results of the factors influencing PFS are shown in **Supplementary Table 2**. The data indicated that sex, age, ECOG-PS, HBV infection, cirrhosis, AST, tumor node, surgery history, and number of previous TACE procedures were not factors associated with PFS ($P > 0.10$). Factors with $P < 0.10$ including Child-Pugh class, BCLC stage, AFP, tumor size, metastasis, PVTT, and treatment were combined into the Cox proportional hazards regression model. Multivariate analysis showed that BCLC stage (C vs. B) (hazard ratio [HR] = 2.14; 95% CI: 1.01–4.51; $P = 0.047$), AFP level (≥400 ng/mL vs. <400 ng/mL) (HR = 2.20; 95% CI: 1.08–4.78; $P =$ **0.048**), tumor size (≥5 cm vs. <5 cm) (HR = 3.25; 95% CI: 1.47–7.19; $P = 0.003$) and treatment (TACE+Sor+ICI treatment vs. TACE+Sor treatment) (HR = 0.11; 95% CI: 0.05–0.26; $P < 0.001$) were independent predictive factors of PFS. Moreover, univariate analyses showed that sex, age, ECOG-PS, HBV infection, cirrhosis, AST, tumor node, surgery history, and number of previous TACE procedures were not factors associated with OS. Multivariate analysis indicated that Child-Pugh class (B vs. A) (HR = 2.36; 95% CI, 1.02–5.46; $P = 0.044$), BCLC stage (C vs. B) (HR = 3.88; 95% CI: 1.56–9.60; $P = 0.003$), treatment (TACE+Sor+ICI treatment vs. TACE+Sor treatment) (HR = 0.24; 95% CI: 0.11–0.55; $P = 0.001$), and ablation after disease progression (yes vs. no) (HR = 0.29; 95% CI: 0.12–0.75; $P = 0.010$) were independent predictive factors of OS.

Subgroup Analysis

As seen in **figures 3** and **4**, in patients with BCLC stage B, the median PFS was 9.4 months (95% CI: 6.8–11.9) in the TACE+Sor group and 21.2 months (95% CI: 15.5–27.00) in the TACE+Sor +ICIs group (log rank = 11.73, $P = 0.001$). The corresponding OS was 20.3 months (95% CI: 13.3–27.4) in the TACE+Sor group and 30.0 months (95% CI: 23.5–36.6) in the TACE+Sor+ICIs group (log rank = 3.30, $P = 0.069$). In patients with BCLC stage C, the median PFS was 4.8 months (95% CI: 3.3–6.2) in the TACE+Sor group and 10.8 months (95% CI: 7.5–14.1)

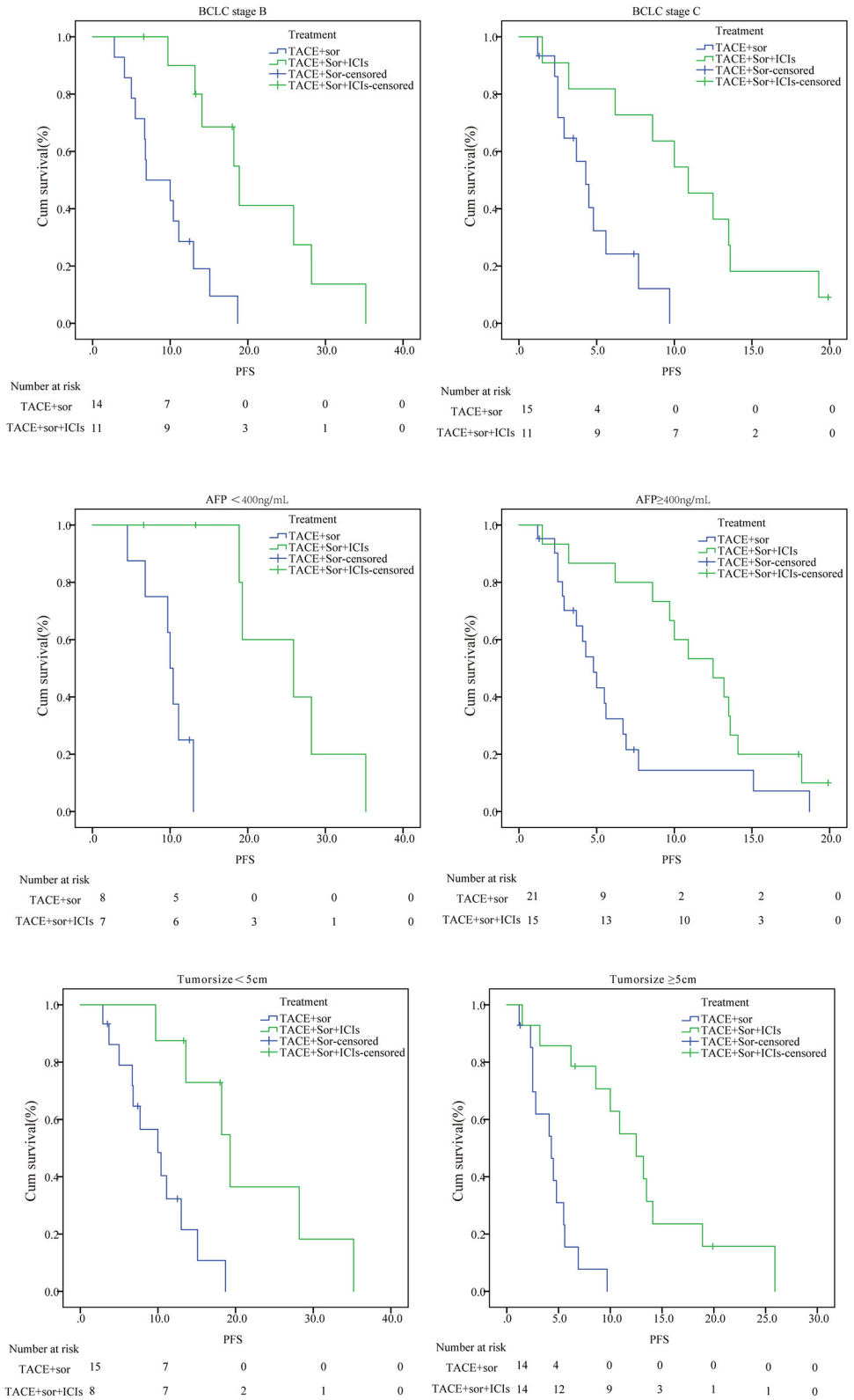

FIGURE 3 | Subgroup analysis of PFS. TACE, transarterial chemoembolization; Sor, sorafenib; ICIs, immune checkpoint inhibitors; PFS, Progression-free survival; OS, overall survival.

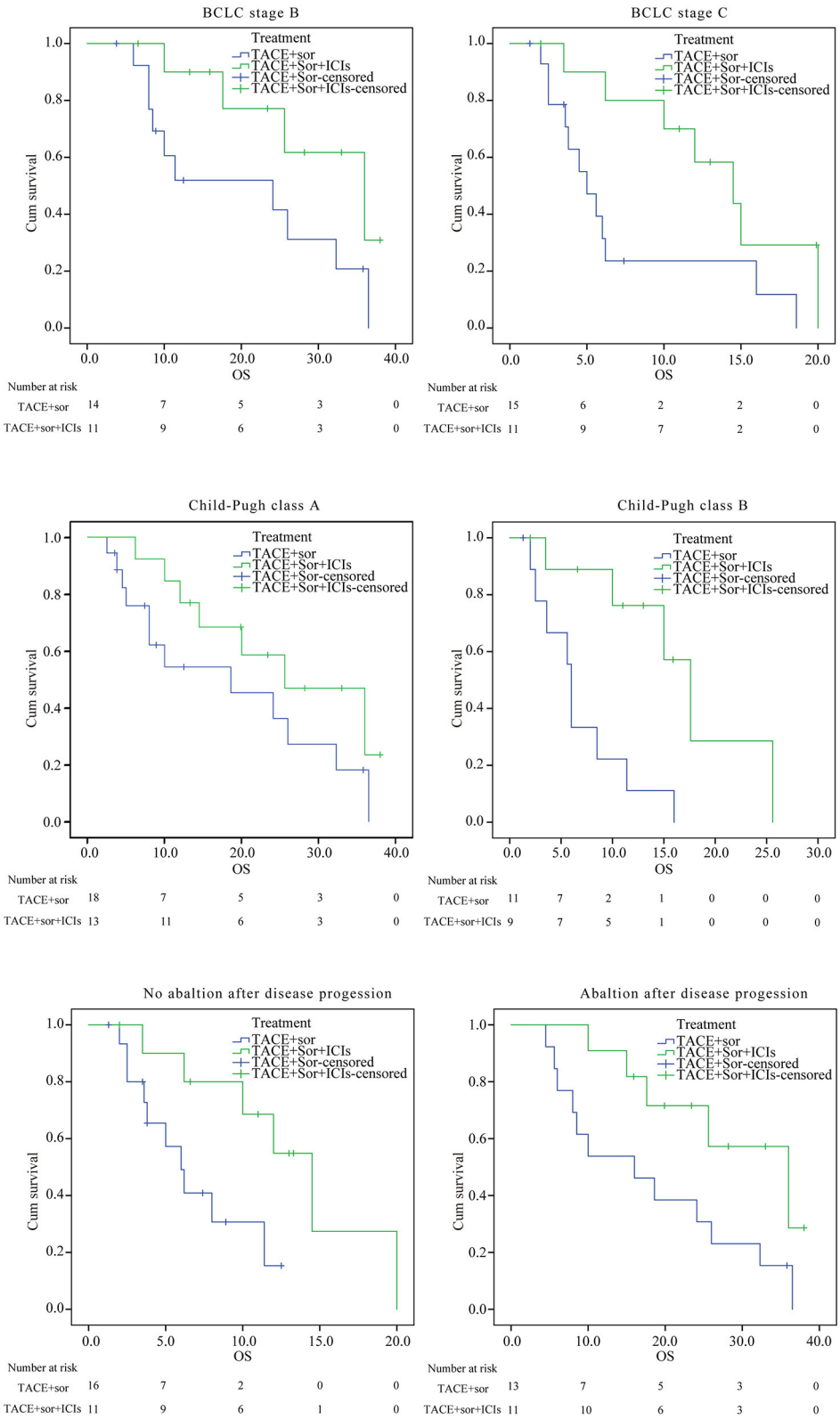

FIGURE 4 | Subgroup analysis of OS. BCLC, Barcelona Clinic Liver Cancer; AFP, alpha-fetoprotein; TACE, transarterial chemoembolization; Sor, sorafenib; ICIs, immune checkpoint inhibitors; PFS, Progression-free survival; OS, overall survival.

in the TACE+Sor+ICIs group (log rank = 9.08, $P = 0.003$). The corresponding OS was 7.3 months (95% CI: 4.0–10.5) in the TACE+Sor group and 13.5 months (95% CI: 9.7–17.3) in the TACE+Sor+ICIs group (log rank = 4.85, $P = 0.028$). In patients with AFP levels lower than 400 ng/mL, the median PFS was 9.8 months (95% CI: 7.8–11.8) in the TACE+Sor group and 25.5 months (95% CI: 19.6–31.4) in the TACE+Sor+ICIs group (log rank = 10.63, $P = 0.001$). In patients with AFP levels higher than 400 ng/mL, the median PFS was 6.2 months (95% CI: 3.9–8.5) in the TACE+Sor group and 11.6 months (95% CI: 8.9–14.2) in the TACE+Sor+ICIs group (log rank = 5.92, $P = 0.015$). In patients with tumor diameters <5 cm, the median PFS was 10.0 months (95% CI: 7.3–12.6) in the TACE+Sor group and 21.6 months (95% CI: 14.4–28.8) in the TACE+Sor+ICIs group (log rank = 8.50, $P = 0.004$). In patients with tumor diameters >5 cm, the median PFS was 4.4 months (95% CI: 3.1–5.6) in the TACE+Sor group and 12.8 months (95% CI: 8.8–16.9) in the TACE+Sor+ICIs group (log rank = 16.64, $P = 0.000$). In patients with Child-Pugh class A, the median OS was 18.6 months (95% CI: 11.8–25.4) in the TACE+Sor group and 25.7 months (95% CI: 19.0–32.4) in the TACE+Sor+ICIs group (log rank = 3.37, $P = 0.066$). In patients with Child-Pugh class B, the median OS was 6.8 months (95% CI: 3.9–9.8) in the TACE+Sor group and 14.1 months (95% CI: 10.1–18.0) in the TACE+Sor+ICIs group (log rank = 6.61 $P = 0.010$). Among patients who did not receive ablation after disease progression, the median OS was 6.9 months (95% CI: 4.8–8.9) in the TACE+Sor group and 13.2 months (95% CI: 9.0–17.4) in the TACE+Sor+ICIs group (log rank = 4.81, $P = 0.028$). Among patients who received ablation after disease progression, the median OS was 17.9 months (95% CI: 11.3–24.4) in the TACE+Sor group and 28.9 months (95% CI: 22.6–35.3) in the TACE+Sor+ICIs group (log rank = 4.08, $P = 0.043$).

DISCUSSION

With a decade of drug development, the therapeutic reversal of immune exhaustion by ICIs has been shown to be effective in advanced HCC. Several published reports have indicated that ICIs elevated the tumor response and prolonged the time to recurrence as well as the OS of advanced HCC. The Check Mate-040 trial showed that patients can clinically benefit from nivolumab at a dose of 3 mg/kg (El-Khoueiry et al., 2017). Like nivolumab, pembrolizumab prolonged the median PFS up to 4.9 months and the median OS up to 12.9 months in advanced HCC patients receiving sorafenib frontline in the KEYNOTE-224 trial (Zhu et al., 2018).

The combination of ICIs and antiangiogenic agents was proposed by investigators due to the additional immunomodulatory effects of antiangiogenic agents (Hilmi et al., 2019). The preliminary results showed that lenvatinib plus pembrolizumab for advanced HCC showed good toxicity tolerance and an objective response rate of 48% (Makker et al., 2017). Other ongoing studies are also focusing on the efficacy and safety of combination therapy, such as nivolumab plus sorafenib or lenvatinib and pembrolizumab plus regorafenib. Thus, the combination of ICIs and sorafenib may be a potential therapy for advanced HCC.

Studies have demonstrated that TACE activates a hypoxic response, promoting the release of vascular endothelial growth factor (VEGF) and other proangiogenic cytokines (Sergio et al., 2008; Viveiros et al., 2019). Additionally, cell necrosis induced by TACE is predicted to be associated with antigen release and the exposure of damage-associated molecular patterns. The application of ICIs can bolster the function of T cells and antigen-presenting cells (APCs) after TACE plus sorafenib therapy. The treatment of TACE combined with sorafenib plus ICIs may obtain a better clinical effect for intermediate and advanced HCC by enabling a robust tumor-specific immune response and inhibiting tumor angiogenesis. In this study, our results revealed that patients who received TACE combined with sorafenib plus ICIs had prolonged OS and PFS compared with those who only received TACE combined with sorafenib alone (median OS: 23.3 vs. 13.8 months, log rank = 6.31, $P = 0.012$; median PFS: 16.26 vs. 7.31 months, log rank = 15.48, $P = 0.000$). As reported by the TACTICS trial, the median PFS of patients who received TACE plus sorafenib was up to 25.2 months, which was much higher than that of patients who received TACE combined with sorafenib plus ICIs or TACE combined with sorafenib alone. The reason is that new intrahepatic lesions were not regarded as PD in the TACTICS trial (Kudo et al., 2020).

Studies have demonstrated that TACE combined with sorafenib or other antineoplastic agents is an independent predictor of prognosis for intermediate and advanced HCC (Wu et al., 2017; Takada et al., 2019; Kudo et al., 2020). In this study, the treatment of TACE combined with sorafenib plus ICIs was a protective factor for PFS and OS in intermediate and advanced refractory HCC patients. The BCLC stage (C vs. B) was an independent predictive factor for PFS and OS. AFP level (≥400 vs. <400 ng/mL) and tumor size (≥5 vs. <5 cm) were risk factors for PFS. Child-Pugh class (B vs. A) was an independent predictive factor for OS. Studies have suggested that other factors, such as AST level, number of nodules, vascular invasion, and metastasis, are also significant predictors of OS or PFS (Peng et al., 2018; Takada et al., 2019). Our data showed that PVTT and metastasis significantly affected OS or PFS in univariate analyses but were adjusted in multivariate analysis. This may be due to the cooperation of PVTT and metastasis in BCLC stage. The small sample size and relatively short follow-up time may also be other reasons. Our data also showed that follow-up treatment was a protective factor for OS after disease progression of intermediate and advanced HCC. The median OS of patients who received ablation was higher than that of patients who did not receive ablation. Among patients who received ablation after disease progression, the median OS of patients treated with TACE combined with sorafenib plus ICIs was higher than that of patients treated with TACE combined with sorafenib alone. These results indicated that ablation after disease progression prolongs the OS of advanced HCC. In addition, our results suggested that patients who received radiotherapy or second-line antiangiogenesis agents had a longer OS. However, the results may also be caused by selection bias, since selected patients who received following treatment tended to have a good performance.

The AEs in this study were mild to moderate and could easily be controlled. The incidences of postembolization syndrome, such as nausea, vomiting, fever, and abdominal pain, and sorafenib-related AEs, such as hand-foot syndrome, hypertension, diarrhea, and alopecia, were similar to those in previous studies of patients treated with TACE combined with sorafenib and TACE or sorafenib alone (Lencioni et al., 2016; Meyer et al., 2017; Ye et al., 2018; Kudo et al., 2020). The incidence of dose reductions or interruptions between the two groups was not significantly different. Three patients who received TACE combined with sorafenib alone suffered from dose reductions or interruptions owing to increased AST levels caused by sorafenib administration. There were four patients who received TACE combined with sorafenib plus ICIs who interrupted sorafenib and ICI administration due to increased aspartate aminotransferase levels (two patients), hypothyroidism (one patient), and rash (one patient). Fortunately, after dose reductions or interruptions and hepatinica, thyroxine or glucocorticoid, all patients returned to normal.

This study indicated that patients who received the combination of TACE with sorafenib plus ICIs had promising outcomes. However, some limitations must be considered. As a retrospective study, it has all of the defects inherent to this type of study design. For example, the background of patients, including financial capability, education, and cognition of liver cancer, have an impact on the choice of therapy by patients and physicians. In addition, the limitation of the sample size of patients and the length of follow-up also had a significant impact on the outcome.

In conclusion, the combination TACE with sorafenib plus ICIs prolongs the PFS and OS of intermediate and advanced refractory HCC patients. TACE with sorafenib plus ICI treatment was a protective predictive factor for PFS, while BCLC stage, AFP level, and tumor size were poor predictive factors for PFS. Child-Pugh class, AFP level, BCLC stage, TACE with sorafenib plus ICI treatment, and follow-up ablation were independent predictive factors for OS. Severe AEs rarely occurred, and we confirmed the clinical safety of using TACE with sorafenib plus ICI treatment. Overall, it is efficient and safe for patients with intermediate and advanced refractory HCC to receive TACE with sorafenib plus ICI therapy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Lishui hospital Zhejiang University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ZZ and JJ designed the research. LZ and SF carried out the study and wrote the first draft of the manuscript. FW and WC helped in the study. XW helped in analyzing the data. MC and QW helped in manuscript writing, editing, and critical evaluation. JJ supervised the project and read and approved the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmolb.2020.609322/full#supplementary-material

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Development and Validation of a Predictive Model for Early Refractoriness of Transarterial Chemoembolization in Patients With Hepatocellular Carcinoma

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Objectives: To develop and validate a predictive model for early refractoriness of transarterial chemoembolization (TACE) in patients with hepatocellular carcinoma (HCC).

Methods: In this multicenter retrospective study, a total of 204 consecutive patients who initially underwent TACE were included. Early TACE refractoriness was defined as patients presented with TACE refractoriness after initial two consecutive TACE procedures. Of all patients, 147 patients (approximately 70%) were assigned to a training set, and the remaining 57 patients (approximately 30%) were assigned to a validation set. Predictive model was established using forward stepwise logistic regression and nomogram. Based on factors selected by logistic regression, a one-to-one propensity score matching (PSM) was conducted to compare progression-free survival (PFS) between patients who were present or absent of early TACE refractoriness. PFS curve was estimated by Kaplan-Meier method and compared by log-rank test.

Results: Logistic regression revealed that bilobar tumor distribution ($p = 0.002$), more than three tumors ($p = 0.005$) and beyond up-to-seven criteria ($p = 0.001$) were significantly related to early TACE refractoriness. The discriminative abilities, as determined by the area under the receiver operating characteristic (ROC) curve, were 0.788 in the training cohort and 0.706 in the validation cohort. After PSM, the result showed that patients who were absent of early TACE refractoriness had a significantly higher PFS rate than those of patients who were present ($p < 0.001$).

Conclusion: This study presents a predictive model with moderate accuracy to identify patients with high risk of early TACE refractoriness, and patients with early TACE refractoriness may have a poor prognosis.

Keywords: hepatocellular carcinoma, treatment failure, predictive, logistic regression, transarterial chemoembolization

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common alimentary malignancy worldwide (Long et al., 2020; Peisen et al., 2020). For patients with HCC, ablative therapy, surgical resection, and liver transplantation are the potentially curative treatments. However, most patients are diagnosed with an advanced stage of disease, and only 20–30% of patients can receive curative treatments (Arizumi et al., 2015; Eilard et al., 2019; Peng et al., 2019; Luedemann et al., 2020). Transarterial chemoembolization (TACE) is the standard therapy for intermediate-stage HCC, which is accepted by several guidelines (Piscaglia and Ogasawara, 2018; Lee et al., 2019). However, it has been reported that not all HCC patients respond to TACE because the patients selected for TACE correspond to a highly heterogeneous population, covering a wide range of tumor burdens, liver function and treatment histories (Maesaka et al., 2020; Xue et al., 2020). Furthermore, repeated TACE procedures could gradually lead to TACE refractoriness, and some patients even show TACE failure at the very beginning of their treatment (Maesaka et al., 2020). For patients with TACE refractoriness, TACE is no longer effective, and those patients are recommended to switch to a systemic therapy, as suggested by the Japan Society of Hepatology (JSH) and the Liver *Cancer* Study Group of Japan (LCSGJ) (Kudo et al., 2014). Therefore, it is of great importance to identify predictive risk factors of early TACE refractoriness so that patients with those factors might switch to systemic therapy earlier to improve their survival.

Therefore, the purpose of the present study is to develop and validate a predictive model for early TACE refractoriness in patients with HCC and compare the progression-free survival (PFS) in patients who are present or absent of early TACE refractoriness.

MATERIALS AND METHODS

Patients

This retrospective study was approved by the institutional review boards of our hospital and in accordance with the Declaration of Helsinki. The requirement for written informed consent was waived by the institutional review boards due to the retrospective nature of the present study.

A total of 610 consecutive patients with unresectable HCC who initially underwent TACE at three hospitals between January 2015 and March 2020 were included. The inclusion criteria were as follows: patients had 1) Eastern Cooperative Oncology Group performance status 0; 2) compensated liver function (Child-Pugh class A or B); and 3) at least two consecutive TACE sessions, or although only one TACE session was performed, complete response (CR) was achieved after the TACE session. The exclusion criteria were as follows: patients had 1) portal venous tumor thrombus (n = 216); 2) distant metastasis (n = 109); 3) lost to follow-up (n = 23); 4) a time interval between the first and second TACE over 3 months (n = 21); 5) follow-up computed tomography (CT) or magnetic resonance (MR) imaging performed after TACE over 3 months (n = 17); 6) infiltrative HCC (n = 12); and 7) having been treated with a combination of TACE and other locoregional therapies such as ablation (n = 8). The flowchart of the study population is shown in **Figure 1**.

Interventions

TACE procedures were discussed with the tumor board prior to administration for each patient. Celiac trunk and superior mesenteric arteriography, as well as indirect portography, were performed to visualize the variations in hepatic arterial anatomy and to evaluate the patency of the portal vein. Either a 2.7 French (Progreat, Terumo Medical Corporation) or a 2.2 French (Carnelian, Tokai Medical Products) coaxial microcatheter was placed into the tumor-feeding arteries with the assistance of cone beam computed tomography (CBCT) if needed. TACE was performed using either drug-eluting beads (DEB) (CalliSpheres Beads, Jiangsu Hengrui Medicine Co., Ltd.) loaded with epirubicin (Shandong New Time Pharmaceutical Co., Ltd.) or up to 20 ml emulsion of iodized oil (Lipiodol, Guerbet Asia Pacific Ltd.) mixed with epirubicin. The oil-epirubicin emulsion was created using the water-in-oil technique by mixing iodized oil with a distilled water solution containing a drug cocktail of dissolved epirubicin at a ratio of 3:1. The dosage of epirubicin in DEB-TACE ranged from 50 to 150 mg and the size of DEBs varied from 100 to 300 um and 300–500 um, while in conventional TACE the dosage of epirubicin was 50–75 mg/m^2 body surface area. In DEB-TACE, no additional embolization was performed after injected of 1–2 g DEB, while in conventional TACE, gelfoam slurries were injected to embolize the proximal tumor feeders after the oil-epirubicin emulsion was injected. The technical endpoint of TACE was defined as the reduction in arterial inflow to the tumor and tumor devascularization. Changes in embolic agents, chemotherapy drugs, or tumor-feeding artery reselection were conducted for the second TACE procedure when an insufficient response after the first TACE occurred.

Data Collection

Among 204 patients, 147 patients from hospital A and hospital B were assigned to a training set, and the remaining 57 patients from hospital C were assigned to a validation set (the training to validation ratio was approximately 7:3). The demographic, laboratory, and radiological data of patients were collected to assess the potential risk factors for early TACE refractoriness. The demographic and laboratory data included age, sex (male/female), Child-Pugh class (A/B), BCLC stage (0-A/B), underlying liver disease, history of resection, initial embolic agents (lipiodol/DEB), initial alpha-fetoprotein (AFP) level (≤400/>400 ug/L), and initial neutrophil to lymphocyte ratio (NLR). The radiological data included tumor distribution (unilobar/bilobar), number of tumors (solitary/2–3/>3), size of the largest tumor, and up-to-seven criteria (within/beyond). Patients who were beyond up-to-seven criteria was defined as: largest tumor diameter [cm] + number of tumors >7 (Mazzaferro et al., 2009; Koroki et al., 2020). Radiological data were independently reviewed by two radiologists with either 22 or 19 years of experience of abdominal imaging, respectively. Both of the radiologists were blinded to the clinical data and were not

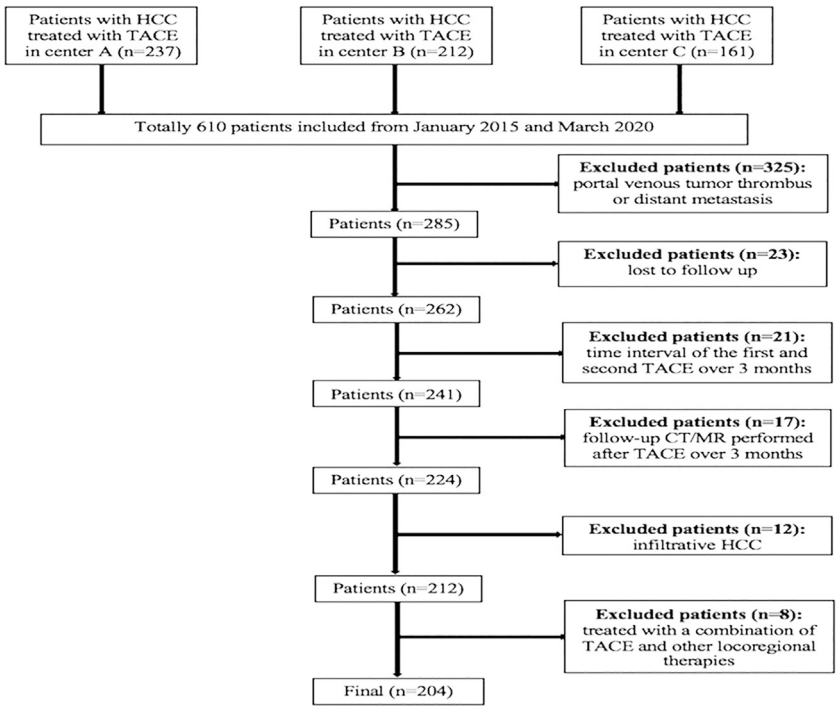

FIGURE 1 | Diagram of the study population.

involved in the treatment. The final results of radiological data were made by the discussion between two radiologists.

Follow-up Schedule and the Definition of TACE Refractoriness

Dynamic CT/MR imaging and laboratory variables were acquired before and after the first and the second TACE sessions. The treatment response of TACE was assessed by using dynamic CT/MR, and residual enhancement of nodules was measured with consideration of the 2019 version of Response Evaluation Criteria in *Cancer* of the Liver (RECICL) (Kudo et al., 2019).

The definition of TACE refractoriness was based on the JSH Consensus Guidelines as follows: 1) intrahepatic lesion: two or more consecutive ineffective responses was observed within the treated tumors (viable lesion>50%) or new lesion occurred in treated area, even after changing the chemotherapeutic agents or reanalysis of the feeding artery on response evaluation CT/MR after 1–3 months following adequate selective TACE; 2) AFP: continuous elevated levels of tumor markers right after TACE; 3) vascular invasion was observed; and 4) extrahepatic spread was observed.

The definition of early TACE refractoriness was that patients presented with TACE refractoriness after initial two consecutive TACE procedures.

PFS Assessment

The PFS was defined as the time interval between date of TACE procedure and death whatever the cause, tumor progression or last clinical follow-up. Tumor progression was assessed according to the 2019 version of RECICL criteria (Kudo et al., 2019), which was defined as tumor enlargement of ≥50%, excluding the area of treatment-induced necrosis in either target lesion or non-target lesion. However, new intrahepatic lesion occurred in non-treated area after TACE was not defined as tumor progression.

Statistical Analysis

The data were shown as the mean with standard deviation (SD), median with interquartile range (IQR), or frequency. To evaluate the inter-reader agreement of radiological data between the two abdominal radiologists, either intraclass correlation coefficient (ICC) analysis (for numerical data) or Kappa test (for categorical data) was performed. Agreement was classified as poor (ICC or Kappa value, 0–0.40), fair to good (ICC or Kappa value, 0.40–0.75), and excellent (ICC or Kappa value, >0.75). In univariate analysis, Pearson's chi-squared test or Fisher's exact test was used to compare categorical variables, while the independent sample t-test or rank-sum (Mann-Whitney) test was used to compare numerical variables. In multivariate analysis, a forward stepwise logistic regression model and nomogram were used. Variables with a p-value less than 0.05 in the univariate analysis were included in the multivariate model, and all those variables were tested by Diagnosis of Collinearity with variance inflation factors less than 5 (VIF < 5). The discrimination of this predictive model was examined by the receiver operating characteristic (ROC) curve, and the goodness of fit was validated by the Hosmer-Lemeshow test, in which a p value > 0.05 indicated good performance. Based on the factors

TABLE 1 | The demographic, radiological and laboratorial characteristics of the patients in training cohort and validation cohort.

Characteristics	Total (n = 204)	Training cohort (n = 147)	Validation cohort (n = 57)	*p* Value
Age (years)	56.5 ± 11.6	57.2 ± 12.5	54.7 ± 8.7	0.163
Gender (%)				0.656
Male	183 (89.7%)	131 (89.1%)	52 (91.2%)	
Female	21 (10.3%)	16 (10.9%)	5 (8.8%)	
Child-pugh class (%)				0.091
A	181 (88.7%)	127 (86.4%)	54 (94.7%)	
B	23 (11.3%)	20 (13.6%)	3 (5.3%)	
BCLC stage (%)				0.840
0-A	123 (60.3%)	88 (59.9%)	35 (61.4%)	
B	81 (39.7%)	59 (40.1%)	22 (38.6%)	
NLR	2.95 (IQR, 3.72)	2.39 (IQR, 1.73)	6.14 (IQR, 4.18)	<0.001
Underlying liver disease (%)				0.035
HBV	172 (84.3%)	119 (81.0%)	53 (92.9%)	
Other	10 (4.9%)	7 (4.8%)	3 (5.3%)	
None	22 (10.8%)	21 (14.2%)	1 (1.8%)	
Initial AFP (%)				0.037
≤400 ug/L	127 (62.3%)	98 (66.7%)	29 (50.9%)	
>400 ug/L	77 (37.7%)	49 (33.3%)	28 (49.1%)	
History of resection (%)				0.127
Presence	26 (12.7%)	22 (15.0%)	4 (7.0%)	
Absence	178 (87.3%)	125 (85.0%)	53 (93.0%)	
Tumor distribution (%)				0.539
Unilobar	132 (64.7%)	97 (66.0%)	35 (61.4%)	
Bilobar	72 (35.3%)	50 (34.0%)	22 (38.6%)	
Number of tumors (%)				0.601
Solitary	123 (60.3%)	88 (59.9%)	35 (61.4%)	
2–3	48 (23.5%)	33 (22.4%)	15 (26.3%)	
>3	33 (16.2%)	26 (17.7%)	7 (12.3%)	
Size of the largest tumor (%)				0.001
≤50 mm	83 (40.7%)	67 (45.6%)	16 (28.1%)	
50–100 mm	75 (36.8%)	57 (38.8%)	18 (31.6%)	
>100 mm	46 (22.5%)	23 (15.6%)	23 (40.3%)	
Up-to-seven criteria (%)				0.006
Within	81 (39.7%)	67 (45.6%)	14 (24.6%)	
Beyond	123 (60.3%)	80 (54.4%)	43 (75.4%)	
Initial embolic agents (%)				<0.001
Lipiodol	102 (50.0%)	60 (40.8%)	42 (73.7%)	
DEB	102 (50.0%)	87 (59.2%)	15 (26.3%)	

Note: HBV, hepatitis B virus; BCLC, Barcelona Clinic Liver Cancer*; NLR, neutrophil to lymphocyte ratio; IQR, inter-quartile range; DEB drug-eluting beads.*

selected by forward stepwise logistic regression, a one-to-one propensity score matching (PSM) was conducted to compare the PFS between patients who were present or absent of early TACE refractoriness. PFS curve was estimated by Kaplan-Meier method and compared by log-rank test.

Statistical analyses were performed with SPSS statistical software (SPSS version 20, International Business Machines Corporation) and R software (version 3.4.2, http://www.R-project.org). A probability value of <0.05 was considered statistically significant.

RESULTS

Demographic and Laboratory Characteristics

Finally, a total of 204 patients were included (183 males and 21 females, with a mean age of 56.5 ± 11.6 years). All TACE procedures achieved technical success according to the Society of Interventional Radiology (SIR) guidelines (Gaba et al., 2016). The diagnosis of HCC was based on pathology (biopsy, n = 12) or on the American Association for the Study of Liver Practice Guidelines (n = 192). There were 181 (88.7%) patients with Child-Pugh class A and 23 patients with Child-Pugh class B (11.3%), 123 patients (60.3%) in BCLC stage 0-A and 81 patients (39.7%) in BCLC stage B. Patients with BCLC-0 or BCLC-A disease received DEB-TACE or conventional TACE for the following reasons: in cases beyond the Milan criteria, liver transplant was contraindicated; presence of portal hypertension or increased bilirubin, hepatectomy was contraindicated according to BCLC staging system; or for HCC lesions in unfavorable location, ablation was technically infeasible. Conventional chemoembolization was initially performed in 102 patients (102/204, 50.0%), and DEB-TACE was also initially performed in 102 patients (102/204, 50.0%). There were 127 patients with initial AFP ≤400 ug/L (62.3%) and

TABLE 2 | The patterns of early TACE refractoriness in patients with HCC.

Characteristics	Total (n = 204)	Training cohort (n = 147)	Validation cohort (n = 57)	*p* Value
Viable lesions >50%, n (%)	47 (23.0%)	28 (19.0%)	19 (33.3%)	0.093
Presence of new lesions, n (%)	7 (3.4%)	4 (2.7%)	3 (5.3%)	0.390
Vascular invasion, n (%)	9 (4.4%)	4 (2.7%)	5 (8.8%)	0.074
Extrahepatic spread, n (%)	5 (2.5%)	5 (3.4%)	0 (0.0)	-
Elevation of AFP, n (%)	31 (15.2%)	24 (16.3%)	7 (12.3%)	0.532

Note: TACE, transarterial chemoembolization; HCC, hepatocellularcarcinoma; AFP, alpha-fetoprotein.

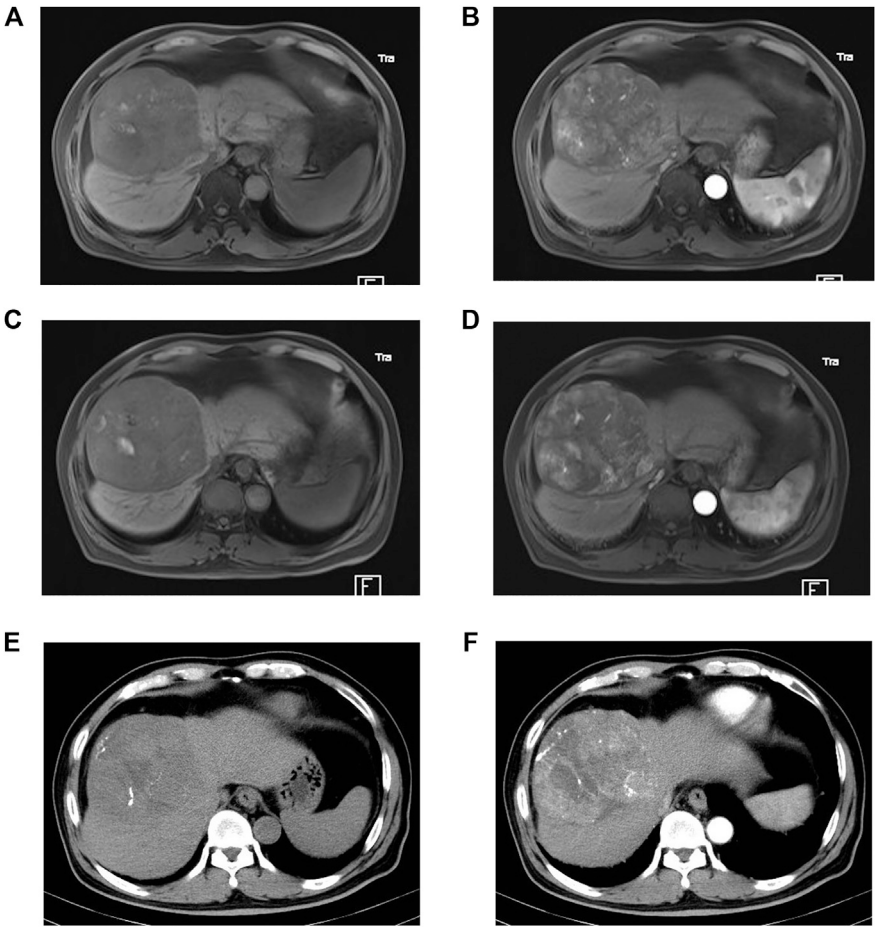

FIGURE 2 | A 69 years old male with hepatocellular carcinoma (HCC) has undergone transarterial chemoembolization (TACE). Early TACE refractoriness is found after two consecutive TACE procedures. The baseline dynamic MR shows an 11 cm tumor with heterogeneous enhancement **(A, B)**. The first follow-up dynamic MR shows a viable tumor >50% **(C, D)**, and the second follow-up dynamic CT also shows a viable tumor >50% **(E, F)**.

77 patients with AFP >400 ug/L (37.7%). The median of NLR was 2.95 (IQR 3.72).

Radiological Characteristics

The inter-reader agreements of radiological data between the two radiologists were all excellent, with Kappa values of 0.947 (tumor distribution) and 0.954 (number of tumors), and the ICC value was 0.838 (size of the largest tumor). Among all patients, the sizes of the largest tumors were ≤50 mm in 83 patients (40.7%), 50–100 mm in 75 patients (36.8%), and >100 mm (22.5%) in 46 patients. Seventy-two (35.3%) patients had tumors with bilobar involvement, and 132 (64.7%) had tumors with unilobar involvement. One hundred twenty-three patients (60.3%) had a single tumor, 48 patients (23.5%) had two or three tumors, and 33 patients (16.2%) had more than three tumors. Eighty-one patients (39.7%) were within up-to-seven criteria, and 123 patients (60.3%) were beyond the up-to-seven criteria. The detailed demographic, radiological and laboratorial characteristics are summarized in **Table 1**.

TABLE 3 | Assessment of potential risk factors of early TACE refractoriness in training cohort.

Characteristics	Absence of TACE refractoriness (n = 99)	Presence of TACE refractoriness (n = 48)	*p* Value	
			Univariate	Multivariate
Age (years)	57.6 ± 11.8	56.4 ± 14.0	0.588	-
Gender (%)			0.899	-
Male	88 (88.9%)	43 (89.6%)		
Female	11 (11.1%)	5 (10.4%)		
Child pugh class (%)			0.432	-
A	84 (84.8%)	43 (89.6%)		
B	15 (15.2%)	5 (10.4%)		
BCLC stage (%)			<0.001	-
0-A	70 (70.7%)	18 (37.5%)		
B	29 (29.3%)	30 (62.5%)		
NLR	2.39 (IQR, 1.84)	2.51 (IQR, 1.78)	0.687	
Underlying liver disease (%)			0.441	-
HBV	83 (83.8%)	36 (75.0%)		
Other	4 (4.0%)	3 (7.5%)		
None	12 (12.2%)	9 (22.5%)		
Initial AFP (%)			0.136	-
≤400 ug/L	70 (70.7%)	28 (58.3%)		
>400 ug/L	29 (29.3%)	20 (41.7%)		
History of resection (%)			0.687	-
Presence	14 (14.1%)	8 (16.6%)		
Absence	85 (85.9%)	40 (83.4%)		
Tumor distribution (%)			<0.001	0.002 (or, 3.251; 95%CI: 1.536–6.883)
Unilobar	79 (79.8%)	18 (37.5%)		
Bilobar	20 (20.2%)	30 (62.5%)		
Number of tumors (%)			<0.001	0.005 (or, 1.894; 95%CI: 1.212–2.961)
Solitary	70 (70.7%)	18 (37.5%)		
2–3	22 (22.2%)	11 (22.9%)		
>3	7 (7.1%)	19 (39.6%)		
Size of the largest tumor (%)			0.021	-
≤50 mm	53 (53.5%)	14 (29.2%)		
50–100 mm	33 (33.3%)	24 (50.0%)		
>100 mm	13 (13.2%)	10 (20.8%)		
Up-to-seven criteria (%)			<0.001	0.001 (or, 3.640; 95%CI: 1.686–7.859)
Within	56 (56.6%)	11 (22.9%)		
Beyond	43 (43.4%)	37 (77.1%)		
Initial embolic agent (%)			0.045	-
Lipiodol	46 (46.5%)	14 (29.2%)		
DEB	53 (53.5%)	34 (70.8%)		

Note: TACE, transarterial chemoembolization; NLR, neutrophil to lymphocyte ratio; IQR, interquartile range; HBV, hepatitis B virus; BCLC, Barcelona Clinic Liver Cancer; AFP, alpha-fetoprotein; DEB, drug-eluting beads.

Potential Predictive Factors of Early TACE Refractoriness

The patterns of early TACE refractoriness in patients with HCC are illustrated in **Table 2**. Totally, there were 73 patients presented with early TACE refractoriness (35.8%, 73/204). A typical patient with early TACE refractoriness is shown in **Figure 2**. In univariate analysis, early TACE refractoriness was associated with BCLC stage ($p < 0.001$), tumor distribution ($p < 0.001$), number of tumors ($p < 0.001$), size of the largest tumor ($p = 0.021$), initial embolic agents ($p = 0.045$), and within/beyond up-to-seven criteria ($p < 0.001$). There was no statistical relationship between early TACE refractoriness and age, gender, Child-Pugh class, underlying liver disease, history of resection, initial AFP level, and NLR level. Multivariate analysis was performed using the significant risk factors determined in the univariate analysis, and within/beyond up-to-seven criteria ($p = 0.001$; odds ratio = 3.640, 95%CI 1.686–7.859), tumor distribution ($p = 0.002$; odds ratio = 3.251, 95%CI 1.536–6.883) and number of tumors ($p = 0.005$; odds ratio = 1.894, 95%CI 1.212–2.961) were independent predictive factors associated with early TACE refractoriness. The results from the univariable analysis performed on the training data set were summarized in **Table 3**.

Predictive Model

A predictive model and nomogram (**Figure 3**) were built on the training set for predicting early TACE refractoriness based on within/beyond up-to-seven criteria, tumor distribution and number of tumors, with an area under the curve (AUC) of 0.788 (95%CI, 0.707–0.868), a sensitivity of 74.4% and a

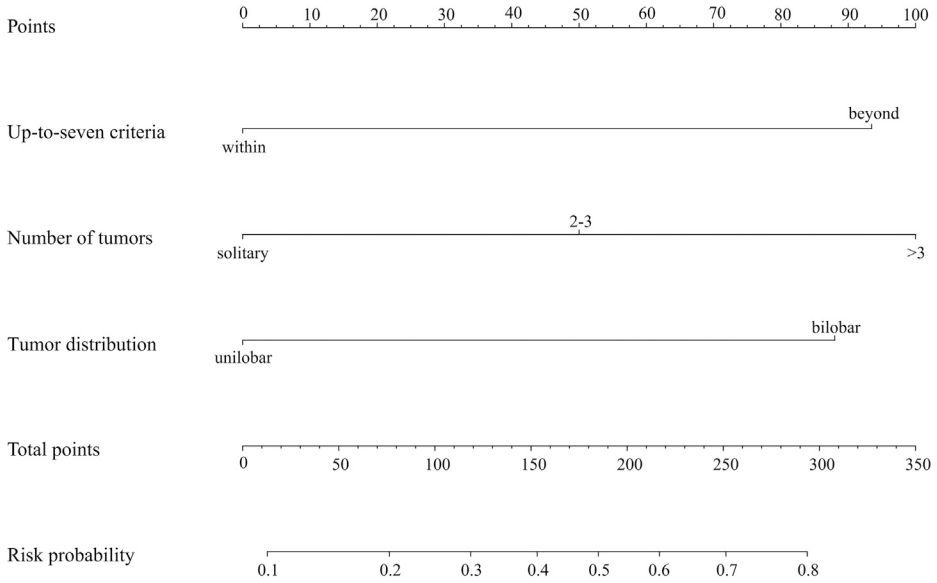

FIGURE 3 | Nomogram to predict early TACE refractoriness, each predictor corresponds to a specific point by drawing a line straight upward to the points axis. Sum of the points is located on the total points axis, and the sum represents the probability of presenting early TACE refractoriness.

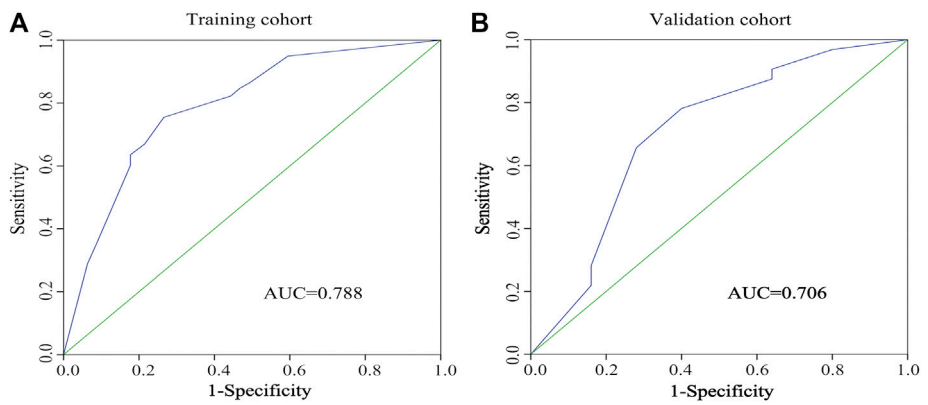

FIGURE 4 | The receiver-operating characteristic (ROC) curve of the regression model in predicting early TACE refractoriness in training cohort **(A)** and validation cohort **(B)**.

specificity of 73.8% (**Figure 4A**). While in the validation set, the AUC was 0.706 (95%CI, 0.564–0.848), with a sensitivity of 78.1% and a specificity of 60.0% (**Figure 4B**). Moreover, satisfactory calibration was confirmed by the Hosmer-Lemeshow test, with p values of 0.236 and 0.539 in the training and validation cohorts.

Comparison of PFS

Based on influencing factors selected by forward stepwise logistic regression including up-to-seven criteria, tumor distribution, and number of tumors, a PSM analysis was performed. After PSM, a total of 96 patients were enrolled, 48 of whom were present early TACE refractoriness, while 48 of whom were absent. There was no difference in baseline characteristics between two groups after PSM (**Table 4**). The median PFS in patients with or without early TACE refractoriness was 133 days (95% CI: 18.2–168.7) and 371 days (95% CI: 269.6–472.4), respectively. Patients who were absent of early TACE refractoriness had a significantly higher PFS rate than those of patients who were present (p < 0.001). The PFS curves of the two groups are shown in **Figure 5**.

DISCUSSION

TACE is the standard and effective therapy for intermediate-stage HCC. However, this course of treatment can be limited in terms of effectiveness as patients present TACE refractoriness (Arizumi et al., 2017; Maesaka et al., 2020). For patients with TACE

TABLE 4 | Demographic, radiological and laboratorial characteristics of the patients after propensity score matching.

Characteristics	Absence of TACE refractoriness (n = 48)	Presence of TACE refractoriness (n = 48)	*p* Value
Age (years)	56.1 ± 12.4	57.3 ± 12.5	0.625
Gender (%)			0.336
Male	41 (85.4%)	44 (91.7%)	
Female	7 (14.6%)	4 (8.3%)	
Child pugh class (%)			0.247
A	39 (81.3%)	43 (89.6%)	
B	9 (18.7%)	5 (10.4%)	
BCLC stage (%)			1.000
0-A	26 (54.2%)	26 (54.2%)	
B	22 (45.8%)	22 (45.8%)	
NLR	2.49 (IQR, 2.69)	2.65 (IQR, 3.58)	0.959
Underlying liver disease (%)			0.281
HBV	35 (72.9%)	41 (85.4%)	
Other	4 (8.3%)	3 (6.3%)	
None	9 (18.8%)	4(8.3%)	
Initial AFP (%)			0.294
≤400 ug/L	27 (56.3%)	32 (66.7%)	
>400 ug/L	21 (43.7%)	16 (33.3%)	
History of resection (%)			0.371
Presence	5 (10.4%)	8 (16.6%)	
Absence	43 (89.6%)	40 (83.4%)	
Tumor distribution (%)			1.000
Unilobar	27 (56.3%)	27 (56.3%)	
Bilobar	21 (43.7%)	21 (43.7%)	
Number of tumors (%)			1.000
Solitary	26 (54.2%)	26 (54.2%)	
2–3	16 (33.3%)	16 (33.3%)	
>3	6 (12.5%)	6 (12.5%)	
Size of the largest tumor (%)			0.638
≤50 mm	14 (29.2%)	18 (37.5%)	
50–100 mm	22 (45.8%)	18(37.5%)	
>100 mm	12 (25.0%)	12 (25.0%)	
Up-to-seven criteria (%)			0.824
Within	15 (31.3%)	14 (29.2%)	
Beyond	33 (68.7%)	34 (70.8%)	
Initial embolic agent (%)			0.063
Lipiodol	32 (66.7%)	23 (47.9%)	
DEB	16 (33.3%)	25 (52.1%)	

Note: TACE, transarterial chemoembolization; NLR, neutrophil to lymphocyte ratio; IQR, interquartile range; HBV, hepatitis B virus; BCLC, Barcelona Clinic Liver Cancer*; AFP, alpha-fetoprotein; DEB, drug-eluting beads.*

refractoriness, ineffective TACE should not be performed repeatedly, and those patients are recommended to switch to systemic therapy, such as sorafenib, as JSH suggested (Kudo et al., 2014). TACE refractoriness occurs almost inevitably, however, it should be noted that even in some patients, refractoriness presents in the very beginning of the TACE procedures (Maas et al., 2020).

In the present study, a predictive model was developed to predict the early TACE refractoriness, and this model was also validated in a validation cohort. The results showed that patients with the characteristics of tumor bilobar distribution, beyond up-to-seven criteria, and more than three tumors were significantly associated with early TACE refractoriness. The first predictor for early TACE refractoriness is beyond up-to-seven criteria. The up-to-seven criteria is one of a criteria for liver transplantation, while it is also used to predict the prognosis after TACE (Mazzaferro et al., 2009; Kimura et al., 2016). In Kimura's study, they showed that the cumulative overall survival (OS) and disease-free survival (DFS) rates after TACE were higher in patients within up-to-seven criteria compared with those beyond the criteria (Kimura et al., 2016). More than three tumors is also an important predictor for early TACE refractoriness. It has been reported by Kim et al. that patients with the feature of multiple tumors (≥5) can significantly increase the risk of suffering TACE refractoriness, and the present study showed a similar finding (Kim et al., 2017). Multiple tumors was an indicator of the tumor burden and may represent the highly aggressive nature of the tumors, which predispose to the development of lesions at different sites. Prognosis of multiple tumors is worse compared to patients with solitary tumors, with five-year OS rates of 29.9% over 58%, respectively (Witjes et al., 2012; Dasari et al., 2020). Thus, patients with this feature may be more likely to present early TACE refractoriness. Another predictor is tumor distribution. To the best of our knowledge, this is the first study identifying bilobar involvement as a predictor for early TACE refractoriness. Bilobar involvement could be viewed as

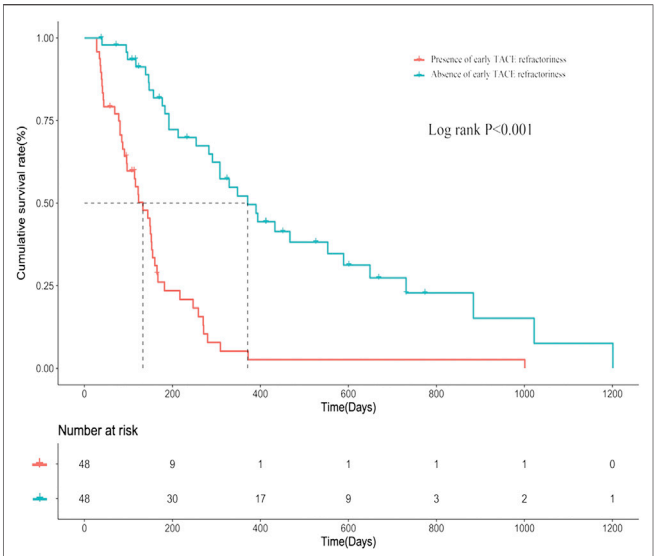

FIGURE 5 | The progression-free survival (PFS) curves of patients who are present/absent of early TACE refractoriness.

intrahepatic metastasis of the primary lesion, reflecting a more aggressive tumor behavior with a higher risk of consequent spread outside the liver (Elmoghazy et al., 2019; Dasari et al., 2020). The study from Elmoghazy et al. had revealed that HCC patients with bilobar involvement tended to have a higher probability of extrahepatic metastasis than those patients without bilobar involvement (Elmoghazy et al., 2019).

In predictive analysis, the regression model and nomogram showed moderate accuracy to predict early TACE refractoriness, with AUCs of 0.788 in the training set and 0.706 in the validation set, respectively. The clinical significance of this study is that it provides a relatively accurate, convenient, and noninvasive method for predicting early TACE refractoriness that is applicable to patients with HCC. Although TACE is recommended for unresectable HCC, not all patients can really benefit from TACE due to the heterogeneous population of unresectable HCC. Moreover, the present study performed a PSM analysis to compare the PFS rate between patients who were present or absent early TACE refractoriness, and the results showed that the PFS rates were significantly declined with patients who were present of early TACE refractoriness ($p < 0.001$), which indicated such patients might have a poor prognosis. Therefore, patients with high risk of early TACE refractoriness should be switched to systemic therapy as early as possible to improve prognosis and this study will certainly help distinguish patients with high risk of early TACE refractoriness.

Despite the valuable results described above, there are several limitations in the present study. Firstly, this is a retrospective study with a relatively small number of patients included and thus may be subject to selection and statistical bias. A prospective study with a relatively large study population should be performed to confirm this finding. Secondly, although those three variables above were tested by Diagnosis of Collinearity with variance inflation factors less than 5 (VIF < 5), there were still some confounding factors among the three variables. Thirdly, the OS of patients is not assessed, although OS is a crucial endpoint of prognosis for clinical study. Due to the retrospective nature of the present study, OS is quite difficult to obtain because some patients may not admit to hospital after tumor progression.

In conclusion, patients with characteristics of beyond up-to-seven criteria, bilobar tumor involvement, and more tumors are independent predictors of early TACE refractoriness, and patients with early TACE refractoriness may have a poor prognosis. Therefore, those characteristics should be taken into consideration when performing TACE.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Second Xiangya Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

TW writes the manuscript. ZZ, TA, and JL provide the patients information and reviewed the patients clinical data. YX provides the concept and edits the manuscript. YX is the main contributor of the study design and concept.

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ORIGINAL RESEARCH
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Development of TACE Refractoriness Scores in Hepatocellular Carcinoma

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Purpose: To identify the independent risk factors for transarterial embolization (TACE) refractoriness and to develop a novel TACE refractoriness score and nomogram for predicting TACE refractoriness in patients with hepatocellular carcinoma (HCC).

Methods: Between March 2006 and March 2016, HCC patients who underwent TACE monotherapy as initial treatment at two hospitals formed the study cohort and validation cohort. The criteria of TACE refractoriness followed the Japan Society of Hepatology 2014 version of TACE refractoriness. In the study cohort, the independent risk factors for TACE refractoriness were identified, and TACE refractoriness score and nomogram were then developed. The accuracy of the systems was validated externally in the validation cohort.

Results: In total, 113 patients from hospital A formed the study cohort and 122 patients from hospital B formed the validation cohort. In the study cohort, 82.3% of the patients (n = 93) developed TACE refractoriness with a median overall survival (OS) of 540 days (95% CI, 400.8–679.1), and the remaining 20 patients in the TACE-non-refractory group had a median OS of 1,257 days (95% CI, 338.8–2,175.2) (p = 0.019). The median time for developing TACE refractoriness was 207 days (95% CI, 134.8–279.2), and a median number of two TACE procedures were performed after refractoriness developed. The independent risk factors for TACE refractoriness were the number of tumors and bilobular invasion of HCC. TACE refractoriness scores <3.5 indicated a lower incidence of TACE refractoriness, whereas scores >3.5 points indicated a higher incidence (p < 0.001). In the validation cohort, 77.9% of the patients (n = 95) developed TACE refractoriness with a median OS of 568 days (95% CI, 416.3–719.7), and a median OS of 1,324 days was observed in the TACE-non-refractory group (n = 27; 95% CI, 183.5–2,464.5).

Conclusions: TACE refractoriness impairs the OS of HCC patients. The number of tumors and bilobular invasion status were independent risk factors for TACE refractoriness. The TACE refractoriness score can be an effective tool and easy approach to predict the risk of TACE refractoriness status.

Keywords: hepatocellular carcinoma, transarterial embolization refractory, risk factors, nomogram, overall survival

INTRODUCTION

Hepatocellular carcinoma is a major health problem worldwide and is especially more commonly seen in the developing countries or regions (Forner et al., 2018). Nearly 80% of HCC in China is first diagnosed at the mid-late stage due to the asymptomatic features of early HCC (Zhou et al., 2018). According to the Barcelona Clinic Liver Cancer (BCLC) staging system (Llovet et al., 1999; Forner et al., 2010), transarterial chemoembolization is recommended as the first-line treatment for patients at intermediate stage (BCLC B) (1). The global HCC BRIDGE (Bridge to Better Outcomes in HCC) study, a multiregional large-scale longitudinal cohort study including 18,031 patients from 14 countries, proved TACE the most widely used approach for HCC across BCLC stages from intermediate to advanced stages (Park et al., 2015).

In general, overall survival in HCC patients treated with TACE reaches 70.3% at 1 year, 51.8% at 2 years, 40.4% at 3 years, and 32.4% at 5 years (Lencioni et al., 2016). Patient's general condition, underlying liver function, tumor response, tumor stage, and treatment technique are factors that can largely influence the treatment outcomes (Bruix, 2002; Xu et al., 2015; Kim et al., 2016).

For the reasons above, the concept of TACE refractoriness has recently drawn much attention. Japan Society of Hepatology defined TACE refractoriness/TACE failure in 2010 and updated in 2014 (Kudo et al., 2011; Kudo et al., 2014) as 1) intrahepatic lesion, which consisted of 1) two or more consecutive insufficient responses of the treated tumor (viable lesion >50%) even after changing the chemotherapeutic agents and/or reanalysis of the feeding artery seen on response-evaluation computed tomography (CT)/magnetic resonance imaging (MRI) at 1–3 months after having adequately performed selective TACE; 2) two or more consecutive progressions in the liver (the number of tumors increased compared to that before the previous TACE procedure) even after having changed the chemotherapeutic agents and/or reanalysis of the feeding artery seen on response-evaluation CT/MRI at 1–3 months after having adequately performed selective TACE; 2) continuous elevation of tumor markers immediately after TACE even though a slight transient decrease is observed; 3) appearance of vascular invasion; or 4) appearance of extrahepatic spread. The International Expert Panel on Interventions in Hepatocellular Carcinoma (EPOIHCC) defines TACE refractoriness as no response after ≥3 TACE procedures within a 6 month period to the same area (Cheng et al., 2014). Since then, several studies found that TACE refractoriness had a significant impact on prognosis (Kudo, 2011; Sieghart et al., 2013; Song et al., 2013; Hiraoka et al., 2015). Therefore, identifying the risk factors for TACE refractoriness and developing a model for predicting prognosis of patients with TACE refractoriness are crucial for HCC patients before being treated with TACE. The purpose of this study was to identify the independent risk factors for TACE refractoriness and to develop a TACE refractoriness score and nomogram for predicting TACE refractoriness in HCC patients receiving TACE monotherapy as initial treatment.

PATIENTS AND METHODS

Patients

Between March 2006 and March 2016, patients with HCC undergoing TACE as an initial treatment at Hospital A were retrospectively studied to form the study cohort (**Figure 1**). From April 2007 to December 2016, patients with HCC who received TACE as initial treatment at Hospital B were retrospectively reviewed as the external validation cohort. The diagnosis of HCC was made as per the diagnosis criteria of European Association for the Study of the Liver (EASL, 2018) Clinical Practice Guidelines: Management of hepatocellular carcinoma (2018 Edition) (2018): 1) in patients with cirrhosis, multiphase enhanced CT or MRI shows hallmark signs of HCC with an α-fetoprotein (AFP) concentration ≥400 μg/L; and 2) in noncirrhotic patients, diagnosis of HCC should be confirmed by pathology.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: 1) age between 18 and 90 years; 2) liver function was suitable for TACE treatment (Child-Pugh A or B level); 3) general condition was suitable for TACE treatment [Eastern Cooperative Oncology Group (ECOG) performance scores = 0]; 4) BCLC stage A or B; and 5) no previous HCC-related treatment. The exclusion criteria were: 1) severe coagulation dysfunction that could not be corrected; 2) severe liver dysfunction (Child-Pugh C) or irreversible liver decompensation; 3) extrahepatic metastasis or vascular invasion; 4) ECOG scores >2; 5) insufficient follow-up data; and 6) history of any other tumor.

Study Objectives

The primary outcome measure was the occurrence of TACE refractoriness, which was defined according to the criteria of the JSH and the Liver Cancer Study Group of Japan (LCSGJ) (Kudo et al., 2014). The second outcome measure was OS. Baseline characteristics, including tumor size, number of tumors, diameter of the largest lesion, extent of tumor, unilobular or bilobular invasion, and vascular invasion, were collected by two

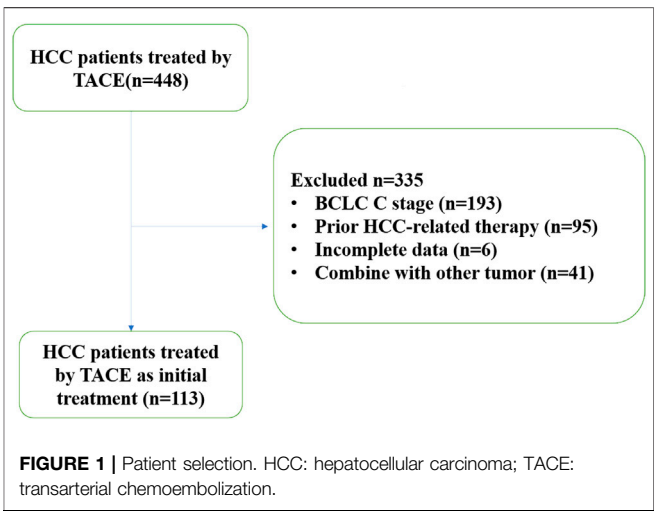

FIGURE 1 | Patient selection. HCC: hepatocellular carcinoma; TACE: transarterial chemoembolization.

TABLE 1 | Baseline characteristics in the study cohort.

Characteristics	TACE-refractory group (*n* = 93)	TACE-non-refractory group (*n* = 20)	*p* Value
Age, yr, mean (range)	61.33 (33–89)	66.30 (36–90)	0.129
Sex (male/female)	83/10 (89%)	18/2 (90%)	1.000
Hepatitis B (yes/no)	67/26 (72%)	17/3 (85%)	0.273
PS (0/1)	65/28 (70%)	16/4 (80%)	0.425
TBIL (μmol/L), mean ± SD	18.19 ± 12.97	16.93 ± 8.64	0.680
ALB (g/L), mean ± SD	36.28 ± 5.31	37.74 ± 5.02	0.263
AST (IU/L), mean ± SD	51.05 ± 62.10	48.25 ± 34.43	0.846
ALT (IU/L), mean ± SD	40.01 ± 36.46	45.10 ± 25.25	0.554
WBC (10^9/L), mean ± SD	5.75 ± 2.23	6.09 ± 2.87	0.547
Hb (g/L), mean ± SD	134.56 ± 18.77	135.05 ± 18.62	0.916
BCLC stage (A/B)	20/73 (22%)	9/11 (45%)	0.029
HKLC stage (I/II/III)	21/32/40	7/9/4	0.133
TN, mean ± SD	3.34 ± 1.61	2.05 ± 1.23	0.001
TD, cm, mean ± SD	7.26 ± 4.31	7.02 ± 3.99	0.819
BI (yes/no)	47/46 (51%)	1/19 (5%)	<0.001

PS: physical status; TBIL: total bilirubin; ALB: albumin; AST: aspartate transaminase; ALT: alanine aminotransferase; WBC: white blood cell count; TN: tumor number; TD: tumor diameter; BI: bilobular invasion; SD: standard deviation.

TABLE 2 | Baseline characteristics in the validation cohort.

Characteristics	TACE-refractory group (*n* = 95)	TACE-non-refractory group (*n* = 27)	*p* Value
Age, yr, mean (range)	59.42 (27–92)	60.41 (36–85)	0.716
Sex (male/female)	77/18 (81%)	19/8 (70%)	0.287
Hepatitis B (yes/no)	53/42 (56%)	18/9 (66%)	0.380
PS (0/1)	75/20 (79%)	18/9 (66%)	0.206
TBIL (μmol/L), mean ± SD	17.37 ± 8.51	20.58 ± 9.07	0.081
ALB (g/L), mean ± SD	39.44 ± 5.02	37.91 ± 4.36	0.167
AST (IU/L), mean ± SD	79.56 ± 205.70	65.33 ± 61.34	0.482
ALT (IU/L), mean ± SD	63.61 ± 153.07	50.32 ± 40.18	0.788
WBC (10^9/L), mean ± SD	6.24 ± 2.55	5.42 ± 1.88	0.123
Hb (g/L), mean ± SD	131.95 ± 20.64	133.44 ± 19.14	0.736
BCLC stage (A/B)	1/94 (1%)	3/24 (11%)	0.034
HKLC stage (I/II/III)	11/40/44	7/13/7	0.074
TN, mean ± SD	3.44 ± 1.09	2.89 ± 1.16	0.032
TD, cm, mean ± SD	8.20 ± 4.20	6.45 ± 3.50	0.051
BI (yes/no)	34/61 (36%)	6/21 (22%)	0.247

PS: physical status; TBIL: total bilirubin; ALB: albumin; AST: aspartate transaminase; ALT: alanine aminotransferase; WBC: white blood cell count; TN: tumor number; TD: tumor diameter; BI: bilobular invasion; SD: standard deviation.

experienced radiologists from multiphase enhanced CT or MRI. Assessment of tumor response after TACE was completed based on the modified Response Evaluation Criteria in Solid Tumors (Lencioni and Llovet, 2010). Clinical data, such as performance status, hepatitis history, cirrhosis history, and ascites, were collected. Serology of AFP, bilirubin, albumin, and white blood cell count were collected as laboratory data.

Treatment Procedure

TACE was performed within 2–3 days after diagnosis of HCC as described. Using the Seldinger technique, an arterial catheter (5-Fr) was inserted into the femoral artery after local anesthesia. The catheter was then advanced in the hepatic artery, and digital subtraction angiography was performed. The tumor-feeding vessels were superselected using the catheter or microcatheter (2.8-Fr) to infuse a suspension containing 20–60 mg of doxorubicin hydrochloride (Adriamycin; Shenzhen Main Luck Pharmaceutical Inc., Shenzhen, China) and 2–20 ml of iodized oil (Lipiodol Ultra-Fluide; Laboratoire Guerbet, Roissy-Charlesde Gaulle, France). Gelfoam sponge embolization was performed following iodized oil embolization. The dosages of doxorubicin and iodized oil were determined by the patient's liver function and tumor characteristics.

Follow-Up

According to the TACE on-demand schedule (Terzi et al., 2012; Zhong et al., 2017), the patients were followed up by dynamic enhanced CT or MRI 4–6 weeks after the procedure. If the lesion had a good response, then no additional treatment was given, and patients were asked to undergo follow-up CT/MRI and α-fetoprotein evaluation in 2–3 months; otherwise, a repeated TACE session was considered. Subsequent TACE was not necessary if patients reached either of the following endpoints: 1) complete devascularization of the lesion; or 2) liver function

TABLE 3 | Baseline characteristics between the study and validation cohorts.

Characteristics	Study cohort (*n* = 113)	Validation cohort (*n* = 122)	*p* Value
Age, yr, mean (range)	62.21 (33–90)	59.64 (27–92)	0.125
Sex (male/female)	101/12 (89%)	96/26 (79%)	0.033
Hepatitis B (yes/no)	84/29 (74%)	71/51 (58%)	0.013
PS (0/1)	81/32 (72%)	93/29 (76%)	0.459
TBIL (μmol/L), median	17.97 ± 12.3	18.08 ± 8.7	0.409
ALB (g/L), mean ± SD	36.54 ± 5.27	39.1 ± 5.05	<0.001
AST (IU/L), mean ± SD	50.56 ± 58.05	76.41 ± 183.62	0.004
ALT (IU/L), mean ± SD	40.91 ± 34.70	60.67 ± 136.3	0.022
WBC (10^9/L), mean ± SD	5.52 ± 2.26	6.06 ± 2.43	0.434
Hb (g/L), mean ± SD	134.65 ± 18.66	132.28 ± 20.25	0.354
BCLC stage (A/B)	29/84 (26%)	4/118 (3%)	<0.001
HKLC stage (I/II/III)	28/41/44	18/53/51	0.144
TN, mean ± SD	3.12 ± 2.62	3.32 ± 1.12	0.423
TD, cm, mean ± SD	7.21 ± 4.24	7.81 ± 4.11	0.279
BI (yes/no)	48/65 (36%)	40/82 (22%)	0.139

PS: physical status; TBIL: total bilirubin; ALB: albumin; AST: aspartate transaminase; ALT: alanine aminotransferase; WBC: white blood cell count; TN: tumor number; TD: tumor diameter; BI: bilobular invasion; SD: standard deviation.

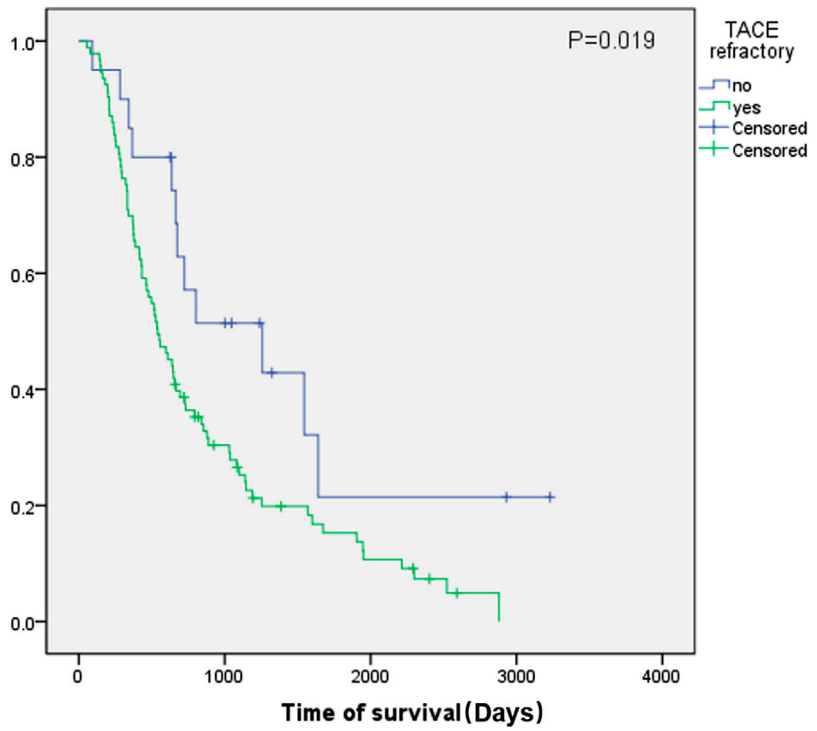

FIGURE 2 | Kaplan-Meier survival curves of the study cohort. Green line: TACE-refractory group. Blue line: TACE-non-refractory group.

not suitable for additional TACE procedure. In these situations, patients were recommended to receive other treatments, such as sorafenib, iodine-125 seed implantation, and supportive therapy. At each follow-up, the patients' imaging study, laboratory data, and general condition were carefully reviewed.

Statistical Analysis

All data were analyzed using SPSS 21.0 for Windows (IMB Corporation, Somers, NY, United States). The categorical variables, such as sex, unilobular or bilobular invasion, BCLC staging, and vascular invasion status, were compared using the χ^2 test or Fisher's exact test. Continuous variables with a normal distribution, such as the number of tumors, tumor diameter, and serum AFP, were compared using the *t* test. A logistic regression model and a neural network were used to identify the independent prognostic factors for TACE refractoriness. A nomogram was estimated based on the conclusion of logistic regression model and by the package

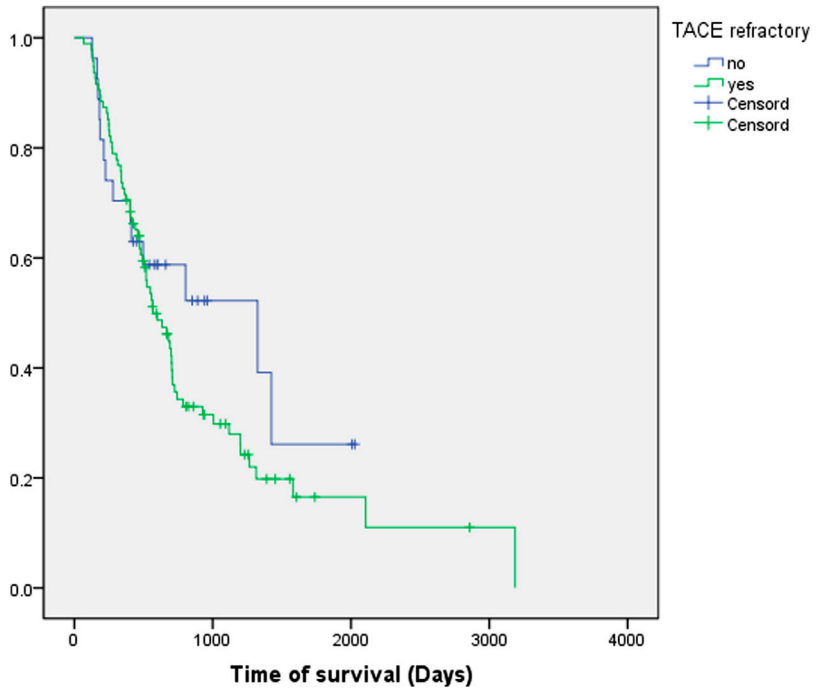

FIGURE 3 | Kaplan-Meier survival curves of the validation cohort. Green line: TACE-refractory group. Blue line: TACE-non-refractory group.

TABLE 4 | Multivariate analysis of prognostic factors of the study cohort.

Variable		Multivariate analysis			TACE refractoriness Score
		OR	95% CI	*p* Value	
TN		1.465	1.183–1.815	0.001	TN×1
BI	No	1.0			0
	Yes	12.572	1.556–101.565	0.018	7.5

TN: tumor number, BI: bilobular invasion.

of rms in R version 3.0.2. A p-value of <0.05 was considered statistically significant.

Establishment of TACE Refractoriness Score and Nomogram

The TACE refractoriness scoring system and nomogram were established based on the identified significant risk factors by univariate analyses. The accuracy of the nomogram was evaluated by the concordance c statistic (C index) in the validation cohort. The C index was assessed using the receiver operating characteristic curve analysis. The C index of the prognostic models is usually between 0.6 and 0.85 (Royston et al., 2009).

RESULTS

Clinical Characteristics of Patients

A total of 113 patients at Hospital A and 122 at Hospital B were enrolled in the study cohort and validation cohort, respectively. The baseline characteristics of the patients in both cohorts were shown in **Table 1**-**3**. No significant difference was observed in baseline characteristics between the TACE-refractory and TACE-non-refractory groups in either the study cohort or validation cohort. Some differences in the baseline characteristics were detected between these two hospitals, including BCLC stage, sex, hepatitis infection status, and liver function. The validation cohort was used to evaluate the accuracy of TACE refractoriness score, so it does not interfere with further analysis.

Kaplan-Meier Survival Curves of Overall Survival

Kaplan-Meier curves were generated for different groups in both the study and validation cohorts. In the study cohort, 82.3% (n = 93) of the patients were TACE-refractory with a median OS of 540 days (95% CI, 400.8–679.1). In the TACE-non-refractory group (n = 20), the median OS was 1,257 days (95% CI, 338.8–2,175.2). The log-rank test showed a significant difference between these two groups (p = 0.019) (**Figure 2**).

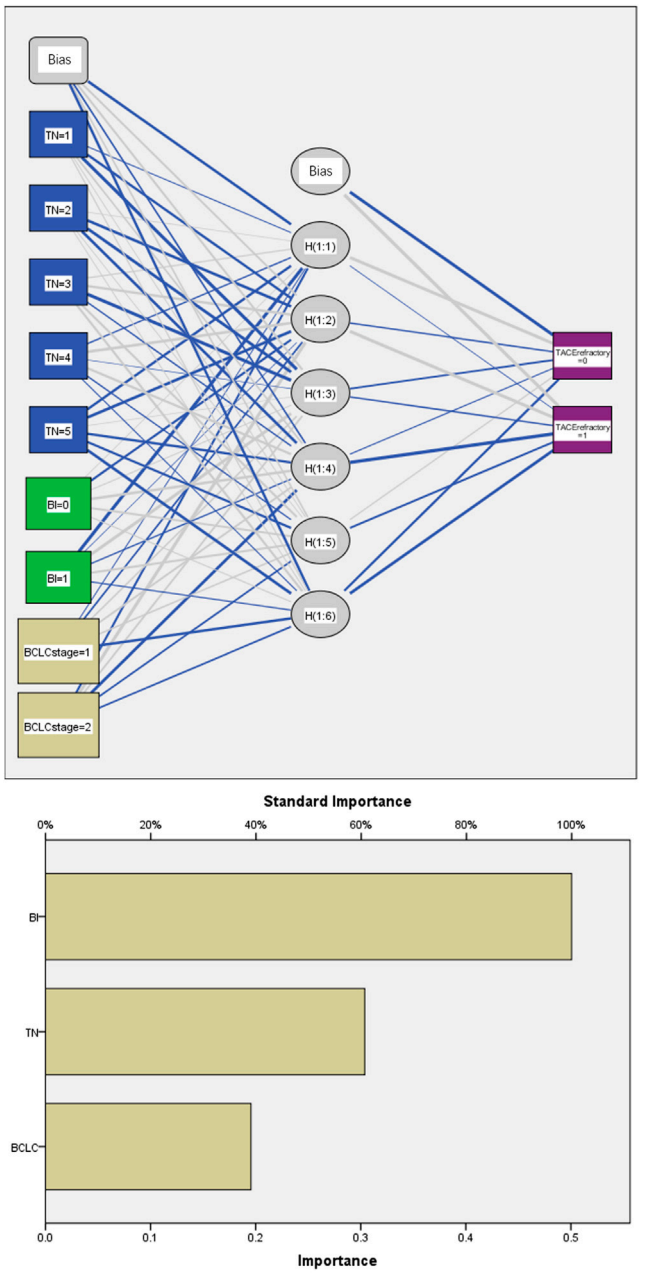

FIGURE 4 | Neural network analysis. BI: bilobular invasion; TN: tumor number. There were six nodes in the hidden layers. A total of 70% of samples were selected for training and the remaining 30% for validation.

In the validation cohort, 77.9% (n = 95) of the patients were TACE-refractory with a median OS of 568 days (95% CI, 416.3–719.7), while the median OS was 1,324 days (95% CI, 183.5–2,464.5) in the TACE-non-refractory group (n = 27) (p = 0.300) (**Figure 3**).

Univariate and Neural Network Analysis

The results of univariate analysis showed that the number of tumors (p = 0.001) and bilobular invasion (p < 0.001) had significant effects on TACE refractoriness. Multi-Layer Perceptron was also used to establish a neural network analysis, with the input variates being the number of tumors, bilobular invasion, BCLC stage, history of hepatitis, and serology indices and output variate being TACE refractoriness. A total of 70% of samples were selected for training, and the remaining 30% were assigned for validation. The outcome of neural network showed the number of tumors and bilobular invasion as top two important indices for TACE refractoriness (**Figure 4**).

Logistic Regression Analysis and Development of TACE Refractoriness Score

The number of tumors and bilobular invasion were entered into a logistic regression analysis (**Table 4**). Both indices remained significant independent prognostic factors for TACE refractoriness. The calculated regression coefficients (B-values) were tripled and rounded to facilitate the calculation of the TACE refractoriness score (**Table 4**). The TACE refractoriness score was the sum of points given to these two variables. This scoring system identified two subgroups with distinct prognoses. Patients with a TACE refractoriness score of 0–3.5 points had a low incidence of TACE refractoriness, whereas those with a score >3.5 points faced a high incidence of TACE refractoriness (p < 0.001). The prognostic performance of TACE refractoriness score was verified in the validation cohort by concordance c statistic. The C-index of this model was 0.80 (95% CI, 0.70–0.90) in the study cohort and 0.65 (95% CI, 0.52–0.78) in the validation cohort.

Prognostic Nomogram for TACE Refractoriness

The prognostic nomogram was created to estimate the possibility of TACE refractoriness based on both significant independent factors (**Figure 5**). In this nomogram, each variable axis displayed a point of an individual patient. The sum of points reflected the likelihood of developing TACE refractoriness.

DISCUSSION

TACE has been the first-line treatment for HCC patients at the intermediate stage. TACE refractoriness, as a signal of poor response, has gained tremendous interest in recent years (Kim et al., 2012; Sieghart et al., 2013; Johnson et al., 2016). It was first described by the JSH in 2010 and then updated in 2014. Later, the EPOIHCC and EASL published their own definitions of TACE refractoriness in 2011 (Park et al., 2013) and 2014 (Raoul et al., 2014), respectively. In this study, the JSH 2014 definition of TACE refractoriness was chosen because it has been widely accepted by radiologists for relatively easy application. As the earliest definition released, many researchers referred to this version for TACE refractoriness, and examples include Hiraoka A. et al. (Hiraoka et al., 2015) and Lee S. et al. (Lee et al., 2017). In addition, the JSH 2014 definition of TACE refractoriness was practical in clinical settings. Radiologists could easily interpret the TACE refractoriness status based on

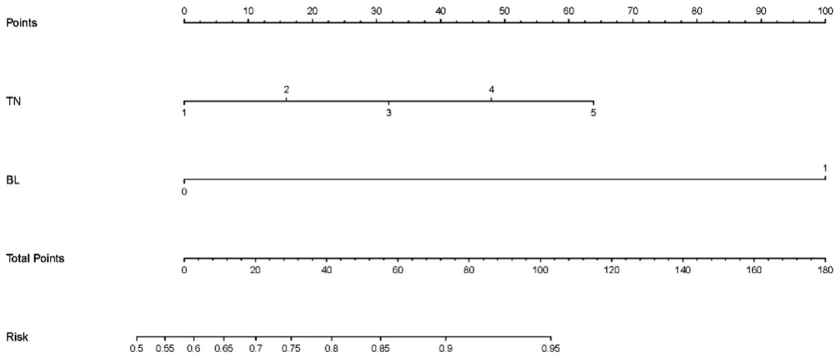

FIGURE 5 | Nomogram for TACE refractoriness. TN: tumor number; BI: bilobular invasion. According to a patient's condition, locate the patient's point on the TN/BI axis and draw a line upwards to find the corresponding point. The sum of these two points (variables) can then be located on the total points axis, and a line is to be drawn downwards to match the likelihood of developing TACE refractoriness.

CT and serum AFP results after patients received two consecutive, adequate TACE procedures.

Our study demonstrated that the OS of the TACE-refractory group was shorter than that of the TACE-non-refractory group in both the study cohort (540 vs. 1,257 days, $p = 0.019$) and validation cohort (568 vs. 1,324 days). These data indicated that TACE refractoriness, which was associated with the number of tumors and bilobular invasion, could contribute to poor prognosis and impair patients' OS (Johnson et al., 2016; Lee et al., 2017).

Similar to many studies, the number of tumors contributed to TACE refractoriness as an independent risk factor in the present work. Many staging systems that use the number of tumors as a crucial index include the Cancer of The Liver Italian Program (CLIP) scoring system, the Chinese University Prognostic Index (CUPI) system, the International Cooperative Study Group on Hepatocellular Carcinoma (ICSGOHC) simple staging system, and the BCLC staging system (Llovet et al., 1999; Llovet and Bruix, 2000; Leung et al., 2002; Vauthey et al., 2002). In the CLIP scoring system, uninodular lesion is worth 0 point with median survival time being 36 months, while multinodular is worth one point with median survival time of 22 months. The CUPI system and the ICSGOHC simplified staging system were based on the TNM staging system, where a single tumor was classified as T1, T2, and T3a and multiple tumors classified as T3b, T3c, and T4. In the BCLC staging system, a single lesion or three lesions with diameter smaller than 3 cm are considered stage A and recommended to be treated by curative methods.

In the current study, bilobular invasion was strongly associated with TACE refractoriness. This finding was also supported by the studies of Lladó, L et al. (Lladó et al., 2000) and Hiraoka, A et al. (Hiraoka et al., 2006) in which bilobular invasion was proved to have prognostic value. The former study included 143 patients treated with TACE and used univariate analysis to clarify the significant effect of bilobular invasion ($p = 0.04$). The latter study published in 2006 depicted the association between bilobular invasion and poor prognosis ($p < 0.05$) in 121 patients.

This present study reported and validated a TACE refractoriness scoring system and a nomogram to predict the occurrence of TACE refractoriness. The tools can be useful in predicting the likelihood of TACE refractoriness by the number of tumors and presence of bilobular invasion. In this scoring system, each tumor counts one point, and bilobular invasion counts 7.5 points. Patients with a TACE refractoriness score over 3.5 points suffered a high incidence of TACE refractoriness. Therefore, this score can act as a simple tool to predict whether the patient suffers a high risk of TACE refractoriness. In the nomogram, the number of tumors and presence of bilobular invasion are assigned with individual points, and their sum becomes the total score to predict the likelihood of TACE refractoriness.

For those who are prone to be TACE-refractory, molecular-targeted therapies such as sorafenib and lenvatinib are recommended by various guidelines (Kudo et al., 2011; Park et al., 2013; Kudo et al., 2014). Some retrospective studies showed the efficacy of sorafenib to prolong OS and time to progression (TTP) (Ikeda et al., 2014; Ogasawara et al., 2014). Ogasawara, S. et al. compared the use of sorafenib and TACE in their study and discovered that both OS (25.4 vs. 11.5 months) and TTP (22.3 vs. 7.7 months) were significantly longer in the sorafenib group than in the TACE group. In the study by Arizumi, T., a similar conclusion was made as OS in the sorafenib and TACE groups was 24.7 and 13.6, respectively (Arizumi et al., 2015).

The main limitation of this study is the relatively small sample size. For this reason, only two variables were included in the TACE refractoriness scoring system and nomogram. The second limitation is that the retrospective nature of this study may have caused recalling bias during the follow-up. Lastly, the baseline characteristics between the two hospitals were not balanced, which could make our conclusion more difficult to understand. However, it proved, on the other hand, that our TACE refractoriness scoring system and nomogram can provide high accuracy for patients with different baseline data.

CONCLUSION

In conclusion, this study showed that TACE refractoriness may impair OS of patients with HCC. The number of tumors and presence of bilobular invasion were independent risk factors for

TACE refractoriness. The successfully developed TACE refractoriness scoring system and nomogram can serve as simple but effective tools for predicting the occurrence of TACE refractoriness before the first TACE procedure. Patients with a TACE refractoriness score >3.5 points are at a higher risk of TACE refractoriness.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by IEC for Clinical Research of Zhongda Hospital, Affiliated to Southeast University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

G-JT, C-XY, LC, B-YZ, and C-FN contributed to study concept and design. G-YZ, QZ contributed to perform TACE procedure. LC, C-XY and Z-CJ contributed to acquisition of clinical data, B-YZ and G-YZ contributed to statistical analysis and data interpretation. C-XY wrote the first draft of the manuscript. HDZ wrote sections of the manuscript. G-JT, B-YZ, H-DZ, and C-FN supervised and oversaw the study. All authors contributed to manuscript revision, read and approved the submitted version.

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Random Survival Forests to Predict Disease Control for Hepatocellular Carcinoma Treated With Transarterial Chemoembolization Combined With Sorafenib

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Objectives: To use baseline variables to predict one-year disease control for patients with hepatocellular carcinoma (HCC) treated with transarterial chemoembolization (TACE) combined with sorafenib as initial treatment by applying a machine learning approach based on the random survival forest (RF) model.

Materials and Methods: The multicenter retrospective study included 496 patients with HCC treated with TACE combined with sorafenib between January 2014 and December 2018. The independent risk factors associated with one-year disease control (complete response, partial response, stable disease) were identified using the RF model, and their predictive importance was determined using the Gini index. Tumor response was assessed according to modified Response Evaluation Criteria in Solid Tumors.

Results: The median overall survival was 15.5 months. A total of 186 (37.5%) patients achieved positive one-year disease control. The Barcelona Clinic Liver *Cancer* (BCLC) stage (Gini index: 20.0), tumor size (≤7 cm, >7 cm; Gini index: 9.0), number of lobes involved (unilobar, bilobar; Gini index: 6.4), alpha-fetoprotein level (≤200 ng/dl, >200 ng/dl; Gini index: 6.1), albumin–bilirubin grade (Gini index: 5.7), and number of lesions (1, >1; Gini index: 5.3) were identified as independent risk factors, with the BCLC stage as the most important variable. The RF model achieved a higher concordance index of 0.724 compared to that for the logistic regression model (0.709).

Conclusions: The RF model is a simple and accurate approach for prediction of one-year disease control for patients with HCC treated with TACE combined with sorafenib.

Keywords: hepatocellular carcinoma, transarterial chemoembolization, sorafenib, disease control, random survival forest

INTRODUCTION

Despite improving surveillance programs, around 80% of hepatocellular carcinomas (HCCs) are first diagnosed at an intermediate or advanced stage according to the Barcelona Clinic Liver *Cancer* (BCLC) staging system (Bray et al., 2018; Forner et al., 2018; Villanueva, 2019). For intermediate-stage HCC, transarterial chemoembolization (TACE) is the standard approach recommended by the American Association for the Study of the Liver Disease (AASLD) and European Association for the Study of the Liver (EASL) guidelines (European Association for the Study of the Liver, 2018; Marrero et al., 2018). According to the BRIDGE study, TACE is the most widely applied method for both intermediate and advanced HCCs in real-world clinical practice (Park et al., 2015). Nevertheless, the prognosis of patients treated with TACE varies from a median survival of 19.4 months generally to around 49.1 months in well-selected patients, which is mainly due to the high heterogeneity of unresectable HCC (Lencioni et al., 2016a; Galle et al., 2017).

Due to the fact that there is an increase in vascular endothelial growth factor after TACE, the combination of TACE with sorafenib, an orally active multikinase inhibitor with antiangiogenic properties, should improve the efficacy of TACE ideally (Li et al., 2004; Wang et al., 2008). Unfortunately, three randomized controlled trials (RCTs) failed to identify significant treatment efficacy and safety for TACE combined with sorafenib compared to TACE monotherapy (Kudo et al., 2011; Lencioni et al., 2016b; Meyer et al., 2017). On the contrary, a recently reported RCT carried out by Kudo et al., the TACTICS trial, demonstrated positive results (Kudo et al., 2019). Notably, a much longer median duration of sorafenib administration was observed in the TACTICS trial compared to that in the previous three negative trials, which might be a key reason for the success of the TACTICS trial (Kudo et al., 2019). Therefore, a longer time of disease control in order to achieve a longer sorafenib administration period is an important factor for patients achieving survival benefit from the combination treatment of TACE and sorafenib (Kudo and Arizumi, 2017).

As mentioned before, high heterogeneity of unresectable HCC leads to the diverse prognosis including the sorafenib administration period for patients treated with TACE combined with sorafenib. The prognosis of HCC is mainly based on tumor burden and liver function. Recently, a machine learning approach, random survival forest (RF), has been applied as an intuitive technique for predicting individual prognosis (Hsich et al., 2011; Hu and Steingrimsson, 2018). It requires little input from the analyst and has ability to easily deal with nonlinear effects and variable interactions, which are major limitations of conventional linear discriminant analysis (Ishwaran et al., 2014). By combining many individual decision trees, RFs form an ensemble method and provide an accurate assessment of variable importance of every individual variable associated with prognosis (Hu and Steingrimsson, 2018).

The present study aimed to predict one-year disease control for unresectable HCC treated with TACE combined with sorafenib by applying an RF model. In addition, the study also evaluated the importance and predictive value of variables in the RF model for a one-year disease control outcome.

MATERIALS AND METHODS

Patients' Criteria

This multicenter retrospective study included patients diagnosed with unresectable HCC according to the AASLD/EASL guidelines and treated with TACE combined with sorafenib as initial treatment between January 2014 and December 2018 at three institutions. The study was approved by the institutional review boards at the three institutions, and the requirement for informed consent was waived due to its retrospective nature. The study was performed in accordance with the Declaration of Helsinki. The inclusion criteria were as follows: 1) 18 years or older with the definite diagnosis of HCC; 2) having an Eastern Cooperative Oncology Group performance score of 0 or 1; 3) not suitable or unwilling to receive curative treatment such as resection, ablation, or transplantation; and 4) no prior HCC-related treatment. Patients were excluded if they had any of the following: 1) Child–Pugh grade C or aspartate transaminase >5 times the upper limit of the normal range and total bilirubin >1.5 times the upper limit of the normal range; 2) inadequate renal, clotting, and hematologic function; 3) accompanying or history of any other primary malignancies; and 4) incomplete or missing clinical and follow-up data. Multidisciplinary discussion was carried out before treatment to determine if TACE combined with sorafenib was the recommended therapy for the patients. Written informed consent regarding the advantages and disadvantages of the combination treatment, including the potential treatment outcomes, treatment-related morbidities, and costs, was obtained from every included patient.

Treatment

Patients included in the study received the conventional TACE procedure, and details on it have been provided in our previous studies (Zhong et al., 2017). Repeat TACE was assessed and provided according to the "on demand" mode: subsequent contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) follow-up was carried out 4–6 weeks after the previous procedure. TACE was discontinued when no vital active tumor lesion(s) was observed during follow-up CT/MRI, and the patient underwent the next contrast-enhanced CT/MRI plus alpha-fetoprotein follow-up every 8–10 weeks. Repeat TACE was evaluated if the contrast-enhanced CT/MRI presented new lesions (Terzi et al., 2012).

Sorafenib (Bayer Healthcare, Leverkusen, Germany) was administered within 3–7 days after every TACE with an initial dose of 400 mg twice daily. It was temporary stopped the day before every TACE. Dose reductions to 200 mg twice daily or 200 mg once daily or temporary interruptions were allowed due to drug-related toxicity. Sorafenib was discontinued in the event of disease progression or unacceptable toxicity.

The primary outcome of the study was one-year disease control, defining patients achieving complete response (CR),

TABLE 1 | Patient characteristics.

Characteristic	Overall (*n* = 496)	Institution A (*n* = 313)	Institution B (*n* = 59)	Institution C (*n* = 124)	*p**
Gender					0.196
Male	427 (86.1%)	269 (85.9%)	47 (79.7%)	111 (89.5%)	
Female	69 (13.9%)	44 (14.1%)	12 (20.3%)	13 (10.5%)	
Hepatitis B					0.428
Yes	417 (84.1%)	268 (85.6%)	49 (83.1%)	100 (80.6%)	
No	79 (15.9%)	45 (14.4%)	10 (16.9%)	24 (19.4%)	
Age (y)					0.019
≤55	321 (64.7%)	217 (69.3%)	33 (55.9%)	71 (57.3%)	
>55	175 (35.3%)	96 (30.7%)	26 (44.1%)	53 (42.7%)	
ALBI grade					0.523
1	251 (50.6%)	163 (52.1%)	26 (44.1%)	62 (50.0%)	
2	245 (49.4%)	150 (47.9%)	33 (55.9%)	62 (50.0%)	
Child–Pugh grade					0.430
A	430 (86.7%)	273 (87.2%)	48 (81.4%)	109 (87.9%)	
B	66 (13.3%)	40 (12.8%)	11 (18.6%)	15 (12.1%)	
Maximum tumor size (cm)					0.417
<5	194 (39.1%)	119 (38.0%)	27 (45.8%)	48 (38.7%)	
5–10	180 (36.3%)	118 (37.7%)	22 (37.3%)	40 (32.3%)	
>10	122 (24.6%)	76 (24.3%)	10 (16.9%)	36 (29.0%)	
Lobes involved					0.852
Unilobar	318 (64.1%)	198 (63.3%)	38 (64.4%)	82 (66.1%)	
Bilobar	178 (35.9%)	115 (36.7%)	21 (35.6%)	42 (33.9%)	
No. of lesions					0.779
1	225 (45.4%)	144 (46.0%)	28 (47.5%)	53 (42.7%)	
>1	271 (54.6%)	169 (54.0%)	31 (52.5%)	71 (57.3%)	
ECOG					0.090
0	482 (97.2%)	307 (98.1%)	58 (98.3%)	117 (94.4%)	
1	14 (2.8%)	6 (1.9%)	1 (1.7%)	7 (5.6%)	
PVTT					0.005
None	309 (62.3%)	194 (62.0%)	44 (74.6%)	71 (57.3%)	
Branch	102 (20.6%)	71 (22.7%)	11 (18.6%)	20 (16.1%)	
Main	85 (17.1%)	48 (15.3%)	4 (6.8%)	33 (26.6%)	
BCLC stage					0.071
B	252 (50.8%)	156 (49.8%)	38 (64.4%)	58 (46.8%)	
C	244 (49.2%)	157 (50.2%)	21 (35.6%)	66 (53.2%)	
AFP (ng/dl)					0.760
≤200	257 (51.8%)	159 (50.8%)	33 (55.9%)	65 (52.4%)	
>200	239 (48.2%)	154 (49.2%)	26 (44.1%)	59 (47.6%)	
AST (U/L)					0.138
≤40	218 (44.0%)	129 (41.2%)	25 (42.4%)	64 (51.6%)	
>40	278 (56.0%)	184 (58.8%)	34 (57.6%)	60 (48.4%)	
ALT (U/L)					0.134
≤40	284 (57.3%)	174 (55.6%)	30 (50.8%)	80 (64.5%)	
>40	212 (42.7%)	139 (44.4%)	29 (49.2%)	44 (35.5%)	

**The chi-square test was used. ALBI, albumin–bilirubin; ECOG, Eastern Cooperative Oncology Group; PVTT, portal vein tumor thrombosis; BCLC, Barcelona Clinic Liver Cancer; AFP, alpha-fetoprotein; AST, aspartate transaminase; ALT, alanine transaminase.*

partial response (PR), or stable disease (SD) according to modified Response Evaluation Criteria in Solid Tumors (mRECIST) with a period no less than 1 year after initial TACE. Tumor response was assessed by two independent radiologists (__ and __) with more than 5 years of experience in diagnostic radiology through the PACS (NEUSOFTPACS/RIS, Shengyang Neusoft Co., Ltd., China). A third radiologist (___) made the final decision in case of disagreement.

Establishment of the RF Model

Variables identified as independently associated with the primary outcome by univariate and multivariate logistic analyses were introduced to establish the RF model. All data were randomly divided into a training set and a validation set with a 5:3 ratio. The RF model is trained by growing a large number of individual trees, and each tree is trained on a random-bootstrap sample from the original cohort (Hsich et al., 2011; Hu and Steingrimsson, 2018). Details on the theory of how the RF model was established have been reported previously (Ingrisch et al., 2018).

Statistical Analysis

Categorical variables are presented as frequencies and percentages, and continuous variables are presented as medians with 95% confidence intervals (CIs) or means with standard deviations. Variables with a *P* value no more than 0.20 in the univariate logistic analysis were considered strong

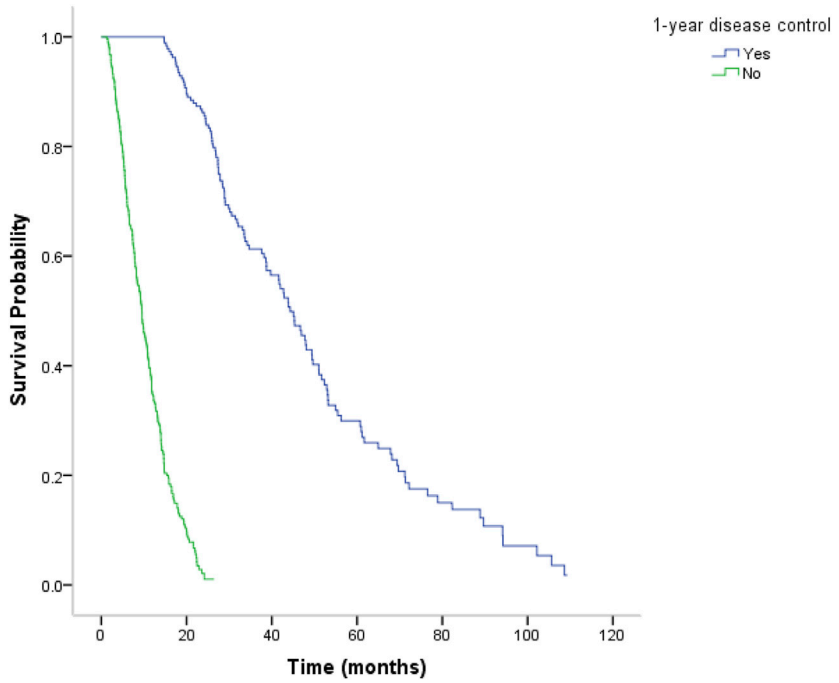

FIGURE 1 | Kaplan–Meier analysis of median overall survival (OS). The median OS was 44.3 months for patients with positive one-year disease control (CR/PR/SD) and was significantly longer for patients with negative one-year disease control (progression disease) (9.5 months) ($P < 0.001$).

risk factors associated with the primary outcome and were put into the multivariate logistic analysis. Variables with *P* values no more than 0.05 were considered independent risk factors associated with the primary outcome. The RF model was established based on the independent risk factors. The predictive performance of the RF model and the traditional logistic model was validated internally using the concordance c statistic (C-index). The Gini index was applied to describe the importance of the variables in the RF model associated with the primary outcome (Jain et al., 2018). Statistical analyses were performed using SPSS version 22.0 software for Windows (IBM Corporation, Somers, NY, United States), and the RF model was established in the R package "randomForest" (https://www.stat.berkeley.edu/~breiman/RandomForests/).

RESULTS

Patient Characteristics

The study included 496 patients (427 males, 69 females; mean age, 54 years; range, 21–81 years), with 313, 59, and 124 patients from institutions A, B, and C, respectively. The baseline characteristics of included patients are presented in **Table 1**. There were 186 (37.5%) patients who achieved CR/PR/SD at least 1 year after initial treatment. The median overall survival (OS) was 15.5 months, with that of 14.8, 25.9, and 14.8 ($p = 0.142$) months in institutions A, B, and C, respectively. The median OS was significantly longer for patients with positive one-year disease control (CR/PR/SD) compared to that of patients with negative one-year disease control (progression disease) (44.3 months vs. 9.5 months; $p < 0.001$) (**Figure 1**). No TACE or sorafenib treatment–related death occurred.

TABLE 2 | Univariate analysis of risk factors associated with one-year disease control.

Variables	HR	95% CI	B-values*	*p* value**
BCLC stage				<0.001
B	1			
C	5.712	3.590–9.087	1.742	
Maximum tumor size (cm)				<0.001
≤7	1			
>7	3.485	2.346–5.177	1.248	
AFP (ng/ml)				0.001
≤200	1			
>200	2.086	1.211–4.237	0.735	
ALBI grade				0.002
1	1			
2	2.024	1.296–3.162	0.705	
No. of lesions				0.003
1	1			
>1	1.744	1.208–2.518	0.556	
Lobes involved				0.006
Unilobar	1			
Bilobar	2.038	1.223–3.396	0.712	
Age (y)				0.110
≤55	1			
>55	0.689	0.437–1.089	−0.372	

**B-values are regression coefficients.*

***Univariate logistic regression analysis was used. BCLC, Barcelona Clinic Liver Cancer; AFP, alpha-fetoprotein; ALBI, albumin–bilirubin.*

TABLE 3 | Multivariate analysis of risk factors associated with one-year disease control.

Variables	HR	95% CI	B-values*	*p* value**
BCLC stage				
B	1			
C	5.657	3.544–9.029	1.733	<0.001
Maximum tumor size (cm)				
≤7	1			
>7	2.387	1.491–3.821	0.870	<0.001
AFP (ng/ml)				
≤200	1			
>200	2.106	1.345–3.297	0.745	0.001
ALBI grade				
1	1			
2	1.906	1.225–2.966	0.645	0.004
No. of lesions				
1	1			
>1	2.218	1.320–3.728	0.797	0.003
Lobes involved				
Unilobar	1			
Bilobar	1.786	1.059–3.014	0.580	0.030

**B-values are regression coefficients.*

***Multivariate logistic regression analysis was used. BCLC, Barcelona Clinic Liver Cancer; AFP, alpha-fetoprotein; ALBI, albumin–bilirubin.*

Strong and Independent Risk Factors Associated With One-Year Disease Control

After univariate logistic analysis using potentially significant variables, seven variables including the BCLC stage, tumor size (≤7 cm, >7 cm), alpha-fetoprotein level (≤200 ng/dl, >200 ng/dl), albumin–bilirubin (ALBI) grade, number of lesions (1, >1), number of lobes involved (unilobar, bilobar), and age (≤55 years, >55 years) were identified as strong risk factors associated with one-year disease control (**Table 2**). Multivariate logistic analysis using these seven strong risk factors was then performed, and six variables including the BCLC stage, tumor size (≤7 cm, >7 cm), alpha-fetoprotein level (≤200 ng/dl, >200 ng/dl), ALBI grade, number of lesions (1, >1), and number of lobes involved (unilobar, bilobar) were finally identified as independent risk factors associated with one-year disease control (**Table 3**).

Establishment of the RF Model and Importance of the Variables in the RF Model

The RF model was established based on the identified independent risk factors (**Figure 2**). The predictive performance of the trained RF model was better than that of the traditional logistic model, with the C-indexes of 0.724 and 0.709, respectively.

The importance of the variables in the RF model is illustrated in **Figure 2**. The BCLC stage showed the highest Gini index (20.0), following tumor size (≤7 cm, >7 cm; Gini index: 9.0), number of lobes involved (unilobar, bilobar; Gini index: 6.4), alpha-fetoprotein level (≤200 ng/dl, >200 ng/dl; Gini index: 6.1), ALBI grade (Gini index: 5.7), and number of lesions (1, >1; Gini index: 5.3).

DISCUSSION

By applying a machine learning approach, the random survival forest model, the present study demonstrated that the BCLC

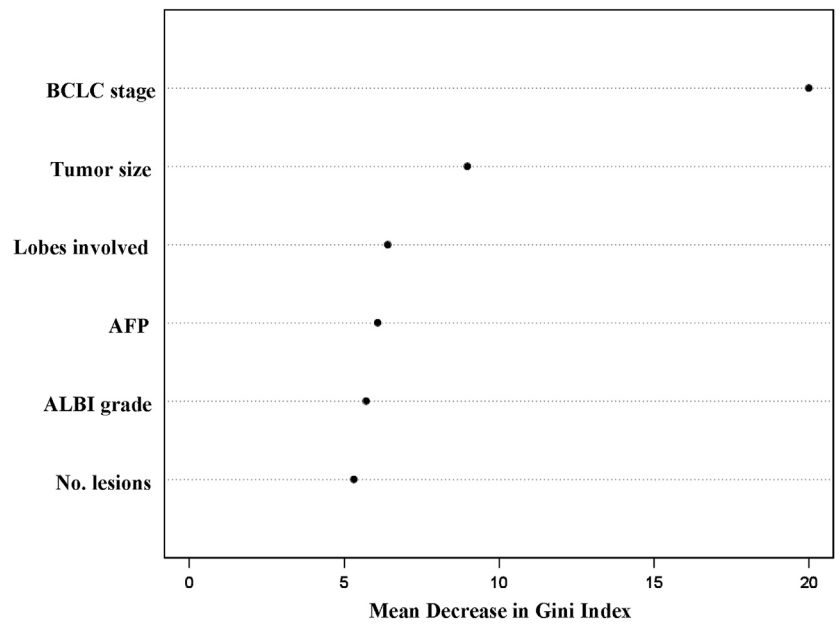

FIGURE 2 | Order of importance of the variables in the random survival forest model for one-year disease prediction. BCLC, Barcelona Clinic Liver *Cancer*; AFP, alpha-fetoprotein; ALBI, albumin–bilirubin.

stage, tumor size, alpha-fetoprotein level, ALBI grade, number of lesions, and number of lobes involved were independent risk factors associated with one-year disease control for unresectable HCC treated with TACE combined with sorafenib. The importance and predictive value of these independent risk factors were assessed and ranked based on the Gini index, with the BCLC stage and number of lesions showing highest and lowest importance and predictive values, respectively. According to the C-index, the predictive performance of the RF model was better than that of the traditional logistic model.

The study identified that the BCLC stage had highest importance and predictive value associated with one-year disease control. Combining TACE with sorafenib, which is the standard recommendation for advanced HCC, should achieve a synergetic effect ideally. Nevertheless, no randomized controlled trial (RCT) has been provided with positive results of this combination therapy for advanced HCC (Park et al., 2019). The only RCT comparing TACE combined with sorafenib vs. sorafenib monotherapy for advanced HCC, the STAH trial, demonstrated that there was no significant survival difference between TACE combined with sorafenib and sorafenib monotherapy for advanced HCC (median OS: 12.8 months vs. 10.8 months; $p = 0.290$) (Park et al., 2019). A relatively short period of sorafenib administration was observed (166 days) for advanced HCC treated with TACE combined with sorafenib in this trial.

Patients with HCC are heterogeneous regarding tumor burden, and previous studies have identified tumor burden as a robust risk factor associated not only with TACE monotherapy but also with TACE combined with sorafenib (Wang et al., 2019). Radiological response rates decrease as the tumor burden increases for patients treated with TACE (Kim et al., 2015). The present study demonstrated that tumor burden including the tumor size, number of lesions, and number of lobes involved were independent risk factors associated with the radiological response rate (one-year disease control) for patients treated with TACE combined with sorafenib.

This study applied the ALBI grade to assess the association between pre-treatment liver function and one-year disease control. The ALBI grade is based solely on two objective variables, which are serum albumin and bilirubin. The ALBI grade was first introduced by Johnson and colleagues in 2015, and it was then identified that its prognostic performance was at least no worse than that of the Child–Pugh grade for patients with HCC treated with various treatments (Johnson et al., 2015). Considering the objectivity and easy application, the ALBI grade is recommended as an alternative method for liver function assessment for HCC (Hiraoka et al., 2019). Patients with unresectable HCC are heterogeneous regarding liver function (Bolondi et al., 2012; Weinmann et al., 2015). The sorafenib administration period is shortened if deterioration of liver function occurs, even though a global real-world study demonstrated that sorafenib is safe and effective for HCC with different liver functions (Marrero et al., 2016). The present study demonstrated that low ALBI grade was an indicator of longer disease control for patients with unresectable HCC treated with TACE combined with sorafenib.

This study has several limitations. First, the retrospective nature of the study might cause selection bias of the included patients. Nevertheless, no significant difference regarding the baseline characteristics except for the age of the included patients between the three institutions was observed. Second, the median OS in institution B was much longer than that in institutions A and C. It might be mainly due to the relatively lower tumor burden of the patients in institution A compared to that in the other two institutions. Third, this study did not analyze independent risk factors associated with longer disease control such as two-year disease control. Fourth, due to the incomplete data, we were unable to collect and analyze the association between dose reduction of sorafenib and treatment outcome. Further work is encouraged to explore the association between dose reduction and prognosis for HCC treated with TACE combined with sorafenib. Fifth, due to the lack of the external validation cohort, the accuracy of the random survival model was just validated internally. Further work should be carried out to validate the accuracy of the random survival model in an independent external cohort. Finally, the study only included patients treated with TACE combined with sorafenib. It is better to include a control group for patients treated with TACE monotherapy to identify the optimal candidates to achieve longer disease control for unresectable HCC treated with TACE combined with sorafenib.

In conclusion, by applying a machine learning approach, the present study establishes a random survival forest model including the BCLC stage, tumor size, alpha-fetoprotein level, ALBI grade, number of lesions, and number of lobes involved to accurately predict one-year disease control for unresectable HCC treated with TACE combined with sorafenib. The predictive performance of the random survival forest model is better than that of the traditional logistic model.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, and further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

This study involving human participants was reviewed and approved by the institutional review boards at The First Affiliated Hospital of Soochow University, Zhongshan Hospital, Fudan University, and The First Affiliated Hospital, Zhejiang University School of Medicine. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors contributed to reviewing and critical revision of the manuscript and approved the final version of the

manuscript. C-FN, LW, X-LZ, and B-YZ contributed to the study concept and design, B-YZ, Z-PY, J-HS, LZ, and Z-HH contributed to acquisition of data, B-YZ and LW contributed to analysis and interpretation of data, B-YZ contributed to statistical analysis, and B-YZ, LW, and C-FN contributed to drafting of the manuscript. The corresponding authors had full access to all of the data and took full responsibility for the veracity of the data and the statistical analyses.

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Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios as Predictors of Outcomes in Patients With Unresectable Hepatocellular Carcinoma Undergoing Transarterial Chemoembolization Plus Sorafenib

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Objectives: To investigate the predictive value of inflammatory biomarkers in patients with unresectable hepatocellular carcinoma (HCC) for outcomes following the combination treatment of transarterial chemoembolization (TACE) plus sorafenib.

Materials and Methods: A total of 314 (270 male and 44 female) treatment-naïve patients with unresectable HCC treated by TACE plus sorafenib between January 2011 and December 2018 were enrolled in the retrospective study. The primary outcome was overall survival (OS). The secondary outcome was progression-free survival (PFS). Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were obtained within 3–7 days before the initial TACE and the median value of the NLR and PLR was considered as the cut-off value.

Results: The median value of NLR and PLR was 2.42 and 100, respectively. The median OS and PFS of the entire cohort were 18.7 months (95% CI: 16.8–20.6) and 9.1 months (95% CI: 8.5–9.8), respectively. The low NLR and PLR group showed improved OS and PFS compared with the high NLR and PLR group [21.8 months (95% CI: 15.2–28.5) vs. 15.4 months (95% CI: 12.4–18.3), $p < 0.0001$; 21.6 months (95% CI: 15.8–27.5) vs. 14.9 months (95% CI: 11.9–17.8), $p = 0.00027$, respectively]. In addition, the low NLR and PLR group also provided a longer PFS than the high NLR and PLR group [10.4 months

Abbreviations: AEs, adverse events; ALBI, albumin-bilirubin; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; CT, computed tomography; EASL, European Association for the Study of the Liver; ECOG, Eastern Cooperative Oncology Group; HCC, hepatocellular carcinoma; IRBs, Institutional Review Boards; mRECIST, modified response evaluation criteria in solid tumors; MRI, magnetic resonance imaging; OS, overall survival; PFS, progression-free survival; TACE, transarterial chemoembolization; VEGF, vascular endothelial growth factor.l.

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(95% CI: 8.9–12.0) vs. 8.1 months (95% CI: 7.1–9.2), $p = 0.00022$; 10.3 months (95% CI: 8.6–11.9) vs. 8.2 months (95% CI: 7.2–9.2), $p < 0.0001$, respectively]. High NLR and PLR at baseline were predictive factors of poor OS ($p = 0.02$ and $p = 0.004$) and PFS ($p = 0.045$ and $p = 0.005$).

Conclusion: This study showed the prognostic value of quantitative inflammatory biomarkers in correlation with OS and PFS in unresectable HCC patients undergoing TACE plus sorafenib treatment.

Keywords: transarterial chemoembolization, sorafenib, platelet count, neutrophils, lymphocytes

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary hepatic tumor and the third leading cause of cancer-related death worldwide (Forner et al., 2018; Kim and Shin, 2019). Transarterial chemoembolization (TACE) is the most widely applied treatment regimen not only in intermediate stage HCC recommended by guidelines but in advanced stage according to the BRIDGE study (Park et al., 2015; European Association for the Study of the Liver, 2018). However, the TACE-induced liver function deterioration and upregulation of vascular endothelial growth factor (VEGF) may lead to tumor recurrence and metastasis, which, in turn, provides unfavorable outcomes in unresectable HCC patients (Wang et al., 2008).

As an oral multikinase inhibitor, sorafenib has been shown to significantly offer clinical benefits in HCC patients with advanced stage (Cheng et al., 2009). Considering that sorafenib suppresses the surge of proangiogenic factors after the administration of TACE, the combination of TACE and sorafenib could have a synergistic effect and improve clinical prognosis in unresectable HCC patients (Pawlik et al., 2011). Although three trials conducted previously (post-TACE trial, SPACE trail, and TACE-2 trial) comparing clinical benefits in the combination therapy with that in TACE monotherapy did not demonstrate any compelling evidence by the addition of sorafenib to TACE, the TACTIS trial indicated that much longer duration of sorafenib administration which may prolong the progression-free survival (PFS) and provide the preservation of the liver function, which could eventually lead to prolongation of overall survival (OS) (Kudo et al., 2011; Lencioni et al., 2016; Meyer et al., 2017; Kudo et al., 2020). However, the high heterogeneity of unresectable HCC may result in heterogeneous therapeutic efficacy in patients treated by the combination of TACE plus sorafenib (Kudo, 2018). It is difficult to predict prognosis in individual patients. Therefore, development of effective biomarkers to identify subpopulations of patients who are most likely to benefit from such a combination therapy is needed (Zhang et al., 2020b). Previous studies showed that sorafenib-related adverse events were associated with favorable prognosis in HCC patients (ref.). Nevertheless, given the high treatment costs, drug-related toxicity, and the harm of ineffective treatment, cheap and readily available predictive biomarkers are of interest.

Concerning the inflammatory and immune environments that contribute to HCC formation and progression, the peripheral blood cells have been reported as a biomarker because neutrophils and high platelets suppress antitumoral immune cell function and induce upregulation of vascular endothelial growth (Zheng et al., 2017a; Schobert et al., 2020). Previous studies showed that high neutrophil-to-lymphocyte (NLR) and platelet-to-lymphocyte (PLR) values are associated with worse OS, which could have the potential to serve as quantitative biomarkers for individual prognosis prediction of HCC patients treated with TACE or sorafenib alone (Chon et al., 2019; He et al., 2019; Hong et al., 2019; Wang et al., 2020). Nevertheless, the same prognostic value of the biomarkers for unresectable HCC patients undergoing the combination therapy of TACE and sorafenib is not well studied. Consequently, the aim of this study was to investigate the effectiveness of the prognostic roles of NLR and PLR in unresectable HCC patients receiving TACE plus sorafenib treatment.

MATERIALS AND METHODS

Patients

This retrospective study was approved by the Institutional Review Board (IRB) at two institutions. All clinical practices and observations were conducted in accordance with the Declaration of Helsinki. The requirement to obtain informed consent was waived due to the retrospective nature. The dual-center (The First Affiliated Hospital of Soochow University and Zhongshan Hospital) study consisted of consecutive treatment-naïve unresectable HCC patients treated with the combination therapy of TACE and sorafenib between January 2011 and December 2018. HCC was diagnosed by pathologic assessment or noninvasive criteria according to the European Association for the Study of the Liver (EASL) guidelines (European Association for the Study of the Liver, 2018). Each treatment decision was assessed by a multidisciplinary consensus, including interventional radiologists, oncologists, liver surgeons, and hepatologists.

The inclusion criteria for this study were as follows: 1) patients ≥18 years old; 2) Eastern Cooperative Oncology Group performance scores ≤1; 3) no prior HCC-related treatment including resection, local ablation, systemic therapy, or TACE; 4) Child-Pugh (CP) liver function stage of A to B7; 5) aspartate aminotransferase (AST) or alanine aminotransferase (ALT) < 5 * upper limit of normal (ULN), serum creatinine <1.5 * ULN, and bilirubin level< 3 mg/dl; and 6) adequate hematologic and

clotting function. The exclusion criteria were as follows: 1) comorbidity with other primary malignancies; 3) prior chemotherapy, radiotherapy, or molecular-targeted HCC therapy; 4) contraindications for embolization or sorafenib; and 5) the administration of sorafenib discontinued more than one month.

Treatment Protocol

All the patients included in this study were treated with the conventional TACE procedure according to institutional standards, and all the TACE procedures were performed by six board-certified interventional radiologists with more than 8 years of experience. Selective or superselective catheterization of the tumor-feeding arteries was introduced with a 2.7 F microcatheter (Progreat; Terumo, Japan) after the diagnostic angiography of the celiac trunk and superior mesenteric artery with a 5 F catheter. Patients received the intra-arterial injection of an emulsion of doxorubicin (10–50 mg) and oxaliplatin (100–200 mg) in ethiodized oil (2–20 ml, Lipiodol Ultra-Fluide; Laboratoire Guerbet, Roissy Charles de Gaulle, France) followed by Gelfoam (Ailikang Inc., Hangzhou, China) particles (350–560 μm) under fluoroscopic guidance until arterial inflow was substantially reduced. After 5 min, another angiography was obtained from the common hepatic artery to verify no residual tumor enhancement. According to the "on-demand" basis in the setting of detecting new or residual tumor tissue (i.e., incomplete necrosis) on follow-up imaging, chemoembolization was repeated.

The initial administration of sorafenib (400 mg, twice daily) was within 1 week after the initial and on-demand chemoembolization. The dose of sorafenib was adjusted (400 mg/day, 400 mg every other day) for drug-related adverse events (AEs), which were based on the Common Terminology Criteria of Adverse Events (CTCAE) version 5.0. The treatment was discontinued when the patients had untreatable progression and unacceptable toxicity.

Outcomes and Follow-Up

All the clinical and radiological data were retrieved from the electronic medical record from the two institutions. NLR was calculated as absolute neutrophil count divided by absolute lymphocyte count measured in the peripheral blood before the initial TACE treatment. PLR was calculated by division of absolute thrombocytes and lymphocytes accordingly. Baseline characteristics, including blood routine examination and biochemical analysis, were obtained 3–7 days before the initial chemoembolization and post-TACE hospitalization and every month outpatient clinical follow-up. The status of patients (alive or dead) was recorded on the medical records or inquired by phone from the family member. Multiphase Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) was performed 1 week before and between 1 and 2 months after the TACE procedure. All CT scans were with 64 or more row systems and all MRI scans were 3 T unit. The evaluation of radiological response was carried out by two radiologists with abdominal imaging experience of more than 5 years. Both of them were blinded to the treatment regimen and patient information. The response of the combination treatment was classified based on the modified response evaluation criteria in solid tumors (mRECIST) (Llovet and Lencioni, 2020).

The primary outcome measurement of this study was OS. OS was defined as the duration of time from the initial TACE treatment to the date of death or the last follow-up (July 31, 2020). The second outcome measurement was PFS, which was defined as the time from the initial chemoembolization to death or radiological progression. Patients who were alive and without progression were censored at the last follow-up period.

Statistical Analysis

Continuous variables and categorical variables were presented as median (interquartile range) and frequencies (percentages), respectively. The median value of the NLR, PLR, and aspartate transaminase (AST)/alanine transaminase (ALT) ratio was considered as the cut-off value. The cut-off values of age, AST, ALT, tumor size, number of nodules, alpha-fetoprotein (AFP), and bilirubin were based on previous studies (Song et al., 2012; Lee et al., 2019; Zhong et al., 2019). The differences of the baseline characteristics were compared between the high and low NLR/PLR groups using the Mann–Whitney *U* test or Fisher's exact test. OS and PFS were plotted using the Kaplan–Meier method and were compared using the log-rank test. Univariate Cox's proportional hazards regression model analysis was performed to determine the factors associated with OS and PFS. Multivariable analysis was carried out on variables that reached $p < 0.05$ at univariable analysis. Considering that NLR and PLR both take into account lymphocyte count, two separate models for NLR (model 1)/PLR (model 2) were developed for the multivariable analyses. The predictive value of NLR and PLR was also assessed by calculating the area under the curve (AUC) from receiver operating characteristic (ROC) curves. The significance level of 5% was used to determine statistical significance. All statistical analyses were performed using the SPSS (version 25.0, IBM Corp.) and R software version 3.2.2 (http://www.r-project.org).

RESULTS

Patient Characteristics

A total of 314 treatment-naïve unresectable HCC patients treated with chemoembolization plus sorafenib were enrolled in this study. Of the included patients, there were 270 (86.0%) males and 44 (14.0%) females with a median age of 55 (range, 26–81) years in the entire cohort. Hepatitis B virus (HBV) (85.7%) was the predominant etiology of liver disease. The number of patients with portal vein invasion and hepatic vein invasion was 106 (33.8%) and 37 (11.8%), respectively. Nearly all patients had a CP A liver function and good performance (ECOG 0). Barcelona Clinic Liver Cancer (BCLC) stage B patients constituted 54.1% of the entire cohort. The median values of NLR and PLR were 2.42 and 100, respectively. The difference in the baseline characteristics of the two NLR/PLR groups was presented in **Table 1**. Compared with the patients in the high NLR group,

TABLE 1 | Comparison of the clinic-laboratory data and demographic features between patients with 1) low NLR and high NLR and 2) low and high PLR.

Characteristic	Overall (*n* = 314)	NLR < 2.42 (*n* = 159)	NLR ≥ 2.42 (*n* = 155)	*p* value	PLR < 100 (*n* = 157)	PLR ≥ 100 (*n* = 157)	*p* value
Gender				0.927			0.034
Male	270 (86.0)	137 (86.2)	133 (85.8)		142 (90.5)	128 (81.5)	
Female	44 (14.0)	22 (13.8)	22 (14.2)		15 (9.5)	29 (18.45)	
Age (years)				0.431			0.366
≤55	165 (52.6)	80 (50.3)	85 (54.8)		87 (55.4)	78 (49.7)	
>55	149 (47.4)	79 (49.7)	70 (45.2)		70 (44.6)	79 (50.3)	
ECOG				0.151			1.000
0	312 (99.4)	159 (100)	153 (98.7)		156 (99.4)	156 (99.4)	
1	2 (0.6)	0 (0)	2 (1.3)		1 (0.6)	1 (0.6)	
Etiology				0.422			0.107
HBV	269 (85.7)	139 (87.4)	130 (83.9)		140 (89.2)	129 (82.2)	
Other	45 (14.3)	20 (12.6)	25 (16.1)		17 (10.8)	28 (17.8)	
Cirrhosis				0.715			0.713
	218 (69.4)	112 (70.4)	106 (68.4)		111 (70.7)	107 (68.2)	
Tumor size (cm)				0.496			0.256
≤5	175 (55.7)	92 (57.9)	83 (53.6)		93 (59.2)	82 (52.2)	
>5	139 (44.3)	67 (42.1)	72 (46.4)		64 (40.8)	75 (47.8)	
No. of nodules				0.501			1.000
<3	160 (51.0)	78 (49.1)	82 (52.9)		80 (51.0)	80 (51.0)	
≥3	154 (49.0)	81 (50.9)	73 (47.1)		77 (49.0)	77 (49.0)	
PVTT				0.001			0.042
	106 (33.8)	39 (24.5)	67 (43.2)		44 (28.0)	62 (39.5)	
Hepatic vein invasion				0.116			0.161
	37 (11.8)	14 (8.8)	23 (14.8)		14 (8.9)	23 (14.7)	
Child-Pugh class				0.980			1.000
A	310 (98.7)	157 (98.7)	153 (98.7)		155 (98.7)	155 (98.7)	
B	4 (1.3)	2 (1.3)	2 (1.3)		2 (1.3)	2 (1.3)	
BCLC stage				<0.001			0.001
B	170 (54.1)	107 (67.3)	63 (40.7)		100 (63.7)	70 (44.6)	
C	144 (45.9)	52 (32.7)	92 (59.3)		57 (36.3)	87 (55.4)	
ALBI grade				0.046			0.191
1	170 (54.1)	97 (61.0)	73 (47.1)		93 (59.2)	77 (49.0)	
2	142 (45.2)	61 (38.4)	81 (52.3)		63 (40.1)	79 (50.3)	
3	2 (0.7)	1 (0.6)	1 (0.6)		1 (0.7)	1 (0.7)	
Tumor distribution				0.282			0.905
Unilobar	210 (66.9)	111 (69.8)	99 (63.9)		106 (67.5)	104 (66.2)	
Bilobar	104 (33.1)	48 (30.2)	56 (36.1)		51 (32.5)	53 (33.8)	
Extrahepatic spread (PVTT excluded)	32 (10.2)	8 (5.0)	24 (15.5)	0.003	10 (6.4)	22 (14.0)	0.039
AFP (ng/dl)				0.070			0.024
≤200	167 (53.2)	93 (58.5)	74 (47.7)		94 (59.9)	73 (45.5)	
>200	147 (46.8)	66 (41.5)	81 (52.3)		63 (40.1)	84 (54.5)	
AST (U/L)				0.003			0.054
≤40	144 (45.9)	86 (54.1)	58 (37.4)		81 (51.6)	63 (40.1)	
>40	170 (54.1)	73 (45.9)	97 (62.6)		76 (48.4)	94 (59.9)	
ALT (U/L)				0.909			0.087
≤40	180 (57.3)	92 (57.9)	88 (56.8)		82 (52.2)	98 (62.4)	
>40	134 (42.7)	67 (42.1)	67 (42.2)		75 (47.7)	59 (37.6)	
Albumin (g/L)				0.595			0.894
≤35	74 (23.6)	35 (22.0)	39 (25.2)		38 (24.2)	36 (22.9)	
>35	240 (76.4)	124 (78.0)	116 (74.8)		119 (75.8)	121 (77.1)	
TBIL (umol/L)				0.107			0.227
≤17.1	242 (77.1)	129 (81.1)	113 (72.9)		116 (73.9)	126 (80.2)	
>17.1	72 (22.9)	30 (18.9)	42 (27.1)		41 (26.1)	31 (19.8)	
AST/ALT				0.178			0.005
≤1.18	156 (49.7)	85 (53.5)	71 (45.8)		91 (58.0)	65 (41.4)	
>1.18	158 (50.3)	74 (46.5)	84 (54.2)		66 (42.0)	92 (58.6)	

AFP, alpha-fetoprotein; ALBI, albumin-bilirubin; ALT, alanine transaminase; AST, aspartate transaminase; BCLC, Barcelona Clinic Liver Cancer; ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PVTT, portal vein tumor thrombus; TBIL, total bilirubin.

those with low NLR had a less advanced-stage disease (BCLC stage, portal vein invasion, and extrahepatic spread), better preserved liver function (albumin-bilirubin, ALBI), and lower value of AST. Additionally, patients in the low PLR group were associated with more males, less advanced-stage disease, lower value of AFP, and lower AST/ALT ratio.

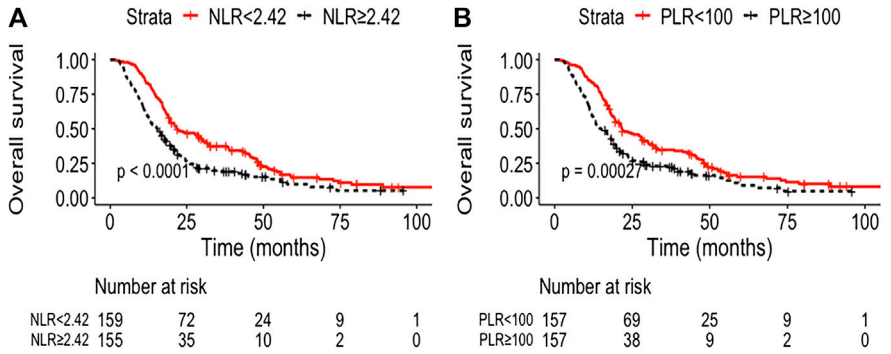

FIGURE 1 | Kaplan–Meier curves of different group analyses of overall survival (OS) according to patients' NLR and PLR level. **(A)** Patients with low NLR vs. high NLR at baseline; **(B)** patients with low PLR vs. high PLR at baseline.

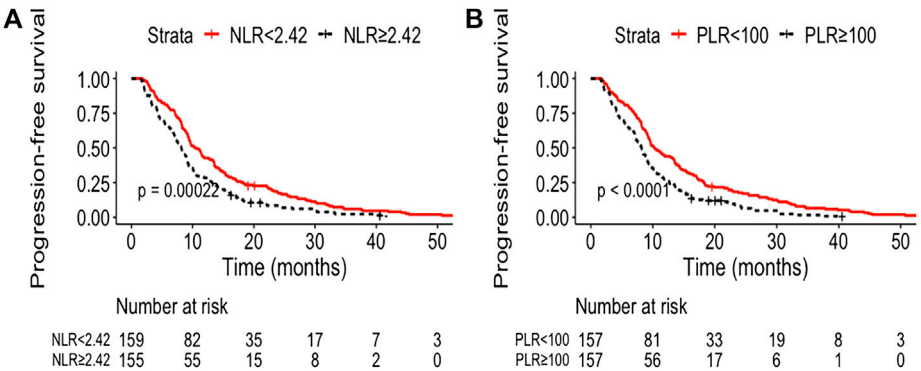

FIGURE 2 | Kaplan–Meier curves of different group analyses of progression-free survival (PFS) according to patients' NLR and PLR level. **(A)** Patients with low NLR vs. high NLR at baseline; **(B)** patients with low PLR vs. high PLR at baseline.

Outcomes

The median follow-up was 21.0 months (95% CI, 19.7–22.4). The median OS and PFS of the entire cohort were 18.7 months (95% CI: 16.8–20.6) and 9.1 months (95% CI: 8.5–9.8), respectively. The low NLR and PLR group showed improved OS compared with the high NLR and PLR group [21.8 months (95% CI: 15.2–28.5) vs. 15.4 months (95% CI: 12.4–18.3), $p < 0.0001$; 21.6 months (95% CI: 15.8–27.5) vs. 14.9 months (95% CI: 11.9–17.8), $p = 0.00027$, respectively] (**Figure 1**). The 1- and 3-year OS rate of the low and high NLR/PLR group was 83.0% vs. 59.4% and 36.6% vs. 18.9% and 83.4% vs. 59.2% and 34.0% vs. 22.2%, respectively. In addition, the low NLR and PLR group also provided a longer PFS than the high NLR and PLR group [10.4 months (95% CI: 8.9–12.0) vs. 8.1 months (95% CI: 7.1–9.2), $p = 0.00022$; 10.3 months (95% CI: 8.6–11.9) vs. 8.2 months (95% CI: 7.2–9.2), $p < 0.0001$, respectively] (**Figure 2**). The 1-year PFS rate of the low and high NLR/PLR group was 42.8% vs. 28.4% and 43.9% vs. 27.4%, respectively. The 1-year AUC of NLR and PLR was 0.684 and 0.681, respectively. The cut-off values of NLR and PLR correspond to sensitivity values of 54.8% and 53.9%, and specificity values of 45.2% and 42.8%, respectively (**Figure 3A**). The 3-year AUC of NLR and PLR was 0.621 and 0.581, respectively. The NLR and PLR correspond to sensitivity values of 45.5% and 46.1%, and specificity values of 42.8% and 55.4%, respectively (**Figure 3B**).

Prognostic Factors

In the univariate survival analysis, tumor size, portal vein invasion, extrahepatic spread, ALBI, AFP, AST, albumin, NLR, PLR and AST/ALT ratio were associated with OS ($p < 0.05$ for all) (**Table 2**). Multivariable Cox regression analysis indicated that in model 1, high NLR, tumor size >5 cm, portal vein invasion, and extrahepatic spread were independent factors associated with poor OS, whereas model 2 showed high PLR, portal vein invasion, AST >40 U/L, and extrahepatic spread as prognostic for poorer OS in patients receiving chemoembolization plus sorafenib (**Table 3**).

The univariate analysis identified tumor size, portal vein invasion, hepatic vein invasion, extrahepatic spread, AFP, AST, albumin, NLR, and PLR as potential prognostic factors for PFS (**Table 4**). The multivariable analysis using model 1 identified tumor size >5 cm, AST >40 U/L, extrahepatic spread, and high NLR as prognostic for shorter PFS, and the model showed similar results with tumor size >5 cm, AST >40 U/L, and extrahepatic spread as prognostic for poorer PFS apart from high PLR (**Table 5**).

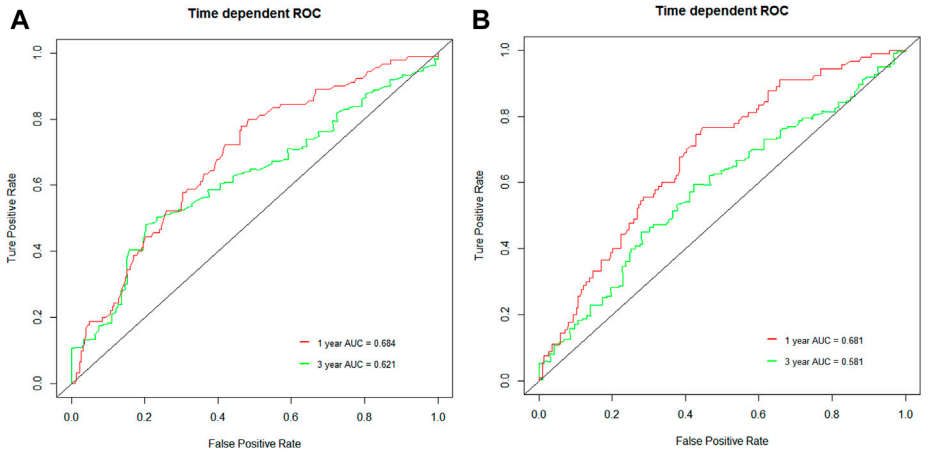

FIGURE 3 | ROC curves of the NLR and PLR in unresectable HCC patients. **(A)** 1-year and 3-year ROC curve of NLR; **(B)** 1-year and 3-year ROC curve of PLR.

TABLE 2 | Univariate analysis of risk factors associated with overall survival.

Characteristic		HR	95% CI	*p* value
Gender	F/M	0.883	0.618–1.261	0.493
Age (years)	>55/≤55	0.813	0.636–1.040	0.099
ECOG	1/0	0.370	0.052–2.655	0.323
Etiology	HBV/others	0.917	0.652–1.290	0.619
Cirrhosis	Yes/no	0.928	0.713–1.209	0.582
Tumor size (cm)	>5/≤5	1.750	1.362–2.249	<0.001
No. of nodules	≥3/<3	1.227	0.961–1.567	0.101
PVTT	Yes/no	1.864	1.429–2.432	<0.001
Hepatic vein invasion	Yes/no	1.283	0.868–1.895	0.211
Child-Pugh class	B/A	1.597	0.593–4.297	0.354
ALBI grade	3/2/1	1.389	1.095–1.762	0.007
Tumor distribution	Bilobar/unilobar	1.146	0.887–1.479	0.297
Extrahepatic spread	Yes/no	2.461	1.673–3.621	<0.001
AFP (ng/dl)	>200/≤200	1.418	1.107–1.817	0.006
AST (U/L)	>40/≤40	1.479	1.155–1.894	0.002
ALT (U/L)	>40/≤40	1.129	0.883–1.443	0.334
Albumin (g/L)	>35/≤35	0.698	0.526–0.926	0.013
TBIL (umol/L)	>17.1/≤17.1	1.089	0.754–1.573	0.648
NLR	≥2.42/<2.42	1.647	1.287–2.109	<0.001
PLR	≥100/<100	1.576	1.231–2.018	<0.001
AST/ALT	>1.18/≤1.18	1.328	1.037–1.701	0.025

AFP, alpha-fetoprotein; ALBI, albumin-bilirubin; ALT, alanine transaminase; AST, aspartate transaminase; ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PVTT, portal vein tumor thrombus; TBIL, total bilirubin; HR, hazard ratio; CI, confidence interval.

DISCUSSION

This study demonstrated that hematological parameters (NLR or PLR) at baseline could predict outcomes of unresectable HCC patients undergoing the combination therapy of chemoembolization plus sorafenib. Patients with NLR <2.42 or PLR <100 had an improved OS or PFS than that in the high NLR or PLR group. Two models, which separately included NLR and PLR, both showed NLR and PLR were strong prognostic factors, indicating that NLR or PLR was useful to differentiate target patients and stratify risk.

More recently, the first-ever positive TACTIS trial showed the superiority of TACE in combination of sorafenib over TACE alone in terms of clinical outcomes, including PFS (25.2 vs. 13.5 months; HR = 0.59; 95% CI: 0.41–0.87; $p = 0.006$) and 1- and 2-year survival rates (96.2% vs. 82.7%; 77.2% vs. 64.6%, respectively), indicating that unresectable HCC patients could benefit from the combination therapy (Kudo et al., 2020). The better outcomes provided by this trial may due to the normalization of feeding arteries, leading to enhancement of TACE efficacy through dense accumulation of embolization agents (i.e., lipiodol mixed with chemotherapeutic drugs followed by Gelfoam). The liver function preservation caused by less TACE repetition and a much longer duration of sorafenib administration could be the other possible explanations (Kudo et al., 2020). However, considering that the biological heterogeneity of unresectable HCC and the tumor microenvironment could hamper treatment efficacy, resulting in heterogeneous prognosis in individuals, new biomarkers are warranted in order to evaluate the impact of the individual immune system activity on tumor progression and susceptibility to the combination therapy of chemoembolization plus sorafenib (Schobert et al., 2020).

As measured in peripheral blood samples, NLR and PLR were considered as indirect markers of systemic inflammatory response and have been evaluated as predictors of recurrence and survival in various malignancies (Templeton et al., 2014; Zheng et al., 2017b). Several meta-analysis studies suggested that high NLR and PLR are associated with an adverse OS in HCC patients undergoing liver transplantation or hepatectomy (Lin et al., 2018; Wang et al., 2018). More recently, a meta-analysis including 5280 HCC patients treated by TACE reported that evaluated NLR and PLR at baseline were significantly correlated with poor OS (HR: 1.81, 95% CI: 1.66–1.97, $p < 0.00001$; HR: 1.56, 95% CI: 1.13–2.16, $p = 0.007$, respectively) (Li et al., 2020). In addition, another meta-analysis indicated that HCC patients with lower NLR at baseline could have a better response to sorafenib than those with higher pretreatment NLR (HR = 1.76, 95% CI: 1.44–2.15, $p < 0.00001$) (Liu et al., 2019). As shown in the present

TABLE 3 | Multivariate Cox proportional hazards regression analysis of NLR and PLR with overall survival.

Characteristic		B	SE B	Wald	HR	95% CI	*p* value
NLR							
Tumor size (cm)	>5/≤5	0.344	0.136	6.434	1.410	1.081–1.840	0.011
PVTT	Yes/no	0.466	0.141	10.950	1.593	1.209–2.099	0.001
Extrahepatic spread	Yes/no	0.708	0.202	12.229	2.029	1.365–3.017	<0.001
NLR	≥2.42/<2.42	0.305	0.131	5.4333	1.356	1.050–1.753	0.020
PLR							
PVTT	Yes/no	0.487	0.140	12.133	1.627	1.237–2.139	<0.001
Extrahepatic spread	Yes/no	0.915	0.197	21.544	2.497	1.697–3.674	<0.001
AST	>40/≤40	0.298	0.129	5.368	1.348	1.047–1.735	0.021
PLR	≥100/<100	0.366	0.128	8.120	1.442	1.121–1.855	0.004

AST, aspartate transaminase; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PVTT, portal vein tumor thrombus; SE, standard error; CI, confidence interval; HR, hazard ratio.

TABLE 4 | Univariate analysis of risk factors associated with progression-free survival.

Characteristic		HR	95% CI	*p* value
Gender	F/M	1.030	0.743–1.428	0.858
Age (years)	>55/≤55	0.911	0.728–1.140	0.415
ECOG	1/0	0.562	0.139–2.273	0.419
Etiology	HBV/others	1.030	0.745–1.423	0.859
Cirrhosis	Yes/no	0.935	0.734–1.193	0.591
Tumor size (cm)	>5/≤5	1.588	1.262–1.997	<0.001
No. of nodules	≥3/<3	1.020	0.813–1.280	0.865
PVTT	Yes/no	1.576	1.234–2.014	<0.001
Hepatic vein invasion	Yes/no	1.477	1.043–2.093	0.028
Child-Pugh class	B/A	1.313	0.488–3.529	0.589
ALBI grade	3/2/1	1.169	0.941–1.452	0.158
Tumor distribution	Bilobar/unilobar	0.915	0.719–1.165	0.472
Extrahepatic spread	Yes/no	2.091	1.442–3.031	<0.001
AFP (ng/dl)	>200/≤200	1.321	1.053–1.656	0.016
AST (U/L)	>40/≤40	1.545	1.229–1.942	<0.001
ALT (U/L)	>40/≤40	1.033	0.822–1.297	0.783
Albumin (g/L)	>35/≤35	0.751	0.575–0.981	0.036
TBIL (umol/L)	>17.1/≤17.1	1.079	0.778–1.499	0.648
NLR	≥2.42/<2.42	1.533	1.220–1.926	<0.001
PLR	≥100/<100	1.599	1.270–2.013	<0.001
AST/ALT	>1.18/≤1.18	1.424	1.135–1.787	0.002

AFP, alpha-fetoprotein; ALBI, albumin-bilirubin; ALT, alanine transaminase; AST, aspartate transaminase; ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PVTT, portal vein tumor thrombus; TBIL, total bilirubin; HR, hazard ratio; CI, confidence interval.

study, lower NLR and PLR were significant predictive factors for better survival in the unresectable HCC patients treated with TACE plus sorafenib (HR = 1.36, 95% CI: 1.05–1.75, $p = 0.02$; HR = 1.44, 95% CI: 1.12–1.86, $p = 0.004$, respectively). The similar prognostic values of NLR and PLR in patients undergoing the combination therapy may result from the following reasons. First, given HBV induces chronic inflammation and immune modulation, patients with either a higher adaptive immune infiltrate (lymphocytes) or lower innate immune infiltrate (neutrophils or platelet) may have a better response to sorafenib. Additionally, previous evidence showed that the immune system and tumor microenvironment could be affected by sorafenib with enhancing T-cell activation and blocking T-cell regulatory function (Bruix et al., 2017). Second, although TACE-induced tumor hypoxia may have an impact on immune cell activity, TACE has the potential to affect the immune system in a positive way by exposing tumor antigens to the immune system (Xue et al., 2015). A recent experimental study illustrated that the specialized subset of T helper lymphocytes (Th17) and its signature cytokine IL-17 were increased after the embolization treatment (Avritscher et al., 2020). Tampaki et al. reported the sTIM-3 level in plasma significantly increased after TACE due to the upregulation, whereas patients with better response had higher posttreatment values (Tampaki et al., 2020).

TABLE 5 | Multivariate Cox proportional hazards regression analysis of NLR and PLR with progression-free survival.

Characteristic		B	SE B	Wald	HR	95% CI	*p* value
NLR							
Tumor size (cm)	>5/≤5	0.303	0.123	6.091	1.354	1.064–1.722	0.014
AST (U/L)	>40/≤40	0.378	0.121	9.712	1.459	1.151–1.850	0.002
Extrahepatic spread	Yes/no	0.378	0.121	9.712	1.459	1.151–1.850	0.002
NLR	≥2.42/<2.42	0.246	0.123	4.013	1.278	1.005–1.626	0.045
PLR							
Tumor size (cm)	>5/≤5	0.271	0.123	4.865	1.312	1.031–1.669	0.027
AST (U/L)	>40/≤40	0.390	0.120	10.643	1.477	1.169–1.868	0.001
Extrahepatic spread	Yes/no	0.735	0.193	14.500	2.086	1.429–3.045	<0.001
PLR	≥100/<100	0.345	0.122	8.063	1.412	1.113–1.792	0.005

AST, aspartate transaminase; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SE, standard error; CI, confidence interval; HR, hazard ratio.

In the present study, the OS and PFS curve showed a cut-off value of median NLR and PLR of 2.42 and 100, which significantly patients' OS and PFS stratified based on NLR and PLR ($p < 0.001$ for all). A recent meta-analysis indicated that 3 was the minimum cut-off value for NLR to play a prognostic value (Liu et al., 2019). However, different ethnic populations and the heterogeneity of the enrolled studies had a greater impact on the consistency of the results. Another study conducted by Wang et al. suggested that baseline NLR >2.4 was an independent prognostic factor of poor OS, which was similar to this study. For the cut-off value of PLR, it was also similar to a previous study which illustrated that lower preoperative PLR (≤100) can predict longer disease-free survival (DFS) and OS for HCC patients undergoing TACE plus radiofrequency ablation (RFA) (Long et al., 2020). Moreover, more patients with less advanced stage were shown in both low NLR and PLR groups in this study; previous evidence also showed the incidence of high pretreatment NLR had a significant association with the presence of portal vein invasion (Li et al., 2020).

It should be noted that there are some limitations in this study. First, select bias may exist in this study due to the retrospective nature. Second, the target population of this study included patients with portal vein invasion, which was a relative contraindication of TACE. However, previous studies showed that advanced HCC patients treated with chemoembolization plus sorafenib could have favorable outcomes compared to those treated with sorafenib monotherapy (Zhang et al., 2020a). Last but not least, this study did not investigate the prognostic value and the other inflammatory and immune biomarkers. Well-designed prospective studies are warranted to evaluate the meaning of the other biomarkers and also conduct a clinically meaningful cut-off value.

In conclusion, this study reported the feasibility and validated two prognostic biomarkers (NLR or PLR) for unresectable HCC patients following the combination therapy of chemoembolization plus sorafenib and indicated that unresectable HCC patients with lower NLR/PLR may have a more favorable outcome than those with high ones. These biomarkers could be easily implemented in routine practice, which may be explored as a paradigm for physicians in the treatment decision.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The First Affiliated Hospital of Soochow University and Zhongshan Hospital, Fudan University. The ethics committee waived the requirement of written informed consent for participation. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors contributed to review and critical revision of the manuscript and approved the final version of the manuscript. QL, C-FN, X-LZ, LZ, Z-PY, Z-HHou, and PH contributed to the study concept and design. LZ, Z-HH, PH, SZ, and M-JY contributed to acquisition of data. LZ, Z-HH, and PH contributed to analysis and interpretation of data. LZ, Z-HH, PH, and S-HZ contributed to statistical analysis. LZ, Z-PY, Z-HH, and PH contributed to drafting of the manuscript. The corresponding author had full access to all of the data and took full responsibility for the veracity of the data and the statistical analyses.

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Corrigendum: Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios as Predictors of Outcomes in Patients With Unresectable Hepatocellular Carcinoma Undergoing Transarterial Chemoembolization Plus Sorafenib

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A Corrigendum on

Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios as Predictors of Outcomes in Patients With Unresectable Hepatocellular Carcinoma Undergoing Transarterial Chemoembolization Plus Sorafenib

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In the published article, there was an error regarding the affiliations for Lei Zhang and Cai-Fang Ni. One of the affiliations was added in error. This has now been removed.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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Efficacy and Safety of Transarterial Chemoembolization in Elderly Patients of Advanced Hepatocellular Carcinoma With Portal Vein Tumor Thrombus: A Retrospective Study

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Objective: The aim of the current study was to evaluate the safety and efficacy of transcatheter arterial chemoembolization (TACE) in elderly patients diagnosed as advanced hepatocellular carcinoma (HCC) accompanied with different types of portal vein tumor thrombosis (PVTT).

Methods: Elderly HCC patients aged 70-year-old and above from January 2015 to December 2019 were included in this retrospective study. Efficacy data including OS, PFS, DCR, and ORR and safety data were collected in the indicated groups. Outcomes of HCC patients in the TACE group were compared with those patients in the best supportive care (BSC) group. Subgroup analyses were also conducted in the patients with different types of PVTT.

Results: Among 245 elderly HCC patients, 124 were enrolled in this study. Out of these, 50.0% (n=62) underwent BSC treatment while 50.0% (n=62) underwent TACE. There were no major differences in the baseline characteristics of the two treatment groups. TACE treatment was associated with better median OS compared with BSC alone (11.30 m *vs.* 7.80 m; $P<0.001$). Subgroup analyses showed that patients with type I and type II PVTT could benefit from TACE compared with BSC, based on that OS was 14.30 m *vs.* 7.80 m ($P=0.007$) and 13.00 m *vs.* 8.00 m ($P=0.002$), respectively. The DCR in the TACE group was 62.90%, and 17.74% in the BSC group (p<0.001). The proportion of ORR in TACE group was 35.48%, while 0.00% in the BSC group (p<0.001). Multivariable analyses showed that patients undergoing TACE treatment had 52% lower odds of mortality compared with patients undergoing BSC treatment (HR: 0.48; 95%CI: 0.32-0.72). Similarly, the media PFS was improved following TACE treatment (7.50 m *vs.* 4.00 m; $P<0.001$). TACE could significantly prolong the PFS in both type I and type II PVTT subgroups, without greatly significant improvement in type III PVTT patients (4.50 m *vs.* 2.70 m; $P=0.103$). Type III PVTT patients in the TACE group had more AEs than type I and type II PVTT patients. According to multivariable analyses, PVTT types

(type III *vs.* type I-II) (HR: 2.18; 95%CI: 1.29-3.70; *P*=0.004), tumor diameter (>5 cm *vs.* ≤5 cm) (HR: 1.94; 95%CI: 1.28-2.93; *P*=0.002), and treatment (TACE *vs.* BSC) (HR: 0.48; 95%CI: 0.32-0.72; *P*<0.001) were independent indicators of overall survival.

Conclusions: In elderly advanced HCC patients with PVTT, palliative TACE treatment can be an accessible effective measure to improve the OS and PFS for both type I and type II PVTT patients.

Keywords: TACE, elderly patients, advanced HCC, PVTT, adverse event

INTRODUCTION

Hepatocellular malignancy is one of the prominent causes of death worldwide. According to the data of GLOBOCAN statistics in 2018, the primary liver cancer ranks sixth among malignant tumors with worldwide new cases of 841,080, and the mortality ranks second with 781,631 liver cancer-related deaths (1). Primary liver cancer is the fourth most common malignancy and the second leading cause of cancer-related death in China. Of note, hepatocellular carcinoma (HCC) is responsible for 85-90% of primary liver cancer. In the past few decades, there has been a tremendous increment in elderly patients not only in China but throughout the world. As life expectancy increases, the management of elderly HCC patients has become a global problem. It is well recognized that the majority of HCC patients are often diagnosed at a late stage (stages B or C) according to the Barcelona Clinic Liver Cancer (BCLC) system and have fewer opportunities to accept radical treatments such as surgical resection, liver transplantation, or percutaneous ablation (2). Furthermore, elderly patients exhibit shorter life expectancy and more comorbidities compared with younger patients, and thereby, physicians are apt to follow more conservative treatment approaches for this population.

At present, transcatheter arterial chemoembolization (TACE) is recommended as an essential first-line palliative choice for the patients who are poor candidates for surgical resection. It has been widely applied for HCC patients with BCLC stage B, or diagnosed as multinodular asymptomatic tumors with an optimal liver function but no macroscopic vascular invasion (MVI) or extrahepatic metastasis. Of note, patients with MVI have a contraindication to TACE (2, 3). Portal vein tumor thrombosis (PVTT) as a common kind of MVI is an important indicator of poor prognosis in HCC patients (4), and occurs in 20-70% of HCC patients, with a low median survival time of only 2-4 months (5, 6). As a result, treatments in elderly patients with HCC and PVTT are limited. Since TACE has a theoretical risk of ischemic damage to normal liver parenchyma, it has long been considered to be contraindicated in HCC patients with PVTT, especially for elderly patients (7).

On the basis of the BCLC groupings, for the patients with advanced HCC and PVTT, the tyrosine kinase inhibitor Sorafenib is regarded as a standard pharmacological therapy (8–10). Similarly, the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) guidelines also recommend Sorafenib. However, they do not recommend TACE for Child-Pugh A or B patients with PVTT, irrespective of the degree of PVTT (11, 12). Of note, medications such as Sorafenib or Lenvatinib are too expensive to be affordable for patients residing in developing countries including China. Furthermore, the rate of tumor response to Sorafenib is modest with less than three months of survival prolongation compared with placebo (9, 10). Even today, TACE is still a meaningful treatment for unresectable HCC patients with PVTT in Asia (13). The Japan Society of Hepatology proposed TACE for HCC patients with PVTT in second-order branches that have a good hepatic function (Child-Pugh A or B) and lack extrahepatic spread (14). Chinese clinical practice guidelines for transarterial chemoembolization of HCC also recommend TACE for HCC patients with PVTT in the following situations: the main portal vein is not completely blocked, or the portal veins are fully blocked but have abundant compensatory collateral circulation or portal blood flow can be recanalized by stenting (15). To date, very few studies have reported the TACE treatment for elderly HCC patients with PVTT.

Moreover, the treatment of TACE for elderly patients remains controversial. For instance, Yau et al. reported that TACE improved both OS and PFS in elderly patients compared with non-elderly patients (16). On the other hand, Cohen et al. showed similar survival patterns between elderly patients and non-elderly patients (17). Overall, the importance to study the TACE treatment cannot be understated due to its efficacy and affordability among elderly HCC patients. To this point, the objective of the current study was to investigate the efficacy and safety of TACE therapy in elderly HCC patients with PVTT, as well as the prognostic difference among the patients with different PVTT types.

MATERIALS AND METHODS

Study Design and Patients

Among elderly HCC patients, about 245 patients with PVTT were reviewed retrospectively from January 2015 to December 2019 in Shanghai East Hospital. Out of them, 124 patients were enrolled in the current study, including 62 receiving best supportive care (BSC) and 62 receiving the TACE treatment. This study was approved by the Ethics Committee of Shanghai East Hospital. Since all the patient identities were anonymized, the requirement for informed consent was waived.

In this study, the inclusion criteria of patients were: 1) 70 years old or older; 2) initially diagnosed as HCC with PVTT or recurrent HCC with PVTT following surgical resection, and could not tolerate surgical resection again or refused further surgery; 3) no history of treatments such as radiofrequency ablation, transplantation, I^{125} seed implantation, percutaneous ethanol injection, TACE, or radiotherapy; 4) no systemic treatment such as Sorafenib, Lenvatinib, or checkpoint inhibitors; 5) liver function is Child-Pugh A or B; 6) European Cooperative Oncology Group performance status (ECOG) is 0-2; 7) adequate hematologic and renal functions; and 8) could be evaluated by Response Evaluation Criteria in Solid Tumors (mRECIST) criteria. The exclusion criteria of patients were: 1) have extrahepatic metastasis; 2) complete main portal vein obstruction without collateral circulation; 3) other cancers; 4) combined with targeted therapy, chemotherapy, or immunotherapy; and 5) have contraindications to arteriography or TACE.

HCC was diagnosed based on pathologic findings and/or the American Association for the Study of Liver Diseases criteria (11). The modified mRECIST criteria was applied to evaluate the response to the therapy (18), clarified as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). The objective response rate (ORR) was defined as (CR +PR)/total cases×100%, and disease control rate (DCR) was defined as (CR+PR+SD)/total cases×100%. The primary endpoint was overall survival (OS), and the secondary endpoint included progress free survival (PFS) and safety. OS and PFS were determined from the time of the initial diagnosis or recurrence of HCC to disease progression or death. PVTT was stratified according to Cheng's PVTT classification system: Type I: Tumor thrombi involving segmental branches of portal vein; Type II: Tumor thrombi involving right/left portal vein; and Type III: Tumor thrombi involving the main portal vein and trunk (19).

TACE Procedure

The TACE procedure was performed as described previously (20). Overall, a selective 5 French catheter was utilized, and visceral angiography was performed out to evaluate the liver cancer feeding artery. The tip of the microcatheter was advanced into the hepatic segmental or tumor-feeding artery. An emulsion of 1-20 ml of lipiodol (Iodinated Oil Injection, Luyin pharmacy, China) and 40 mg of doxorubicin hydrochloride were administered into the feeding vessels. However, if the flow of the tumor feeding artery did not lower after injecting 20 ml, we continued to inject 150-1400 μm gelatin sponge to embolize the vessel, until we could observe a significantly slower rate of flow. The general treatment cycle of TACE was 4-6 weeks and then other TACE cycles were conducted out according to the results of the follow-up contrast-enhanced CT or MRI.

Follow-Up

All patients were reevaluated one month after TACE. TACE would be repeated with an interval of 4-6 weeks if necessary. If the tumor had no viability based on contrast-enhanced CT or the patients had contradictions to TACE, the treatment of TACE would not be performed. The follow-up program included vital signs, serum AFP, liver function, coagulation function, chest CT scan, and abdominal contrast-enhanced CT or MRI scan every 6-8 weeks for the first year and every 3 months thereafter. Side effects of TACE were reported according to NCI-CTCAE version 5.0. All patients were followed up until death or until June 30, 2020.

Statistical Analyses

Demographic characteristics and clinical outcomes were compared among patients undergoing TACE or BSC treatment. All categorical and continuous data were analyzed using descriptive statistics. Categorical variables were reported as total count and frequencies (%) while continuous variables were reported as median and interquartile ranges (IQR). Bivariate analyses were performed using the chi-squared test or Fisher exact test (2-tailed) for categorical variables, as appropriate, to assess the differences in the TACE and BSC treatment groups. Means for continuous variables that were normally distributed were compared using independent samples t-test. Survival curves were analyzed using the Kaplan-Meier method and compared using the log-rank test. The bivariate and multivariable Cox proportional hazard modeling was performed to identify independent prognostic factors based on adjusted hazard ratio and its associated 95% confidence interval (CI). Model selection was based on the stepwise technique to assess factors associated with overall survival. All statistical analyses were performed using IBM SPSS 23.0, and $P<0.05$ was considered statistically significant.

RESULTS

Patient Demographics and Clinical Characteristics

Between January 2015 and December 2019, 245 elderly patients with unresectable HCC and PVTT undergoing treatment with TACE or BSC were collected, of which 124 were included in the final analysis (**Figure 1**). 50.0% (n=62) of the patients were treated with BSC, and 50.0% (n=62) was treated by the TACE therapy. Demographic characteristics were comparable among both the groups ($P>0.05$). Similarly, tumor size, tumor number, and total bilirubin were comparable among both the groups. The median levels of the ALT, AST, and D-Dimer among elderly HCC patients were higher in the BSC group compared with the TACE group (median BSC *vs.* TACE, ALT: 55.5 *vs.* 35.5, AST: 71.0 *vs.* 34.5, D-Dimer: 3.66 *vs.* 2.58; all $P<0.05$) (**Table 1**).

Safety

The most common AEs related to TACE treatment observed in this study were fever (45.16%), liver dysfunction (41.94%), abdominal pain (35.48%), and anorexia (33.87%), and D-Dimer elevation (33.87%) (**Table 2**). Nausea, fatigue, ventosity, and diarrhea were also observed frequently. There is one upper GI hemorrhage (1.61%) after TACE treatment, which was under control after treatment. Subgroup analyses revealed that type III

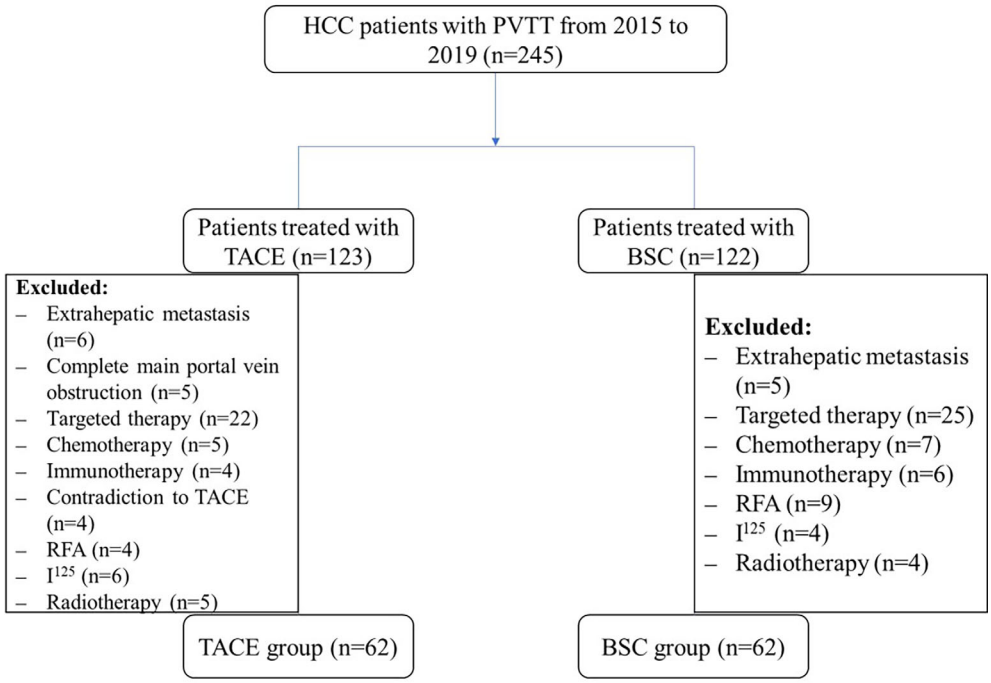

FIGURE 1 | Flowchart of elderly HCC patients with PVTT recruitment.

PVTT patients suffered more liver dysfunction, D-Dimer elevation, and ventosity than type I or type II PVTT patients (P<0.0167). Most of the AEs were grade 1/2 and well tolerated. The most common grade 3/4 AEs was fever and occurred in 10 (16.13%) patients. All these findings returned to the pre-treatment levels within less than one month after TACE.

Efficacy

Overall Survival

As shown in **Figure 2**, the median overall survival (OS) of elderly patients in the TACE and BSC groups was 11.30 months (95%CI: 9.636-12.964) and 7.80 months (95%CI: 6.748-8.852), respectively (P<0.001). In subgroup analyses, the median OS of type I PVTT patients was 14.30 months (95%CI: 11.492-17.108) and 7.80 months (95%CI: 3.875-11.725) in the TACE and BSC groups, respectively (P=0.007). Interestingly, in the type II PVTT group, the median OS of the TACE and BSC groups were 13.00 months (95%CI: 10.539-15.461) and 8.00 months (95%CI: 6.987-9.103), respectively (P=0.002). However, the OS of type III PVTT patients in the TACE group was poorer than the BSC group, which were 4.50 months (95%CI: 3.313-5.687) and 7.00 months (95%CI: 5.239-8.761), respectively (P=0.176).

Progress Free Survival

The median PFS was 4.00 months (95%CI: 3.625-4.375) for HCC patients with BSC treatment and was 7.50 months (95%CI: 6.284-8.716) for HCC patients treated with TACE (P<0.001). The TACE treatment prolonged the PFS in the three subgroups. In comparison of TACE and BSC, the PFS of type I PVTT patients were 8.00 months (95%CI: 5.485-10.515) and 6.00 months (95%CI: 3.973-8.027), respectively (P=0.003). For type II PVTT patients, the PFS in the TACE and BSC groups were respectively 7.50 months (95%CI: 5.152-9.848) and 4.00 months (95%CI: 3.574-4.426) (P=0.005). Type III PVTT patients could also benefit from TACE treatment. The PFS of type III PVTT patients in the TACE group was 4.50 months (95%CI: 2.337-6.663), while in the BSC group was 2.70 months (95%CI: 2.083-3.317), without great significance (P=0.103) (**Figure 3**).

Response Rate

The response rate was separately 35.48% in the TACE group and 0.00% in the BSC group and the response rate of TACE was significantly better (P<0.001). The DCR was separately62.90% in the TACE group and 17.74% in the BSC group (P<0.001). The proportions of CR, PR, SD, and PD were 3.22%, 32.26%, 27.42%, and 37.10% in the TACE group while 0.00%, 0.00%, 17.74%, and 82.26% in the BSC group. The waterfall plot figure was indicated in **Figure 4**, showing the response rate of HCC patients with PVTT in the TACE group.

Bivariate and Multivariable Cox Proportional Hazard Modeling Analyses

On bivariate analyses, the clinical factors such as PVTT types, ECOG performance status, tumor size, treatments (TACE *vs.* BSC), and tumor numbers were associated with the overall survival. On the multivariable regression analyses, it was found that the patients with type III PVTT had 118% higher hazards of mortality than those with type I-II PVTT (HR: 2.18; 95%CI:

TABLE 1 | Baseline characteristics of elderly HCC patients in the BSC group and the TACE group.

Variables	BSC group (n=62)	TACE group (n=62)	*P* value
Gender			
Male n(%)	51 (82.25)	53 (85.48)	0.63
Female n(%)	11 (17.75)	9 (14.52)	
Age*	75 (73, 78)	74 (72, 78)	0.33
PVTT type			
Type I n(%)	21 (33.87)	28 (45.16)	0.44
Type II n(%)	28 (45.16%)	23 (37.10)	
Type III n(%)	13 (20.97%)	11 (17.74)	
Child-Pugh			
A n(%)	58 (93.54)	59 (95.16)	0.70
B n(%)	4 (6.56)	3 (4.84)	
ECOG			
0 n(%)	11 (17.74)	18 (29.03)	0.20
1 n(%)	49 (79.03)	40 (64.52)	
2 n(%)	2 (3.23)	4 (6.45)	
Tumor size (cm)*	6.0 (4.0 - 7.0)	5.0 (4.0 – 7.0)	0.26
Tumor number			
single n(%)	4 (6.45)	3(4.84)	0.697
multiple n(%)	58 (93.55)	59(95.16)	
Hepatitis			
Hepatitis B n(%)	62 (100.00)	61(98.39)	0.32
Hepatitis C n(%)	0 (0.00)	1(1.61)	
AFP			
≥400 n(%)	17 (27.4%)	15 (24.2%)	0.68
<400 n(%)	45 (72.6%)	47 (75.8%)	
Total bilirubin*	14.5 (11.0, 20.0)	14.0 (10.0, 21.0)	0.89
Albumin*	38.5 (35.0, 40.0)	39.0 (36.0, 41.0)	0.28
ALT*	55.5 (34.0, 80.5)	35.5 (49.0, 21.0)	**<0.001**
AST*	71.0 (37.0, 85.5)	34.5 (23.0, 50.0)	**<0.001**
D-Dimer*	3.66 (2.09, 4.98)	2.58 (1.27, 3.88)	**0.039**

**data are median. p < 0.05 was considered statistically significant. Demographic characteristics were comparable among both the groups (p>0.05). The median levels of the ALT, AST, and D-Dimer among elderly HCC patients were higher in the BSC group compared with the TACE group (all p < 0.05).*

ECOG, Eastern Cooperative Oncology Group; AFP, Alpha–fetoprotein; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase.

Bold values marked the significant P values (P < 0.05).

1.29-3.70; P = 0.004). Interestingly, elderly HCC patients receiving the TACE treatment had 52% lower hazards of mortality than those receiving the BSC treatment (HR: 0.48; 95%CI: 0.32-0.72; P = 0.004). Based on these findings, multivariable Cox proportional hazards regression analyses revealed that PVTT types (type III *vs.* type I-II) (HR: 2.18; 95%CI: 1.29-3.70; *P*=0.004) and tumor diameter (>5 cm *vs.* ≤5 cm) (HR: 1.94; 95%CI: 1.28-2.93; *P*=0.002) were independent indicators of overall survival (**Table 3**).

DISCUSSION

In China, there is an increasing trend in the incidence of HCC patients. With the prolongation of life expectancy, treatment of elderly HCC patients has been recently a new challenge for global healthcare system. Due to the high prevalence of hepatitis B infection and low screening rate of early-stage liver cancer, most of HCC patients are usually diagnosed at an advanced stage. Among them, PVTT incidence can be as high as 70% (6). In addition, the patients suffering from advanced HCC with PVTT exhibit a poor prognosis, with median survival of only 2-4 months (21). Sorafenib is currently recommended as the standard first-line treatment by the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) for Child-Pugh A or B patients with PVTT (11, 12). However, the SHARP study revealed that the survival benefit of advanced HCC patients with Sorafenib administration was less than three months (9). In 2018, the randomized phase III non-inferiority trial REFLECT study revealed that the non-inferiority had achieved with a median OS of 13.6 months in the Lenvatinib group and 12.3 months in the Sorafenib group. Median PFS was 7.4 months and 3.7 months in Lenvatinib and Sorafenib groups, respectively. The study has met both the primary and secondary end points (22). Thereby, Europe, the Middle East, and Africa (EMEA), the American Food and Drug Administration (FDA), and National Medical Products Administration (NMPA) in China have approved Lenvatinib for the first-line treatment of both young and elderly unresectable HCC. Although Lenvatinib and Sorafenib could improve the survival of advanced HCC, these drugs have not yet been covered by the common medical insurance in most regions of China. The cost of Lenvatinib is as high as 48,000 yuan

TABLE 2 | Adverse events related to the TACE treatment. Data are presented as number (%) of patients.

AE n(%)	All events							Grade 1-2	Grade 3-4
	Total (n=62)	PVTT I (n=28)	PVTT II (n=23)	PVTT III (n=11)	*P* value*				
					I *vs* II	I *vs* III	II *vs* III		
Fever	28 (45.16)	9 (32.14)	11 (47.83)	8 (72.73)	0.388	0.033	0.271	18 (29.03)	10 (16.13)
Liver dysfunction	26 (41.94)	8 (28.57)	8 (34.78)	10 (90.91)	**0.764**	**0.001**	**0.003**	19 (30.65)	7 (11.29)
Abdominal pain	22 (35.48)	8 (28.57)	8 (34.78)	6 (54.55)	0.764	0.068	0.151	22 (35.48)	0 (0.00)
Anorexia	21 (33.87)	8 (28.57)	6 (26.09)	7 (63.64)	1.000	0.068	0.060	21 (33.87)	0 (0.00)
D-Dimer elevation	21 (33.87)	5 (17.86)	6 (26.09)	10 (90.91)	**0.514**	**<0.001**	**0.001**	21 (33.87)	0 (0.00)
Nausea	17 (27.42)	7 (25.00)	5 (21.74)	5 (45.45)	1.000	0.262	0.232	17 (27.42)	0 (0.00)
Fatigue	17 (27.42)	7 (25.00)	6 (26.09)	4 (36.36)	1.000	0.694	0.692	16 (25.80)	1 (1.61)
Ventosity	14 (22.58)	3 (10.71)	5 (21.74)	6 (54.55)	**0.442**	**0.008**	**0.114**	14 (22.58)	0 (0.00)
Diarrhea	5 (8.06)	2 (7.14)	2 (8.70)	1 (9.09)	1.000	1.000	1.000	5 (8.06)	0 (0.00)
Upper GI hemorrhage	1 (1.61)	0	0	1 (9.09)	–	0.282	0.324	1 (1.61)	0 (0.00)

**p<0.0167 was considered statistically significant. Type III PVTT patients suffered more liver dysfunction, D-Dimer elevation, and ventosity than type I or type II patients.*

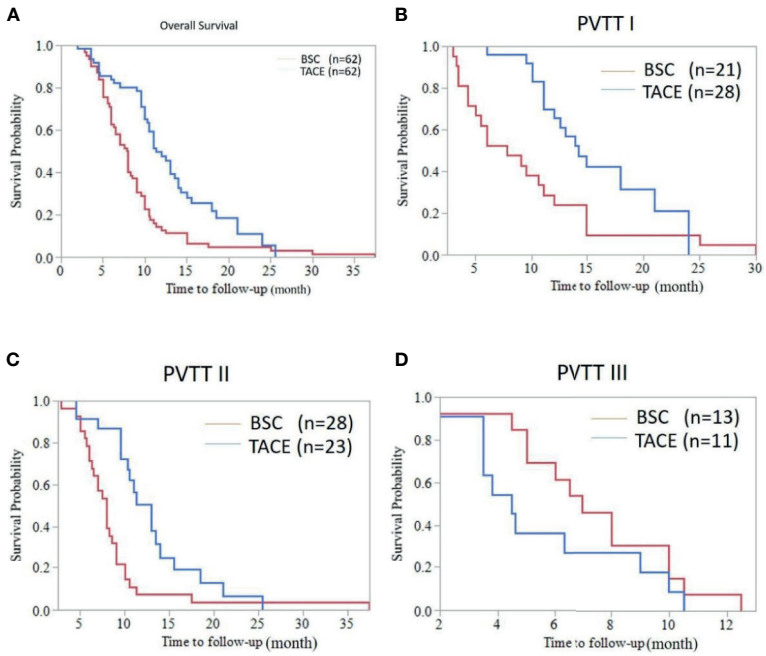

FIGURE 2 | Overall survival curve of HCC patients with PVTT accepting the TACE or BSC treatment. **(A)** Whole population survival curve for the TACE group and the BSC group (median OS [months], 11.30 (9.636-12.964) *vs.* 7.80 (6.748-8.852); $P < 0.001$). **(B)** Survival curve of HCC patients with type I PVTT in the TACE group and the BSC group (OS, 14.30 (11.492-17.108) *vs.* 7.80 (3.875-11.725); $P=0.007$). **(C)** Survival curve of HCC patients with type II PVTT in the TACE group and the BSC group (OS, 13.00 (10.539-15.461) *vs.* 8.00 (6.987-9.103); $P=0.002$). **(D)** Survival curve of HCC patients with type III PVTT in the TACE group and the BSC group (OS, 4.50 (3.313-5.687) *vs.* 7.00 (5.239-8.761); $P=0.176$).

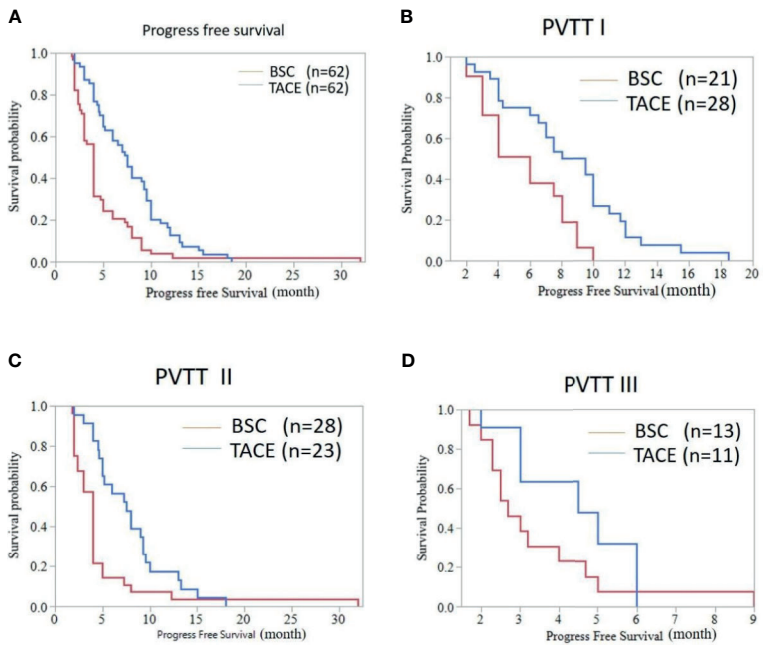

FIGURE 3 | Progress free survival curve of HCC patients with PVTT accepting the TACE or BSC treatment. **(A)** Whole population (median PFS [months], 7.50 (6.284-8.716) *vs.* 4.00 (3.625-4.375); $P < 0.001$). **(B)** Patients with type I PVTT (PFS, 8.00 (5.485-10.515) *vs.* 6.00 (3.973-8.027); $P=0.003$). **(C)** Patients with type II PVTT (PFS, 7.50(5.152-9.848) *vs.* 4.00 (3.574-4.426); $P=0.005$). **(D)** Patients with type III PVTT (PFS, 4.50 (2.337-6.663) *vs.* 2.70 (2.083-3.317); $P=0.103$).

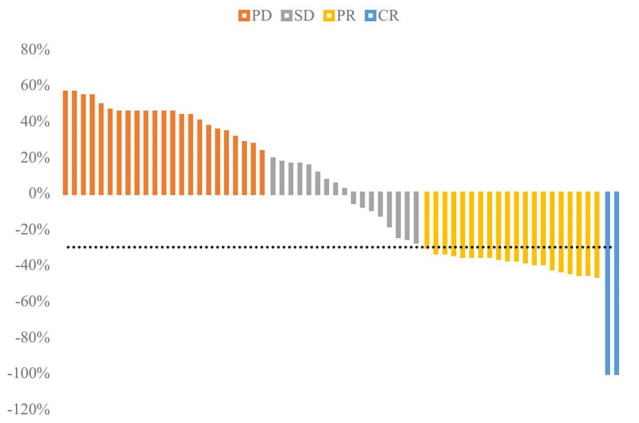

FIGURE 4 | Waterfall plot of response rate in the TACE group. The disease control rate (DCR) was 62.90%, and objective response rate (ORR) was 35.48%.

(nearly $7,000) per month, which limits its clinical application due to poor affordability. Compared to these drugs, TACE as an effective treatment measure with a low cost still plays an important role in advanced HCC patients. Although international guidelines do not recommend TACE for HCC patients with PVTT, TACE is still widely applied in clinical practice in Asia and recommended by both Chinese and Japanese guidelines (14, 15).

However, presently, there is no clear consensus on the efficacy of TACE for elderly HCC patients with PVTT. As such, we conducted this retrospective study to compare the survival in HCC patients treated with TACE *versus* BSC. The current study revealed that HCC patients above the age of 70 years old and receiving the TACE treatment had 52% times lower hazards of mortality compared with those treated with the BSC treatment. Subgroup analyses showed that the prognosis and response rate of the TACE treatment in HCC patients with different PVTT types were different. Compared with the BSC group, the TACE group significantly prolonged the OS and PFS of type I and type II PVTT patients. In contrast, among the type III PVTT patients, the results of PFS were comparable between the two groups. However, the OS of HCC patients with type III PVTT were even worse in the TACE group than that of the BSC group. The reason might be that the patients with type III PVTT were more likely to have TACE-related adverse effects and were at a later stage of the tumor, and thus they could not benefit from the TACE treatment.

Similar studies reported controversial results for the efficacy of the TACE treatment in HCC patients with type III PVTT. Liang et al. reported no survival benefit of TACE for the patients with the main portal vein thrombosis (20), while Yuan J et al. reported TACE benefit of type III PVTT patients (23). The discordances among the results of such clinical studies may be reduced to the selection bias among the subjects in different clinical studies, or the different regimens for the TACE treatment. These data suggested that large scale phase III clinical trials may be required to verify how TACE treatment could benefit advanced HCC patients with type III PVTT. Similar to our results, Chung et al. and Bai et al. proposed that HCC with type I/II PVTT could benefit from the TACE treatment in both young and elderly patients (24, 25). Based on the previous results and our research, the TACE treatment could be selected for elderly HCC patients with type I and type II PVTT if there are no contradictions, in order to find the chance of survival benefit.

The safety analysis showed that, although the adverse effects such as fever, abnormal liver function, and abdominal pain increased in the TACE group, most of them were grade 1 to 2

TABLE 3 | Bivariate and multivariable Cox proportional hazard modeling analyses for overall survival.

Variable	Bivariate analysis			Multivariate analysis		
	HR	CI	*P* value	HR	CI	*P* value
Gender(F/M)	1.12	0.68- 1.86	0.660			
Age(<75/≥75)	1.27	0.87- 1.85	0.218			
Child-Pugh(B/A)	1.36	0.63- 2.95	0.431			
PVTT type(III/I-II)	2.46	1.47- 4.09	**<0.001**	2.18	1.29-3.70	**0.004**
ECOG(1-2/0)	2.41	1.50- 3.86	**<0.001**			
Tumor size(>5cm/≤5cm)	1.91	1.29- 2.83	**0.0012**	1.94	1.28-2.93	**0.002**
Albumin(≥35/<35g/L)	0.98	0.62- 1.52	0.909			
TACE *vs* BSC	**0.61**	**0.42- 0.89**	**0.010**	0.48	0.32-0.72	**0.004**
Total bilirubin(≥20/<20umol/L)	1.59	1.06- 2.38	**0.024**			
Hepatitis (C/B)	0.72	0.10- 5.17	0.741			
AFP(<400/≥400 ng/ml)	0.81	0.53- 1.24	0.342			
Tumor number(≥3/<3)	2.37	1.03- 5.44	**0.042**			
ALT(<40/≥40U/L)	0.83	0.57- 1.23	0.359			
AST(<60/≥60U/L)	0.94	0.64- 1.39	0.759			
D-Dimer (<0.55/≥0.55mg/L)	1.06	0.62- 1.79	0.841			

Elderly HCC patients received the TACE treatment had 52% lower hazards of mortality compared with the BSC group patients (p<0.001). Multivariable Cox proportional hazards regression analysis revealed that PVTT types (type III vs type I-II) and tumor diameter (>5cm vs ≤ 5cm) (p<0.005) were independent indicators of overall survival.
ECOG, Eastern Cooperative Oncology Group; AFP, Alpha–fetoprotein;ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; TACE, Transarterial Chemoembolization.
Bold values marked the significant P values (P < 0.05).

according to CTCAE 5.0 and could return to normal within one month. Subgroup analyses revealed that the incidences of adverse effects were higher in the type III PVTT group, especially liver dysfunction, D-Dimer elevation, and ventosity with great significance. It should be noted that D-Dimer obviously was upregulated following TACE treatment, especially in the type III PVTT group, which indicated possible hypercoagulation following the TACE treatment. Therefore, more attention should be paid to monitor the coagulation after surgery to avoid the possibility of embolism due to tumor-related or TACE-related hypercoagulation.

Bivariate analyses revealed that PVTT types, ECOG performances status, tumor size, tumor numbers, and treatment groups (TACE *vs*. BSC) were correlated with advanced HCC. On multivariable analyses, it was found that the type I and type II PVTT, tumor size (≤5 cm), and patients treated with TACE were advantageous independent indicators of HCC patients' overall survival. Overall, the data suggested the necessity to fully evaluate the risks and benefits of HCC patients in the clinical practice, in order to make a suitable strategy to maximize the benefits of patients.

Currently, the combination strategies of TACE with other treatments, such as TACE combined with surgery, radiotherapy, seed implantation, Sorafenib administration, and other treatment modes are being discussed (23, 25–29). However, the subjects and results of these clinical studies varied greatly. Therefore, it is also necessary to explore the optimal combination strategies of TACE and other treatments, in order to maximize the benefits to the patients.

There were some limitations to the study. First, it is a retrospective study, and there may be a selection bias during the enrollment of subjects. Second, it is a single-center study with a small sample size which could not be fully representative, and thus large-scale phase III clinical trials or multicenter study are recommended to confirm these results. Third, the evaluation of clinical effects on basis of mRECIST criteria may be biased because of the investigator-independent factors. Independent Review Committee (IRC) is still in need of future studies to increase the generalizability.

In conclusion, the retrospective study showed that palliative TACE treatment could prolong the OS and PFS of elderly advanced HCC patients with type I and type II PVTT without severe adverse events. The elderly patients diagnosed as HCC had 52% lower hazards of mortality compared with those treated in the BSC group. For these patients who could not afford the standard first-line drugs such as Lenvatinib or Sorafenib, TACE is still an accessible effective measure.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Shanghai East Hospital. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

JL and WH collected the clinical data. QT processed the statistical data. JX drafted the manuscript. JX and QT revised the final manuscript. All authors contributed to the article and approved the submitted version.

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A Radiomics Signature-Based Nomogram to Predict the Progression-Free Survival of Patients With Hepatocellular Carcinoma After Transcatheter Arterial Chemoembolization Plus Radiofrequency Ablation

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Objective: The study aims to establish an magnetic resonance imaging radiomics signature-based nomogram for predicting the progression-free survival of intermediate and advanced hepatocellular carcinoma (HCC) patients treated with transcatheter arterial chemoembolization (TACE) plus radiofrequency ablation

Materials and Methods: A total of 113 intermediate and advanced HCC patients treated with TACE and RFA were eligible for this study. Patients were classified into a training cohort (n = 78 cases) and a validation cohort (n = 35 cases). Radiomics features were extracted from contrast-enhanced T1W images by analysis kit software. Dimension reduction was conducted to select optimal features using the least absolute shrinkage and selection operator (LASSO). A rad-score was calculated and used to classify the patients into high-risk and low-risk groups and further integrated into multivariate Cox analysis. Two prediction models based on radiomics signature combined with or without clinical factors and a clinical model based on clinical factors were developed. A nomogram comcined radiomics signature and clinical factors were established and the concordance index (C-index) was used for measuring discrimination ability of the model, calibration curve was used for measuring calibration ability, and decision curve and clinical impact curve are used for measuring clinical utility.

Results: Eight radiomics features were selected by LASSO, and the cut-off of the Rad-score was 1.62. The C-index of the radiomics signature for PFS was 0.646 (95%: 0.582–0.71) in the training cohort and 0.669 (95% CI:0.572–0.766) in validation cohort. The median PFS of the low-risk group [30.4 (95% CI: 19.41–41.38)] months

was higher than that of the high-risk group [8.1 (95% CI: 4.41–11.79)] months in the training cohort (log rank test, z = 16.58, $p < 0.001$) and was verified in the validation cohort. Multivariate Cox analysis showed that BCLC stage [hazard ratio (HR): 2.52, 95% CI: 1.42–4.47, $p = 0.002$], AFP level (HR: 2.01, 95% CI: 1.01–3.99 $p = 0.046$), time interval (HR: 0.48, 95% CI: 0.26–0.87, $p = 0.016$) and radiomics signature (HR 2.98, 95% CI: 1.60–5.51, $p = 0.001$) were independent prognostic factors of PFS in the training cohort. The C-index of the combined model in the training cohort was higher than that of clinical model for PFS prediction [0.722 (95% CI: 0.657–0.786) vs. 0.669 (95% CI: 0.657–0.786), $p < 0.001$]. Similarly, The C-index of the combined model in the validation cohort, was higher than that of clinical model [0.821 (95% CI: 0.726–0.915) vs. 0.76 (95% CI: 0.667–0.851), $p = 0.004$]. The calibration curve, decision curve and clinical impact curve showed that the nomogram can be used to accurately predict the PFS of patients.

Conclusion: The radiomics signature was a prognostic risk factor, and a nomogram combined radiomics and clinical factors acts as a new strategy for predicted the PFS of intermediate and advanced HCC treated with TACE plus RFA.

Keywords: nomogram, prediction, progression-free survival, transarterial chemoembolization plus radiofrequency ablation, hepatocellular carcinoma

INTRODUCTION

Primary liver cancer (PLC) is the sixth most common malignant cancer and the fourth leading cause of cancer-related death worldwide (Bray et al., 2018). A total of 854,000 new cases are diagnosed every year, and almost 50% of PLCs come from China, which places a heavy burden on Chinese health care (Chen et al., 2018). Hepatocellular carcinoma (HCC) accounts for 75–85% of PLC, which cause a heavy burden of death in China. Approximately 70% of patients with HCC are diagnosed with advanced HCC and thus have missed the best opportunity for surgery (European Association for the Study of the Liver, 2018). The 5 years recurrence rate of HCC is as high as 70%. Local interventional strategies such as transarterial arterial chemoembolization (TACE), radiofrequency ablation (RFA) and radioactive particle implantation are widely used in the treatment of advanced HCC (Makary et al., 2020). The combination of TACE and RFA therapy has a synergistic cytotoxic effect on HCC and results in better local tumour control and longer survival than TACE or RFA alone (Peng et al., 2013; Shimose et al., 2019; Chang et al., 2020; Yuan et al., 2021).

TACE pretreatment reduces or eliminates the "heat sink" caused by blood flow during the RFA procedure and clearly shows lesion contours on CT because of lipiodol deposition. These factors may broaden the extent of coagulation necrosis and ultimately result in better outcomes (Iezzi et al., 2016). In addition, the tissue oedema induced by TACE pretreatment enlarges the necrotic area produced by RFA, thereby reducing local recurrence and increasing the safety of the ablation procedure. Use of the TACE procedure after RFA was shown to have a therapeutic effect on the sublethal heating zone of the tumour (Iezzi et al., 2016), which was the main source of local recurrence (Su et al., 2018; Tan et al., 2019). However, the 1-, 3- and 5-years local tumor progression rates reached 9, 40, 55%, respectively (J. H. Kim et al., 2011). What's more, as a malignant tumor with obvious heterogeneity, the therapeutic effects of TACE combined with RFA were significantly various. Therefore, development of an accurate prediction model for the progression-free survival (PFS) of these patients is urgently needed.

Efforts have been made to select appropriate candidates for TACE and RFA treatment based on the Barcelona Clinic Liver Cancer (BCLC) staging system and on histopathologic grade, AFP and performance status as well as vascular invasion (Makary et al., 2020). In addition, many risk assessment models have been established to predict recurrence after TACE or RFA treatment (Canale et al., 2018; Wang et al., 2019; Han et al., 2020; Kobe et al., 2020). However, candidate patient selection and prognostic assessment models for patients with intermediate and advanced HCC who have been treated with TACE plus RFA have rarely been reported. Thus, an accurate prognostic prediction model is needed for HCC patients after TACE combined with RFA treatment.

Radiomics is a field that involves tumour segmentation, feature extraction and radiomics model building using the high-throughput mining of quantitative image features extracted from images such as magnetic resonance imaging (MRI) and computed tomography (CT) as well as positron emission computed tomography (Lambin et al., 2017; Mayerhoefer et al., 2020). Radiomics approaches have been identified as efficient strategies for predicting the risk of recurrence and survival in HCC patients (Guo et al., 2019; Hui et al., 2018; Kim et al., 2018; Peng et al., 2018). Radiomics analysis has been reported to have specific advantages. It can provide additional, reliable information that radiologists cannot obtain through analysis with the naked eye and can avoid some mistakes made by radiologists who draw conclusions based on

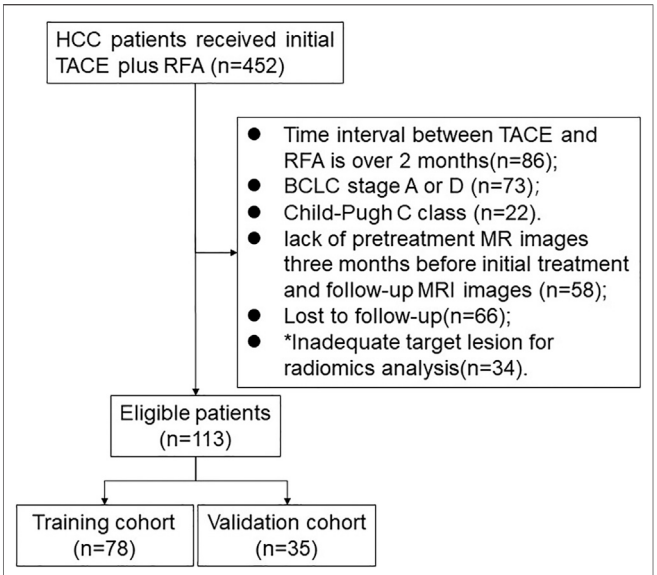

FIGURE 1 | Flowchart of patient selection. * Note: The inadequate target lesion for radiomics analysis is defined as the diameter of lesion less than 1 cm.

their own personal experience. A previous study suggested that a radiomics nomogram served as a non-invasive preoperative prediction method and exhibited favourable predictive accuracy for microvascular invasion in patients with HBV-related HCC (J. Peng et al., 2018). A study by Kim et al. (2018) indicated that the combination of radiomics signature and clinical factors could accurately predict the survival of TACE-treated HCC patients. Our previous study also indicated that the radiomics and clinical indicator-based predictive nomogram can well predict tumor response in intermediate-advanced HCC (Kong et al., 2021).

This study aimed to develop and verify a novel radiomics model including a radiomics signature and clinical risk factors that can be used to predict the PFS of intermediate and advanced HCC patients treated with a combination of TACE and RFA.

MATERIALS AND METHODS

Study Design and Patient Population

This retrospective study was approved by the institutional ethics committee. A total of 452 HCC patients who received initial TACE plus RFA from January 2010 to December 2018 were initially included. The exclusion scheme is shown in **Figure 1**. The exclusion criteria were as follows: (I) time interval between TACE and RFA of more than 2 months (n = 86) (II) BCLC stage A or D (n = 73) (III) Child-Pugh class C (22) (IV) lack of available pretreatment MR images 3 months before initial treatment and of follow-up MRI images (n = 58) (V) lost to follow-up (n = 66) (VI) target lesion inadequate for radiomics analysis (n = 34). Finally, 113 patients were analysed in this study.

MR Scanning

All patients were scanned using a 3.0 T MR scanner (Philips Medical Systems, Eindhoven, Netherlands) before and after TACE plus RFA treatment. Dynamic contrast MRI images were obtained by injecting dimeglumine gadopentetate (Guangzhou Kangchen Pharmaceutical Co. Ltd.) at a dose of 0.1 mmol per kilogram body weight. The scanning parameters were as follows: 1) spectral presaturation with inversion recovery T2-weighted sequence (3,000/200 ms repetition time/echo time (TE), 7 mm slice thickness, 1 mm interslice gap, 200 × 195 matrix size); 2) breath-hold unenhanced and contrast-enhanced mdIXON-T1WI (water) sequence (3.6/1.31/2.2 ms repetition time/TE1/TE2; 400–314 mm view field, 5 mm slice thickness; −2.5 mm slice gap; 224 × 166 matrix size) for four dynamic phases: hepatic arterial phase (15 s), portal venous phase (50 s), substantial period phase (90 s) and delayed phase (180 s); and 3) breath-hold diffusion-weighted echoplanar sequence (2,500/64 ms repetition time/TE; 400–343 mm field view; 7 mm section thickness; 1 mm intersection gap; 116 × 97 matrix size; b value = 0 and 800 s/mm^2). MRI images were exported in DICOM format.

TACE Procedure

All 113 eligible patients were successfully treated with TACE. All TACE procedures were performed by interventional radiologists with no less than 10 years of clinical experience according to the practice guidelines. Briefly, the femoral artery was punctured at the groin area in the region showing the strongest pulsation using the Seldinger technique. A 2.7-Fr microcatheter (Progreat; Terumo, Japan) was carefully inserted into the blood supply artery of the lesion, and the chemotherapeutic and embolic agents were injected through the microcatheter under DSA. Angiography was performed before the end of embolization to confirm satisfactory embolization of the feeding artery.

RFA Procedure

Percutaneous RFA was described in our previous study (W. Liu et al., 2020). Briefly, under the guidance of CT, the appropriate needle path and depth were calculated. After local anaesthesia at the puncture site and path using 1–2% lidocaine (5–15 ml), an RF electrode was inserted into the target lesion. The RF current was emitted for 10–15 min by an RF generator depending on the increased impedance. Overlap ablations were performed within the effective ablation range covering 0.5–1.0 cm outside the edge of the target lesion. Needle path ablation was performed to avoid bleeding and implant metastasis.

Follow-Up

PFS was the end point of this study. PFS was defined as the time from the date of TACE until the date of relapse (relapse refers to intrahepatic recurrence or extrahepatic metastasis) or until the date on which the patient was last known to be free of relapse. The first outpatient clinic visits were conducted within 4–6 weeks after TACE plus RFA treatments and every 2–3 months thereafter MRI images were obtained and used to track tumour progression.

Serum AFP levels, liver function and blood cell levels were also tested.

Features Extraction and Selection

Two experienced radiologists with more than 10 years of experience in clinical HCC diagnosis independently and semi-automatically conducted delineation of the volume of interest (VOI) using ITK-SNAP (www.itksnap.org). All preoperative contrast-enhanded T1-weighted images were then imported into Artificial Intelligence Kit software (A.K., GE Healthcare, China) to obtain quantitative VOI information. A total of 396 radiomics features divided into five categories, including histogram, form factors, grey-level co-occurrence matrix (GLCM), grey-level size zone matrix (GLSZM) and run-length matrix (RLM), were extracted.

Intra-Observer and Interobserver Reproducibility

Intra- and interclass correlation coefficients (ICCs) were applied to evaluate the intra- and inter-observer agreement of VOI segmentation and radiomics feature extraction. The two readers obtained the radiomics features twice with at least a 1-month interval between the two readings, and the inter-observer agreement and intraclass correlation were computed. An ICC value greater than 0.75 was considered to indicate good agreement.

Radiomics Model Building and Accuracy Evaluation

The features were then standardized and normalized by the Z score method, and abnormal values were replaced with the median of the parameter. Before dimension reduction, all patients were randomly divided into a training cohort (n = 78 cases) and a validation cohort (n = 35 cases) for radiomics model building and verification, respectively. The least absolute shrinkage and selection operator (LASSO) logistic regression algorithm was further conducted for feature selection in the training cohort, and a rad-score was calculated for each patient based on the selected radiomics features in both the training and validation cohorts using Cox-regression. The predictive accuracy of the radiomics signature was evaluated based on the area under the curve (AUC) of the receiver operator characteristic (ROC) curve in the training cohort and verified in the validation cohort. An optimal cut-off value of the rad-score was calculated based on Youden index and used to classify the patients into a high-risk group and a low-risk group for recurrence-free survival analysis. The PFS in the two groups was analysed by the Kaplan-Meier method, and the difference was calculated using the log-rank test.

Clinical Model and Combined Model Building

Univariate Cox proportional hazard regression analysis was applied to select the clinical characteristics with p-values < 0.05. The potential clinical characteristics related to recurrence-free survival from the univariate analysis were identified by multivariate Cox proportional hazard analysis. The clinical model was established using the characteristics with p-values < 0.05 in multivariate analysis. Again, the radiomics signature were integrated into the clinical model to develop a combined model, which include clinical prognostic factors and radiomics signature. The AUC of each model was calculated to quantify predictive accuracy in both the training cohort and the validation cohort. Finally, a nomogram using radiomics signature and clinical factors from clinical model to generate a probability for individuals was built and verified by the concordance index (C-index), calibration curve, decision curve and clinical impact curve.

Statistical Analysis

All statistical analyses were performed using SPSS software (SPSS version 22.0) and R software (R version 4.0.3). p-values < 0.05 were defined as statistically significant.

Categorical variables are shown as frequencies, and continuous variables are presented as mean and standard deviation or as median and 95% confidence interval (CI) values. Categorical variables were compared using the χ^2 test and Fisher's exact test. Student's t-test was used to determine the significant difference between the training and validation groups for normally distributed data; otherwise, the Mann-Whitney test was used. Feature selection was performed using the LASSO logistic regression model, which was conducted by 10-fold cross-validation. Univariate analysis using Cox's proportional hazards regression model was applied to select the variables for multivariate analysis. The concordance index (C-index) was used to evaluate the discrimination of the radiomics, clinical and combined prediction models. The agreement between predictions from the model and observed outcomes were measured by calibration curve, assessed by calculating the Hosmer–Lemeshow goodness-of-fit test. The clinical usefulness of the prediction models was estimated based on decision curves and clinical impact curve.

RESULTS

Patient Demographics and Clinical Characteristics

A total of 113 patients were eligible for this study; among these, 80 patients (70.79%) had confirmed tumour recurrence according to the clinical final observer outcome. The median PFS was 13.20 months, and the 95% CI was 9.78–16.61. The overall 1-, 2-, and 3-years cumulative PFS rates of the patients were 57.54, 31.34, and 20.37%, respectively. There were no significant differences in sex, age, HBsAg, cirrhosis, BCLC stage, Child-Pugh classification, AFP level, tumour diameter, node number, metastasis, portal vein thrombosis, TACE-RFA procedures and time interval between TACE and RFA treatment in the training and validation cohorts (**Table 1**).

Radiomics Feature Extraction and Radiomics Model Building

Satisfactory inter- and intra-observer reproducibility of feature extraction was achieved. The intra-observer ICC calculated based on two measurements ranged from 0.81 to 0.93, and the interobserver agreement between the two readers ranged from 0.79 to 0.90. The extracted features are shown in **Figure 2**. Eight

TABLE 1 | Characteristic of patients in the training and validation cohorts.

Characteristic	Training cohort (*n* = 78)	Validation cohort (*n* = 35)	Test value	*p* value
Gender	—	—	1.85	0.17
Male	73(93.58%)	30(85.71%)	—	—
Female	5(6.42%)	5(14.29%)	—	—
Age	—	—	1.18	0.28
<55 y	29 (37.66%)	17 (48.57%)	—	—
≥55 y	33 (62.34%)	18 (51.43%)	—	—
Child-Pugh class	—	—	1.59	0.21
A	56(71.79%)	29(82.86%)	—	—
B	22(28.21%)	6(17.14%)	—	—
Cirrhosis	—	—	1.06	0.30
Yes	70(89.74%)	29(82.86%)	—	—
No	8(10.26%)	6(17.14%)	—	—
HBsAg	—	—	0.42	0.52
Positive	72(93.31%)	31(88.57%)	—	—
Negative	6(6.69%)	4(11.43%)	—	—
BCLC stage	—	—	1.30	0.25
B	49(47.43%)	18(54.28%)	—	—
C	29(52.57%)	17(45.71%)	—	—
Tumor size (cm)	5.99 ± 2.10 cm	5.70 ± 1.89 cm	0.51	0.48
≤5 cm	38(48.71%)	20(57.14%)	0.69	0.41
>5 cm	40(51.29%)	15(42.86%)	—	—
Node	—	—	0.73	0.39
single	19(24.36%)	6(17.14%)	—	—
multiple	59(75.64%)	29(82.86%)	—	—
AFP	—	—	2.86	0.09
<200 ng/ml	19(24.36%)	14(40.00%)	—	—
≥200 ng/ml	59(75.64%)	21(60.00%)	—	—
Metastasis	—	—	0.23	0.63
Yes	14(17.94%)	5(14.28%)	—	—
No	64(82.06%)	30(85.72%)	—	—
PVTT	—	—	0.08	0.78
Yes	16(20.51%)	8(22.86%)	—	—
No	62(79.49%)	27(77.14%)	—	—
TACE-RFA procedures	1.76 ± 1.00 times	1.97 ± 1.01 times	0.09	0.30
one time	41(52.56%)	14(40.00%)	2.17	0.15
No less than two times	37(47.43%)	21(60.00%)	—	—
Time interval	—	—	2.13	0.14
<14 d	33(42.31%)	20(57.14%)	—	—
≥14 d	45(57.69%)	15(42.83%)	—	—
PFS	12.57(9.08~16.05)	17.73(9.70 ~ 25.77)	0.18	0.65

Abbreviations: HBsAg, hepatitis B surface antigen; BCLC stage, Barcelona Clinic Liver Cancer; AFP, Alpha-fetoprotein; PVTT, Portal vein tumor thrombus; PFS, progression free survival.

radiomics features were selected by the LASSO algorithm. A radiomics signature based on the eight radiomics features was built, and the formula for calculating the Rad-score for each patient was as follows: Rad-score = 0.1889–0.7190×Variance–0.5262×ClusterShade_angle0_offset7+0.0325×Correlation_AllDirection_offset4+0.2151 ×Correlation_angle90_offset7 + 0.8843×GLCMEntropy_angle90_offset4–0.2489×HaralickCorrelation_AllDirection_offset7_SD-1.3472×InverseDifferenceMoment_AllDirection_offset1_SD + 1.7838×LongRunEmphasis_angle90_offset4. The cut-off value of the rad-score was 1.62, and this value was used to classify the patients into high-risk and low-risk groups.

Performance of the Radiomics Model

He C-index of the radiomics signature for PFS was 0.646(95%: 0.582–0.71) in the training cohort and 0.669(95% CI: 0.572–0.766) in validation cohort. In the training cohort, the median PFS was 30.4 (95% CI: 19.41–41.38) months in the low-risk group, while the median PFS was 8.1 (95% CI: 4.41–11.79) months in the high-risk group. Compared with the high-risk group, the PFS of the low-risk group was significantly longer (log rank test, z = 16.58, $p < 0.0001$, **Figure 3A**). In the validation cohort, the median PFS was 31.50 (95% CI: 16.99–46.00) months in the low-risk group, while the median PFS was 10.43 (95% CI: 3.07–27.62) months in the high-risk group. Compared with the high-risk group, the PFS of the low-risk group was significantly longer (log rank test, z = 7.90, $p = 0.0049$, **Figure 3B**).

Univariate and Multivariate Analyses for Prognostic Factors of PFS

Univariate Cox regression analysis showed that among the candidate variables, five variables, including BCLC stage, tumour size (≥5 cm vs. <5 cm), AFP (≥200 ng/ml vs. <200 ng/ml), interval (<14 days vs. ≥14 days) and radiomics signature (high-risk vs. low-risk), had *p* values <0.05. Multivariate analyses

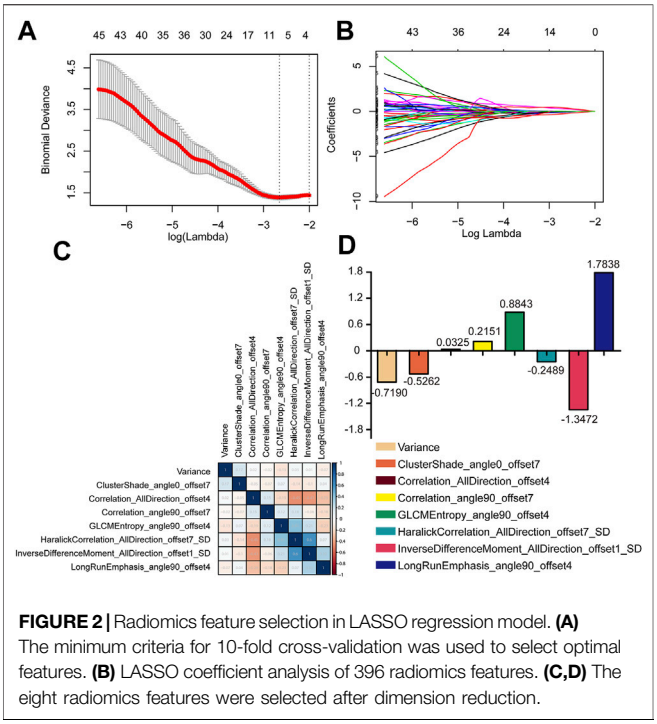

FIGURE 2 | Radiomics feature selection in LASSO regression model. **(A)** The minimum criteria for 10-fold cross-validation was used to select optimal features. **(B)** LASSO coefficient analysis of 396 radiomics features. **(C,D)** The eight radiomics features were selected after dimension reduction.

suggested that BCLC stage (hazard ratio (HR): 2.52, 95% CI: 1.42–4.47, $p = 0.002$), AFP level (HR: 2.01, 95% CI: 1.01–3.99, $p = 0.046$), time interval (HR: 0.48, 95% CI: 0.26–0.87, $p = 0.016$) and radiomics signature (HR 2.98, 95% CI: 1.60–5.51, $p = 0.001$) were independent prognostic factors of PFS (**Table 2**).

Accuracy of Radiomics Signature Combined With Clinical Factors

The C-index of the combined model in the training cohort was higher than that of clinical model [0.722 (95% CI: 0.657–0.786) vs. 0.669 (95% CI: 0.657–0.786), $p < 0.001$]. similarly, The C-index of the combined model in the validation cohort, was higher than that of clinical model [0.821 (95% CI: 0.726–0.915) vs. 0.76 (95% CI: 0.667–0.851), $p = 0.004$].

A Novel Nomogram for Individual PFS Prediction

A radiomics nomogram based on the combined model including the radiomics signature and clinical characteristics was established (**Figure 4A**). The calibration curve showed that the nomogram-predicted PFS was consistent with the observed PFS at 12 months and at 24 months (**Figures 4B–E**). These results indicate that the nomogram has a good predictive effect for the PFS of patients with intermediate and advanced HCC. The decision curve results showed that at threshold probabilities of up to 18%, the nomogram with the radiomics signature alone provided a greater benefit than the "no treatment" or "all treatment" strategies. At threshold probabilities of up to 25%, the discrimination ability of the combined nomogram was better than that of the nomogram with clinical factors alone (**Figure 4F**). Moreover, the clinical impact curve also showed that the nomogram offered good net benefit for the identification of patients who were likely to suffer recurrence (**Figure 4G**).

DISCUSSION

Locoregional therapies, including transarterial chemoembolization (TACE) and ablations, are proposed to postpone disease progression (Palmer et al., 2020). Therapy that combines TACE and RFA has been recommended and has proven to be an effective selection for intermediate and advanced HCC (Z. W. Peng et al., 2013). However, the outcomes are not satisfactory for all individuals. Therefore, it is urgent to identify patients who may truly experience a clinical benefit. In this study, we developed a model for predicting PFS based on a radiomics signature and clinical factors. We found that the PFS of patients with a low-risk radiomics signature was higher than that of patients with a high-risk radiomics signature. The C-index of the combined model in the training cohort was higher than that of clinical model [0.722 (95% CI: 0.657–0.786) vs. 0.669 (95% CI: 0.657–0.786), $p < 0.001$]. The C-index of the combined model in the validation cohort, was higher than that of clinical model [0.821 (95% CI: 0.726–0.915) vs. 0.76 (95% CI: 0.667–0.851), $p = 0.004$]. The model combining the radiomics signature and clinical factors provided a better evaluation of PFS than the model that considered clinical factors alone.

A radiomics approach was applied to identify more heterogeneous information from images. Radiomics signature extracted from ultrasonic, CT and MRI images have been found to predict recurrence in HCC patients who receive surgery (Akai et al., 2018), liver transplantation (Guo et al., 2019) or TACE (Song et al., 2020) as well as ablation (C. Yuan et al., 2019). In this study, a total of 396 radiomics features extracted by AK software were classified into five clusters: 42 histogram-based features, nine form factor-based features, 11 GLSZM-based features, 180 RLM-based features and 154 GLCM-based features. Histograms and form factor-based features were used to describe the grey distribution and the geometric characteristics of the region of interest. The GLSZM-, RLM- and GLCM-based features reflect the spatial arrangement of the colour or intensity of lesions. Eight potential predictors were selected from the 396 features by shrinking the regression coefficients with the LASSO method. Especially, variance is a parameter of histogram and represents the average of the squared differences from the Mean of image. Cluster Shade_angle0_offset7 is a texture parameter, which groups the similar samples according to their position and, optionally, normal into clusters. Correlation_AllDirection_offset4 is the Correlation that measures the similarity of the grey levels in neighboring pixels, telling how correlated a pixel is to its neighbor over the whole image. Correlation_angle90_offset7 and Haralick Correlation_AllDirection_offset7_SD is the parameters that measure the degree of similarity of the gray level of the image in the row or column direction, representing the local grey level correlation. the greater their value, the greater the correlation. GLCM Entropy_angle90_offset4 shows the amount of information of the image that is needed for the image compression. Inverse Difference Moment_AllDirection_offset1_SD is the local homogeneity. It is high when local gray level is uniform and inverse GLCM is high. Long Run Emphasis_angle90_offset4 is a Parameter of RLM

TABLE 2 | Univariate and Multivariate analysis of factors associated with PFS.

Characteristic	Univariate analysis				Multivariate analysis			
	HR	HR	(95% CI)	*p* value	HR	HR	(95% CI)	*p* value
Age (years) (≥55 years vs <55 years)	0.71	0.45	1.11	0.14	—	—	—	—
Gender (Male vs Female)	0.68	0.29	1.58	0.37	—	—	—	—
Child-Pugh class (B vs. A)	1.43	0.88	2.32	0.15	—	—	—	—
BCLC stage (C vs. B)	2.10	1.22	3.62	**0.007**	2.52	1.42	4.47	**0.002**
Tumor size (≥5 cm vs. <5 cm)	1.83	1.07	3.14	**0.027**	—	—	—	—
Node (single vs. multiple)	1.26	0.73	2.17	0.41	—	—	—	—
AFP (≥200 ng/ml vs. <200 ng/ml)	2.58	1.31	5.06	**0.006**	2.01	1.01	3.99	**0.046**
HBsAg status (positive vs. negative)	1.69	0.68	4.20	0.26	—	—	—	—
Cirrhosis status (yes vs. no)	1.07	0.55	2.09	0.84	—	—	—	—
Metastasis (yes vs. no)	1.37	0.77	2.42	0.28	—	—	—	—
PVTT (yes vs. no)	1.41	0.82	2.42	0.22	—	—	—	—
TACE-RFA procedures	0.81	0.626	1.04	0.10	—	—	—	—
Time interval (<14 days vs. ≥14 days)	0.48	0.27	0.84	**0.010**	0.48	0.26	0.87	**0.016**
Radionics signatures (high-risk vs. low-risk)	3.26	1.79	5.93	**0.000**	2.98	1.60	5.51	**0.001**

Abbreviations: HBsAg, hepatitis B surface antigen; BCLC stage, Barcelona Clinic Liver Cancer; AFP, Alpha-fetoprotein; PVTT, Portal vein tumor thrombus; PFS, progression free survival. Note: The p *value marked bold indicated statistical significance.*

that is generated for each sample image segment as different directions. A Rad-score based on the eight features was calculated for each patient. The cut-off of the Rad-score was calculated, and the patients were classified into high-risk and low-risk groups. Previous studies demonstrated that the Rad-score could predict the recurrence of HCC patients after TACE treatment. We also found that the AUCs for PFS of the radiomics signature of patients in the training and validation cohorts were as high as 0.83 and 0.81, respectively, indicating good sensitivity and specificity for PFS prediction. In addition, the PFS of the low-risk group was higher than that of the high-risk group, further confirming that radiomics signature could be an option for PFS evaluation of HCC patients treated with TACE and RFA.

The BCLC staging system and AFP level have been proven to be efficient in predicting the survival of liver cancer. Consistent with previous studies (Ren et al., 2019; Shimose et al., 2019), we also found that BCLC stage and AFP were related to the PFS of HCC patients treated with TACE and RFA by univariate and multivariate analysis. Other clinical factors such as tumour size, the presence of multifocal lesions, HBV status and neoplasm grade have been recommended for use in evaluating the short- and long-term outcomes of liver cancer. Studies have indicated that tumour size is an independent prognostic factor for recurrence in HCC patients receiving TACE with or without RFA (Sieghart et al., 2015; H. Liu et al., 2016). In contrast, a study by Peng (Z. W. Peng et al., 2013) showed that tumour size was not associated with recurrence-free survival of HCC. In this study, we also found that tumour size was excluded from the multivariate model and was not a prognostic factor for intermediate or advanced HCC. These discrepancies may be due to selection bias.

Studies have demonstrated that TACE shows synergistic effects with RFA in improving tumour response and survival in intermediate or advanced HCC patients (Z. W. Peng et al., 2013; P. Yuan et al., 2021; W. Liu et al., 2020). However, consensus regarding the optimal time interval between TACE

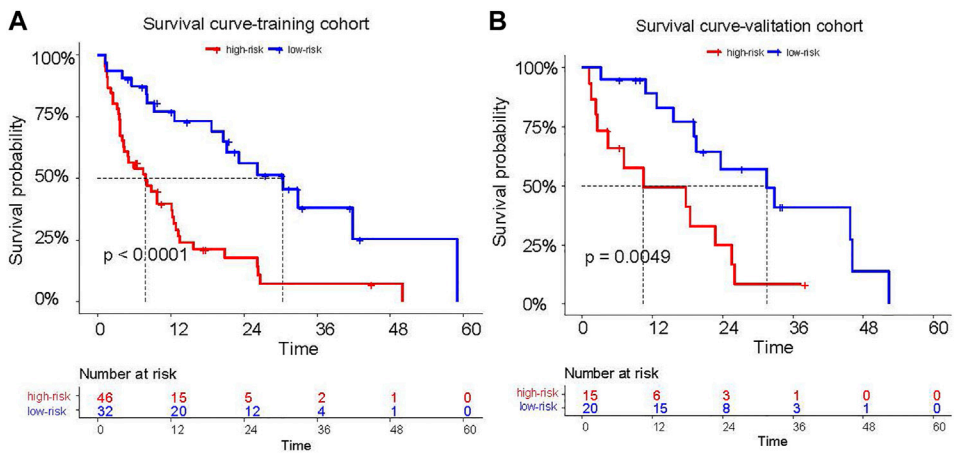

FIGURE 3 | The PFS of patients who were classified into high risk and low risk patients according to the cutoff of the Rad-score **(A,B)**.

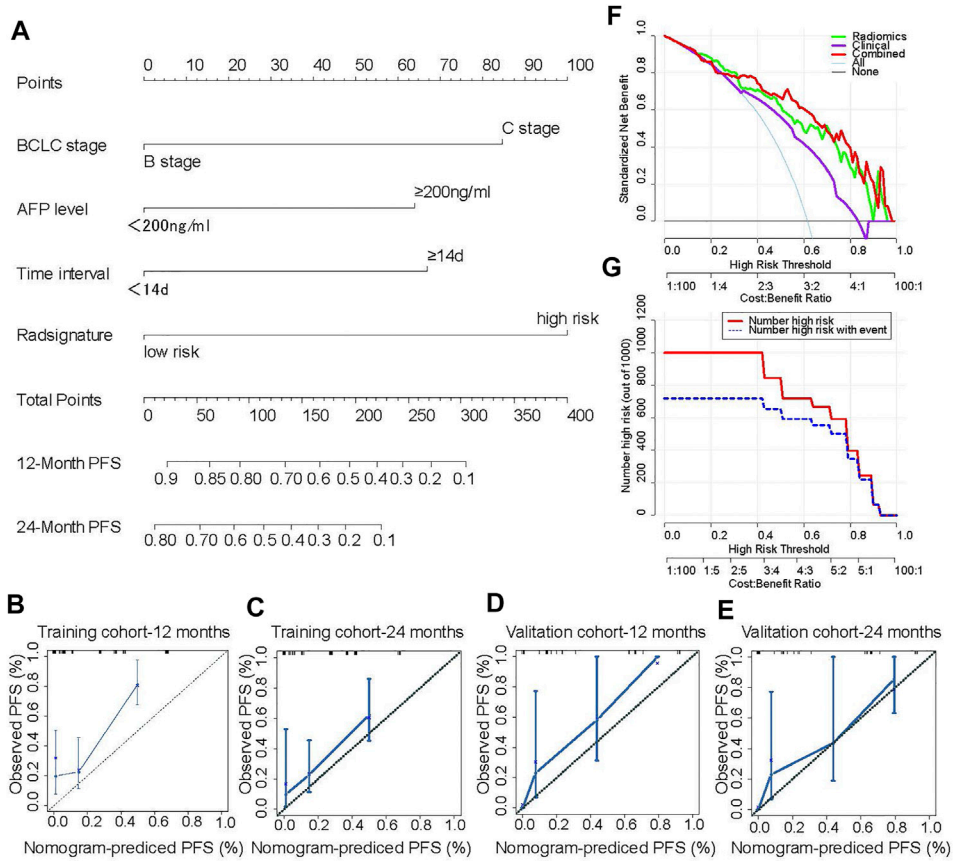

FIGURE 4 | A novel nomogram for individual PFS prediction. A novel nomogram based the radiomics signatures and clinical factors that was developed in the training cohort. The probability values of PFS for each HCC patient at 12 and 24 months were showed **(A)**. The corresponding calibration curve as well as the C-index were conducted to compared the probability of PFS between the nomogram prediction and actual observation in the training and validation cohorts **(B–E)**. The net benefit for each model was calculated by using decision curve, which the abscissa indicated the threshold probability and the ordinate represented the net benefit **(F)**. The consistency between nomogram predicted recurrence and actual recurrence was compared by using clinical impact curve **(G)**. The number of patients with high-risk for recurrence who were classified as high risk by the by the model at each threshold probability was calculated (red line) and the number of patients who truly suffer from recurrence was showed at each threshold probability (blue line).

and RFA for balancing efficacy and safety remains unclear. A shorter time interval might increase the potential risk of liver dysfunction. Conversely, a longer time interval favours recovery of liver function but may prolong the hospital stay and might decrease the local efficacy of the combination therapy. It is suggested that the use of a time interval between 1 and 30 days could amplify the synergistic effects of the combination therapy (Choe et al., 2014; Iezzi et al., 2016). In this study, we also showed that an interval of 14 days or less was a predictive factor for PFS, consistent with the study by Peng (Z. W. Peng et al., 2013).

A nomogram was used to provide individual predictions; the accuracy of the predictions was verified by calculating the C-index and AUC, and they were analysed based on the calibration curve, the decision curve and the clinical impact curve (Guo et al., 2019; C. Yuan et al., 2019; Song et al., 2020; Zheng et al., 2020). In this study, three factors (BCLC stage, AFP and time interval) with or without a radiomics signature were used to develop clinical and combined prediction models. The AUC of the combined model was higher than that of the clinical model in both the training and the validation cohorts, indicating better performance of the combined model. Thus, a nomogram incorporating the above four factors was constructed. The nomograms in the training and validation cohorts showed favorable consistency, with C-index values of 0.722 (95% CI: 0.657–0.786) and 0.821 (95% CI: 0.726–0.915), respectively. The decision curve suggested that when the threshold probability was approximately 18%, application of the nomogram with the radiomics signature alone for PFS prediction provided a greater benefit than the non-treatment or overall-treatment strategies. The probability of PFS based on a clinical model ranges from 38 to 82%. The net benefits of the radiomics signature alone and the combined nomogram were higher than that of the clinical model alone. Moreover, the outcome was verified by a clinical impact curve.

Admittedly, this study has certain limitations. As a single-centre and retrospective study, there was inevitable selection bias. Almost half of HCC patients in this study were in BCLC stage C, which have great impact on the outcome owing to the fact that the first-line treatment for these patients should be systemic therapy rather than the combination of TACE and RFA. The PFS evaluation did not comprehensively reflected the overall biological behaviours of these patients, especially who with extrahepatic metastasis. A more accurate evaluation for these patients received TACE and RFA treatment should be further investigated in future study. Besides, the sample size was relatively small, and larger datasets and external validation are urgently needed to validate our findings. In the future, large-sample, multicentre cohorts will be examined to verify the prognostic significance of these radiomics signature.

CONCLUSION

This study provides a radiomics signature-based nomogram of PFS prediction for intermediate and advanced HCC patients treated with TACE and RFA. The radiomics signature was a prognostic risk factor, and the nomogram combined radiomics and clinical factors acts as a new strategy for predicting the PFS of intermediate and advanced HCC treated with TACE plus RFA. These results could be used to facilitate HCC treatment management.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of Lishui Hospital Zhejiang University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ZZ, JT, and JJ designed the research. SF and LZ conducted the study and wrote the first draft of the manuscript. LL, YX, FW, and WC helped conduct the study. XW helped analyse the data. JZ, MC, and QW helped with manuscript writing, editing, and critical evaluation. JJ supervised the project and approved the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmolb.2021.662366/full#supplementary-material

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Hepatic Resection *Versus* Transarterial Chemoembolization for Intermediate-Stage Hepatocellular Carcinoma: A Cohort Study

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Background: The selection criteria for hepatic resection (HR) in intermediate-stage (IM) hepatocellular carcinoma (HCC) are still controversial. We used real-world data to evaluate the overall survival (OS) in treatment with HR or transarterial chemoembolization (TACE).

Methods: In total, 942 patients with IM-HCC were categorized into the HR group and the TACE group. OS was analyzed using the Kaplan–Meier method, log-rank test, Cox proportional hazards models, and propensity score-matched (PSM) analysis. Curve smoothing was performed through the generalized additive model. The interaction test was performed to evaluate the impact of HR on OS concerning risk factors. Also, we used multiple imputation to deal with missing data.

Results: In total, 23.0% (n = 225) of patients received HR. At a median OS of 23.7 months, HR was associated with improved OS in the multivariate analysis [hazard ratio (HzR) = 0.45, 95%CI = 0.35–0.58; after PSM: HzR = 0.56, 95%CI = 0.41–0.77]. Landmark analyses limited to long-term survivors of ≥6 months, ≥1 year, and ≥2 years demonstrated better OS with HR in all subsets (all $p < 0.05$). After PSM analysis, however, HR increased the risk of death by 20% (HzR = 1.20, 95%CI = 0.67–2.15) in the subgroup of patients with lactate dehydrogenase (LDH) ≤192 U/L (p for interaction = 0.037). Furthermore, the significant interaction was robust between the LDH and HR with respect to the 1-, 3-, and 5-year observed survival rates (all $p < 0.05$).

Conclusion: HR was superior to TACE for intermediate-stage HCC in patients with LDH levels >192 U/L. Moreover, TACE might be suitable for patients with LDH levels ≤192 U/L.

Keywords: real-world study (RWS), lactate dehydrogenase (LD), surgical resection, liver cancer (LC), chemoembolization (TACE)

HIGHLIGHTS:

- Hepatectomy was superior to transarterial chemoembolization (TACE) for BCLC-B hepatocellular carcinoma (HCC).
- Hepatectomy increased 20% risk of death for LDH <192 U/L after matching.
- A significant interaction was robust between LDH and hepatectomy with respect to the 1-, 3-, and 5-year observed survival rates.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths worldwide and the fifth cause of death in China (1). According to the Barcelona Clinic Liver Cancer (BCLC) staging system, the most widely used scheme, patients with early-stage (stages 0 and A) cancer are suitable for hepatic resection (HR), while intermediate-stage (IM) HCC patients are recommended for transarterial chemoembolization (TACE) (2). Compared with conservative treatment for IM-stage (stage B) HCC, patients treated with TACE have better 2-year overall survival (OS) (3). After selecting the criteria of Bolondi et al. (4), it was shown that patients with stage B1 or B2 cancer have higher 5-year survival rates (21.4% *vs.* 13.9%) (5). Subsequently, the subgroup of IM-HCC patients who benefit from TACE was identified through numerous criteria, including the Assessment for Retreatment with TACE (ART) score (6), the alpha-fetoprotein (AFP), BCLC, Child–Pugh, and response (ABCR) score (7), and the albumin–bilirubin (ALBI) grade (8), among others. Although the highly selected HCC patients have a median survival of 51.5 months (9), the role of TACE is challenged by HR.

A meta-analysis including 18 high-quality studies was recently performed to compare the survival outcomes of 5,986 patients after HR and TACE. The authors found that both stage B and stage C patients showed significantly better OS for HR than for TACE (10). However, a controversial evidence has emerged that HR is superior to TACE only in the subgroup of IM-HCC patients with a lower mortality risk (11–15), such as those in BCLC stages B1/B2 (12, 13). Although the subgroup of IM-HCC patients has been selected using predictive models with a median overall survival (mOS) of 61.3 months, which patients are more suitable for HR is still controversial. Interestingly, Cucchetti et al. (16) performed a regret-based decision curve analysis (Regret-DCA) to choose HR or TACE for IM-HCC patients. In this study, HR should be offered to patients with a 3-year mortality risk <35%, but the optimal strategy (HR *vs.* TACE) is still unclear when the mortality risk is between 35% and 70%. Although numerous subgroups have been identified, more promising biomarkers are urgently needed in order to choose better therapy.

To deal with this issue, we conducted a real-world propensity score-matched cohort study to compare HR and TACE in the treatment of intermediate-stage HCC.

METHODS AND PATIENTS

Patient Selection

The clinical and biological data in our study had been previously published in full (17). In this study, we mainly focus on the derivation cohort from the Sun Yat-sen University Cancer Center (SYSUCC) between January 2007 and May 2012. Details of the inclusion criteria are shown in **Supplementary Figure S1**. A total of 979 patients were included in the derivation cohort. In this cohort, 37 (3.8%) patients were excluded for refusing to receive treatment, and 942 patients were included into the final analysis, with TACE (717/979, 73.2%) or surgical resection (225/979, 23.0%) as the first-line treatment. A total of 805 patients were afforded second-line treatments after the initial treatment at the second follow-up visit (n = 597 after TACE; n = 208 after HR). According to the decision of the multidisciplinary teams, second-line therapy for these 805 patients included ablative therapies (n = 66, 8.2%), surgical resection (n = 38, 4.7%), repeated TACE (n = 172, 21.4%), other therapies (n = 5, 0.6%), or best supportive care (n = 524, 65.1%).

The Ethics Committee of SYSUCC approved the study protocol (2017-FXY-129). Because this was a retrospective study, informed consent was waived.

Diagnosis, Treatment, and Follow-Up

For patients treated with HR, the HCC diagnosis was confirmed by histopathological examination of surgical samples. In contrast, for the patients receiving TACE, the diagnosis was established by the combination of the serum level of alpha-fetoprotein (AFP; over 400 ng/ml) and clinical imaging, which included ultrasonography, computed tomography, or magnetic resonance imaging. If the diagnosis was uncertain based on imaging and the AFP level, a needle biopsy was performed.

Based on the decisions of the multidisciplinary teams, the optimal treatment plan was adopted for each HCC patient. The indications for HR in IM-HCC patients were appropriate residual liver volume determined by computed tomography. For patients without cirrhosis, 30% remnant liver volume after HR was considered adequate. However, for those with chronic hepatitis, cirrhosis, and severe fatty liver, the remnant volume should be more than 50%. Liver resection should not be carried out among intermediate or advanced cirrhosis patients and those with poor liver function (Child–Pugh C). Patients who satisfied the indications for HR were treated by surgical resection, unless the patient requested TACE.

During the initial treatment period, for the first 2 years, patients were followed up every 2 or 3 months to check whether complete remission was achieved. The frequency gradually decreased to every 3–6 months after 2-year remission.

Variables and Definition

Patients were stratified into a hepatic resection (HR) group and a transarterial chemoembolization (TACE) group. HR was defined as surgical therapy for the lesions in hepatic segments or lobes. Clinically, patients with good liver function and less tumor loading are usually suitable for HR. TACE was defined as

chemoembolization of the hepatic artery. The categorical variables consisted of gender, Child–Pugh class (A or B), intrahepatic tumor number (three or less or more than three), and both lobes with lesions (no or yes). Continuous variables, such as age, the diameter of the main tumor, AFP, C-reactive protein (CRP), LDH, hemoglobin (Hgb), white blood cell (WBC) count, and platelet (PLT) level, were also regarded as categorical variables. AFP and PLT were transformed into the Log_{10} scale because of their left skewness. All variables were examined at baseline before any anticancer treatment. The endpoint of interest was OS, which was defined as the time from diagnosis to death by any cause. BCLC stage B and CNLC (China Liver Cancer staging) HCC were defined as follows (18, 19):

BCLC stage B: Two to three lesions, at least one of more than 3 cm in diameter, or more than three lesions of any diameter. Eastern Cooperative Oncology Group (ECOG) PS 0 and Child–Pugh class A or B. Without blood vessel invasion and extrahepatic metastases.

CNLC stage IIa: Two to three lesions, of which at least one is more than 3 cm in diameter. ECOG PS 0–2 and Child–Pugh class A or B. Without blood vessel invasion and extrahepatic metastases.

CNLC stage IIb: More than three lesions of any diameter. ECOG PS 0–2 and Child–Pugh class A or B. Without Blood vessel invasion and extrahepatic metastases.

Statistical Analyses

To compare differences in the baseline characteristics between the HR and TACE groups, we compared the categorical variables using the chi-square test and the continuous variables using the Mann–Whitney test.

Firstly, survival was calculated using the Kaplan–Meier method, and univariate comparisons were performed using the log-rank test and unadjusted Cox models. Also, multivariable Cox proportional hazards models were adjusted for factors such as the Child–Pugh class, the diameter of the main tumor, location of lesions, intrahepatic tumor number, AFP, LDH, and the PLT level.

Subsequently, to account for potential biases favoring the administration of HR to patients with more favorable baseline prognoses, sequential landmark analyses were performed to evaluate survival with HR or TACE for patients with a minimum of ≥6 months, ≥1 year, and ≥2 years survival from diagnosis. Interaction and stratified analyses were performed for the covariates selected *a priori*, including the Child–Pugh class, diameter of the main tumor, location of lesions, intrahepatic tumor number, CNLC stage, AFP, LDH, and PLT level. To further explore the interaction, curve smoothing was performed between LogLDH and the observed mortality at 1, 3, and 5 years through a generalized additive model.

Sensitivity Analysis

Finally, we applied three approaches to evaluate the core results in a sensitivity analysis. To minimize potential bias, propensity score (PS)-matched analyses were performed to compare the outcomes of TACE and HR. One-to-one matching (TACE *vs.* HR) without replacement was completed using the nearest-neighbor match on the logit of the PS (derived from age, diameter of the main tumor, location of lesions, intrahepatic tumor number, AFP, Hgb, LDH, WBC, CRP) (all $p < 0.05$ in **Table 1**). The caliper width was 0.02 times the standard deviation of the logit of the PS.

We also used multiple imputation (MI) to maximize statistical power and eliminate bias, which may occur if the confounders with missing data were excluded from the analysis. The MI was based on five replications and the Markov chain Monte Carlo method in the MI procedure in R to account for missing data on Child–Pugh class, diameter of the main tumor, location of lesions, intrahepatic tumor number, PLT, AFP, and LDH. We then created an MI cohort to perform sensitivity analyses using complete-case analysis.

To eliminate the effects of ablative therapies and surgical resection on the second-line treatment, we built a secondary cohort based on the MI cohort without those therapies. All the multivariable Cox analyses mentioned above were repeated in the PS, MI, and secondary cohorts.

Statistical analysis was performed using Empower (X&Y Solutions, Inc., Boston, MA, USA; www.empowerstats.com) and R software (version 3.4.3). A p-value <0.05 was considered significant.

RESULTS

Descriptive Characteristics

After excluding those who refused to receive treatment ($n = 33$), a total of 942 HCC patients were included in the derivation cohort: 563 patients (59.8%) with CNLC stage IIb (480 patients for TACE and 83 patients for HR) and 379 patients (40.2%) with stage IIa (237 patients for TACE and 142 patients for HR). All patients had good performance status (ECOG PS 0). After first-line treatment with TACE, 46 of 597 patients (6.6%) had invasion of the portal vein or its branch ($n = 38$), hepatic veins ($n = 6$), or of the vena cava/atrium ($n = 2$) and 53 patients (8.9%) had distant metastasis, while 36 patients (6.0%) showed lymph node metastasis at the second follow-up visit.

In the derivation cohort, patients with HR were younger, had shorter diameter of the main tumor, lower hematological indicators (AFP, CRP, Hgb, LDH, and WBC), less frequent intrahepatic tumor number, and with lesions of both lobes (all $p < 0.05$), which are shown in **Table 1**. The majority of the patients (825/942, 87.6%) had hepatitis B virus (HBV) infection, which was treated with nucleos(t)ide analog therapy. The difference in the HBV infection rates was not significant between the HR and TACE groups.

Survival Analysis for the Entire Cohort

As shown in **Figure 1**, the mOS for the entire cohort was 23.7 months (95%CI = 20.4–27.2 months). The mOS rates were 18.5 months (95%CI = 16.9–20.3 months) for the TACE group *versus* 67.4 months (95%CI = 46.7–NA) for the HR group ($p < 0.0001$). After PS matching, the difference in the mOS rates between the TACE (29.9 months, 95%CI = 22.5–38.9) and HR (67.4 months, 95%CI = 44–NA) groups was still significant ($p < 0.0003$).

TABLE 1 | Baseline characteristics between the transarterial chemoembolization (TACE) and hepatic resection (HR) groups in the derivation cohort.

	Treatment		*p*-value
	TACE (*n* = 717)	HR (*n* = 225)	
Age (years)	53.9 ± 12.3	50.9 ± 12.6	0.001
Gender			0.802
Male	654 (91.2%)	204 (90.7%)	
Female	63 (8.8%)	21 (9.3%)	
HBV infection			0.132*
No	18 (2.8%)	2 (1.0%)	
Yes	622 (97.2%)	203 (99.0%)	
Child–Pugh class			0.302
A	613 (85.5%)	186 (82.7%)	
B	104 (14.5%)	39 (17.3%)	
Diameter of main tumor (cm)	7. 5 ± 3.8	6.4 ± 2.8	<0.001
Location of lesions			<0.001
Unilobar	254 (35.4%)	148 (65.8%)	
Bilobar	463 (64.6%)	77 (34.2%)	
Intrahepatic tumor number			<0.001
≤3	237 (33.1%)	142 (63.1%)	
>3	480 (66.9%)	83 (36.9%)	
AFP (ng/ml)			0.014
<25	180 (26.5%)	75 (35.2%)	
≥25	500 (73.5%)	138 (64.8%)	
CRP (mg/L)			<0.001
<10	318 (45.4%)	72 (32.4%)	
≥10	382 (54.6%)	150 (67.6%)	
Hgb (g/L)			<0.001
<120	148 (20.8%)	71 (31.6%)	
≥120	562 (79.2%)	154 (68.4%)	
LDH (U/L)			<0.001
<245	356 (50.1%)	152 (67.6%)	
≥245	354 (49.9%)	73 (32.4%)	
WBC (10^9/L)			<0.001
<11	611 (86.9%)	162 (74.0%)	
≥11	92 (13.1%)	57 (26.0%)	
PLT (10^9/L)			0.249
<150	381 (53.7%)	111 (49.3%)	
≥150	328 (46.3%)	114 (50.7%)	

Numbers that do not add up to 942 are attributable to missing data. Chi-square test was performed for categorical measures and the Kruskal–Wallis test for continuous measures. HBV, hepatitis B virus; AFP, alpha-fetoprotein; CRP, C-reactive protein; Hgb, hemoglobin; LDH, lactate dehydrogenase; WBC, white blood cell; PLT, platelet.
**Fisher's exact probability test.*

In the univariable analysis focusing on the entire cohort (**Table 2**), the Child–Pugh class (*vs.* A: HzR = 1.28, 95% CI = 1.01–1.62), diameter of the main tumor (*vs.* <5: HzR = 2.28, 95%CI = 1.86–2.80), location of lesions (*vs.* unilobar: HzR = 1.50, 95%CI = 1.26–1.79), intrahepatic tumor number (*vs.* ≤3: HzR = 1.55, 95%CI = 1.30–1.86), AFP level (*vs.* <25: HzR = 1.63, 95%CI = 1.33–2.00), LDH level (*vs.* <245; HzR = 1.61, 95% CI = 1.36–1.92), and the PLT level (*vs.* <150: HzR = 1.33, 95% CI = 1.12–1.57) were significantly associated with survival (all $p < 0.05$). These variables were included in further analyses.

Subsequently, all seven variables were included in the multivariable analysis shown in **Table 3**. In model I, the adjusted hazard ratio (aHR) was 0.43 (95%CI = 0.34–0.55) for liver resection compared to TACE. To explore the nonlinearity of the confounding factor, the diameter of the main tumor, LogAFP, LDH, and LogPLT were regarded as continuous variables in model II. Compared with TACE, hepatectomy reduced the risk of death by 55% (aHR = 0.45, 95%CI = 0.35–0.58). After PS matching, hepatic resection was still superior to TACE (aHR = 0.56, 95%CI = 0.41–0.77).

Sequential landmark analysis revealed statistically significant improvement in OS with HR for patients surviving over 6 months (HzR = 0.45, 95%CI = 0.35–0.58), 1 year (HzR = 0.46, 95%CI = 0.34–0.62), and 2 years (HzR = 0.52, 95%CI = 0.33–0.79) (**Figure 2** and **Supplementary Table S2**). In the stratified analyses (**Figure 3** and **Supplementary Tables S3** and **S4**), the magnitude of the association between HR and better survival was more significant for patients with higher LDH (*vs.* the bottom tertile; p for interaction = 0.006) and higher PLT (*vs.* the bottom tertile; p for interaction = 0.037) levels. After PS matching, however, only for patients with higher LDH levels was there a significant interaction. In the subgroup of patients with LDH <192 U/L (bottom tertile), HR increased the risk of death by 20% (HzR = 1.20, 95%CI = 0.67–2.15). The HzRs were 0.50 (95%CI = 0.30–0.84) and 0.26 (95%CI = 0.14–0.47) in the subgroups of middle tertile (192 < LDH < 255) and top tertile

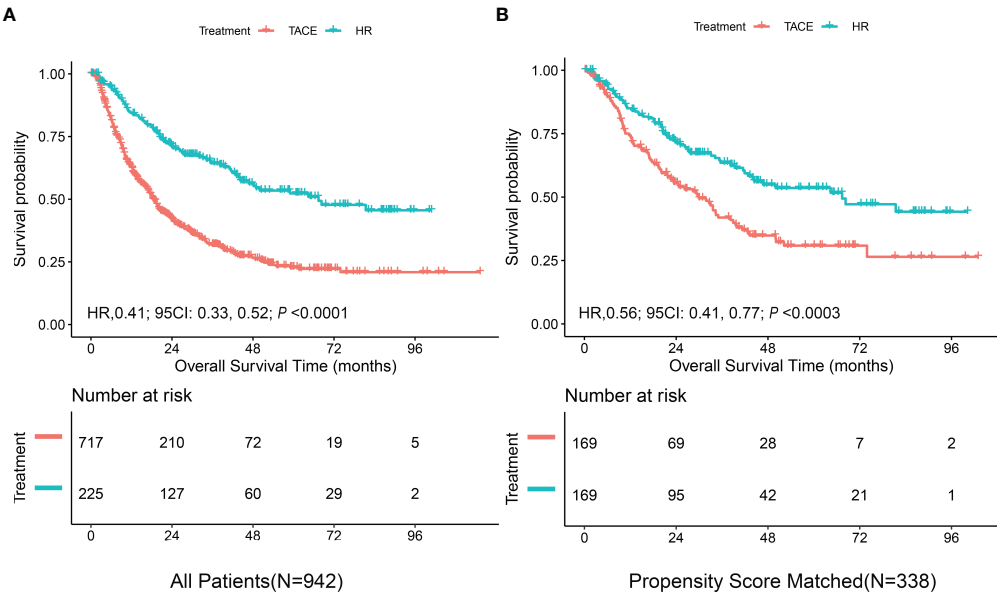

FIGURE 1 | Kaplan–Meier curves of overall survival in the derivation cohort stratified by hepatic resection (HR) and transarterial chemoembolization (TACE). **(A)** All patients. **(B)** Propensity score-matched patients.

(LDH ≥ 255), respectively. No significant interactions were observed between the effects of TACE and Child–Pugh class, diameter of the main tumor, location of lesions, intrahepatic tumor number, CNLC stage, and AFP.

Sensitivity Analysis

After MI, HR remained associated with better OS using multivariable Cox regression on the imputed dataset (**Table 3**). The aHRs were 0.44 (95%CI = 0.35–0.56) for model I and 0.47 (95%CI = 0.37–0.60) for model II. Furthermore, the cohort results were still consistent in the MI cohort after excluding the patients with liver resection and ablative therapy as second-line treatments (**Table 3**).

After PS matching of the dataset of derivation cohort, there were no significant differences between the HR and TACE groups (both groups, $n = 169$), as shown in **Supplementary Table S1** and **Figure S2**. The median survival in hepatic resection patients was 67.4 months (95%CI = 44–NA) and that in TACE patients was 29.9 months (95%CI = 22.5–38.9 months). Compared with TACE, liver resection continued to be associated with improved OS (HzR = 0.56, 95%CI = 0.41–0.77, $p < 0.0003$) (**Figure 1B**). The C-statistic of the receiver operating characteristic (ROC)-calculated PS was 0.66 (95%CI = 0.60–0.72).

Besides, the 1-, 3-, and 5-year observed survival rates were 76.9%, 52.7%, and 46.7% for the TACE group and were 85.8%, 68.6%, and 63.3% for the HR group, respectively. When the LDH level was <192 U/L, however, the mortality rates for HR patients were 2.89 times (95%CI = 0.71–11.81), 1.20 times (95% CI = 0.54–2.65), and 1.22 times (95%CI = 0.57–2.62) *versus* those in the TACE group at 1, 3, and 5 years (**Supplementary Table S4**). The significant interaction was robust between the LDH level and HR concerning the 1-, 3-, and 5-year observed survival rates (all $p < 0.05$) (see **Figure 4** and **Supplementary Table S5**).

DISCUSSION

In this large-scale, real-world data, we found that the OS for HR was significantly better than that for the TACE counterpart, which was consistent with previous literature (10, 20). Interestingly, Toshifumi et al. (11) also reported that liver resection reduced the risk of death by 44% after PS matching (HzR = 0.56). Notably, this finding remained marked after adjusting for crucial clinical confounders. When the LDH level increased, the magnitude of the association between liver resection and better survival was more significant. After PS matching, however, hepatic resection was associated with worse survival compared with TACE, but not significantly. To the best of our knowledge, this is the first observation of a significant interaction between the effect of HR and the LDH level.

TACE had been recommended as the first-line treatment for unresectable IM-HCC (18). However, whether surgery should be recommended for resectable BCLC-B HCC patients with good liver functional reserve remains a great controversy. In clinical practice from the Asia-Pacific region (21), intrahepatic lesions of more than three tumors, both lobes with tumors, or satellite nodules were not contraindicated for surgical resection of multinodular HCC. Based on the tumor burden, numerous subgroups (11–14) had been identified for the selection of favorable treatments. The previous study showed that a higher LDH level was associated with worse outcomes after hepatectomy or TACE (22). A correlation was also

TABLE 2 | Univariate analysis of prognostic factors in the derivation cohort.

	Statistics	Death
Age (years)		
<55	465 (49.36%)	1
≥55	477 (50.64%)	0.95 (0.80–1.13)
Gender		
Male	858 (91.08%)	1
Female	84 (8.92%)	1.15 (0.85–1.56)
Child–Pugh class		
A	799 (84.82%)	1
B	143 (15.18%)	1.28 (1.01–1.62)
Diameter of main tumor (cm)		
<5	300 (31.85%)	1
≥5	642 (68.15%)	2.28 (1.86–2.80)
Lesions of lobe		
Unilobar	402 (42.68%)	1
Bilobar	540 (57.32%)	1.50 (1.26–1.79)
Intrahepatic tumor number		
≤3	379 (40.23%)	1
>3	563 (59.77%)	1.55 (1.30–1.86)
AFP (ng/ml)		
<25	255 (28.56%)	1
≥25	638 (71.44%)	1.63 (1.33–2.00)
CRP (mg/L)		
<10	390 (42.30%)	1
≥10	532 (57.70%)	1.19 (0.99–1.41)
Hgb (g/L)		
<120	219 (23.42%)	1
≥120	716 (76.58%)	0.98 (0.80–1.20)
LDH (U/L)		
<245	508 (54.33%)	1
≥245	427 (45.67%)	1.61 (1.36–1.92)
WBC (10^9/L)		
<11	773 (83.84%)	1
≥11	149 (16.16%)	1.06 (0.85–1.34)
PLT (10^9/L)		
<150	492 (52.68%)	1
≥150	442 (47.32%)	1.33 (1.12–1.57)

Numbers that do not add up to 942 are attributable to missing data.
AFP, alpha-fetoprotein; CRP, C-reactive protein; Hgb, hemoglobin; LDH, lactate dehydrogenase; WBC, white blood cell; PLT, platelet.

demonstrated between high serum LDH levels and a high tumor volume, a high percentage of necrosis, or an aggressive phenotype for gastric and pancreatic cancer (22, 23). In this study, we found a subgroup in which HR was superior to TACE for IM-HCC: those with LDH levels >192 U/L. Its underlying mechanism is still unclear, and one possible reason might be that surgery reduced the recurrence risk by removing larger lesions with a more aggressive phenotype.

Our study has some strengths. Firstly, we created a propensity score-matched cohort to minimize potential bias. Secondly, our

TABLE 3 | Hepatic resection [*vs.* transarterial chemoembolization (TACE)] and multivariate hazard ratios of overall survival with 95% CIs in Barcelona Clinic Liver Cancer (BCLC) stage B hepatocellular carcinoma (HCC).

	N[a]	Not adjusted	Model I[b]	Model II[a]
Before PS matching	522/876	0.41 (0.33–0.52)	0.43 (0.34–0.55)	0.45 (0.35–0.58)
After MI	553/942	0.41 (0.37–0.46)	0.44 (0.35–0.56)	0.47 (0.37–0.60)
Minus (HR+AT)[c]	382/701	0.39 (0.31–0.51)	0.42 (0.32–0.55)	0.45 (0.34–0.60)
After PS matching	157/338	0.56 (0.41–0.77)	–	–

Numbers that do not add up to 942 are attributable to missing data.
PS, propensity score; MI, multiple imputation; HR, hepatic resection; AT, ablative therapy
[a]*This model was adjusted for Child–Pugh class (A or B), diameter of main tumor (in centimeters), location of lesions (unilobar or bilobar), intrahepatic tumor number (three or less or more than three), LogAFP (in nanograms per milliliter), LDH (in units per liter), and LogPLT (10^9/L).*
[b]*This model was adjusted for Child–Pugh class (A or B), diameter of main tumor (<5 or ≥5 cm), location of lesions (unilobar or bilobar), intrahepatic tumor number (three or less or more than three), and AFP (<25 or ≥25), LDH (<245 or ≥245), and PLT (<150 or ≥150) levels.*
[c]*This cohort excluded patients with HR and AT as second-line treatments after MI.*

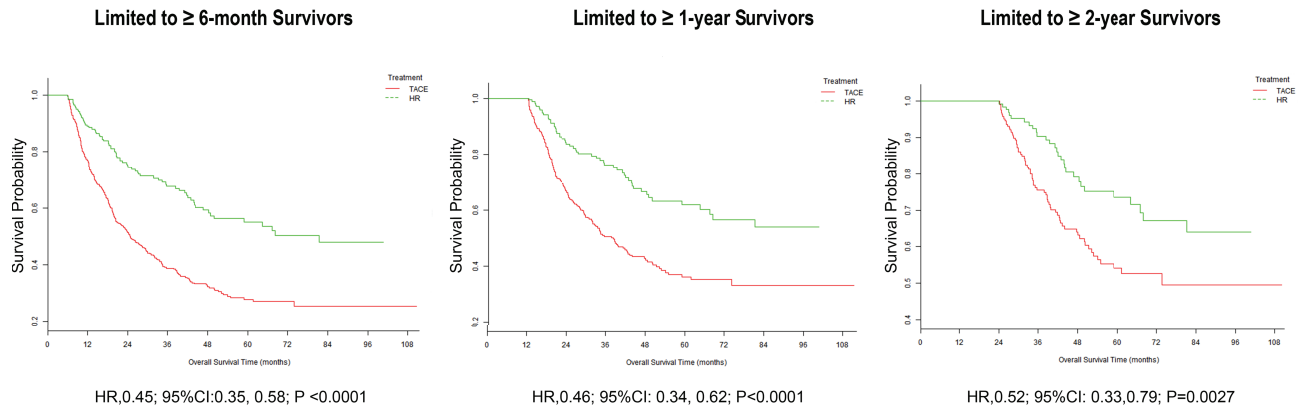

FIGURE 2 | Landmark analyses of overall survival for long-term (≥6 months, ≥1 year, and ≥2 years) survivors.

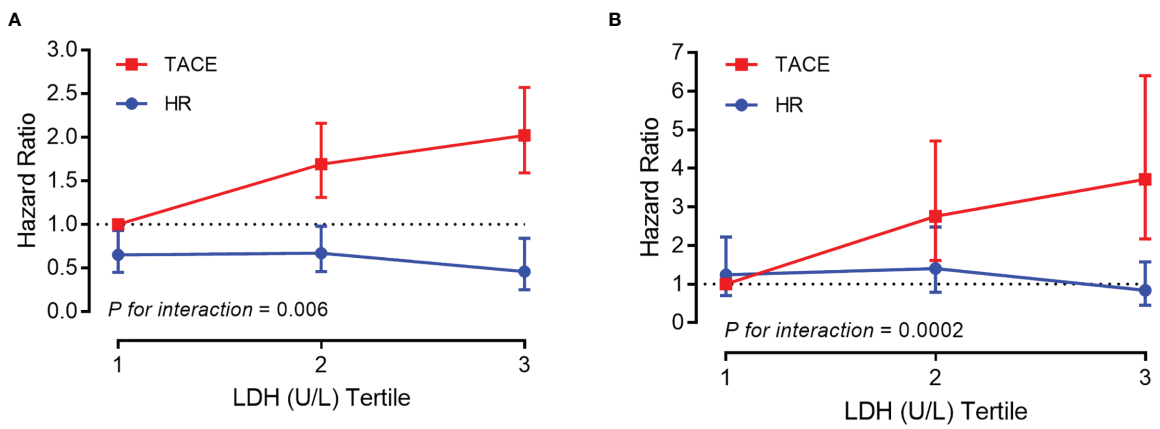

FIGURE 3 | Association between overall survival and platelet count/lactic dehydrogenase stratified by tertile before **(A)** and after **(B)** propensity score (PS) matching [*vs.* transarterial chemoembolization (TACE) in the bottom tertile].

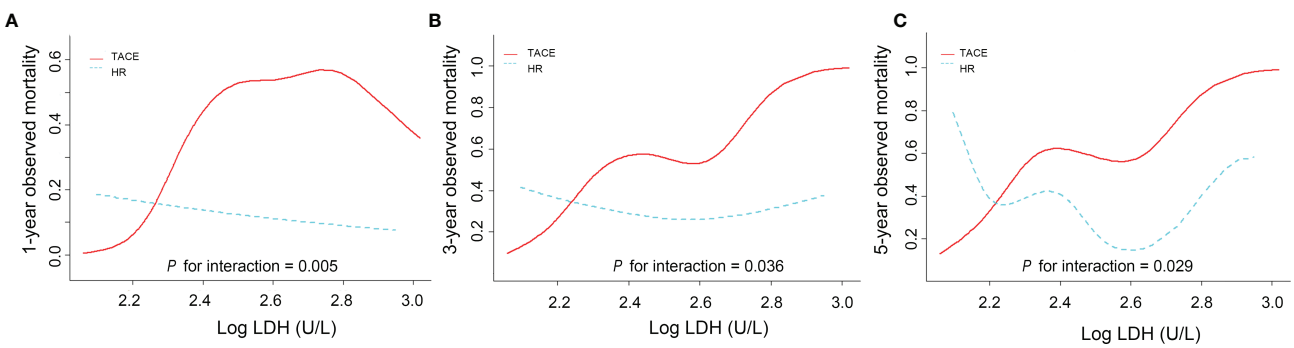

FIGURE 4 | Curve smoothing between LogLDH and observed mortality at 1 **(A)**, 3 **(B)**, and 5 **(C)** years stratified by hepatic resection (HR) and transarterial chemoembolization (TACE).

study provided new insights into the selection of appropriate HCC patients for treatment with surgical resection. Hematological indicators, such as the LDH level, should be promising biomarkers.

Our study also has several limitations. Firstly, this is a retrospective cohort with real-world data. Residual bias and unmeasured confounders were unavoidable, even if we had used PS matching to eliminate inherent differences between the two groups. The results after PS matching and MI revealed that the bias from confounders and missing data might have overestimated the advantage of surgical resection. On the contrary, this would make the benefit from TACE treatment more significant in those with LDH levels ≤192 U/L. Secondly, because this is a secondary analysis, the surgical program (radical *vs.* palliative and laparoscopic *vs.* open) was unclear. Differences in the cirrhosis rates, portal hypertension, and the MELD (Model for End-Stage Liver Disease) scores between the two groups were unknown, although the PS matching results were consistent. Thirdly, this study focused on populations from East Asia with hepatitis B between January 2007 and May 2012. Thus, our conclusions might not be applicable to Western populations. With the development of more aggressive surgical treatments, the cutoff value of LDH should be further explored. In the future, the interaction between the effect of HR and the LDH levels should be validated in a randomized control trial and in larger-scale real-world data in various populations.

CONCLUSION

Hepatic resection was superior to TACE for intermediate-stage HCC in patients with LDH levels >192 U/L. Moreover, TACE might be suitable for patients with LDH levels ≤192 U/L.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Sun Yat-sen University Cancer Center, which approved the study protocol (2017-FXY-129). Written informed consent for participation was not required for this study, in accordance with the national legislation and the institutional requirements.

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 the 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 agreed to be accountable for all aspects of the work. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.618937/full#supplementary-material

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Superselective Transarterial Chemoembolization for Unresectable or "Ablation Unsuitable" Hepatocellular Carcinoma in the Caudate Lobe: A Real World, Single-Center Retrospective Study

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Objectives: To analyze the clinical outcomes of Transarterial chemoembolization (TACE) for unresectable or "ablation unsuitable" hepatocellular carcinoma (HCC) in the caudate lobe (CL) found at initial presentation in clinical practice.

Methods: Fifty-eight patients with HCC-CL undergoing conventional TACE from January 2015 to January 2020 were enrolled in our medical center. Overall survival (OS), progression-free survival (PFS), tumor response rate and major complication rates were analyzed. Multivariate analyses for potential clinical and radiologic factors were performed by using the Cox proportional hazard model.

Results: The median OS was 23 months (95%CI: 18.1-27.9), and the median PFS was 11 months (95%CI: 7.4-14.6). The 1-, 3-, and 5-years OS rates were 66.5%, 31.9% and 15.7%, respectively. The 0.5, 1-, and 3-years PFS rates were 60.3%, 44.5% and 6.3%, respectively. Objective response rate was 53.4% and disease control rate was 79.3%. The most serious complication was bile duct injury, with an incidence of 3.4%. Multivariable analysis revealed that total bilirubin, Barcelona Clinic Liver Cancer stage, nonselective chemoembolization and TACE session were four significant factors associated with OS.

Conclusions: Superselective TACE treatment might be associated with better survival benefits in unresectable or "ablation unsuitable" HCC in the CL without macroscopic vascular invasion (MVI) and adequate liver function, compared with the non-selective TACE group, and should be considered as an important reliable therapy for surgeons and interventional radiologists.

Keywords: transarterial chemoembolization, hepatocellular carcinoma, caudate lobe, overall survival, progression-free survival

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common malignancy, with incidence and mortality rates ranking sixth and third in the world, respectively (1). Cases of HCC arising or involving the caudate lobe (HCC-CL) are relatively rare, and its incidence is reported to be between 1.9% and 12.4% (2–7). Although the implementation of surveillance programs for high-risk populations and advances in imaging diagnostic technology have increased the diagnosis rate of HCC-CL (8, 9), about 80% of patients have tumors that are unresectable owing to either severe hepatitis-related cirrhosis or tumor invasion of the intrahepatic vessels (10). Surgical resection and percutaneous radiofrequency ablation (RFA) can be used as a curative treatment for HCC-CL (11, 12). However, resection of the HCC-CL is a challenging task for accomplished surgeons owing to the tumor's deep location that is adjacent to the inferior vena cava and hepatic vein and narrow surgical margin (2, 3, 6, 13, 14). Radiofrequency therapy cannot be performed safely under certain circumstances, such as thermal injury of adjacent structure, heat sink effect (near major vessels), and limited tumor necrosis range (15). Although some radical treatments or combination treatments have favorable clinical benefits in several studies (16–18), the five-year recurrence rate of HCC is still high.

Therefore, intra-arterial therapy which causes tumor necrosis through the occlusion of blood flow and the slow release of chemotherapeutic drugs into tumors is the mainstay palliative treatment options recommended by the Barcelona Clinic Liver Cancer (BCLC) guidelines for unresectable or recurred HCC with large diameters or multiple intrahepatic lesions even for portal vein tumor thrombus in some Asia-Pacific regions (19–23). Kim HC et al. (24) reported that selective chemoembolization *via* the caudate artery for solitary caudate lobe HCC is possible in most patients and a critical factor in longer overall survival and progression free survival. Won Seok Choi et al. (25) demonstrated that most tumor-feeding arteries supplying HCCs in the caudate lobe could be found by C-arm CT. Although many studies (18, 26–28) have reported the efficacy of TACE, RFA or combined treatment on HCC-CL, the reported tumor diameter or size is often relatively small, which is inconsistent with real-world data, because most HCC are already in the middle and advanced stages when they are diagnosed (29). Therefore, the present retrospective study was conducted to evaluate the effectiveness and safety of transcatheter arterial chemoembolization (TACE) for HCC arising or involving the caudate lobe found at initial presentation in the real world.

Abbreviations: HCC, Hepatocellular carcinoma; CL, Caudate lobe; R-CL, Right-Caudate lobe; L-CL, Left-Caudate lobe; MVI, macroscopic vascular invasion; RFA, Radiofrequency ablation; BCLC, Barcelona Clinic Liver Cancer; TACE, Transarterial chemoembolization; ECOG, the Eastern Cooperative Oncology Group; mRECIST, Modified Response Evaluation Criteria in Solid Tumors; CR, Complete response; PR, Partial response; SD, Stable disease; PD, Progressive disease; ORR, Objective response rate;OS, Overall survival; PFS, Progression-free survival; CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; CT, Computed tomography; MR, Magnetic resonance; AFP, Alpha-fetoprotein; SD, standard deviation; CI, confidence intervals; HR, hazard ratio; EASL, European Association for the Study of the Liver; AASLD, American Association for the Study of Liver Disease; DSA, Digital subtraction angiography; HBV, Hepatitis B virus; VEGF, Vascular endothelial growth factor; HIF, Hypoxia inducible factor.

METHODS

Study Design and Patient Selection

This was a retrospective study and performed according to the guidelines of the Helsinki Declaration. This study was approved by Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology. The written informed consent was waived due to the retrospective nature of this study.

As a result of searching radiologic database of our hospital, a total of 924 consecutive patients had a tumor arising or invading the caudate lobe confirmed by imaging or biopsy in our medical center between January 2015 and January 2020. We finally included 58 patients whose target lesions were in the caudal lobe or the caudal lobe of the liver was violated that could be measured at initial chemoembolization. The patient enrolment and categorization flow chart were shown in **Figure 1**.

The inclusion criteria were as follows: (1) HCC was measurable and unresectable or "ablation unsuitable" arising or involving the caudal lobe, which means the tumor was located near the inferior vena cava and peripheral gastrointestinal tract and had limited ablation or resection range; (2) Liver function status at Child-Pugh class A or B; (3) the Eastern Cooperative Oncology Group

(ECOG) performance status of 0 or 1; (4) No severe coagulopathy or ascites (e.g., platelets ≥50,000/ml, prothrombin time ratio ≥ 50%); (5) No previous treatment; (6) Available medical records. Exclusion criteria were: (1) Incomplete clinical data; (2) Tumor thrombus in the main portal vein, inferior vena cava; (3) Extrahepatic metastasis at preprocedural imaging study; (4) Severe liver dysfunction (Child-Pugh class C); (5) Patients were received other combination therapy (**Figure 1**).

TACE Procedure

TACE was performed according to our institutional standard protocol and has been previously reported (30, 31). All operators had at least eight years of experience in performing TACE procedures. TACE was performed using transfemoral arterial access route with a micro-puncture system by placing a 5F vascular introducer (Cook, Bloomington, Indiana, USA) and celiac or superior mesenteric arteriography was carried out to assess the arterial anatomy, tumor supplying vessel and patency of the portal vein. A 2.6-Fr microcatheter (Terumo, Japan) was inserted into the tumor donor arteries as superselectively as possible to identify the staining and arteries feeding the target lesions. First, an emulsion of 5-20 mL lipiodol (Lipiodol Ultrafluido, Guerbet, France) mixed with 20-60 mg doxorubicin hydrochloride (Hisun Pharmaceutical Co. Ltd., Zhejiang, China) was injected into tumor feeding branch of the hepatic artery. Then the gelatin sponge particles (300–500 um, Cook, Bloomington, Indiana, USA) mixed with contrast material were administered into the tumor-feeding arteries until stasis of the arterial flow was achieved.

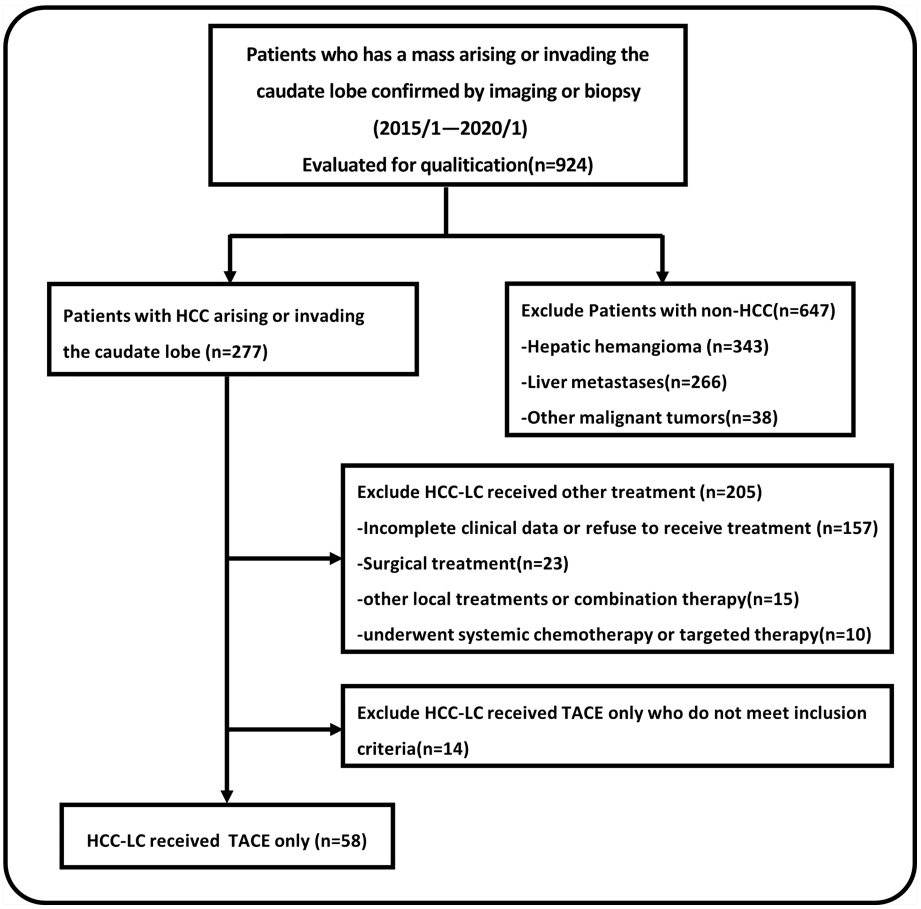

FIGURE 1 | Patient enrolment and categorization flow chart.

Definition and Evaluation of Data

Selective chemoembolization was defined as using microcatheter systems to catheterize each tumor's feeding blood vessel branches in segment or subsegment and transport chemotherapy drugs mixed with lipiodol-based regimens followed by embolic agents, and no residual tumor staining was present (32). Modified Response Evaluation Criteria in Solid Tumors (mRECIST) was used to evaluate treatment response by two interventional radiologists (33), that was carried out at the 1-1.5 month after the first TACE procedure and then every 2 or 3 months until the time of progression or death. Objective tumor regression (ORR) referred to (complete response) CR or PR (partial response). Disease control rate (DCR) was used to represent the portion of patients who reach CR+PR+SD (stable disease). The diagnosis of macroscopic vascular invasion (MVI) was based on standard radiological imaging prior to treatment based on liver vessel structure and prognosis for different location of vascular tumor thrombus, including portal and hepatic vein tumor and its branch thrombus (34). Overall survival (OS) and progression-free survival (PFS) were calculated for each patient from the date of the first TACE to the date of death or the last follow up, and to the date of tumor progression (intrahepatic recurrence or new intrahepatic or extrahepatic lesions developed) or the last follow up, respectively. When a residual or recurrent tumor was detected, decisions about additional treatment were made according to the recurrence pattern, underlying liver function, and overall clinical condition of the patient.

Adverse events and complications after therapy were identified and described according to the Society of Interventional Radiology Classification system for Complications by Outcome (35). Major complications were defined as events leading to death and disability.

Follow-Up and Repeated TACE

All patients were followed up until October 2020. Follow-up contents included laboratory tests, contrast-enhanced computed tomography (CT) or magnetic resonance (MR) imaging examination were performed 4-6 weeks after the treatment. Once local progression or intrahepatic metastasis occurs, palliative TACE treatment was given "on demand" until it is intolerable. Imaging (contrast-enhanced CT or MR) and laboratory examinations were performed every 2–3 months for patients, follow-up continued until the patient died or the end point of this study's follow-up.

Statistical Analyses

All statistical analyses were performed by using SPSS software (version 24.0; IBM, Armonk, New York). Quantitative data were expressed as mean ± standard deviation (SD), while qualitative data were represented by proportion. OS and PFS were plotted by using Kaplan–Meier method. Calculate 95% confidence intervals (CI) for median OS, median PFS, and hazard ratio (HR). Univariate analysis was performed using log-rank test, in which variables with P less than 0.1 were included in the multivariate analysis, which was implemented with the Cox proportional hazard regression model. All statistical tests were two tailed, and $P< 0.05$ regarded as significant difference.

RESULTS

Study Population and Patient Characteristics

From January 2015 to January 2020, a total of 924 patients have a tumor arising or invading the caudate lobe as a result of searching radiologic database of our hospital and 866 patients were excluded because they did not meet the study requirements, as shown in **Figure 1**. Among them, 277 patients were diagnosed as HCC that depended on the diagnostic criteria of the European Association for the Study of Liver (EASL) and the American Association for the Study of Liver Disease (AASLD) (11, 12). Prior to these patients undergoing initial TACE, the treatment strategy was recommended by the multidisciplinary tumor board. 157 cases were excluded because most cases were taken from the hospital outpatient system and imaging reports suggested the presence of HCC in the caudal lobe, but there was no hospitalization or the follow-up data missed. Finally, a total of 58 patients were included in this study, the detailed baseline clinical characteristics of the 58 patients were summarized in **Table 1**. In general, the majority of patients who underwent TACE had hepatitis B virus infection (81%), Child-Pugh A liver function (84.5%), with large tumor sizes (7.5 ± 4.0, range 2-16.5cm) and the middle and advanced patients (86.2% in total). Among these patients, 32 patients whose target lesions originated in the caudate lobe, and the tumors in the other 26 patients invaded the caudate lobe and were unresectable. Nearly half of patients with caudate lobe HCC have multiple blood supply (46.6%) and multiple tumors (44.8%). Superselective chemoembolization *via* a 2.6-Fr microcatheter was achieved in 49 (84.5%) of the 58 patients. Nonselective chemoembolization was performed in 9 (15.5%) patients because of failed catheterization of the tumor-feeding vessels that were not clearly indicated on digital subtraction angiography (DSA) and extraordinarily technical difficulty owing to the tumor-feeding vessels' small caliber or the acute angulation. **Supplementary Table 1** showed that there was no significant statistical difference ($P > 0.05$) in baseline data between the two groups except gender and total bilirubin. It means that the two groups are comparable without selection bias. Repeated TACE could continue to be used "on demand" when the multidisciplinary tumor board considered that TACE was promising for the control of intrahepatic lesions,

TABLE 1 | Baseline clinical characteristics of patients.

Characteristic	Patients with TACE treatment (No, %; Mean ± SD)
Gender	
Male	42 (72.4%)
Female	16 (27.6%)
Age (y)	55.4 ± 12.1
Hepatitis	
Hepatitis B	47 (81%)
Other	11 (19%)
Albumin (g/L)	37.8 ± 5.2
Total bilirubin (μmol/L)	20.7 ± 15.8
Platelet count (10^9/L)	175.7 ± 88.7
ALT(IU/L)	43.8 ± 31.6
AST (IU/L)	52.9 ± 41.7
Prothrombin time(s)	14.0 ± 1.9
ECOG	
0	54 (93.1%)
1	4 (6.9%)
AFP (ng/ml)	
>400	21 (36.2%)
≤400	37 (63.8%)
Liver cirrhosis	
Absent	26 (44.8%)
Present	32 (55.2%)
Ascites	
Absent	51 (87.9%)
Present	7 (12.1%)
BCLC stage	
A	8 (13.8%)
B	35 (60.3%)
C	15 (25.9%)
Child-Pugh score	
A	49 (84.5%)
B	9 (15.5%)
Macroscopic vascular invasion	
Yes	12 (20.7%)
No	46 (79.3%
Tumor size (cm)	
Mean ± SD	7.5 ± 4.0
Range	2-16.5
Number of tumors	
1	32 (55.2%)
>1	26 (44.8%)
Tumor-feeding artery	
Single	31 (53.4%)
Multiple	27 (46.6%)
Superselective embolization	
Yes	49 (84.5%)
No	9 (15.5%)
TACE sessions	
1	10 (17.2%)
2 or more	48 (82.8%)
Tumor location	
Spigel lobe	29 (50.0%)
Paracaval portion	23 (39.7%)
Caudate process	6 (10.3%)
Origin of tumor/tumor distribution	
CL	32 (55.2%)
R-CL	20 (34.5%)
L-CL	6 (10.3%)

SD, Standard deviation; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; PT, Prothrombin time; AFP, Alpha fetoprotein; ECOG, Eastern Cooperative Oncology Group; CL, Caudate lobe; R-CL, Right-Caudate lobe; L-CL, Left- Caudate lobe.

such as localized progression or metastasis in the liver. The average number of TACE sessions was 5 (1-15). The median follow-up period was 17.5 months (range, 1–65 months). Before the final follow-up, 49 (84.5%) patients had experienced varying degrees of disease progression. 21 (36.2%) patients were deemed TACE intolerable (extensive extrahepatic spread, diffuse liver metastasis), 15 received targeted drug therapy and 6 received the best supportive treatment in this circumstance. A total of 39 (67.2%) people died due to extensive metastases or liver failure during the observation period.

Treatment Response and Complications

The treatment response at the first follow-up CT or MR was CR in 6 patients (10.3%), PR in 25 patients (43.1%), and SD in 15 patients (25.9%). ORR was 53.4% and DCR was 79.3%. No treatment-related deaths occurred in this study. Two patients had bile duct related complications, with an incidence of 3.4%. Percutaneous biliary intervention has been needed in symptomatic patients. Common minor complications occurred in 18 patients (31.0%), including 15 patients (25.9%) with fever, 13 patients (22.4%) with elevated total bilirubin, 7 patients (12.1%) with elevated serum alanine aminotransferase or aspartic aminotransferase levels, 4 patients (6.8%) with abdominal pain, 6 patients (10.3%) with nausea and vomiting. These symptoms lasted 2–7 days and were relieved by symptomatic treatment before discharge. No other serious complications occurred.

Overall Survival and Progression-Free Survival Analysis

The median OS was 23 months (95%CI: 18.1-27.9). The median PFS was 11 months (95%CI: 7.4-14.6). The 1-, 3-, and 5-years OS rates were 66.5%, 31.9%, and 15.7%, respectively. The 0.5, 1-, and 3-years PFS rates were 60.3%, 44.5% and 6.3% respectively. Survival curves of patients were shown in **Figure 2**. The median OS was 27 months (95% CI 17.7 months, 36.3 months) for superselective chemoembolization in HCC-CL and 6 months (95% CI 3.1 months, 8.9 months) for non-selective chemoembolization (P = 0.001) (**Figure 3A**). Meanwhile, the median PFS in the two groups was 13 months (95% CI 8.1 months, 17.9 months) and 6 months (95% CI 4.6 months, 7.4 months), respectively (P = 0.032) (**Figure 3B**).

Prognostic Factors Affecting OS and PFS

The risk factors for OS and PFS were analyzed in the **Table 2**. Multivariate analyses identified TBIL (HR = 1.033, 95% CI: 1.004–1.063, P = 0.027), BCLC stage (HR = 6.796, 95% CI:

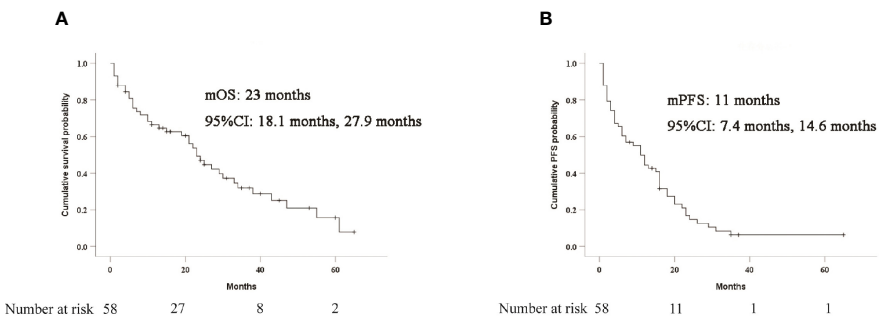

FIGURE 2 | Kaplan-Meier curves of survival outcomes after TACE treatment in all patients.

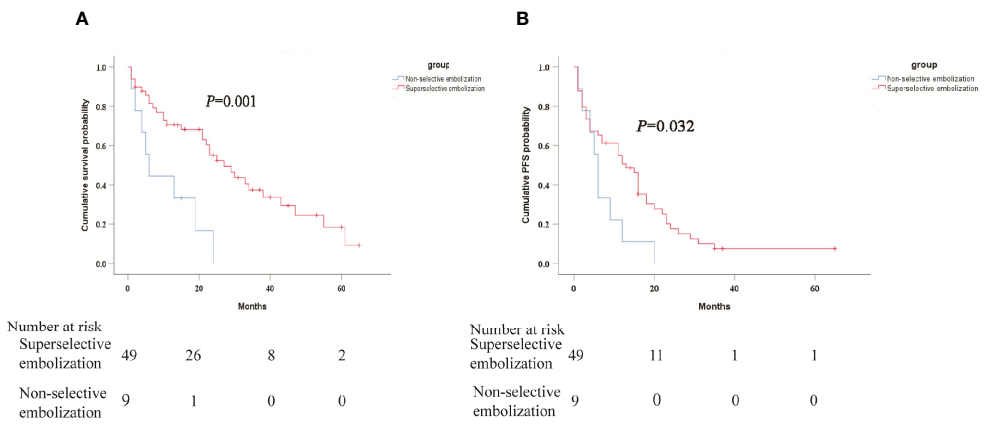

FIGURE 3 | Kaplan-Meier curves of subgroup analysis survival outcomes by selective chemoembolization.

TABLE 2 | Univariate analysis of prognostic factors for overall survival (OS) and progression-free survival (PFS).

Variables	OS		PFS	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Gender				
Male	1		1	
Female	0.954 (0.462, 1.970)	0.899	1.010 (0.544, 1.877)	0.974
Age (y)	0.975 (0.947, 1.003)	0.081	0.996 (0.971, 1.021)	0.729
Hepatitis				
Hepatitis B	1		1	
Other	0.575 (0.223, 1.482)	0.252	0.539 (0.241, 1.206)	0.133
Albumin (g/L)	0.970 (0.912, 1.030)	0.319	0.988 (0.936, 1.043)	0.662
Total bilirubin (μmol/L)	1.022 (1.001, 1.044)	0.045	1.012 (0.992, 1.033)	0.254
Platelet count (10^9/L)	1.001 (0.996, 1.005)	0.759	1.001 (0.997, 1.004)	0.602
ALT(IU/L)	1.010 (1.001, 1.019)	0.038	1.008 (0.999, 1.017)	0.074
AST (IU/L)	1.006 (1.000, 1.012)	0.056	1.004 (0.999, 1.009)	0.105
Prothrombin time, INR	1.028 (0.890, 1.188)	0.703	0.936 (0.816, 1.072)	0.339
ECOG				
1	1		1	
0	0.464 (0.107, 2.023)	0.307	0.416 (0.123, 1.402)	0.157
AFP (ng/ml)				
>400	1		1	
≤400	0.462 (0.241, 0.885)	0.020	0.424 (0.231, 0.777)	0.006
Liver cirrhosis				
Present	1		1	
Absent	1.188 (0.626, 2.255)	0.598	1.255 (0.719, 2.191)	0.424
Ascites				
Present	1		1	
Absent	1.106 (0.429, 2.848)	0.835	1.699 (0.701, 4.118)	0.240
BCLC stage				
A	1		1	
B	2.365 (0.710, 7.872)	0.161	2.868 (1.110, 7.405)	0.030
C	5.970 (1.614, 22.089)	0.007	6.196 (2.056, 18.670)	0.001
Child-Pugh score				
B	1		1	
A	0.725 (0.317, 1.660)	0.447	1.145 (0.536, 2.446)	0.727
Macroscopic vascular invasion				
Yes	1		1	
No	0.334 (0.157, 0.710)	0.004	0.396 (0.193, 0.811)	0.011
Tumor size (cm)	1.089 (1.005, 1.179)	0.036	1.093 (1.017, 1.174)	0.016
Number of tumors				
>1	1		1	
1	0.731 (0.387, 1.379)	0.333	0.504 (0.284, 0.893)	0.019
Tumor-feeding artery				
Multiple	1		1	
Single	0.976 (0.513, 1.854)	0.940	0.788 (0.453, 1.372)	0.400
Superselective embolization				
Yes	1		1	
No	3.530 (1.527, 8.163)	0.003	0.610 (0.347, 1.070)	0.085
Child-Pugh score				
A	1		1	
B	4.634 (1.346, 15.935)	0.015	2.156 (1.019, 4.562)	0.044
TACE sessions				
2 or more	1		1	
1	2.083 (0.939, 4.622)	0.071	1.232 (0.595, 2.550)	0.575
Tumor location				
Spigel lobe	1		1	
Paracaval portion	1.540 (0.771, 3.079)	0.221	1.547 (0.850, 2.814)	0.153
Caudate process	1.071 (0.362, 3.173)	0.901	1.261 (0.511, 3.109)	0.614
Origin of tumor				
CL	1		1	
R-CL	1.548 (0.789, 3.034)	0.203	1.513 (0.821, 2.789)	0.184
L-CL	1.350 (0.395, 4.609)	0.632	1.263 (0.483, 3.303)	0.634

HR, Hazard ratio; CI, Confidence interval; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; PT, Prothrombin time; AFP, Alpha fetoprotein; ECOG, Eastern Cooperative Oncology Group; CL, Caudate lobe; R-CL, Right-Caudate lobe; L-CL, Left- Caudate lobe.

1.200-38.482, P = 0.030), nonselective chemoembolization (HR = 5.512, 95% CI: 1.905-15.947, P = 0.002) and TACE session (HR = 3.998, 95% CI: 1.465–10.909, P = 0.007) as four significant factors associated with OS **(Table 3)**. Single tumor (HR = 0.439, 95% CI: 0.214–0.900, P = 0.025) was a unique factor for PFS in multivariate analysis **(Table 4)**.

TABLE 3 | Multivariate analysis of prognostic factors for overall survival (OS).

Variables	HR (95% CI)	*P* value
Age (y)	0.980 (0.950, 1.010)	0.190
Total bilirubin (μmol/L)	1.033 (1.004, 1.063)	0.027
ALT(IU/L)	0.997 (0.985, 1.010)	0.653
AST (IU/L)	1.002 (0.993, 1.012)	0.640
AFP (ng/ml)		
>400	1	
≤400	0.610 (0.254, 1.467)	0.270
BCLC stage		
A	1	
B	6.796 (1.200, 38.482)	0.030
C	6.543 (0.377, 113.401)	0.197
Macroscopic vascular invasion		
Yes	1	
No	0.391 (0.046, 3.323)	0.390
Tumor size (cm)	1.007 (0.905, 1.121)	0.894
Superselective embolization		
Yes	1	
No	5.512 (1.905, 15.947)	0.002
Child-Pugh score		
A	1	
B	0.511 (0.139, 1.879)	0.312
TACE sessions		
2 or more	1	
1	3.998 (1.465, 10.909)	0.007

HR, Hazard ratio; CI, Confidence interval.

TABLE 4 | Multivariate analysis of prognostic factors for progression-free survival. (PFS).

Variables	HR (95% CI)	*P* value
ALT(IU/L)	1.000 (0.989, 1.011)	0.974
AFP (ng/ml)		
>400	1	
≤400	0.521 (0.249, 1.089)	0.083
BCLC stage		
A	1	
B	2.275 (0.606, 8.542)	0.223
C	1.928 (0.246, 15.102)	0.532
Macroscopic vascular invasion		
Yes	1	
No	0.293 (0.052, 1.650)	0.164
Tumor size (cm)	1.020 (0.930, 1.120)	0.671
Number of tumors		
>1	1	
1	0.439 (0.214, 0.900)	0.025
Superselective embolization		
Yes	1	
No	1.902 (0.817, 4.425)	0.136
Child-Pugh score		
A	1	
B	0.438 (0.163, 1.173)	0.100

HR, Hazard ratio; CI, Confidence interval.

Subgroup Analysis

The median OS was 27 months (95% CI 19.5 months, 34.5 months) in patients without MVI and 6 months (95% CI 2.7 months, 9.3 months) in patients with MVI (P = 0.003), as shown in **Figure 4A**. Meanwhile, the median PFS was 13 months (95% CI 9.4 months, 16.6 months) and 4 months (95% CI 1.8 months, 6.2 months), respectively (P = 0.006) **(Figure 4B)**. The median OS was 29 months (95% CI 18.8 months, 39.2 months) for CL alone and 19 months (95% CI 9.4 months, 28.6 months) for R-CL,23 months (95% CI 0 months, 49.5 months) for L-CL, respectively (P = 0.426) **(Figure 5A)**. The median PFS was 12 months (95% CI 6.5 months, 17.5 months) for CL alone and 9 months (95% CI 0 months, 19.1 months) for R-CL, 4 months (95% CI 0 months, 17.2 months) for L-CL, respectively (P = 0.376) **(Figure 5B)**

DISCUSSION

Although there were a large number of studies confirming that surgery and percutaneous ablation treatment could bring survival benefits to some patients with HCC in the caudate lobe (2, 16, 17, 26), these treatment options remained difficult to achieve and had higher local recurrence rate owing to its deep location, adjacent major vessels and limited therapeutic margin (5, 7, 15, 36, 37). Our study demonstrated that in the real world, for some selected patients with good liver function and high tumor burden, TACE could have significant benefits of survival and disease control.

To our knowledge, there are currently a few studies confirming the efficacy of TACE in some patients with early-stage HCC-CL under the advances in microcatheters and DSA-guided devices (3, 24, 27, 28). Lu et al. (38) reported that early diagnosis and active treatment could result in long-term survival and HCC-CL had results similar to those of chemoembolization for HCC in other segments, while Terayama et al. (39) reported that chemoembolization for caudate lobe HCC was associated with a high local recurrence rate. Kim et al. (24) reported that the overall survival rates of patients with solitary HCC-CL with a median diameter of 2.5 cm undergoing initial TACE, at 3 and 5 years were 65% and 56%, which were comparable to those after surgical resection. Choi et al. (25) reported the use of C-arm CT enabled more accurate selective chemoembolization, which may result in lower cumulative local recurrence rates, but the tumor states of patients were usually small, not in line with clinical practice, so it cannot represent the vast majority of patients with caudate lobe liver cancer.

This study not only included patients whose tumor originating in the caudate lobe, but also patients whose caudate lobe was violated by the tumor of left and right lobes. In our study, there were more initially treated patients whose tumor burden was more than 5cm in diameter and had large blood vessels invaded, which was in consistent with the actual treatment process for HCC patients in the Asia-Pacific region. Similar to the baseline data of the population included in this study, the latest TACTICS trial (40) reported patients with

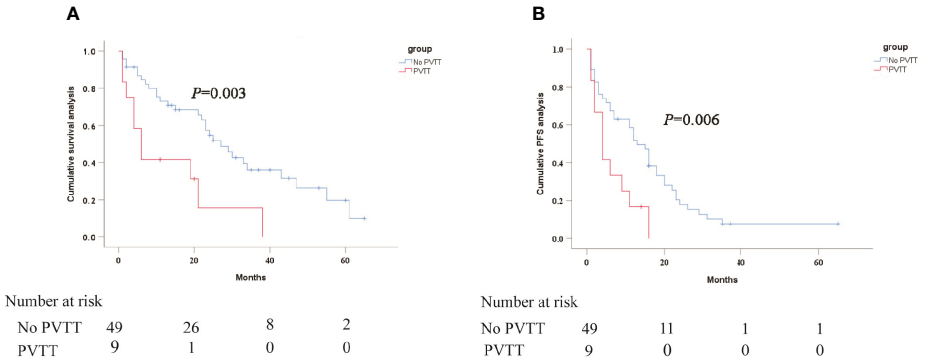

FIGURE 4 | Kaplan-Meier curves of subgroup analysis survival outcomes by MVI.

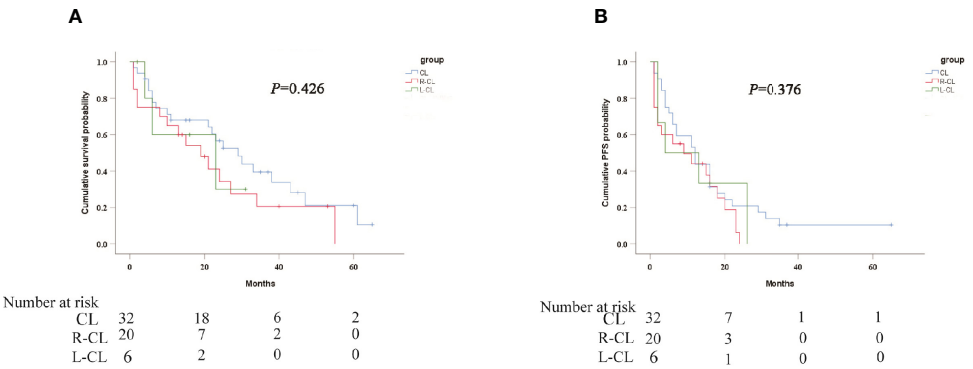

FIGURE 5 | Kaplan-Meier curves of subgroup analysis survival outcomes by tumor distribution.

unresectable HCC undergone TACE alone had a median PFS 13.5 months, comparable to this research 11.0 months. OS at 1 year was longer than that in this study (82.7% *vs.* 66.5%). A meta-analysis, including a total of 10,108 patients treated with conventional TACE, reported that the overall survival rates at 1,3, and 5 years were 70.3%, 40.4%, and 32.4%, respectively (20). Its long-term prognosis is better than this study, showing caudate liver cancer may have a worse prognosis, or baseline condition included in the study are worse.

More retrospective studies or prospective randomized controlled trials (RCT) comparing caudate and non-caudal liver cancers need to be carried out to verify this hypothesis.

Multivariate analysis showed that nonselective chemoembolization, high TBIL, BCLC stage B and once TACE session seemed to have a greater impact on OS. Single tumor was a unique factor for PFS in multivariate analysis. It was confirmed by other researches (5, 7, 36) that attention should be not only paid to accurate super-selective intubation, but also to the number of tumors, liver function of patients, repeat TACE. Early combination of molecular targeted drugs may improve long-term efficacy.

The results of subgroup analyses showed that the application of superselective chemoembolization and the absence of portal vein invasion could have greater OS and PFS benefits regardless of whether it originated from the caudate lobe or invaded the caudate lobe. This was possibly due to mild chemoembolization would cause the increase of vascular endothelial growth factor (VEGF) and hypoxia inducible factor (HIF), cause tumor recurrence or TACE resistance/failure (22). At the same time, portal vein invasion may indicate a poor prognosis and the possibility of distant metastasis. More cases needed to be accumulated to confirm this part of the issue.

To note, the characteristics showed that most of the patients with a higher tumor burden in HCC-CL were given superselective TACE treatment. For unresectable caudate lobe liver cancer, the long-term effect is acceptable, but the cumulative recurrence rate in 3 years is as high as 93.7%. Repeated TACE treatment may have good prognostic value for survival.

This study had some limitations. First, this was a retrospective study, there may be some inevitable selection biases. Findings from this study should be further expanded to multicenter to obtain higher-level medical evidence. Second, due to included patients with HCC-CL were often inoperable or ablation treatment, there is a certain degree of heterogeneity. Better stratification for these patients was needed to be done in a bigger cohort study. Besides, it was worth noting that the

analyzed patients were coming from a single center in China which was known to have a high incidence of hepatitis B virus (HBV) associated HCCs. Given most of them are intermediate and advanced HCCs, the final research results may not be applicable to other regions or countries. Meanwhile, the present study lacks a control group to compare it with other treatment modality, and further studies are needed.

CONCLUSION

In conclusion, our study indicated that, superselective TACE treatment might be associated with better survival benefits in unresectable or "ablation unsuitable" HCC in the caudate lobe without MVI and adequate liver function, compared with the non-selective TACE group, and should be considered as an important reliable therapy for surgeons and interventional radiologists.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LY, HZ, XK, and KQ collected the patients' data. LY drafted the manuscript. LY, YR, and CZ revised the manuscript. KQ, YR, and LC analyzed and interpreted the data. CZ made substantial contributions to the conception of the work. LY, YR, and CZ made substantial contributions to the design of the work, and have revised the manuscript substantively. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.678847/full#supplementary-material

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ORIGINAL RESEARCH
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Purified PTEN-Long Induces Liver Cancer Cells to Undergo Autophagy and Apoptosis

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Background: PTEN-Long is a translational variant of phosphatase and tensin homolog deleted on chromosome 10 (PTEN). This tumor suppressor is frequently lost or mutated and even it has been shown as the determinant in several human tumors. Therefore, we will determine the significant roles of PTEN-Long in the development of liver cancer.

Methods: In the present study, we characterized the antitumor effects of PTEN-Long and PTEN in proliferation, migration of HepG2 cells, apoptosis and autophagy in liver cancer cells. To extends, we have also measured the effects of purified PTEN and PTEN-Long in the above index of HepG2 cells.

Results: PTEN and PTEN-Long were ectopic-expressed in HepG2 cells, and their phenotypic effects were recorded. As expected, there was less expression of PTEN-Long and PTEN in liver cancer samples than in paired normal tissues. Ectopic expression of PTEN-Long or PTEN significantly decreased the proliferation and migration of HepG2 cells and increased apoptosis. PTEN ectopic-expression increased the number of GFP-/RFP+-LC3 puncta and levels of beclin-1 and LC3BII/LC3BI, suggesting autophagy induction. Purified PTEN-Long freely entered cells, decreased proliferation, and increased autophagy and apoptosis, while purified PTEN did not.

Conclusions: Our results identify an antitumor function of purified PTEN-Long and suggest its potential utility for liver cancer treatment.

Keywords: PTEN-Long, autophagy, apoptosis, liver, cancer

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INTRODUCTION

Primary liver cancer is the sixth most common cancer worldwide, and hepatocellular carcinoma (HCC) accounts for 80% of such cancers (1). Data from China suggest that hepatocellular carcinoma's morbidity and mortality rates rank fourth and third among malignant tumors, respectively (2). Surgical resection is effective for the treatment of early-stage liver cancer. Unfortunately, approximately 80% of liver cancer patients already have advanced disease at presentation. For these reasons, liver cancer represents a major therapeutic challenge, and further research focused on the molecular mechanisms of HCC tumorigenesis is essential.

Cell death is a complex process that is carefully regulated. As the first recognized programmed cell death process, the role and regulatory network of apoptosis have gradually become clear nowadays (3). However, apoptosis is not the only factor that determines the fate of cell death. In

recent years, autophagy, known as type II programmed cell death process, has been shown to co-regulate cell death with apoptosis. In some cases, autophagy inhibits apoptosis and is a cell survival pathway, but autophagy itself can also induce cell death, or acts together with apoptosis and as a backup mechanism to induce cell death in the case of apoptosis defects. These two pathways are correlated and regulated by each other in different environment (4). The study and utilization of these interactions will be beneficial to further reveal the pathogenesis of liver cancer.

The gene encoding the phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*) is frequently lost or mutated in many late-stage tumors (5). It is the second most prevalent genetic mutation found in several human tumors, including liver cancer. *PTEN* encodes a protein of 403 amino acid residues; it is a tumor suppressor characterized as a dual-specificity protein with both lipid phosphatase and protein phosphatase activities. The protein employs trisphosphate (PIP3) as its primary substrate, which is hydrolyzed to phosphatidylinositol (4, 5)-bisphosphate (PIP2) (6, 7). PTEN blocks the phosphatidylinositol 3-kinase (PI3K) signaling pathway via its lipid phosphatase activity in which PTEN dephosphorylates phosphatidylinositol (3,4,5)-trisphosphate [PI(3,4,5)P3] to form phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2], inhibiting AKT and its downstream signaling pathways; in this manner, the protein inhibits cell growth, proliferation, and survival (8–11). These findings suggest that PTEN represents a critical node in tumor development and might be helpful as a potential therapeutic target in tumor treatment. Nevertheless, its use in clinical trials is limited by the non-secretory nature of PTEN. Its use also presents the risk of the generation of new tumors induced by adenovirus-mediated gene transfer (6).

Recently, two isoforms of PTEN have been identified as translational variants of PTEN, PTEN-Long, and PTENα (7, 12). PTEN-Long is superior to PTEN depending on its specific region, translated from an alternative start site within the 5'-coding region of *PTEN* mRNA. It contains a 173 amino acid-residue domain at its *N* terminus (13). This enhanced region characterizes PTEN-L as a secreted protein, and it is detected in human serum and plasma, unlike the non-secretory protein PTEN (14). Furthermore, the lipid phosphatase and protein phosphatase activities of PTEN-Long are comparable to that of PTEN, and it can display higher activities than PTEN (15). PTEN-Long can be actively secreted from cells and enter other cells, inhibiting PI3K signaling both *in vitro* and *in vivo*. The protein might be involved in the alternatively translated region, including a polyarginine stretch with homology to known cell-permeable peptides (8, 16, 17). Recent studies reported that levels of PTEN-Long are significantly lower in tumor tissues than in normal tissues. Upregulated expression of PTEN-Long inhibits the proliferation of breast cancer and renal cell adenocarcinoma cells and induces tumor regression in murine models of cancer, suggesting that PTEN-Long may serve as a therapeutic target in cancer (13, 18). Nevertheless, the roles of PTEN-Long in the development of liver cancer are unknown.

TABLE 1 | Clinicopathologic features of HCC patients.

		%
Age (y)	45–80	
Sex		
Male	10	40%
Female	15	60%
Tumor size (cm)	1.6–16	
Histology		
HCC	25	100%
Grade		
I	13	52%
II	2	8%
IIIA	8	32%
IVA	1	4%
IVB	1	4%
T classification		
T1	13	52%
T2	2	8%
T3	9	36%
T4	1	4%
N classification		
N0	23	92%
N1	2	8%
M classification		
M0	24	96%
M1	1	4%

Here, we study the effects of PTEN-Long on the HCC-derived cell line HepG2. Moreover, we compared the effects of purified PTEN and PTEN-Long on cell migration, apoptosis, and autophagy. Our results demonstrate the potential antitumor activity of purified PTEN-Long, which was not shared by PTEN, suggesting the possible significance of the former as a therapeutic target.

MATERIALS AND METHODS

Tissues

Liver cancer tumor tissues and paired normal tissues ($n = 25$ pairs) were obtained from the affiliated Hospital of Ningbo University, LiHuili Hospital. The LiHuili Hospital Ethics Committee, Ningbo Medical Treatment Center, approved this study. Normal tissues were extracted approximately 1 cm from the tumor margin, and all tissues were immediately stored at −80°C. All diagnoses were histopathologically confirmed as having HCC (**Table 1**).

Cell Line

HepG2 cells were purchased from the Shanghai Cell Bank, Chinese Academy of Sciences. Cells were cultured in DMEM/high glucose medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum and 100

U/ml penicillin/streptomycin. Cells were maintained in a humidified incubator.

Expression Plasmids

The mammalian expression plasmids pcDNA 3.1, pcDNA 3.1-PTEN, and pcDNA 3.1-PTEN-Long were described in a previous study (18). JpExpress404-PTEN-V5/His and JpExpress404-PTEN-Long-V5/His were gifts from Ramon Parsons (Addgene plasmids # 49420 and # 49417).

Generation of Stable Transfectants

HepG2 cells were transfected in the presence of Lipofectamine 2000 (Life Technology, CA, USA) according to the manufacturer's protocol. Briefly, cells (2×10^5 cells per well) in six-well plates (Nunc, Roskilde, Denmark) were transfected when they reached 80%−90% confluency. Mock transfection with the empty plasmid (pcDNA3.1) served used as a control. The transfection mixtures were diluted in Opti-MEM Reduced Serum Medium (Life Technology), and the HepG2 cells were incubated in this mixture for 6 h. After transfection, the cells were cultured for 2 weeks in 400 μg/mL G418 to generate stable transfectants.

Western Blot Analysis

Tumor and normal adjacent tissues were crushed with a mortar under liquid nitrogen and suspended on ice in lysis buffer, and the BCA Assay was used (Bio-Rad Laboratories, California, USA) to determine protein concentrations.

Harvested cells (1×10^6) were homogenized in 100 μl RIPA lysis buffer (Solarbio, Beijing, China) supplemented with 1× protease inhibitor cocktail. Proteins were separated using SDS-PAGE and transferred onto polyvinylidene fluoride membranes (PVDF, Immobilon P; Millipore, Billerica, MA, USA). After blocking in 5% low-fat milk powder in phosphate-buffered saline/Tween-20 (PBST) for 60 min, primary antibodies were incubated with the membranes at 4°C overnight. The antibodies were as follows: anti-PTEN (138G6), anti-AKT (C67E7), anti-p-AKT (Ser473) (D9E), anti-Cleaved caspase 3 (D175), anti-PRAS40 (D23C7), anti-p62/SQSTML (5114S), anti-LC3 (D3U4C), anti-BECLIN-1 (D40C5), anti-Bcl-xl (2764), anti-Bax (2772), anti-p-PRAS40 (Thr246) (D4D2) (Cell Signaling Technology, Danvers, MA, USA), and anti-GAPDH (sc-47724; Santa Cruz Biotechnology, Inc., Dallas, TX, USA). After washing, membranes were incubated with the appropriate secondary antibodies (Santa Cruz Biotechnology) at room temperature

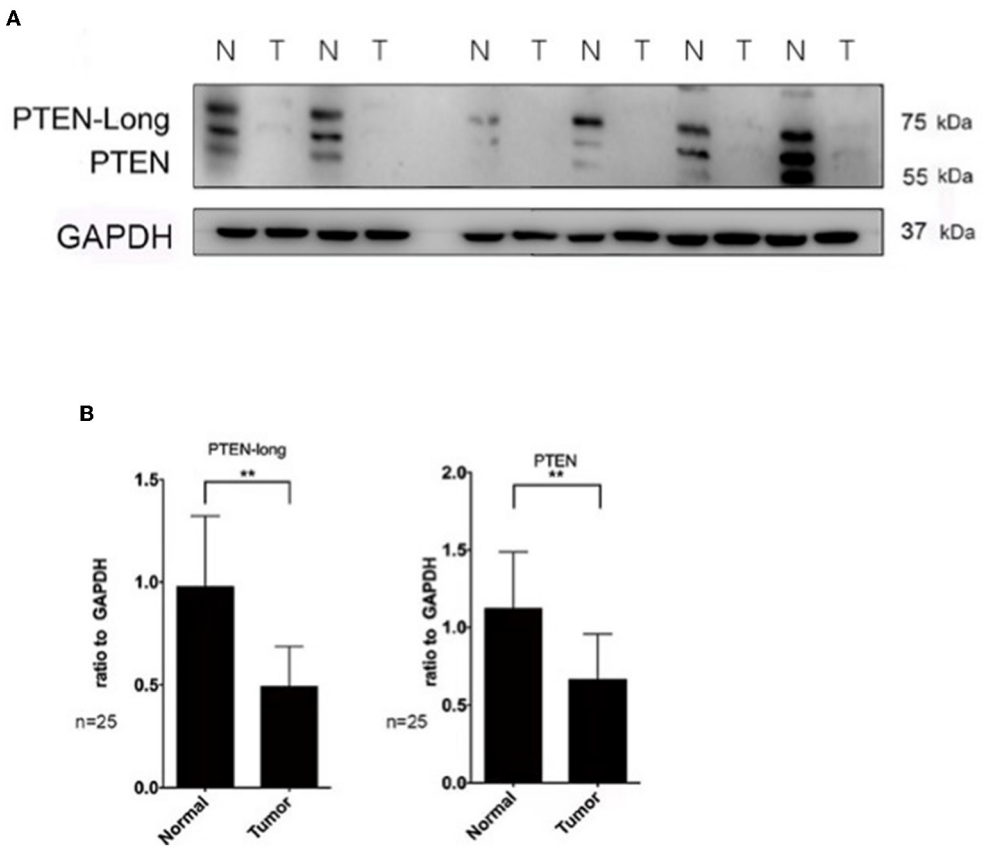

FIGURE 1 | The expression of PTEN-Long is significantly reduced in hepatocellular carcinoma. Western blot analysis of PTEN and PTEN-Long expression in tumor and adjacent normal tissues. **(A)** Immunoblot of six randomly selected PTEN hepatocellular tumors and the corresponding normal tissues (*N* = normal, T = tumor). **(B)** The ratios of PTEN-Long to GAPDH and PTEN to GAPDH in 25 pairs of samples are presented (**$P < 0.01$).

for 45 min. Immunocomplexes were visible using an enhanced chemiluminescence detection system. Western blot results were analyzed using ImageJ (NIH).

Cell Proliferation Assays

MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assays were performed according to the manufacturer's protocol (Promega Madison, WI, USA). Briefly, after 2,000 cells per well were seeded into 96-well plates, they were incubated at 37°C for 24, 48, 72, or 96 h. Absorbance was measured at 490 nm using a Microplate Reader (Bio-Rad Laboratories).

In vitro Scratch Assays

Stably transfected HepG2 cells were cultured in 6-well plates and starved for 24 h. A linear scratch wound was introduced into the cell monolayer using a 200-μL pipette tip. The cells were washed with PBS 2–3 times and subsequently cultured in fresh medium without FBS for 72 h. Scratches were observed using an inverted microscope immediately (0 h) and after 72 h.

Apoptosis Assays

According to the product specification, Apoptosis was measured using a PE Annexin V Apoptosis Detection Kit I (BD, Biosciences, Franklin Lakes, NJ, USA). After labeling with Annexin V, cells (≥ 20,000 cells per sample) were analyzed using flow cytometry (BD Biosciences).

Autophagic Flux Assays

Cells were infected with the stubRFP-lensGFP-LC3B Lentivirus (19, 20) (GeneChem, Shanghai, China). Images were taken using a confocal microscope 72 h after infection.

Protein Purification

Proteins were purified from *Escherichia coli* BL21 (DE3; Invitrogen, Carlsbad, CA, USA) transformed by a plasmid

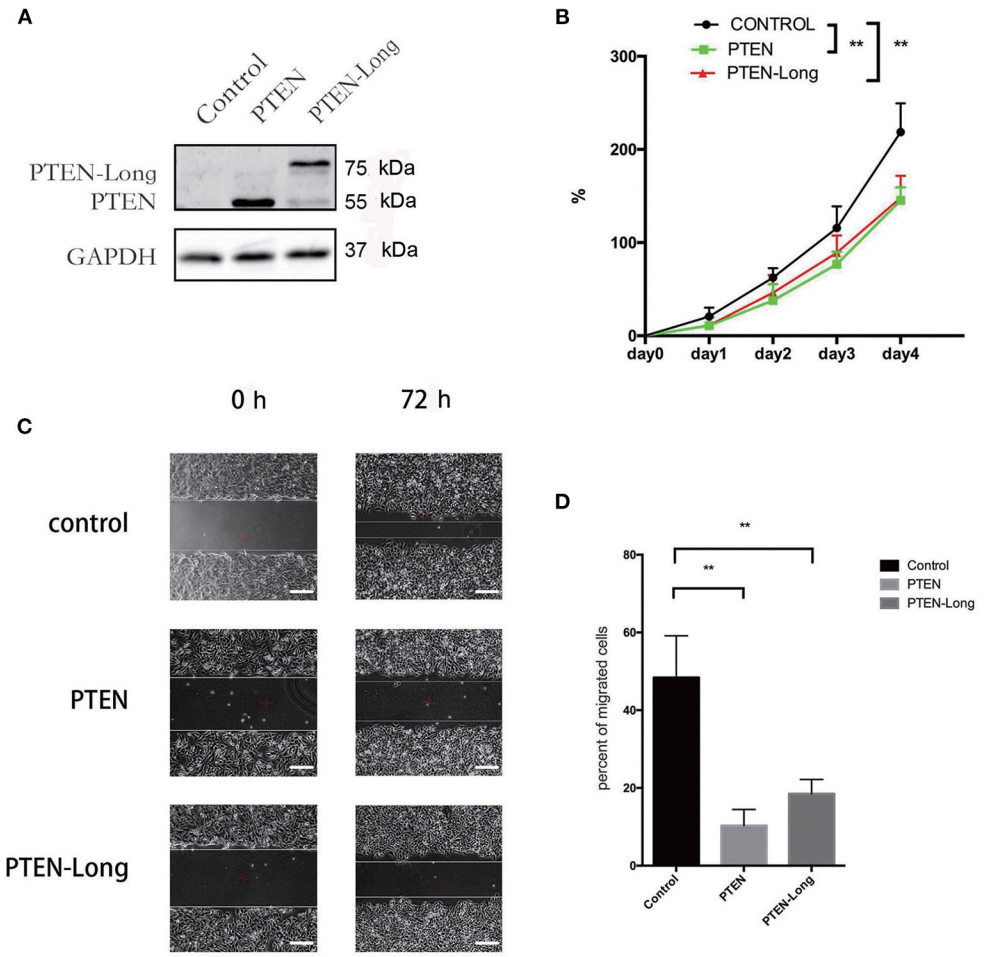

FIGURE 2 | PTEN-Long inhibits cell proliferation and migration. HepG2 cells were selected for at least 2 weeks by incubation with 400 μg/mL G418 after transfection. **(A)** Western blot analysis of lysates prepared from HepG2 cells. **(B)** Of cell proliferation analysis by MTT assay (**$P < 0.01$). **(C)** Cell migration analysis by scratch assay. Scale bar = 200 μm. **(D)** ImageJ analysis of the invaded area (**$P < 0.01$).

encoding JpExpress-PTEN or JpExpress-PTEN-Long. After inductive expression of protein using 0.1 mM isopropyl β-D-1-thiogalactopyranoside (Sigma–Aldrich, St. Louis, MO, USA) for 4.5 h at 21°C, the protein was extracted from bacteria sonicated in lysis buffer (500 mM NaCl, 25 mM Tris, pH 7.5). This was followed by centrifugation at 30,000 × *g* for 30 min, and *E. coli* lysates were filtered through 0.22-μm filters and passed through an AKTA Prime Plus. Proteins were subsequently resolved using SDS-PAGE and quantified using the BCA Assay.

Statistical Analysis

Experiments were performed at least three times. Each value was expressed as the mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance and a two-sample independent *t*-test SPSS v17.0 (SPSS Inc; Chicago, IL). $P < 0.05$ indicated significant difference.

RESULTS

PTEN-Long Expression Is Significantly Reduced in HCC

Western blot analysis revealed that the levels of PTEN-Long and PTEN were reduced by approximately 50% ($P < 0.01$) and 60% ($P < 0.01$) in paired tumor tissues vs. adjacent normal liver tissues ($n = 25$ pairs) (**Figures 1A,B**).

PTEN-Long Inhibits the Migration and Invasion of HepG2 Cells

To investigate the role of PTEN-Long in HCC, HepG2 cells were transfected with pcDNA3.1 plasmids harboring sequences encoding PTEN-Long or PTEN. Western blot analysis revealed ectopic expression of PTEN-Long and PTEN by the transfectants (**Figure 2A**). The growth rates of the PTEN-Long or PTEN transfectants were significantly different from those of the mock-transfected controls 72 and 96 h after transfection ($P < 0.01$). There was no significant difference between the growth rates of PTEN-Long or PTEN transfectants (**Figure 2B**). The scratch assay showed that the migration of each PTEN-Long or PTEN transfectant was inhibited compared with those of the controls (**Figures 2C,D**).

PTEN-Long Induces HepG2 Cells to Undergo Autophagy

To study the effect of PTEN-Long on autophagy, cells infected with the stubRFP-lensGFP-LC3B lentiviral vector were subjected to autophagic flux analysis. There were significantly more GFP+/RFP+ puncta in PTEN-Long or PTEN ectopic expressing cells than in controls, suggesting that autophagosomes formed (**Figure 3A**). Compared with the controls, the PTEN-Long and PTEN ectopic-expressing cells contained more autolysosomes, suggesting autophagy induction. Western blot analysis of the expression of the autophagy-related proteins p62, beclin-1, and LC3BII/I revealed that the expression of p62 was significantly reduced, while the levels of BECLIN-1 and the ratios of LC3BII/LC3BI levels were significantly increased in the PTEN-Long and PTEN transfectants compared with controls, suggesting increased autophagic activity (**Figure 3B**).

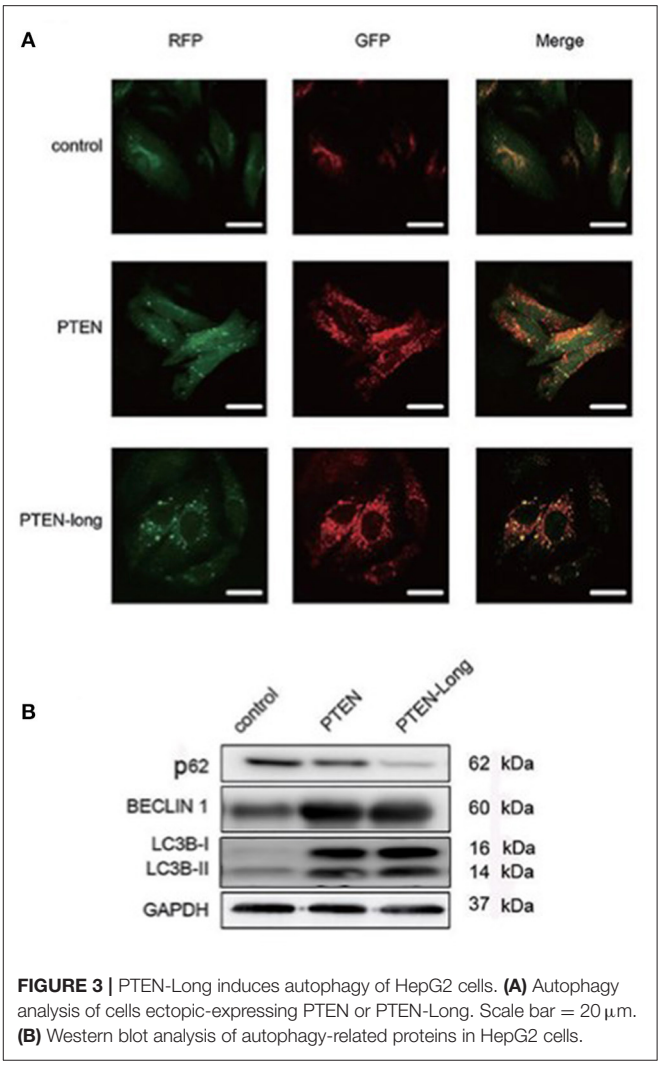

FIGURE 3 | PTEN-Long induces autophagy of HepG2 cells. **(A)** Autophagy analysis of cells ectopic-expressing PTEN or PTEN-Long. Scale bar = 20 μm. **(B)** Western blot analysis of autophagy-related proteins in HepG2 cells.

PTEN-Long Induces HepG2 Cells to Apoptosis

The ectopic expression of PTEN-Long and PTEN induced HepG2 cells to undergo apoptosis (**Figure 4A**). The proportions of apoptotic cells in cultures of the PTEN and PTEN-Long transfectants were approximately 3-fold higher than cultures of the control cells (**Figure 4B**). Levels of the apoptosis-related proteins cleaved caspase 3 and Bax were increased, whereas expression level of BCL-XL was decreased compared with those of the control cells (**Figure 4C**).

PTEN-Long Suppresses PI3K/AKT Signaling in HepG2 Cells

PTEN suppresses the classical PI3K/AKT pathway. Therefore, we investigated the effect of PTEN-Long on this pathway in HepG2 cells. The levels of p-AKT (Ser473), p-PRAS40

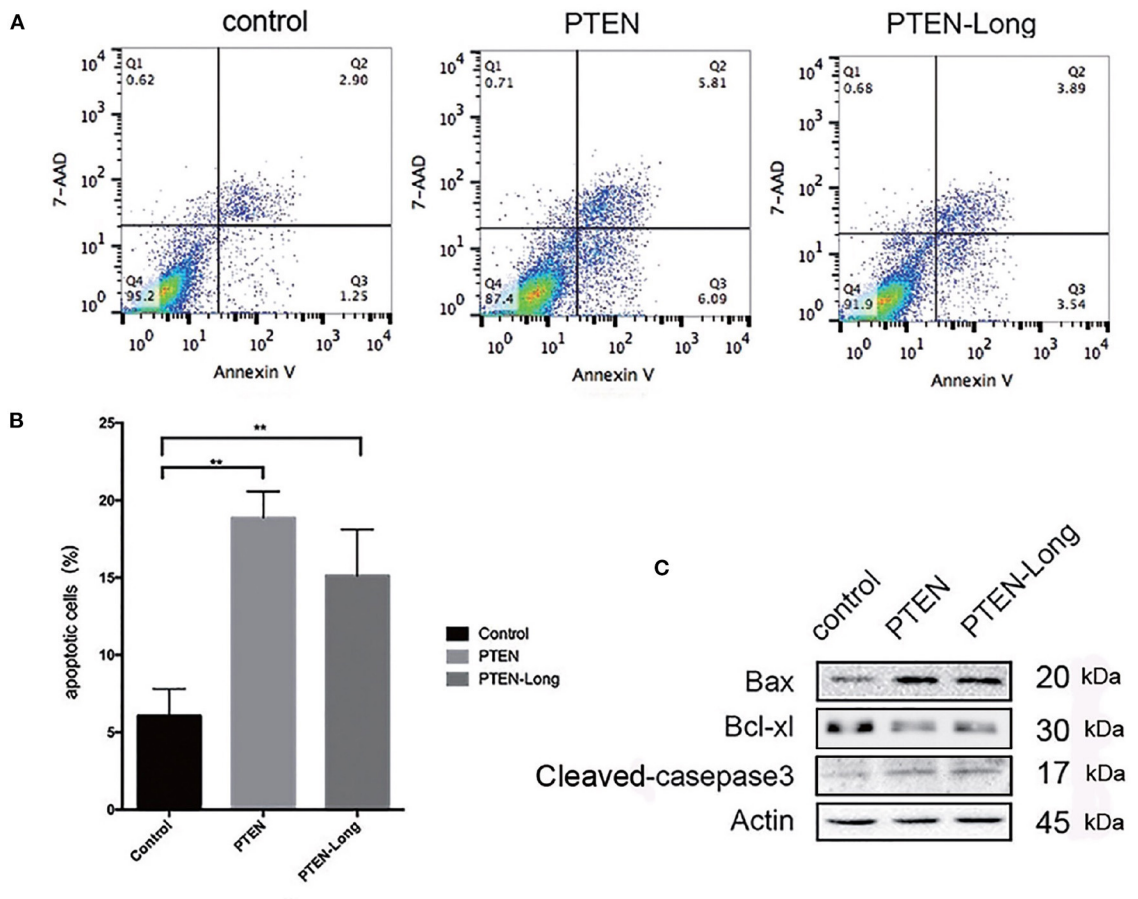

FIGURE 4 | PTEN-Long induces apoptosis of HepG2 cells. **(A)** Flow cytometric analysis of apoptosis. **(B)** The proportion of apoptotic cells (Annexin V+, 7-ADD-) calculated using FlowJo (**$P < 0.01$). **(C)** Western blot analysis of apoptosis-related proteins in HepG2 cells.

(Thr246), and p-mTOR (Ser2448) were significantly lower in the PTEN-Long- and PTEN transfectants than in control cells (**Figure 5**), suggesting the suppression of PI3K/AKT pathway activity. However, there were no significant differences between PI3K/AKT signaling in the PTEN and PTEN-Long transfectants.

Purified PTEN-Long, but Not PTEN, Inhibits Cell Migration and Induces HepG2 Cells to Undergo Autophagy and Apoptosis

The generation of purified PTEN-Long and PTEN is shown in **Figure 6A**. PTEN-Long inhibited the growth of HepG2 cells in a concentration-dependent manner, and the most significant difference was detected when cells were treated with 75 nM PTEN-Long, while no effects were observed in cells treated with purified PTEN (**Figure 6B**). Similarly, treatment with PTEN-Long, not PTEN, significantly inhibited cell migration (**Figures 6C,D**) and induced apoptosis in a concentration-dependent manner (**Figures 6E,F**). We detected increased levels of autophagy- and apoptosis-related proteins and inhibition of PI3K/AKT signaling (**Figure 6G**). At the same, we found that downregulated AKT was negatively regulated with autophagy- and apoptosis-related proteins, which means PTEN-Long might upregulate autophagy and apoptosis process through inhibition of PI3K/AKT signaling (**Figure 6H**).

DISCUSSION

Liver cancer is one of the most prevalent cancers worldwide. Surgery is the primary treatment for liver cancer, but it is mainly administered to patients with early-stage disease. Unfortunately, the few available treatments for advanced liver cancer are insufficient. Although chemotherapy has been used for over 30 years to treat liver cancer, definitive evidence of prolonged survival time is lacking (21, 22). Resistance to chemotherapeutic drugs remains a significant barrier and often leads to treatment failure (15). There are more opportunities for targeted therapy with advanced knowledge of liver cancer genetics and liver cancer-related molecular pathways. The PTEN-PI3K-AKT pathway has been a compelling target in the clinical trials of cancer treatment (22). Activation of the PTEN-PI3K-AKT signaling pathway is involved in normal cell

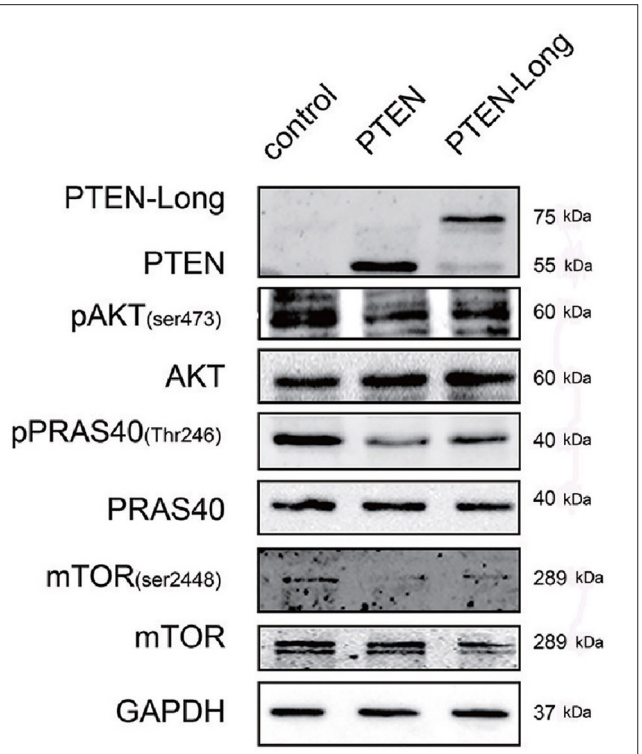

FIGURE 5 | PTEN-Long inhibits PI3K-AKT signaling. Western blot analysis of PI3K-AKT signaling-related proteins in HepG2 cells ectopic-expressing PTEN or PTEN-Long.

proliferation, survival, and migration; however, its abnormal activation promotes cancer cell growth (11). Expression levels of mTOR and its downstream P70S6K in the PI3K-AKT-mTOR signaling pathway are usually upregulated in liver cancer compared to paracancerous and normal liver tissues (20). In this study, we showed that the deficiency of PTEN and increased levels of p-AKT and p-mTOR were associated with tumor grade, intrahepatic metastasis, vascular invasion, TNM stage, and high Ki-67 labeling index in liver cancer or HepG2 cells, emphasizing again that PTEN can act as a tumor suppressor through inhibition of the proliferation and migration of HCC via PTEN-PI3K-AKT pathway.

Despite evidence to suggest that PTEN serves as a potential anti-tumor therapeutic molecule, there are drawbacks associated with using PTEN as a gene target. For example, adenovirus technology can directly increase the levels of PTEN (16, 23); however, this technique has adenovirus-associated side effects (5). Furthermore, PTEN expression can be increased indirectly using microRNAs (16, 24–26). Although microRNAs inhibit tumor growth by increasing PTEN expression, they are not currently used as medications due to their broad off-target effects. Considering the difficulties of delivering a therapeutic vector containing PTEN to target cells in gene therapy, PTEN-Long could be efficiently delivered anywhere via the circulation without any vector.

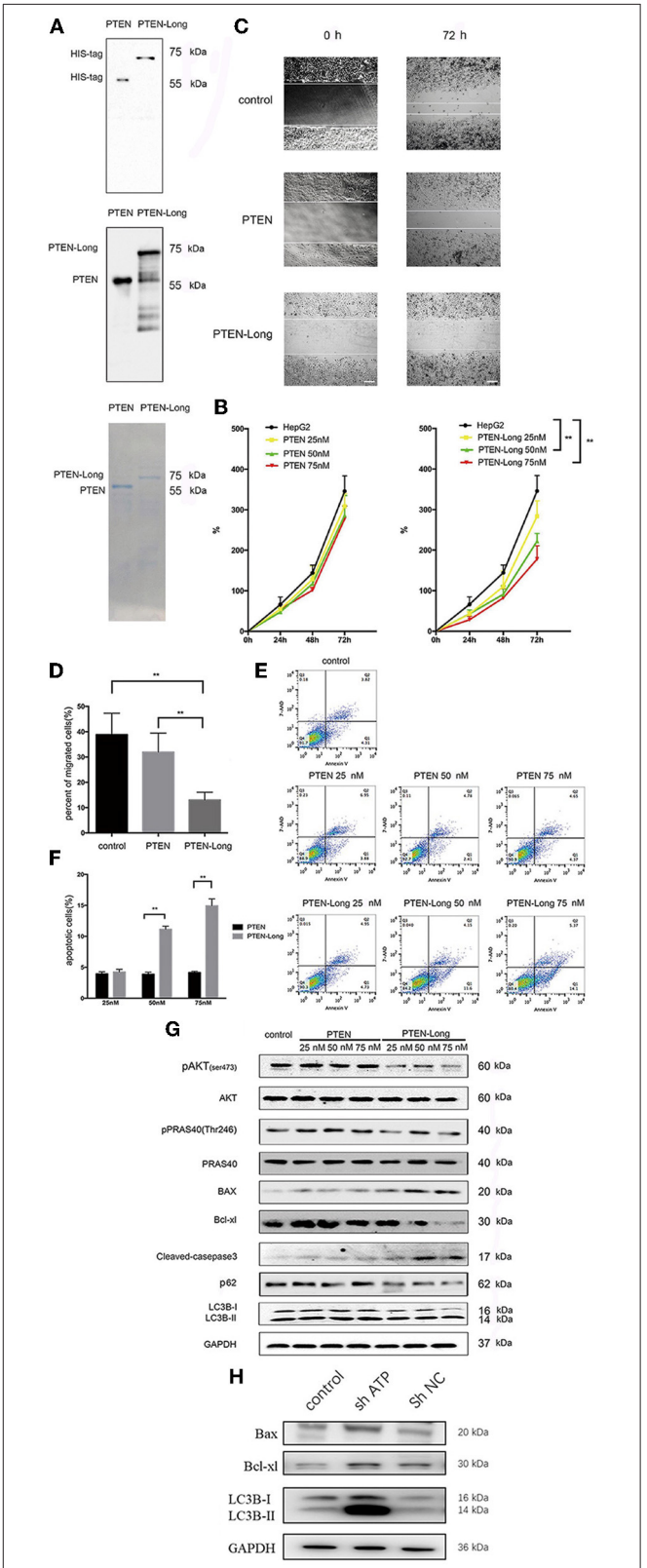

FIGURE 6 | Purified PTEN-Long induces autophagy and apoptosis of HepG2 cells. **(A)** Western blot analysis of purified protein PTEN isoforms. **(B)** Cell

(Continued)

FIGURE 6 | proliferation analysis by MTT assay (**$P < 0.01$). **(C)** Cell migration analysis by scratch assay. Scale bar = 100 μm. **(D)** ImageJ was used to analyze the invaded area indicated in **(C)** (**$P < 0.01$). E. Flow cytometric analysis of apoptosis. **(F)** The proportions of apoptotic cells (Annexin V+, 7-AAD-) indicated in **(E)** were calculated using FlowJo (**$P < 0.01$). **(G)** Western blot analysis of protein expression after treatment of HepG2 cells with purified PTEN or PTEN-Long. **(H)** Western blot analysis of autophagy- and apoptosis- relative protein expression after suppressing of AKT expression.

As the critical role of classical PTEN, a member-permeable protein, PTEN-Long can act on the PI3K-AKT-mTOR pathway and enter neighboring cells following its secretion from cells, dephosphorylating PIP3, antagonizing PI3K-AKT signaling and inducing cell death in renal cell carcinoma (18). In the present study, we demonstrated that protein expression of PTEN-Long was reduced or completely lost in liver cancer patients at high frequency, suggesting that it plays an essential role in liver cancer via PTEN-Long-PI3K-AKT pathway through inhibiting tumor cell proliferation, migration and inducing apoptosis and autophagy. These effects are similar to the significant role of PTEN in the development of liver cancer.

The PI3K/AKT/mTOR pathway is involved in tumor formation, cell cycle progression, cell cycle progression, survival, and even apoptosis. Apoptosis occurs by two pathways, the "exogenous" and "endogenous" pathways mediated by death receptors and mitochondria, respectively. These pathways clear damaged or redundant cells, indicating it as an essential target of cancer (27). These two pathways are related to the Bcl-2 family and mitochondrial proteins (28); Bcl-2 is usually maintained by PI3K activity in the cytoplasm, where it regulates the degree of Bax translocation to mitochondria (29). The PI3K/AKT/mTOR pathway is a modulator of autophagy (30). In the present study, we observed that treatment with purified PTEN-Long suppressed levels of p-AKT, p-PRAS40, and p-mTOR with upregulated expression of Bax and the activity of apoptosis-related proteins in HepG2 cells. PTEN-Long downregulated expression of Bcl-2, but also changed expression of autophagy-related proteins (p62, beclin-1, and LC3BII/I), suggesting that apoptosis induction and autophagy may be mediated by suppression of the PI3K/AKT/mTOR pathway. More importantly, exogenously added PTEN-Long to HepG2 cells resulted in reduced p-AKT, p-PRAS40, and p-mTOR, which inhibited tumor cell proliferation and migration without any significant change induced in HepG2 cells after treatment with exogenous PTEN protein. In summary, our findings suggest that PTEN-Long participates in the development of liver cancer, and it might serve as a functional tumor suppressor protein.

DATA AVAILABILITY STATEMENT

We have approved the statement that the data presented in this article is publicly available, and further queries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical approval was not provided for this study on human participants because the Ethics Committee had been not established in that time. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HL and LT: conception and design. HL: administrative support. SZ and JZ: provision of study material or patients. LT and ZX: collection and assembly of data. QM and LT: data analysis and interpretation. All authors wrote the manuscript and approved the final version of the manuscript.

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Corrigendum: Purified PTEN-Long induces liver cancer cells to undergo autophagy and apoptosis

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The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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