

The predictive values of serum dickkopf-1 and circulating tumor cells in evaluating the efficacy of transcatheter arterial chemoembolization treatment on hepatocellular carcinoma

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Abstract

In this study, we aim to explore the values of serum dickkopf-1 (DKK1) and circulating tumor cells (CTCs) in predicting the efficacy and prognosis of transcatheter arterial chemoembolization (TACE) treatment on patients with hepatocellular carcinoma (HCC). We did a retrospective analysis on 155 HCC patients who underwent TACE treatment. The patients were divided into response group (complete response and partial response) and nonresponse group (stable disease and progressive disease), and their changes in serum DKK1 and CTCs after TACE were recorded. Receiver operating characteristic (ROC) curve and survival analysis were used to assess the predictive values of DKK1 and CTCs for TACE efficacy and long-term prognosis of HCC. We found that the levels of preoperative DKK1 and CTCs in patients with HCC had a moderate positive correlation ($r=0.54$). After TACE treatment, the serum DKK1 and CTCs in the response group were significantly decreased compared to pretreatment levels ($P<.05$), whereas the nonresponse group showed significantly increased serum DKK1 and CTCs levels ($P<.05$). The largest area under the curve (AUC) was achieved when using $>0.02\ \mu\text{g/L}$ reduction in DKK1 level after 4 weeks of TACE to predict the efficacy of TACE treatment (AUC = 0.913, 95% confidence interval: 0.856–0.952, $P<.001$), with the sensitivity of 78.26% and the specificity of 88.07%. The overall survival, disease-free survival, and 5-year survival rates were all significantly lower in the patients with positive preoperative levels of serum DKK1 and CTCs. COX multivariate regression analysis showed that Eastern Cooperative Oncology Group score, and preoperative levels of serum DKK1 and CTCs are independent influencing factors for the prognosis of patients with HCC. Overall, our results demonstrated that serum DKK1 and CTCs levels were good biomarkers for predicting the efficacy and prognosis of TACE treatment in patients with HCC. Moreover, these parameters exhibited different characteristics, and might have different potential applications.

Abbreviations: AFP = α -fetoprotein, AUC = area under receiver operating characteristic curve, BCLC = Barcelona Clinic Liver Cancer, CR = complete response, CTCs = circulating tumor cells, DKK1 = dickkopf-1, ELISA = enzyme-linked immunosorbent assay, EpCAM = epithelial cell adhesion molecule, FISH = fluorescence in situ hybridization, HCC = hepatocellular carcinoma, mRECIST = modified Response Evaluation Criteria in Solid Tumors, OS = overall survival, PD = progressive disease, PFS = progression-free survival, PR = partial response, SD = stable disease, TACE = transcatheter arterial chemoembolization.

Keywords: circulating tumor cells, dickkopf-1, hepatocellular carcinoma, prognosis, therapy response

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignant disease and the third leading cause of cancer-related death in China.^[1] Surgical intervention (including local hepatectomy

and liver transplantation) is still the most effective treatment method, with the possibility of radical cure. Due to the low sensitivity of α -fetoprotein (AFP) (only 25%–65%)^[2] and the lack of health care awareness, >60% of patients with HCC are, however, already in advanced disease stages or with multiple lesions in cirrhotic/fibrotic

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Written informed consent was obtained from all individual participants included in the study.

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liver at the time of diagnosis, and thereby losing the opportunity of surgical resection.^[3] Currently, Transcatheter arterial chemoembolization (TACE) is the preferred method for treating the inoperable patients with advanced HCC. Randomized controlled trials have shown that TACE can improve the survival rate and life quality of patients with HCC.^[4] Not all the patients with HCC that meet the requirements for TACE can, however, benefit from this treatment, mainly due to the disease heterogeneities from tumor burden, liver function, disease etiology, etc.^[5] Also, repeated TACE can exaggerate liver damage and abnormal liver functions. Therefore, it is highly important to evaluate and predict the efficacy of TACE in early stage and screen out the patients who can benefit from it. Currently, there is no widely accepted serum biomarkers to predict the therapy response and long-term prognosis of TACE treatment.^[6]

AFP is a traditional serum biomarker for HCC diagnosis, therapeutic efficacy evaluation, and prognosis assessment, but its sensitivity and specificity are unsatisfactory. The American Association for the Study of Liver Diseases diagnostic criteria published in 2010 have removed AFP as a diagnostic criterion for HCC.^[7] With the widespread use of gene expression and liquid biopsy techniques, dickkopf-1 (DKK1)^[8] and circulating tumor cells (CTCs)^[9] are becoming the novel biomarkers with potential advantages over AFP. DKK1 is a negative regulator in Wnt signaling pathway. It plays an important role in cell proliferation, differentiation, survival, and apoptosis. Yu et al^[10] showed that DKK1 mRNA level was significantly higher in recurrent HCC samples compared to nonrecurrent samples, and high DKK1 was a marker for poor prognosis of HCC. CTCs are tumor cells that have been detached from the primary or metastatic lesions and released into peripheral blood circulation. These tumor cells can circulate through the bloodstream, travel to different tissues or organs, and result in distant metastasis.^[11] A number of studies have shown that the absolute numbers of CTCs and its changes after therapies are associated with survival and treatment response in multiple malignant tumors (breast, colon, prostate cancers, and HCC).^[12] It is, however, still unclear that how DKK1 and CTCs change after TACE treatment, whether their changes can be used to predict therapy response and long-term prognosis, and which one is a better biomarker. Therefore, in this study, we aim to explore the values of DKK1 and CTCs for predicting the efficacy and prognosis of TACE by conducting a retrospective case-control study and survival analysis.

2. Materials and methods

2.1. Clinical data

A retrospective analysis was conducted on the patients with HCC who underwent TACE treatment from February 2012 to January 2014 at our hospital. All patients received imaging and histopathology examination. Their diagnosis and treatment followed the standards of diagnosis and treatment for HCC formulated by the Bureau of Medical Administration, National Health and Family Planning Commission of China in 2017.^[13] The patients did not receive surgery, radiotherapy, chemotherapy, or other antitumor treatments before. Their lesions were measurable and evaluable, with no evidence of distant metastasis. The patients volunteered to participate in the regular follow-up study. Clinical staging was based on Tumor/Lymph Node/Metastasis (TNM) system and the Barcelona Clinic Liver Cancer (BCLC) staging system.^[14] Exclusion criteria: iodine allergy; dysfunctions in liver, kidney, or blood coagulation; main portal

vein completely embolized by tumor; reduced collateral vessel formation; active hepatitis or severe infection; extensive distant metastasis; estimated survival of <3 months; dyscrasia or multiple organ failure; severe anemia; or significant reduction in white blood cells and platelets. The following information of the enrolled patients were recorded: clinical and imaging data, history of chronic hepatitis or cirrhosis, laboratory blood parameters, and serum DKK1 and CTCs levels before TACE and 1 and 4 weeks after TACE. The study was approved by the ethics committee of our hospital, and informed consents were obtained from the patients.

2.2. Treatment procedure

The Seldinger puncture technique was used to puncture the femoral artery, and the 5F catheter (Cook Medical, Bloomington, IN) was delivered to common hepatic or superior mesenteric artery under digital subtraction angiography. Angiography was performed to determine the variations of blood vessels, the presence of arteries in tumor blood supply, and the dyeing conditions. When the superselected catheterization reached the blood supply vessel of tumors, 30 mg epirubicin (Pfizer, New York, NY) was added to 5 to 20 mL of super liquid iodized oil for chemoembolization. If the remaining tumor feeding arteries were still abundant, embospheres with different diameters (Merit Medical Inc, South Jordan, UT) were selected for supplementary embolism. Routine hepatic treatment was performed after surgery.

2.3. Detection of serum DKK-1, CTCs levels, and their evaluation criteria

Peripheral venous blood was taken from all patients on an empty stomach. Serum levels of DKK-1 and CTCs were measured before TACE, and at 1 and 4 weeks after surgery. Serum DKK1 level was detected by enzyme-linked immunosorbent assay. The kit was purchased from Shanghai Raygene Company, Shanghai. DKK1 $\geq 2.15 \mu\text{g/L}$ was determined as positive.^[8] Serum CTCs were detected by immunomagnetic beads combined with fluorescence in situ hybridization (FISH). The morphology of enriched cells was analyzed under fluorescent microscope based on chromosome 8 staining (Centromere-enumeration probes 8, Beijing Lyle Biotechnology Company, Beijing, China), leukocyte surface marker staining (CD45-AF594 fluorescent antibody, German Miltenyi Biotec Company, Bergisch Gladbach, Germany), and nuclear staining (4',6-diamidino-2-phenylindole [DAPI], Beijing Lyle Biotechnology Company). The criteria were positive DAPI staining, no overlapped nuclei; abnormal chromosome 8 amplification, FISH signals ≥ 3 , no overlapped signal under 40X object; and no positive staining for blood-derived leukocyte surface antigen marker. The result was positive when 1 or more CTCs were found.

2.4. Treatment efficacy evaluation

Tumor measurements were performed by 2 qualified radiologists. When there was inconsistency in tumor measurement or response evaluation, a final decision was made by consensus. According to the modified Response Evaluation Criteria in Solid Tumors (mRECIST),^[15] the tumor response was classified as complete response (CR): the enhancement in arterial phase computed tomography (CT) disappeared for all target lesions; partial

response (PR): the total long diameters of all target lesions were reduced by $\geq 30\%$; progressive disease (PD): the total long diameters of all target lesions were increased by at least 20%, and the absolute increase of total long diameter was >5 mm or new lesions appeared; and stable disease (SD): neither PR nor PD. The patients with tumor responses of CR or PR after TACE were defined as response group, and the patients with SD or PD were defined as a nonresponse group.

2.5. Follow-up and prognosis evaluation

On the 4th week after the first TACE treatment, the patients received CT and/or magnetic resonance imaging (MRI), blood AFP measurement, liver and kidney function test, and blood routine examination. If imaging examination showed that the iodized oil deposition in liver tumors were dense, tumor tissue was necrotic, no tumor size increase, and no new lesions, then there was no need for another TACE treatment. These patients were followed up every 3 months. Their blood AFP levels and CT and/or MRI scan results were used to determine whether there was recurrence and/or metastasis, and whether another TACE treatment was needed. The follow-up was mainly based on outpatient service and telephone survey. The follow-up ended on June 30, 2018. Overall survival (OS) is the time from postsurgery to death of any reason; progression-free survival (PFS) is the time from postsurgery to initial tumor progression or death of any reason.

2.6. Statistical analysis

Statistical analysis was performed using SPSS 23.0 software. The data with normal distribution were expressed as mean \pm standard deviation ($\bar{x} \pm s$), and the non-normally distributed data were expressed by medians (P25, P75). The differences of serum DKK1 and CTCs levels before and after TACE were analyzed by Kruskal-Wallis test. The count data were expressed as frequency and percentage, and the comparison between groups was performed by χ^2 test. The receiver operating characteristic (ROC) curve was established to analyze the values of changes in serum DKK1 and CTCs after TACE for predicting the tumor response and prognosis of patients with HCC. The area under ROC curve (AUC) was compared by z test. The multivariate analysis of 2 categorical variables was conducted by logistic regression. Survival curves and univariate analysis were conducted using Kaplan-Meier method, and the differences were analyzed by log-rank test. The prognostic factors found to be significant in the univariate analysis were subjected to multivariate analysis with the Cox proportional hazards regression model. $P < .05$ in a 2-tailed test was considered statistically significant.

3. Result

3.1. Clinical characteristics of the patients

A total of 155 patients with HCC met the inclusion criteria. The average age was 58.0 ± 9.3 years (28–83 years). Male patients accounted for 67.3% (120/155), chronic hepatitis patients accounted for 93.5% (145/155), and cirrhosis patients accounted for 87.1% (135/155). The clinical data and pathological features are shown in Table 1. The levels of serum DKK1 and CTCs before TACE were 5.87 (3.06, 8.75) $\mu\text{g/L}$ and 2 (0, 3), respectively, and these 2 parameters had a moderate positive correlation ($r = 0.54$, $P < .001$).

Table 1

Baseline characteristics of studied subjects (n = 155).

Variables	No. (%)
Age, y	
≤ 60	88 (56.8%)
> 60	67 (43.2%)
Sex	
Male	120 (77.4%)
Female	35 (22.6%)
Family heredity history (yes)	57 (36.8%)
Chronic hepatitis (yes)	145 (93.5%)
Liver cirrhosis (yes)	135 (87.1%)
Portal vein cancerous thrombosis (yes)	28 (18.1%)
Alpha-fetoprotein level (>400 ng/mL)	39 (25.2%)
Primary tumor site	
Left lobe	19 (12.3%)
Right lobe	117 (75.5%)
Bilobar	16 (10.3%)
Tumor size (mRECIST standard)	
≥ 5 cm	94 (60.6%)
< 5 cm	61 (39.4%)
Morphological type	
Massive	99 (63.9%)
Nodule	56 (36.1%)
Child-Pugh classification	
A	89 (57.4%)
B	66 (42.6%)
BCLC stage	
A	59 (38.1%)
B	21 (13.5%)
C	75 (48.4%)
TNM stage	
I	52 (33.5%)
II	54 (34.8%)
III	43 (27.7%)
IV	6 (3.9%)
ECOG performance status	
0	80 (51.6%)
1	60 (38.7%)
2	15 (9.7%)

BCLC=Barcelona Clinic Liver Cancer, ECOG=Eastern Cooperative Oncology Group, mRECIST=modified Response Evaluation Criteria in Solid Tumors.

3.2. The changes of serum DKK1 and CTCs after TACE treatment

After TACE treatment, 70.3% (109/155) of the enrolled patients had response (CR or PR) and 29.6% (46/155) did not show response (SD or PD). The serum DKK1 and CTCs levels were measured before TACE, and at 1 and 4 weeks after TACE.

As shown in Table 2, the serum DKK1 level before TACE was significantly lower in the response group than in the nonresponse group ($P < .001$), but the ratios of positive patients were not significantly different ($P > .05$). For the response group, the DKK1 levels and the ratio of positive patients were significantly reduced after 1 and 4 weeks of TACE treatment, as compared with the preoperative levels ($P < .001$); moreover, the DKK1 levels after 4 weeks of treatment were significantly lower than the levels after 1 week of treatment ($P = .026$), but the proportion of positive patients did not change much ($P > .05$). For the nonresponse group, there was no significant difference in the proportion of positive patients or DKK1 levels at all time points ($P > .05$); moreover, the DKK1 levels after 1 week of treatment

Table 2
Changes of serum dickkopf-1 levels in response and nonresponse group before and after transcatheter arterial chemoembolization treatment.

Group	Response group (n = 109)		Nonresponse group (n = 46)	
	Value, $\mu\text{g/L}$	Positive cases (%)	Value, $\mu\text{g/L}$	Positive cases (%)
Before TACE	4.73 (2.23, 7.14)	87 (79.8%)	8.43 (5.78, 9.84)	39 (84.8%)
1 wk after TACE	2.76 (1.89, 4.80)*	63 (57.8%)*	7.81 (5.24, 9.80)	42 (91.3%)
4 wk after TACE	2.22 (1.89, 4.35) ^{†,‡}	66 (60.6%) [†]	8.47 (5.95, 10.25) ^{†,‡}	43 (93.5%)

* The difference between 1 week after TACE and before TACE is statistically significant.

[†] The difference between 4 weeks after TACE and before TACE is statistically significant.

[‡] The difference between 4 weeks after TACE and 1 week after TACE is statistically significant.

TACE = transcatheter arterial chemoembolization.

was not significantly changed compared to the preoperative levels ($P = .096$), and the DKK1 levels after 4 weeks of treatment were significantly increased compared to both the preoperative levels ($P = .036$) and the levels after 1 week of treatment ($P < .001$).

As shown in Table 3, the serum CTCs and the proportion of positive patients before TACE were significantly lower in the response group, as compared to the nonresponse group ($P < .001$). For the response group, there was no significant difference in the proportion of positive patients at all time points ($P > .05$), but the CTCs levels were significantly reduced after 1 week of TACE treatment ($P < .001$); moreover, the CTCs levels after 4 weeks of TACE was significantly lower than preoperative levels ($P < .001$). For the nonresponse group, there was no significant difference in the proportion of positive patients at all time points ($P > .05$); the CTCs levels after 1 week of treatment were not significantly changed after 1 week of treatment ($P = .683$), but were significantly increased after 4 weeks of treatment, as compared to the levels after 1 week of treatment ($P = .001$) and the preoperative levels ($P < .001$).

3.3. The predictive values of changes in serum DKK1 and CTCs levels after TACE treatment

The changes of serum DKK1 and CTCs after 1 and 4 weeks of TACE treatment were calculated and recorded as $\Delta\text{DKK1-1}$, $\Delta\text{DKK1-2}$, $\Delta\text{CTCs-1}$, and $\Delta\text{CTCs-2}$. The corresponding ROC curves are drawn (Fig. 1) to predict the response status of patients with HCC after TACE treatment. $\Delta\text{DKK1-1}$, $\Delta\text{DKK1-2}$, $\Delta\text{CTCs-1}$, and $\Delta\text{CTCs-2}$ were -1.08 ($-2.24, -0.06$) $\mu\text{g/L}$, -0.98 ($-2.23, 0.08$) $\mu\text{g/L}$, and 0 ($-1, 0$) and 0 ($-1, 1$) respectively. The predictive values of DKK1 changes after 1 and 4 weeks ($\Delta\text{DKK1-1}$, $\Delta\text{DKK1-2}$) for TACE were better than CTCs ($\Delta\text{CTCs-1}$, $\Delta\text{CTCs-2}$), and the area under the curve was significantly

different (Fig. 1, $P = .025$ and $< .001$). Moreover, the predictive value of DKK1 changes after 4 weeks of TACE was better than 1 week, and the area under the curve was significantly different (Fig. 1, $P < .001$). When the reduction of DKK1 level was $> 0.02 \mu\text{g/L}$ after 4 weeks of TACE treatment ($\Delta\text{DKK1-2}$), its sensitivity for predicting postoperative response was 78.26%, the specificity was 88.07%, and the AUC was 0.913 [95% confidence interval (CI): 0.856–0.952, $P < .001$].

Logistic regression analysis was used to analyze and correct the effects of patient's age, sex, family history, chronic hepatitis history, cirrhosis, portal vein thrombosis, blood AFP level, Eastern Cooperative Oncology Group (ECOG) score, primary tumor location, tumor size, morphological classification, Child-Pugh classification, BCLC staging, and TNM staging. The results showed that the changes of DKK1 after 4 weeks of TACE ($\Delta\text{DKK1-2}$) was an independent risk factor for postoperative response ($\beta = 1.94$, odds ratio = 6.957, 95% CI: 3.385–14.298, $P < .001$).

3.4. Survival analysis of patients with HCC after TACE treatment

The median follow-up time was 32 months, and the follow-up rate was 89.7% (139/155). Among all the patients, 16 of them did not complete follow-up due to the loss of connection, and 98 patients died. The median survival time of all the patients was 37 months, and the 1-, 3-, and 5-year survival rates were 82.0%, 51.5%, and 29.4%, respectively.

The median OS of the patients with positive serum DKK1 before TACE was 32 months, and their 1-, 3-, and 5-year survival rates were 78.5%, 43.4%, and 21.1%, respectively, which were significantly lower than the DKK1-negative patients, whose median OS was 70 months, and the 1-, 3-, and 5-year survival

Table 3
Changes of serum circulating tumor cells levels in response and nonresponse group before and after transcatheter arterial chemoembolization treatment.

Group	Response group (n = 109)		Nonresponse group (n = 46)	
	Value	Positive cases (%)	Value	Positive cases (%)
Before TACE	1 (0, 3)	71 (65.1%)	3 (2, 4)	44 (95.7%)
1 wk after TACE	1 (0, 2)*	63 (57.8%)	3 (2, 4)	45 (97.8%)
4 wk after TACE	1 (0, 2) [†]	65 (59.6%)	3 (2, 5.5) ^{†,‡}	45 (97.8%)

* The difference between 1 week after TACE and before TACE is statistically significant.

[†] The difference between 4 weeks after TACE and before TACE is statistically significant.

[‡] The difference between 4 weeks after TACE and 1 week after TACE is statistically significant.

TACE = transcatheter arterial chemoembolization.

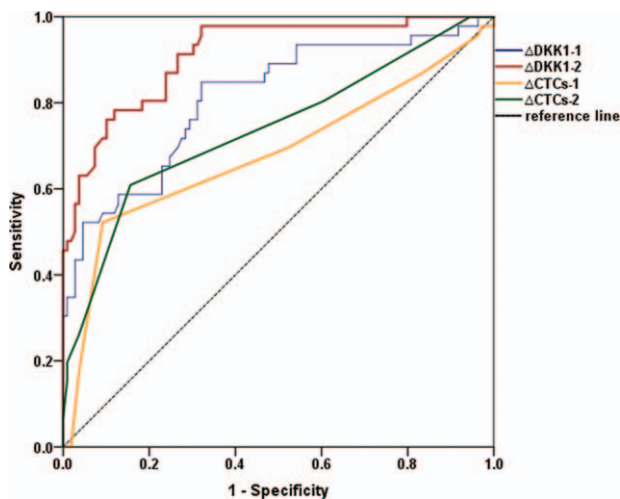


Figure 1. The values of serum DKK1 and CTCs changes for predicting transcatheter arterial chemoembolization (TACE) efficacy. CTC=circulating tumor cell, DKK1=dickkopf-1.

rates were 92.6%, 84.7%, and 62.6%, respectively ($\chi^2 = 13.511$, $P = .001$) (Fig. 2A). The median PFS of the patients with positive serum DKK1 before TACE was 24 months, and their 1-, 3-, and 5-year PFS rates were 58.0%, 31.3%, and 14.1%, respectively, which were significantly lower than the DKK1-negative patients, whose median PFS was 70 months, and the 1, 3, and 5-year PFS rates were 82.1%, 64.1%, and 52.8%, respectively ($\chi^2 = 11.525$, $P = .001$) (Fig. 2B).

The median OS of the patients with positive and negative serum CTCs before TACE were 28 and 63 months, respectively. The 1-, 3-, and 5-year OS rates were 76.2%, 34.5%, and 12.6% for positive patients and 94.9%, 89.5%, and 67.1% for negative patients, respectively; the differences were statistically significant ($\chi^2 = 35.771$, $P < .001$) (Fig. 3A). The median PFS of positive and negative patients were 15 and 63 months, respectively. The 1-, 3-, and 5-year PFS rates were 50.9%, 14.1%, and 9.5% for positive patients and 94.9%, 86.9%, and 67.3% for negative patients,

respectively; the differences were statistically significant ($\chi^2 = 45.513$, $P < .001$) (Fig. 3B).

Univariate analysis was used to analyze the effects of clinical characteristics on the prognosis of patients with HCC after TACE treatment. The results showed that age, portal vein thrombosis, blood AFP level, tumor size (mRECIST criteria), morphological classification, Child-Pugh staging, BCLC staging, TNM staging, and ECOG score were also prognostic factors for these patients ($P = .000-.010$). Moreover, high serum DKK1 and CTCs before TACE treatment were correlated with male, portal vein cancerous thrombosis, bilobar (primary tumor site), tumor size ≥ 5 cm, massive morphological type, Child-Pugh classification, BCLC and TNM stage, and ECOG performance status ($r = 0.163-0.749$, all $P < .05$, Table 4), indicating that high serum DKK1 and CTCs are also prognostic factors for HCC after TACE treatment. In addition, COX multivariate regression analysis showed that ECOG score and the preoperative serum levels of DKK1 and CTCs were the independent influencing factors for the prognosis of these patients, and the hazard ratios were 3.223 (95% CI: 1.753–5.925), 1.993 (95% CI: 1.019–3.896), and 2.844 (95% CI: 1.411–5.734), respectively (all $P < .05$).

4. Discussion

The BRIDGE study^[16] showed that, in real-world practice, TACE is the most widely used and preferred treatment for patients with HCC, but the efficacy and long-term prognosis of the patients with HCC received TACE are highly heterogeneous.^[15] In this study, the response rate (CR + PR) after TACE was 70.3%, and the median OS was 37 months, which was consistent with the BRIDGE study.^[16] Therefore, identifying the patients who can benefit from TACE in early stage could help improve their prognosis. Currently, the existing algorithms for assessing the efficacy and prognosis of HCC treatment (such as BCLC staging, Okuda grading system, mRECIST criteria) usually focus on the clinical symptoms, physical status, tumor status (tumor size, tumor number, vascular invasion, lymph node metastasis, etc), liver function, and imaging features of the patients, but often ignore the molecular characteristics of the HCC tumors.^[17] DKK1 and CTCs are potential serum biomarkers for HCC

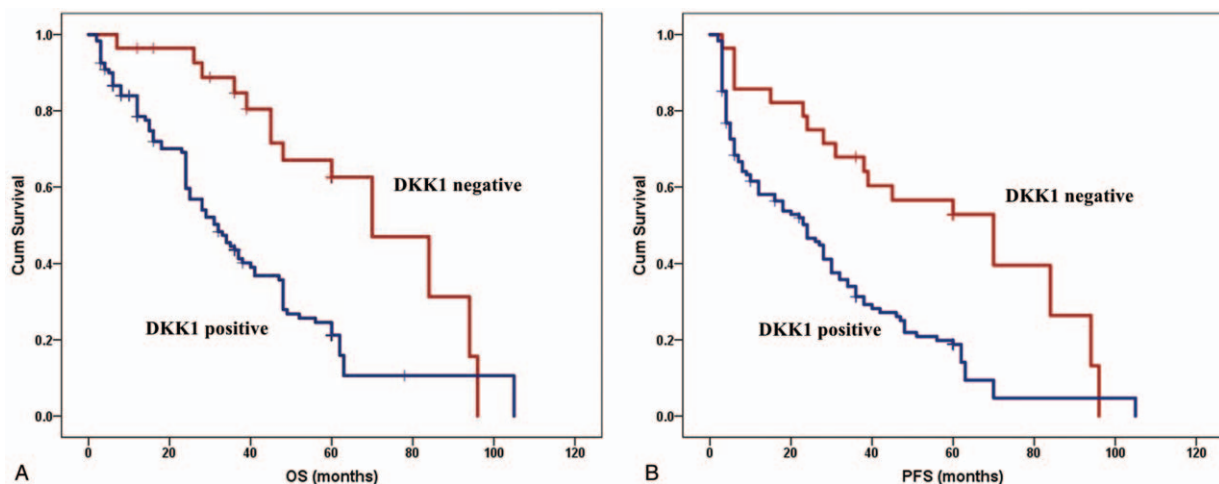


Figure 2. Survival analysis of the patients with hepatocellular carcinoma (HCC) with positive and negative serum DKK1 levels before transcatheter arterial chemoembolization (TACE). A, Overall survival (OS) of the 2 groups. B, Progression-free survival (PFS) of the 2 groups, red curve is DKK1-negative group, blue curve is DKK1-positive group. DKK1=dickkopf-1.

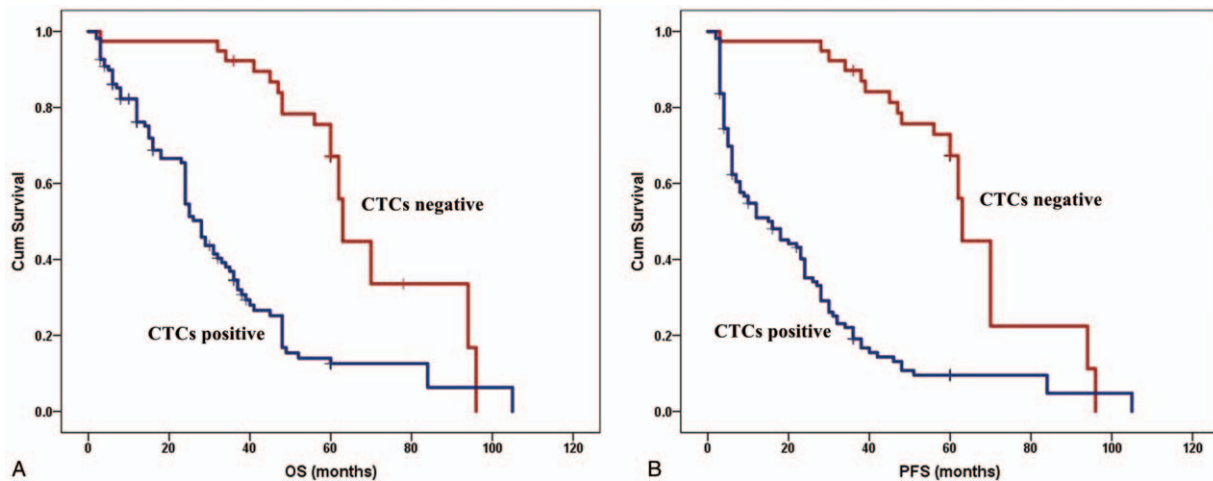


Figure 3. Survival analysis of the patients with hepatocellular carcinoma (HCC) with positive and negative serum CTCs levels before transcatheter arterial chemoembolization (TACE). A, OS of the 2 groups. B, PFS of the 2 groups, red curve is CTCs-negative group, blue curve is CTCs-positive group. CTC circulating tumor cells, OS=overall survival, PFS=progression-free survival.

diagnosis and prognosis, and have been widely studied in recent years. They reflect the biological characteristics of tumor invasion and metastasis. They are, however, rarely used for evaluating the efficacy and prognosis of TACE treatment in patients with HCC. In this study, we dynamically recorded the changes of serum DKK1 and CTCs levels in patients with HCC with TACE treatment, and explored their values for predicting TACE efficacy and prognosis.

DKK1 was highly expressed in the tumors and serum of HCC patients. Several studies^[10,18] have shown that the level of serum DKK1 is closely related to HCC tumor size, Edmondson-Steiner grade, venous infiltration, and tumor invasion, indicating that it is a potential biomarker for monitoring HCC recurrence and metastasis. The underlying mechanism might be related to the regulation of noncanonical Wnt pathway. In this study, we found that the preoperative serum DKK1 levels of patients with HCC

who achieved CR or PR after TACE treatment were significantly lower than the nonresponse group (SD and PD). The serum DKK1 level gradually decreased after TACE, and when the reduction in serum DKK1 level at 1 month after TACE was $>0.02 \mu\text{g/L}$, its sensitivity and specificity of predicting tumor response were 78.26% and 88.07%, which were better than CTCs. These results suggest that the change of serum DKK1 after treatment is a sensitive parameter for predicting the efficacy of TACE in patients with HCC.

Also, it has been found^[10] that DKK1-positive patients have poor long-term prognosis, and the patients with HCC with high DKK1 expression have significantly lower 5-year OS and disease-free survival rate compared to DKK1-negative patients (46.0% and 18.0% vs 66.0% and 59.8%, $P=.002$). These results are consistent with our study, suggesting that serum DKK1 level is also a biomarker for predicting long-term prognosis in patients

Table 4
Correlation analysis between serum dickkopf-1 and circulating tumor cells and clinical characteristic of hepatocellular carcinoma patients before transcatheter arterial chemoembolization treatment.

Variables	DKK1, $\mu\text{g/L}$		CTCs	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age	-0.072	.373*	0.036	.652*
Sex (female/male)	0.163	.043†	0.254	.001†
Family heredity history (no/yes)	0.068	.399	0.082	.311
Chronic hepatitis (no/yes)	-0.107	.184	-0.111	.168
Liver cirrhosis (no/yes)	0.063	.439	-0.014	.867
Portal vein cancerous thrombosis (no/yes)	0.557	.000†	0.341	.000†
Alpha-fetoprotein level	0.234	.004 ^{*,†}	0.064	.435*
Primary tumor site (left/right/bilobar)	0.208	.010†	0.185	.023†
Tumor size (mRECIST standard) (<5/≥5 cm)	0.506	.000†	0.354	.000†
Morphological type (nodule/massive)	0.311	.000†	0.281	.000†
Child-Pugh classification (A/B)	0.318	.000†	0.252	.002†
BCLC stage (0-A/B/C)	0.541	.000†	0.543	.000†
TNM stage (I/II/III/IV)	0.749	.000†	0.582	.000†
ECOG performance status (0/1/2)	0.518	.000†	0.445	.000†

BCLC=Barcelona Clinic Liver Cancer, CTC=circulating tumor cell, DKK1=dickkopf-1, ECOG=Eastern Cooperative Oncology Group, mRECIST=modified Response Evaluation Criteria in Solid Tumors.

* Pearson correlation analysis was used; the remaining variables were analyzed by spearman correlation.

† There was statistical significance.

with HCC. In addition, we also found that the preoperative levels of serum DKK1 and CTCs were only moderately correlated ($r=0.54$), and the HR value of DKK1 after COX multivariate regression analysis was lower than CTCs, indicating that its value of predicting long-term prognosis of HCC was slightly lower than CTCs, and they have different biological mechanisms.

Because of the abundant blood supply to liver, blood transfer is the main mode of metastasis after HCC treatment, and is also an important influencing factor for prognosis. Thus, the appearance of CTCs is an important evidence for HCC metastasis. Currently, the detection of CTCs usually uses an immunomagnetic enrichment technique based on epithelial cell adhesion molecule (EpCAM). Most HCC cells, however, do not express EpCAM,^[19] and EpCAM is often lost in tumor cells during the epithelial mesenchymal transition process,^[20] which can possibly result in no detection of CTCs. In this study, we specifically obtained CTCs by amplifying chromosome 8 (CEP8) with negative enrichment and identified them by in FISH using CEP8/CD45/DAPI markers. We found that the serum CTCs in nonresponse group were significantly higher than the response group, regardless of before or after TACE; moreover, the serum CTCs in nonresponse group continued to increase over time. In addition, TACE is a local treatment that causes tumor necrosis by embolizing the artery for tumor blood supply. Although embolization can reduce the further release of CTCs into the blood, it cannot affect the CTCs that are already in the blood. Thereby, the area under curve of using serum CTCs changes to predict the efficacy of TACE treatment was significantly lower than DKK1. The CTCs in peripheral blood can develop into metastases under certain conditions after arriving at new organs through blood circulation.^[21] Several studies have shown that^[22] CTCs are key factors for predicting HCC recurrence, metastasis, and long-term prognosis, which are also consistent with our results. We found that the patients with HCC with positive serum CTCs had a median OS and PFS of only 28 and 15 months after TACE treatment, and their 5-year OS rate and PFS rate were 12.6% and 9.5%, respectively; the risk ratio of these patients was 2.844 after COX regression analysis, which was higher than DKK1, suggesting that the risk of poor prognosis in serum CTCs-positive patients was higher than that in DKK1-positive patients.

5. Conclusions

In summary, the serum levels of DKK1 and CTCs are effective biomarkers for predicting the efficacy and long-term prognosis of TACE treatment in patients with HCC. There is only a moderate positive correlation between the 2 parameters, indicating that the mechanisms of producing DKK1 and CTCs by HCC tumors are different. We analyzed the changes of serum DKK1 and CTCs levels after TACE treatment, and found that when the changes of serum DKK1 was $>0.02 \mu\text{g/L}$ after 1 month of TACE treatment, its area under curve for predicting tumor response was the largest, and the sensitivity and specificity were the highest. Moreover, although the prognosis of CTCs-positive and DKK1-positive patients were both poor, the risk ratio of CTCs-positive patients was greater than DKK1-positive patients, suggesting that the risk of poor prognosis in serum CTCs-positive patients was higher.

6. Limitations

First, this study is a retrospective study from 1 institution and with small sample size; thereby, a larger sample size is needed to

further understand the relationship between serum DKK1 and CTCs. Second, due to the high incidence of chronic hepatitis and cirrhosis in patients with HCC in China, the enrolled group included 38.1% of BCLC stage A patients, resulting in a slightly higher OS and 5-year survival rate than the literatures.

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