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Evaluating the predictive power of circulating tumor cells for the prognosis of transarterial chemoembolization treatment on patients with advanced hepatocellular carcinoma

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Abstract

Explore the predictive power of Circulating Tumor Cells (CTCs) for evaluating the prognosis of transarterial chemoembolization (TACE) treatment on advanced hepatocellular carcinoma (HCC) patients, and use it to construct a prediction model.

We retrospectively analyzed 43 patients with Barcelona Clinic Liver Cancer stage C HCC who underwent TACE treatment.

The survival time of 43 advanced HCC patients were 2 to 60 months, with the median survival time of 12 months, 1-, 3-, and 5-year survival rates were 42.9%, 9.0%, and 3.6%, respectively. The OS of patients with high level of CTCs before TACE (CTC1 > 2) was significantly lower than that of patients with low level of CTCs (8 vs 12 months, $P = .040$), but there was no significant difference in PFS between the 2 groups ($P = .926$). Meanwhile, there was no significant difference in OS and PFS between patients with high level CTCs and those with low level CTCs at 1 week and 4 weeks after TACE (P all > .05). In univariate and multivariate Cox regression analysis, the number of lesions and CTC before TACE were the independent influencing factors for prognosis in these patients, and the HR was 3.01 and 1.20, respectively (all $P < .05$). The area under curve of COX regression model to predict OS increased with the increase of follow-up time, ranging from 0.56 to 0.85.

The CTCs number before TACE is an effective biomarker for predicting the OS of advanced HCC patients. The joint prediction model based on CTCs and tumor number can effectively predict the prognosis of patients with advanced HCC.

Abbreviations: AUC = the area under the curve, BCLC = Barcelona Clinic Liver Cancer, CI = confidence interval, CTCs = circulating tumor cells, EpCAM = epithelial cell adhesion molecules, HCC = hepatocellular carcinoma, TACE = transarterial chemoembolization.

Keywords: circulating tumor cells, hepatocellular carcinoma, model, prognosis

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1. Introduction

Primary liver cancer is the 6th most common malignant tumor and the 4th leading cause of cancer-related death in the world.[1] In China, the hepatocellular carcinoma (HCC) standardized mortality rate is 25.26/ 100,000, which is the second leading cause of tumor-related death in China.[2] The onset of HCC is hard to detect, and its development is very rapid. More than 60% of patients already have local progression or distant metastasis at the time of treatment,[3] which is diagnosed as HCC stage C based on the Barcelona Clinic Liver Cancer (BCLC) clinical staging system.[4] These patients are seriously ill, and the disease progresses very fast. Without effective intervention, these patients' survival time is very short. According to Chinese guideline for diagnosis and treatment of primary liver cancer (2017 Edition), transarterial chemoembolization (TACE) is recommended for HCC patients in BCLC stage C with Child-Pugh class A or B liver function.[5] Studies have shown that TACE can improve the overall survival of patients with nonresectable HCC by inducing tumor necrosis and reducing lesion size.[6] However, due to the large heterogeneity in tumor burden (tumors that have macroscopic vascular invasion, extrahepatic spread, etc) and clinical symptoms of BCLC stage C patients, their prognosis are quite different.[7] Thus, a simple, reliable and accurate prognostic prediction model can help with the personalized management and treatment of HCC patients.

However, there is currently no widely accepted serum biomarkers that can predict the long-term prognosis after TACE.[8]

Circulating tumor cells (CTCs) are the tumor cells that are released from primary or metastatic tumors to the peripheral blood or lymphatic system and eventually grow in the bone marrow, lymph nodes, or metastasized organs.[9] Studies have found that the CTCs number before treatment and its change after treatment are closely related to the therapeutic efficacy and prognosis of various epithelial solid tumors (breast cancer, colon cancer, prostate cancer, etc.).[10,11] However, there are few applications of CTCs in HCC. The reason might be that the FDA-certified CTCs detection system (Cell Search system) is an immunomagnetic enrichment technology based on epithelial cell adhesion molecules (EpCAM). Unlike other epithelial tumors, most liver cancer cells do not express EpCAM; moreover, during the formation and development of metastases, some tumor cells will undergo epithelial mesenchymal transformation (EMT) and lose some cellular phenotypes including EpCAM.[12] Our previous study found that the CTCs detection method based on abnormal amplification of chromosome 8 (CEP8) can be used in HCC.[13] However, whether CTCs detection can predict the prognosis of patients with advanced HCC after TACE treatment and whether there is an optimal detection time is currently unknown. Therefore, we recorded the CTCs levels of patients with advanced HCC in our center before and after TACE treatment and analyzed the predictive value of CTCs for patient prognosis and survival time through follow-up; base on the results, we tried to build a simple model to predict the prognosis of patients with advanced HCC.

2. Materials and methods

2.1. Clinical data

This study is a retrospective cohort study. We retrospectively analyzed the patients diagnosed with BCLC stage C primary liver cancer diagnosed by imaging and histopathology and received TACE treatment in our hospital from July 2016 to May 2018. The inclusion criteria were:

(1) The diagnosis conformed to the standardization of diagnosis and treatment for hepatocellular carcinoma established by the Bureau of Medical Administration, National Health, and Family Planning Commission of China in 2017[5];
(2) 2). The patients had not received any surgery, radiotherapy, chemotherapy, and other treatments.
(3) Patients had measurable or evaluable lesions, and there was no radiographic evidence of distant metastasis;
(4) Digital subtraction angiography (DSA) contrast showed no lateral hepatic branch blood supply for the tumor.
(5) Patients voluntarily participated in this study and were willing to accept regular follow-up.

Exclusion criteria:

(1) Iodine allergy,
(2) Hepatic tumor rupture and bleeding, and there was obvious shunt in hepatic artery-portal vein or hepatic artery-hepatic vein;
(3) Tumors lacked blood supply;
(4) There was occlusion at the second hepatic portal or upper hepatic IVC;
(5) Patient was older than 80 years old;
(6) Severe anemia or severely reduced white blood cells and platelets.

The clinical and imaging data, chronic hepatitis/cirrhosis history, laboratory hematology indicators, and peripheral blood CTCs number before TACE, 1 week and 4 weeks after TACE were recorded. All patients signed the informed consent form. The study followed the Declaration of Helsinki and was approved by the Ethics committee of our hospital.

2.2. The procedure of TACE

The Seldinger technique was used to puncture the femoral artery cannula, and angiography on the abdominal aorta and superior mesenteric artery was performed under DSA (Philips UNIQ-FD20 digital subtraction angiography machine). Epirubicin (Pfizer, New York) and iodized oil were used to conduct chemoembolization on the tumor supply artery, microcatheter, and embolizing microspheres (Merit Medical Inc., South Jordan) were used during the operation if necessary. Each patient received TACE at least twice, and the interval between 2 TACE operations was 1–2 months. Routine hepatoprotective treatment was performed after TACE.

2.3. Peripheral blood CTCs detection and positive judgment criteria

Peripheral venous blood was collected from all patients on empty stomach. Serum CTCs were measured before TACE, and at 1 week, 4 weeks after TACE, which were recorded as CTC1, 2, 3, respectively. Serum CTCs were detected by negative enrichment combined with immunofluorescence in situ hybridization. The specific detection process was: collecting peripheral blood, separating plasma, lysing red blood cells, removing white blood cells, making slide smear, immunofluorescence staining, and observation under microscope. Under a fluorescence microscope, we analyzed the morphology of the enriched cells based on chromosome 8 (Centromere-enumeration probes 8, Jiangsu Leier Biotechnology Company), leukocyte surface marker staining (CD45-AF594 fluorescent antibody, Miltenyi Biotec Company, Germany) and nuclear staining (DAPI, Jiangsu Lyell Biological Technology Company). The CTC judgment criteria were: cells were round and long ellipse, the long diameter was $> 10 \mu m$, the probe signal points in nucleus ≥ 3, no hematogenous white blood cell surface antigen. The positive cell was marked with red circle, as shown in Figure 1. CTCs ≥ 1 was considered positive.

2.4. Follow-up and prognosis assessment

Patients were followed-up every 3 months after TACE. Outpatient follow-up was the main form, including blood routine, liver and kidney function test, blood AFP, abnormal prothrombin (DCP), and reexaminations with liver enhanced CT / MRI and ultrasound contrast to understand the tumor conditions. The follow-up was mainly based on outpatient service and telephone surveys. The follow-up ended on June 30, 2018. Overall Survival (OS) is the time from post-surgery to death of any reason; progression-free survival (PFS) is the time from post-surgery to initial tumor progression or death of any reason.

2.5. Statistical analysis

Continuous variables were expressed as mean ± standard deviation when they followed normal distribution, and P50 (P25, P75) when they were not normally distributed. Categorical
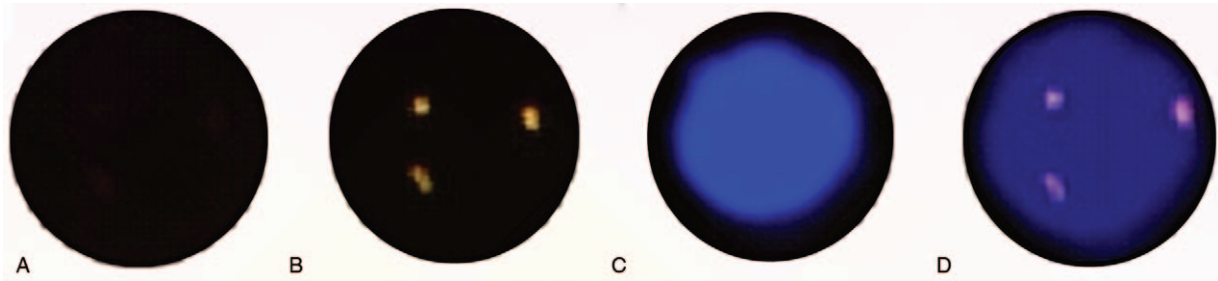
Figure 1. Circulating tumor cells (CTCs) from patients with hepatocellular carcinoma (CD45$^-$/CEP8$^+$/DAPI). CEP8: Centromere-enumeration probes 8, DAPI: 4, 6-diamidino-2-phenylindole dye.

variables were expressed as frequency (%). For comparisons between groups, continuous variables were tested using unpaired Student-t test or Mann-Whitney nonparametric test, and categorical variables were tested using Pearson chi-square test or Fisher exact test.

Survival curves and univariate analyses were conducted using the Kaplan-Meier method, and the differences were analyzed by the log-rank test. Multivariate Cox regression method was used to establish the prediction model (The variable introduction standard was $P < .2$). The best model parameters were selected according to the minimum Akaike's information criterion (AIC), and calculate hazard ratio (HR) and 95% confidence interval (CI). The time-dependent the area under the curve (AUC) of the model was drawn based on the methods proposed by Chambless and Diao.[14] All analyses were performed using R 3.4.3 (http://www.R-project.org). $P < .05$ was considered statistically significant.

3. Results

3.1. Clinical characteristics

A total of 43 patients with BCLC stage C primary liver cancer met the inclusion criteria. The average age of the patients was 59.6 ± 9.7 years (44–77 years); males accounted for 79.1% (34/43); cirrhosis accounted for 79.1% (34/43); embolization at portal vein trunk or branch accounted for 30.2% (13/43); ascites accounted for 34.9% (15/43). 86.0% (37/43) of the patients were positive for peripheral blood CTCs before TACE, and the CTCs median was 2.0 (1.0, 3.0). The peripheral blood CTCs median was 2.0 (1.0–3.0) and 3.0 (2.0–4.0) at 1 week and 4 weeks after TACE. The clinical characteristics, laboratory indicators, and peripheral blood CTCs of the study group are shown in Table 1.

3.2. Survival analysis of HCC patients after TACE treatment

The median follow-up time was 9 months, and the follow-up rate was 100.0%, and 32 patients died. Of the 32 deaths, 17 deaths (53.1%) due to HCC, 6 deaths (18.8%) due to liver failure, 9 deaths (28.1%) due to upper gastrointestinal hemorrhage and hepatic encephalopathy. The median survival time of all the patients was 12 months (range from 2 to 60 months), and the 1-, 3-, and 5-year survival rates were 42.9%, 9.0%, and 3.6%, respectively (see Fig. 2). According to the median of CTC1, CTC2 and CTC3, we transformed them into binary variables and drew survival curves for each group (see Figs. 3 and 4 and Table 2). It

was found that the OS of patients with high level of CTCs before TACE (CTC1 > 2) was significantly lower than that of patients with low level of CTCs (8 vs 12 months, $P = .040$, Fig. 3A), but there was no significant difference in PFS between the 2 groups ($P = .926$). Meanwhile, there was no significant difference in OS and PFS between patients with high level CTCs and those with low level CTCs at 1 week and 4 weeks after TACE (P all > .05), Table 2.

3.3. Univariate and multivariate Cox regression analysis

In univariate Cox regression analysis, possible variables related to prognosis include family heredity history, liver cirrhosis, portal vein cancerous thrombosis, ECOG performance status, number of lesions, length of the largest tumor, AFP and CTC1 (all $P < .2$, See Table 3). In COX multivariate regression analysis, the effects

Table 1	
Clinical characteristics, laboratory indicators, and peripheral blood CTCs of the patients.	
Parameters	**Value**
Age (r)	59.6 ± 9.7
Male (%)	34 (79.1%)
Family heredity history (yes)	11 (25.6%)
Liver cirrhosis (yes)	34 (79.1%)
Chronic hepatitis (yes)	38 (88.4%)
Portal vein cancerous thrombosis (yes)	13 (31.7%)
Combined ascites (yes)	15 (36.6%)
Child-Pugh classification	
A	22 (51.2%)
B	21 (48.8%)
ECOG performance status	
1	28 (65.1%)
2	15 (34.9%)
Number of lesions	
≤3	32 (74.4%)
>3	11 (25.6%)
Length of the largest tumor (cm)	6.7 (5.3–10.0)
AFP (ng/ml)	154.8 (5.1–1636.1)
CA199 (U/ml)	15.2 (8.5–24.5)
CA125 (U/ml)	25.7 (11.4–52.1)
CTC1	2.0 (1.0–3.0)
CTC2	2.0 (1.0–3.0)
CTC3	3.0 (2.0–4.0)

AFP = Alpha-fetoprotein, CA125 = Carbohydrate antigen 125, CA199 = Carbohydrate antigen 199, CTC1 = peripheral blood CTCs before TACE, CTC2 = peripheral blood CTCs at 1 week after TACE, CTC3 = peripheral blood CTCs at 4 weeks after TACE, ECOG = Eastern Cooperative Oncology Group.
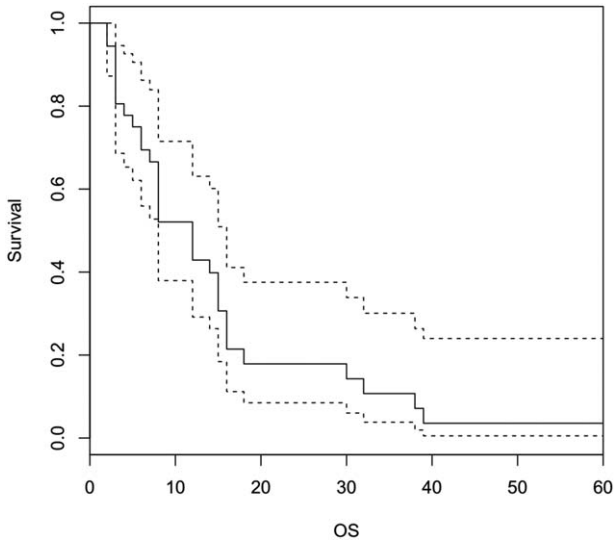
Figure 2. Survival curve of advanced hepatocellular carcinoma patients after TACE treatment. The solid line is the survival curve, and the dashed line is the 95% confidence interval.

from family heredity history, liver cirrhosis, portal vein cancerous thrombosis, ECOG performance status, length of the largest tumor and AFP were corrected, and the results showed that the number of lesions and CTC1 were the independent influencing factors for prognosis in these patients, and the HR was 3.01 (95% CI: 1.12 - 8.12) and 1.20 (95% CI: 1.01 - 31.42), respectively (all $P < .05$). See Table 3.

The Cox regression model is as follows: $1.10273 \times$ (Number of lesions $\leq 3 = 0$, Number of lesions $> 3 = 1$) $+ 0.17827 \times CTC1$. Then draw the model-dependent time-dependent AUC (Fig. 5). Utilizing this model, AUC to predict OS increased with the increase of follow-up time, ranging from 0.56 to 0.85, which indicates that our distributed Cox model works properly for prediction (AUC > 0.5) by estimating parameters β.

4. Discussion

The advanced HCC (BCLC stage C) represents a unique clinical challenge. The prognosis and treatment decision usually depends on the extent of the vascular invasion and/or metastatic disease, the severity of underlying cirrhosis, and the patient's condition.[15] The large sample studies, prospective studies, randomized controlled studies, SHARP studies,[16] Oriental studies[17] all showed that sorafenib could significantly prolong the overall survival and progression-free survival of advanced HCC patients,
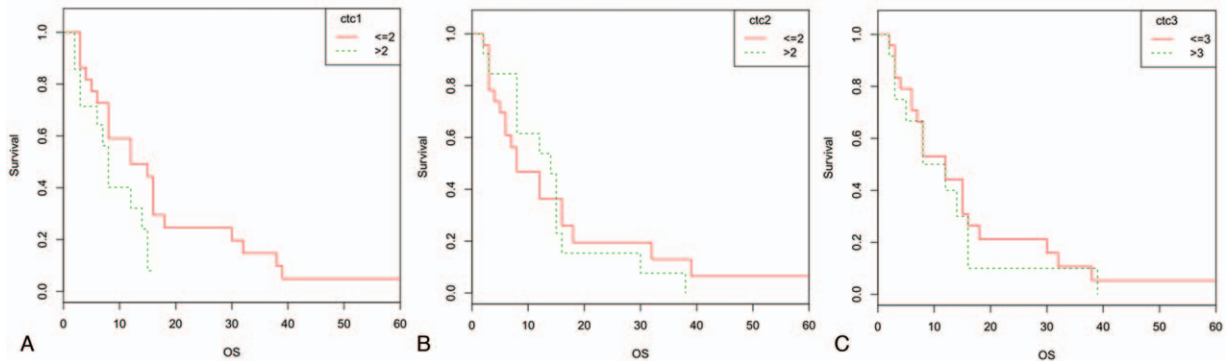

Figure 3. A-3C Influence of serum CTCs level before and after TACE treatment on overall survival (OS) of advanced hepatocellular carcinoma (HCC) patients. 3A: Serum CTCs before TACE and OS of advanced HCC patients, 3B: Serum CTCs after 1 week after TACE and OS of advanced HCC patients, 3C: Serum CTCs after 4 weeks after TACE and OS of advanced HCC patients.
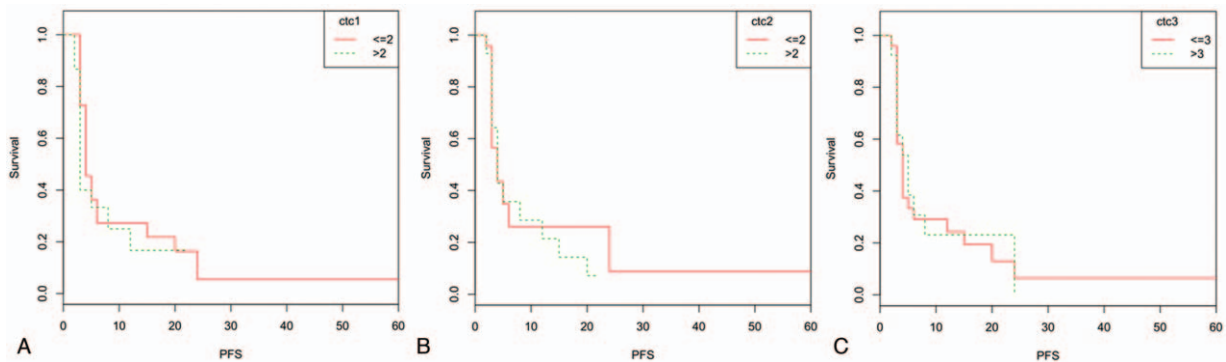

Figure 4. A-4C Influence of serum CTCs level before and after TACE treatment on progression-free survival (PFS) of advanced hepatocellular carcinoma patients. 4A: Serum CTCs before TACE and PFS of advanced HCC patients, 4B: Serum CTCs after 1 week after TACE and PFS of advanced HCC patients, 4C: Serum CTCs after 4 weeks after TACE and PFS of advanced HCC patients.

Table 2

Effect of peripheral blood CTCs levels on prognosis of HCC patients before and after TACE treatment.

	CTC1		CTC2		CTC3	
	≤2	>2	≤2	>2	≤3	>3
Median OS (months)	12*	8*	8	14	12	10
Median PFS (months)	4	3	4	4	4	5

CTC1 = peripheral blood CTCs before TACE, CTC2 = peripheral blood CTCs at 1 week after TACE, CTC3 = peripheral blood CTCs at 4 weeks after TACE, OS = overall survival, PFS: progression-free survival.
*indicates $P < 0.05$

but the prolonged survival was only 2.8 months (SHARP study)[16] or 2.4 months (Oriental study).[17] In China, TACE is still 1 of the effective treatment options for advanced HCC according to the guidelines.[5] In this study, the median survival time of the enrolled patients after TACE treatment was 12 months, higher than the sorafenib treatment group in the SHARP study (10.7 months)[16] and the Oriental study (6.5 months).[17] Also, we found that, although the included patients were all BCLC stage C and were treated with TACE, there was a large variation in overall survival between different patients (2 to 60 months). This obvious heterogeneity was consistent with the previous reports.[18] Therefore, if we can select the advanced HCC patients who can benefit from TACE treatment, it will help to realize the individualized treatment of HCC patients.

CTCs are malignant tumor cells present in the blood circulation, and its number is closely related to tumor burden, tumor blood supply, and tumor invasiveness.[19] Dynamic detection of CTCs before and after treatment can help in predicting the efficacy of HCC treatment and monitoring tumor recurrence. Sun et al[20] found that the peripheral blood CTCs of HCC patients were significantly reduced after surgery, and the

recurrence rate of the patients with continuous CTCs <2 was significantly lower than those with CTCs≥2. Huang et al[21] also suggested that CTCs number at different time points before and after TACE treatment was an important prognostic parameter for HCC recurrence. However, some studies[22] also found that TACE treatment could cause peripheral blood CTCs to increase in HCC patients. The progression-free survival of the patients with elevated CTCs was not significantly different from those with unchanged CTCs. In our study, we found that for patients with BCLC stage C HCC, median OS of patients with CTC1 > 2 was significantly shorter than patients with CTC1 ≤ 2 (8 vs 12 months), and the CTCs measured after treatment (CTC2 and CTC3) could not predict survival and prognosis, which might be because that TACE treatment could cause increased release of CTCs.[22,23]

Tumor burden in terms of tumor size and number play an essential role in determining the survival outcomes. Zhao Y et al[24] found that for patients with advanced-stage HCC, the number of tumors ≥3 was significantly associated with decreased survival and was used for determining the risk scores. Zhao P et al[25] also found that tumor number was associated with

Table 3

Univariate and Multivariate Cox regression analysis of predicting patient prognosis.

Exposure	Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (years)	0.98 (0.95, 1.02)	0.290	—	—
Male (%)	0.83 (0.35, 1.94)	0.666	—	—
Family heredity history (yes)	1.74 (0.76, 3.99)	0.193	—	—
Liver cirrhosis (yes)	1.80 (0.76, 4.22)	0.180	—	—
Chronic hepatitis (yes)	0.76 (0.29, 2.02)	0.587		
Portal vein cancerous thrombosis (yes)	1.93 (0.85, 4.37)	0.114	—	—
Combined ascites (yes)	1.44 (0.67, 3.10)	0.346	—	—
Child-Pugh classification				
A	1.0		—	—
B	1.54 (0.74, 3.21)	0.244	—	—
ECOG performance status				
1	1.0		—	—
2	1.91 (0.82, 4.46)	0.137	—	—
Number of lesions				
≤3	1.0		1.0	
>3	2.66 (1.15, 6.17)	0.022*	3.01 (1.12, 8.12)	.029*
Length of the largest tumor (cm)	1.08 (0.99, 1.19)	0.096	—	—
AFP (ng/mL)	1.00 (1.00, 1.00)	0.143	—	—
CA199 (U/mL)	1.01 (1.00, 1.02)	0.243	—	—
CA125 (U/mL)	1.00 (1.00, 1.00)	0.265	—	—
CTC1	1.22 (1.05, 1.43)	0.012*	1.20 (1.01, 1.42)	.040*
CTC2	1.13 (0.93, 1.37)	0.213	—	—
CTC3	1.08 (0.87, 1.34)	0.502	—	—

AFP = alpha-fetoprotein, CA125 = carbohydrate antigen 125, CA199 = carbohydrate antigen 199, CTC1 = peripheral blood CTCs before TACE, CTC2 = peripheral blood CTCs at 1 week after TACE, CTC3 = peripheral blood CTCs at 4 weeks after TACE, ECOG = Eastern Cooperative oncology group.
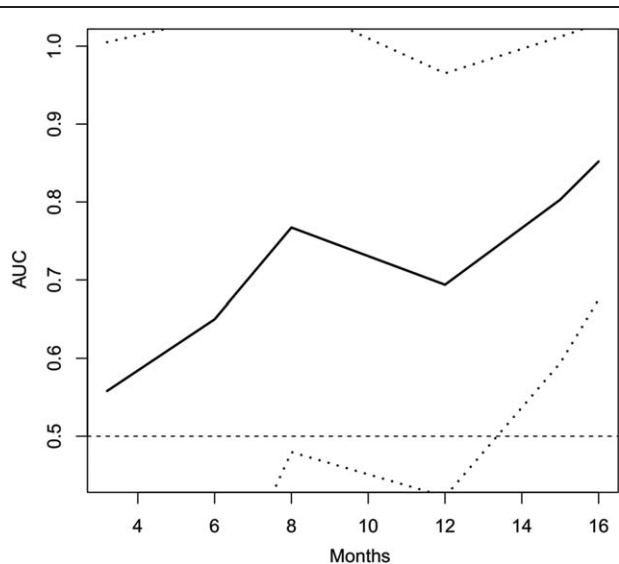*indicates $P < .05$.

Figure 5. The time-dependent AUC of the Cox regression model, where the solid line and the dotted line represent AUC and random chance, respectively. AUC increased with the increase of follow-up time, ranging from 0.56 to 0.85, which indicates that our distributed Cox model works properly for prediction (AUC > 0.5) by estimating parameters β.

progression-free-survival after TACE treatment. Our study found that the number of lesions greater than 3 was 1 of the independent prognostic factors for patients with advanced HCC. The potential cause may be related to the number of nodules ≥2 was associated with worse CR.[26]

Several prognostic algorithms (HAP, modified HAP, ART, ABCR score, 6 and12 criteria, et al) have shown potential to address the clinical heterogeneities of patients with intermediate-stage HCC, and facilitate the early identification of patients with poor prognostic features, who may need alternative treatments or best supportive care.[27] However, these HCC prognostic algorithms were developed based on highly heterogeneous patients, and their prognostic values for TACE-treated HCC patients are still controversial.[28] Moreover, the existing algorithms are mostly aimed at patients with mid-term HCC (ie, BCLC-B), with very few can predict the prognosis of advanced HCC patients, and their application has been limited in clinical practice.[29] Edeline J et al constructed the SAP (Sorafenib Advanced HCC Prognosis) scoring system based on the HAP scoring system to stratify the prognosis of advanced HCC patients after sorafenib treatment (AUC = 0.675–0.732).[29] Zhao Y et al[24] utilized six factors (Child-Pugh class, PVTT, extrahepatic metastasis, tumor size, number of HCC nodules and alpha-fetoprotein) to construct the risk score model, the AUC to predict 1 year survival was 0.7 (95%CI 0.7–0.8). However, the Cox regression model constructed by the tumor number and CTC before TACE was simple and easy to operate and had a high predictive effect (AUC: 0.56–0.85) on OS of patients with advanced HCC.

4.1. Limitations

There are some limitations to our study. First, this study is a single-center retrospective study. Although TACE is 1 of the treatment options for patients with BCLC stage C HCC, according to China's HCC diagnosis and treatment guidelines, the enrolled population is still small. It still cannot completely rule out the risk of overfitting.

Therefore, large sample, prospective, and randomized control studies are required to externally validate the model. Second, CTCs in peripheral blood has different phenotypes, and the biological characteristics of different CTCs phenotypes are significantly different. Investigating the predictive value of different CTCs phenotypes on therapeutic efficacy and survival prognosis of HCC patients is our future research direction.

5. Conclusions

In summary, TACE is an effective treatment for advanced HCC patients, but the prognosis is quite heterogeneous. The number of peripheral blood CTCs before TACE is an effective biomarker for predicting the OS of advanced HCC patients. The prognostic prediction model constructed based on CTCs before TACE and tumor number can effectively predict the OS of patients with advanced HCC.

Author contributions

Conceptualization: Jun Deng, Jun Li.
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Validation: Jun Deng, Jun Li.
Writing – original draft: Jun Deng, Jun Li.
Writing – review & editing: Jun Deng, Jun Li.

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