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Circulating Tumor Cells Predict Prognosis of Patients with Hepatocellular Carcinoma

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Abstract

Background: Peripheral blood Circulating Tumor Cells (CTCs) was detected and their prognostic significance was investigated in HCC patients undergoing hepatectomy.

Materials and Methods: A total of 137 patients were recruited for the study. The time points for blood collection were one day before the operation, and at 1 month, 2 months after surgery. Preventative adjuvant Transarterial Chemoembolization (TACE) treatment was performed in 26 HCC patients 1 month after liver resection. CTCs were detected by the method of Isolation by Size of Epithelial Tumor cells (ISET).

Results: The incidence rate for preoperative CTC detection was 73%. The CTC count in 5 mL of blood ranged between 1 and 45, and the mean and median CTC counts were 4.19 and 5 cells, respectively. The results indicated that a preoperative CTC cut-off value of 5 showed the most significant power to predict recurrence. A preoperative CTC ≥ 5 was an independent risk factor for recurrence (P<0.001). The group of patients receiving hepatectomy and TACE sequential therapy showed a more significant decrease in the ΔCTC count (mean ΔCTC, -1.73 *vs*. -0.66, P=0.019) and a longer Disease-Free Survival (DFS) (median, 16.4 months *vs*. 11.4 months) and lower recurrence rates (46.2% *vs*. 64.9%) than the patients who underwent hepatectomy alone (P=0.036).

Conclusion: Preoperative CTC ≥ 5 is a predictor for tumor recurrence after resection. Preventively performing TACE after hepatectomy for those patients with so-called high-risk factors leads to decreased CTC counts and improved DFS.

Keywords: Circulating Tumor Cells; Isolation by Size of Epithelial Tumor Cells; Hepatocellular Carcinoma; Hepatectomy

Introduction

Hepatocellular carcinoma is one of the most common malignancies worldwide, and its high mortality makes it the second leading cause of cancer death [1]. The dismal prognosis of HCC has improved significantly over the last decade due to increased knowledge of HCC behavior, improvements in staging systems and multiple therapeutic options [2]. Nevertheless, the prognosis of HCC remains very poor due to the high incidence of recurrence and metastasis, and the 5-year recurrence rate after curative treatment remains high (70%), with 15% of HCC patients developing extrahepatic metastasis [3,4]. One important reason is that tumor cells are able to penetrate the microvasculature, disseminate through the bloodstream to other sites and finally form metastatic tumors. At present, the diagnosis of HCC still relies on imagological diagnosis, tissue biopsy and tumor markers including AFP. Although AFP is the main biological indicator for early screening and postoperative monitoring, it is not a sensitive and specific indicator. In more than 30% patients, AFP is consistently negative for the whole course of disease, but is abnormally elevated in liver cirrhosis and chronic hepatitis etc. [5]. In addition, tumor heterogeneity, described by different genomic profiles in both "space and time" in anatomically different areas, may facilitate tumor evolution and adaptation and single tumor biopsy may fail to monitor the therapeutic response in a real-time manner [6,7]. Therefore, it is imperative to develop novel approaches for early screening, postoperative monitoring and continuous surveillance of treatment response.

In the 1860s, Ashworth discovered CTCs in peripheral blood [8]. These cancer cells that are

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shed from the original tumors circulate in the blood stream and can generate a new metastasis. Thus, they have been vividly described as the "seeds" of tumors. CTC detection is superior to tissue biopsy in that a sample can be obtained in a convenient and minimally invasive manner during the whole course of disease. CTCs are considered to be a significant critical factor in recurrence and metastasis in HCC [9]. In recent years, CTC detection has been the most intensively investigated hotspot and at present, the Cell Search System is the gold standard for detecting and counting CTCs [10]. The Cell Search System used to detect tumor cells relies on magnetic beads coated with anti-EpCAM monoclonal antibodies to immune magnetically capture tumor cells away from the peripheral blood cells. Due to only approximately 35% of HCC cases expressing EpCAM, the investigation of the clinical relevance of CTCs in HCC lags behind breast cancer, prostate cancer and lung cancer [11,12]. In 1999, Vona et al. [13] used ISET to detect CTCs in HCC patients, and demonstrated how tumor micro-emboli diffuse into the peripheral blood during surgery. ISET method don't subject to the express of EpCAM and identify CTCs by analyzing the morphology and molecular characterization of the circulating tumor cells. Therefore, this represents a novel approach to investigate the biological characteristics of HCC and obtain more information on preoperative diagnosis and postoperative recurrence.

Therefore, this paper aimed to investigate the relationship between preoperative peripheral CTCs and prognosis in HCC patients undergoing curative resection. In addition, we wanted to explore whether the detection of postoperative CTCs enabled the evaluation of the therapeutic efficacy of preventative adjuvant TACE.

Materials and Methods

Patients and specimens

A total of 137 patients undergoing curative resection were enrolled consecutively from November 2016 to October 2019 at the Hepatic Surgery Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. The inclusion criteria were (1) definitive pathological diagnosis of HCC based on the guide criteria; (2) curative resection, defined as complete macroscopic removal of the tumor with negative (R0) margins; (3) twenty-six patients were preventatively performed adjuvant Transarterial Chemoembolization (TACE) treatment in HCC patients with so-call high risk factors 1 month later after liver resection [14]. (4) Age between 18 and 80 years. The exclusion criteria were (1) distant metastasis; (2) Child-Pugh C liver disease. In addition, 18 patients with benign liver disease were enrolled as negative controls. All surgical procedures were performed following the same surgical principles. The institutional review board approved the study protocol, and all patients provided written informed consent.

Surgical methods

All surgeries were accomplished by a team that was able to competently implement hepatectomy. All enrolled patients with Portal Vein Tumor Thrombosis (PVTT) were Type I, II and no Type III and IV. TypeI: Tumor thrombus involving segmental branches of the portal vein or above; Type II: Tumor thrombus involving the right/left portal vein; (Cheng's new classification system) [15,16]. The different therapeutic schedules of PVTT were schemed according to the corresponding different types. Type I was treated by segmental hepatectomy. Hemihepatectomy was performed for the type II.

Postoperative adjuvant TACE

Some studies have indicated that postoperative adjuvant TACE treatment could benefit patients suffering from HCC tumors that are larger than 10 cm in diameter and show macroscopic vascular invasion, satellite lesion or multiple nodules [17-19]. Similarly, some studies have observed PVTT is an independent prognostic factor after surgical operation [20-22]. Therefore, when observing the above biological characteristics, twenty-six patients were identified, in whom TACE was performed 1 month after surgery. All TACE procedures were performed by the radiology department using digital subtraction angiography guidance. Four weeks after surgery, when the liver function of the patient was normal and the Child-Pugh score was A, the Seldinger technique was used for TACE. Specifically, a catheter was placed into the hepatic artery through the femoral artery and then hepatic angiography was performed to detect any obvious tumor stains in the remnant liver. Finally, pharmorubicin (20 mg to 40 mg) and lipiodol (2 mL to 10 mL) were infused through the catheter. The dosage of lipiodol and doxorubicin injected by the doctor was determined by the body surface area and underlying liver function. After 1 month of follow-up evaluation, a CT scan was performed to evaluate lipiodol deposition.

CTC detection

The time points for collecting blood were one day before the operation, on the 1 and 2 month after surgery. Five-milliliter blood samples were drawn to detect CTC by the ISET method. This involves blood filtration and analysis by microscopy using standard histopathological/cytumorphological criteria [13,23]. The ISET technique-membrane filtration and separation of tumor cells-is based on differences in size and deformability between tumor cells and blood cells. The specific tumor cell stain also identifies the captured CTCs. In this study, the ISET technique combined with blood filtration was applied to isolate CTCs, and histopathological criteria were used to analyze the CTCs by microscopy [24,25]. The ISET instrument filtered the blood to capture CTC by a polycarbonate membrane with an 8 μm pore. An experienced cancer cytologist identified the degree of malignancy using histopathological criteria. CTCs were defined with respect to the following six characteristics: a) abnormal karyotypes, such as lobulated nuclei; b) cell diameter larger than 15 μm; c) irregular, dented or shriveled nuclear borders; d) nucleus-to-cytoplasmic ratio >0.8; e) giant nucleoli and f) non-homogeneous nuclear staining. Cells meeting at least 4 of these criteria were identified as CTCs. In addition, if giant nucleoli or abnormal karyotypes appeared and at least 2 other criteria were met, the cells were also identified as CTCs.

Follow-up and tumor recurrence

Postoperative patient surveillance was performed regularly by telephone or re-examination. Recurrence was diagnosed by computed tomography scans, magnetic resonance imaging, digital subtraction angiography, and elevated serum alpha-fetoprotein level. Follow-up was terminated on October 30th, 2019. The time to recurrence was defined as the interval between resection and the diagnosis of intrahepatic recurrence and/or extra hepatic metastasis (the end points) [26]. All patients received curative resection.

Statistical analysis

Data are presented as the mean ± SD. Receiver Operating Characteristic (ROC) curve analysis was used to evaluate the predictive value of the preoperative peripheral blood CTC count for the early recurrence. A chi-squared test, Fisher's exact test and Student's t-test were used for comparison between groups where appropriate. CTC counts between pre- and post-TACE were analyzed

Table 1: Clinical Characteristics of 137 patients.

Clinical characteristics	No. of patients
Age years	Mean 53 ± 12 Median 55
Sex	
Male	123
Female	14
ALT (U/ml)	37 ± 30
AST (U/ml)	30 ± 15
TBIL (μmol/l)	Mean 15.9 ± 9.2 Median 13.4
Child-Pugh score	
A	129
B	8
HBsAg	
Positive	114
Negative	23
Liver cirrhosis	
No	31
Yes	106
ICG15 min (%)	Mean 7.1 ± 3.9 Median 6.6
AFP (ng/mL)	
<400	96
>400	41
Tumor diameter (cm)	Mean 5.5 ± 3.9 Median 4.5
No. of tumor	
Singe	108
Multiple	29
Portal vein tumor thrombosis	
No	109
Yes	28
BCLC stage	
0+A	78
B+C	59

ALT: Alanine Transaminase; AST: Aspartate Aminotransferase; TBiL: Total Bilirubin; HBsAg: Hepatitis B surface Antigen; ICG R15 min (%): Indocyanine Green 15 minutes Retention Rate; AFP: Alpha Fetoprotein; BCLC: Barcelona Clinic Liver Cancer staging system

using a paired-samples t-test. Survival analysis was calculated using the Kaplan-Meier statistical method. Differences between survival curves of different groups were tested using the univariate log-rank test. Multivariate Cox model was used to search for independent prognostic factors for prognosis and death. P<0.05 was considered statistically significant. Statistical analyses were performed with SPSS version 19.0.

Results

Patient characteristics of 137 HCC patients

Table 1 demonstrates the clinical and tumor characteristics of the 137 patients with HCC. The mean patient age was 53 ± 12 years (range, 21 to 74 years). The patients were 89.8% (123/137) male and 10.2% (14/137) female. Hepatitis B surface antigen (HBsAg) was positive in 83.2% (114/137), and six patients were positive for the hepatitis C virus (HCV). 77.4% (106/137) had liver cirrhosis and 29.9% (41/137) had an AFP level >400 ng/ml. Portal vein tumor thrombosis was present in 20.4% (28/137) of the patients. Tumor stage was stratified by the Barcelona Clinic Liver Cancer (BCLC) staging system. Of these patients, stage 0+A was 56.9% (78/137). Eight patients (5.8%) had hepatic function of Child-Pugh score B and received short-term liver protective therapy before surgery; the remaining patients were at Child-Pugh score A.

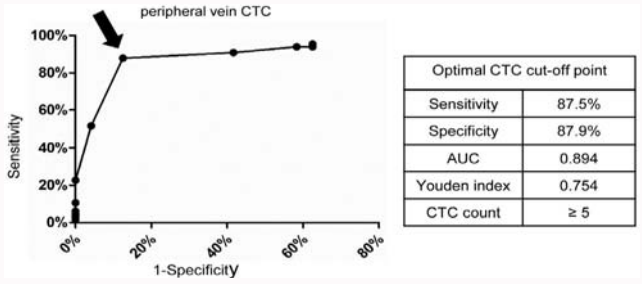

Figure 1: Receiver Operating Characteristic (ROC) curve analysis for range of Circulating Tumor Cell (CTC) cutoff values. Preoperative peripheral vein CTC cutoff for prognosticating early recurrence.

Preoperative blood CTC count predicted early recurrence

We defined recurrence within 1 year after surgery as early recurrence [27]. The optimal CTC cut-off value for predicting early recurrence was determined by subjecting the data to ROC curve analysis to incorporate both the sensitivity and specificity. The ROC curve shown in (Figure 1) was used to analyze CTC optimal cut-off values. When preoperative CTC was 5, the sensitivity was 87.5%, the specificity was 87.9%, the AUC was 0.894 and the Youden index was 0.754 (P<0.001, 95% CI, 0.82 to 0.96).

Preoperative peripheral blood CTC counts and correlation with clinical characteristics

The correlation between preoperative CTC counts and clinical characteristics is shown in (Table 2). As can be seen in the table, larger tumor diameters (≥ 5 cm) (P=0.002), multiple tumors (P=0.003), incomplete encapsulation (P=0.006), poorer tumor differentiation (P<0.001), microvascular invasion (P=0.001), portal vein tumor thrombosis (P=0.001), satellite lesions (P<0.001), poorer BCLC stage (P<0.001) and a higher recurrence rate (P<0.001) were more frequently observed when preoperative CTC counts ≥ 5 were detected (Table 2). The univariate log-rank test and multivariate Cox analysis showed that increased levels of AFP, tumor size, tumor number, tumor encapsulation, tumor differentiation, Ki67, microvascular invasion, portal vein tumor thrombosis and satellite lesions were unfavorable prognostic variables for recurrence (P<0.05). In addition, microvascular invasion and CTC count were independent risk variables for recurrence (P<0.05) (Table 3).

The preoperative CTC count and their prognostic significance

Preoperative blood sample CTC counts for HCC and benign tumor patients are shown in (Figure 2A). Eighteen patients with benign hepatic tumors had 0 CTC. The difference in the mean blood CTC 5 mL levels between the recurrence group and the non-recurrence group was statistically significant (5.6 ± 5.4 *vs.* 2.1 ± 2.2, P<0.001). Patients with CTC counts ≥ 5 had a significantly shorter DFS (median, 10.46 months versus not reached) and higher recurrence rates (77.9% *vs.* 44.9%) than those with a CTC of <5 (P<0.001) (Figure 2B).

Table 2: Clinical Characteristics of HCC Patients and Correlation with CTC.

Clinical characteristics	No. of patients	CTC<5	CTC ≥ 5	*P*
Age, years				
>50	87	39	48	0.095
≤ 50	50	29	21	
Sex				
Male	123	59	64	0.274
Female	14	9	5	
ALT (U/L)				
>40	28	14	14	0.567
≤ 40	109	54	55	
AST (U/L)				
>40	32	15	17	0.439
≤ 40	105	53	52	
AFP (ng/ml)				
>400	41	7	34	**<0.001**
≤ 400	96	61	35	
Child-Pugh score				
A	129	66	63	0.142
B	8	2	6	
HBsAg				
Positive	114	55	59	0.31
Negative	23	13	10	
Liver cirrhosis				
No	31	17	14	0.325
Yes	106	51	55	
ICG 15 min (%)				
>10	27	13	14	0.534
≤ 10	109	54	55	
Largest tumor size, cm				
>5	65	23	42	**0.002**
≤ 5	72	45	27	
No. of tumors				
single	108	61	47	**0.003**
multiple	29	7	22	
Tumor encapsulation				
No	59	21	38	**0.006**
Yes	78	47	31	
Tumor differentiation stage				
I, II	78	50	28	**<0.001**
III, IV	59	18	41	
Ki67				
≤ 20	96	59	37	**<0.001**
>20	41	9	32	
Microvascular invasion				
No	78	54	24	**<0.001**
Yes	59	14	45	
Portal vein tumor thrombosis				
No	109	62	47	**0.001**
Yes	28	6	22	
Satellite lesion				
Yes	115	66	49	**<0.001**
No	22	2	20	
BCLC stage				
0+A	78	51	27	**<0.001**
B+C	59	17	42	
Recurrence				
Yes	84	19	65	**<0.001**
No	53	49	4	

ALT: Alanine Transaminase; AST: Aspartate Aminotransferase; AFP: Alpha Fetoprotein; HBsAg: Hepatitis B surface Antigen; ICG R15 min (%): Indocyanine Green 15 minutes retention rate; PVTT: Portal Vein Tumour Thrombosis; BCLC: Barcelona Clinic Liver Cancer staging system; CTC: Circulating Tumour Cells

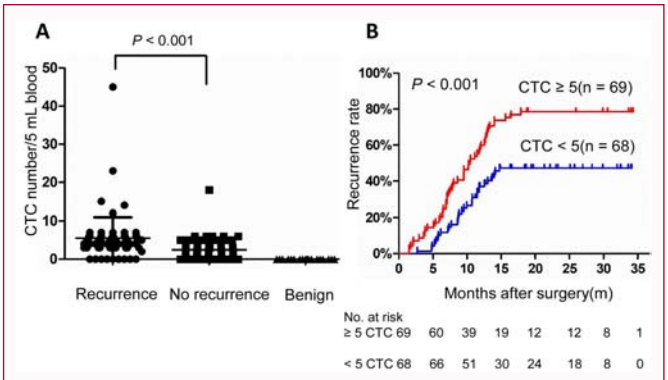

Figure 2: CTCs correlate significantly with recurrence. (A) Distribution of CTCs in recurrent patients, non-recurrent patients and benign liver disease patients (P<0.001). (B) Kaplan-Meier analysis for time to recurrence in HCC patients with CTC count <5 or ≥ 5 preoperatively.

The changes of postoperative CTC counts after adjuvant TACE and their prognostic significance

Twenty-six HCC patients with so-called high risk factors received preventatively performed adjuvant TACE treatment 1 month after liver resection. The change in CTC counts between 1 month and 2 months after surgery were analyzed to evaluate the influence of TACE on CTC counts. Ladder plots of Figure 3A displayed CTC counts at 1 and 2 months after surgery for 111 patients undergoing liver resection. Figure 3B illuminated CTC counts at pre-TACE and post-TACE for 26 patients undergoing hepatectomy and TACE sequential therapy. ΔCTC was defined as the CTC difference value between post-TACE and pre-TACE. CTC counts decreased more significantly in the group receiving hepatectomy and TACE sequential therapy than in the group of patients receiving hepatectomy alone (mean ΔCTC, -1.73 *vs.* -0.66, P=0.019) (Figure 3C). Patients in whom we performed surgery and TACE had a significantly longer DFS (median, 16.4 *vs.* 11.4 months) and lower recurrence rates (46.2% *vs.* 64.9%) than those patients in whom we performed surgery alone (P=0.036) (Figure 3D).

Discussion

Early screening and radical hepatectomy can improve the overall survival of HCC patients [28,29]. At present, surgeons consider hepatectomy and liver transplantation the optimal therapies to improve prognosis in HCC. Unfortunately, the high recurrence rate (50% to 70% at 5 years) is still discouraging [30]. The significant reason for the dismal prognosis is residual micro metastases which are derived from hidden metastasis. In addition, previous studies have shown that cancer cells probably dislodge from the primary focus

Table 3: Univariate and multivariate Cox proportional regression analysis of factors associated with recurrence.

Variables	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	P	HR (95% CI)	P
Age, >50 years *vs.* ≤ 50 years	1.514 (0.943~2.431)	0.086	NA	NA
Sex, male *vs.* female	0.881 (0.425~41.825	0.733	NA	NA
HBsAg, positive *vs.* negative	1.750 (0.927~3.301)	0.084	NA	NA
Liver cirrhosis, yes *vs.* no	1.174 (0.696~1.978)	0.548	NA	NA
Child-Pugh score, B *vs.* A	2.039 (0.935~4.443)	0.073	NA	NA
ALT, ≤ 40 U/L *vs.* >40 U/L	1.152 (0.684~1.942)	0.595	NA	NA
AST, ≤ 40 U/L *vs.* >40 U/L	1.544 (0.961~2.481)	0.072		
AFP, ≤ 400 ng/mL *vs.* >400 ng/mL	2.953 (1.902~4.548)	<0.001	0.876 (0.367~2.090)	0.765
ICG15min (%) ≤ 10 *vs.* >10	0.768 (0.439~1.343)	0.355		
Largest tumor size , ≤ 5 cm *vs.* >5 cm	1.762 (1.1442~2.731)	0.01	1.478 (0.868~2.516)	0.15
No. of tumors, multiple *vs.* single	2.776 (1.740~4.428)	<0.001	1.406 (0.767~2.577)	0.27
Tumor encapsulation, none *vs.* Complete	1.616 (1.051~2.484)	0.029	0.989 (0.544~1.765)	0.97
Edmondson stage, III-IV *vs.* I-II	1.597 (1.040~2.4525)	0.032	1.259 (0.753~2.103)	0.38
Ki67 (%), ≤ 20 *vs.* >20	1.903 (1.229~2.947)	0.004	0.565 (0.313~1.022)	0.059
Microvascular invasion, yes *vs.* no	5.197 (3.250~8.310)	<0.001	3.049 (1.744~5.330)	<0.001
PVTT, yes *vs.* no	3.160 (1.977~5.049)	<0.001	1.508 (0.732~3.108)	0.266
Satellite lesion, yes *vs.* no	2.328 (1.424~3.805)	0.001	0.558 (0.295~1.055)	0.073
BCLC stage, 0+A *vs.* B+C	2.875 (1.848~4.472)	<0.001	1.165 (0.579~2.346)	0.668

ALT: Alanine Transaminase; AST: Aspartate Aminotransferase; AFP: Alpha Fetoprotein; HBsAg: Hepatitis B Surface Antigen; ICG R15 min (%): Indocyanine Green 15 minutes Retention Rate; BCLC: Barcelona Clinic Liver Cancer staging system

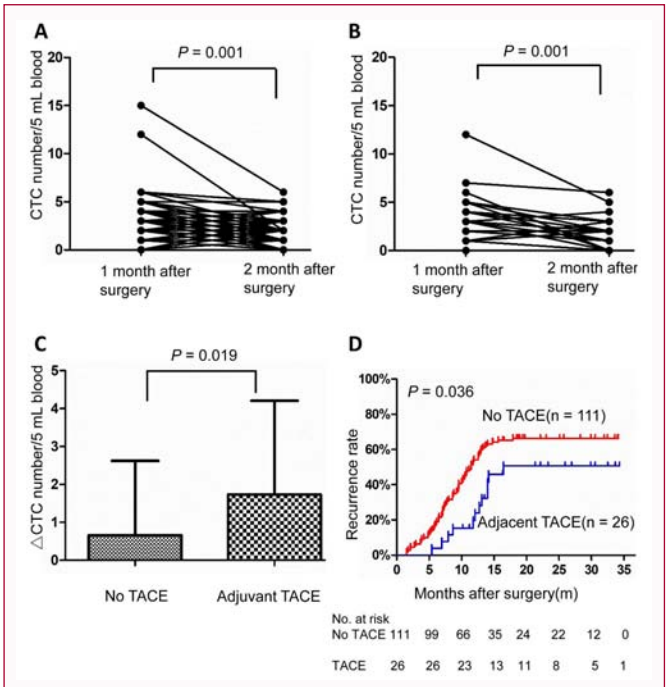

Figure 3: Changes in CTC count indicate treatment response to TACE. (A) Ladder plots displaying 1 month and 2 month after surgery CTC counts for 111 liver resection patients; (B) Ladder plots displaying 1 month and 2 month after surgery CTC counts for 26 hepatectomy and TACE sequential therapy patients. (C) Change of CTC counts in adjuvant TACE subgroup and no TACE subgroup. (D) (B) Kaplan-Meier analysis for time to recurrence in HCC patients with adjuvant TACE or no TACE.

into the portal venous circulation when surgeons perform resection and rotate the liver [31,32]. Unfortunately, routine diagnostic tools, including AFP and imagological diagnosis, are unable to identify high risk HCC patients [27]. In line with virtually all solid tumors, HCC displays characteristics of inherent intra tumor genetic heterogeneity, especially in the metastases [33,34]. Traditional methods make it difficult to mirror response to treatment and disease progression. Previous studies have shown that CTCs may play an important role in the metastasis cascade in breast, colon and prostate cancers [35]. Therefore, it is imperative to develop CTC detection approaches for early screening, postoperative monitoring and continuous surveillance of treatment response.

In this study, we found that patients with preoperative CTC counts ≥ 5 were inclined to possess the attributes related to recurrence, including a larger tumor diameter, multiple tumors, incomplete encapsulation, poorer tumor differentiation, microvascular invasion, portal vein tumor thrombosis, satellite lesions and a poorer BCLC stage. Moreover, our data indicated that CTC levels were an independent risk variable for recurrence. Logically, higher recurrence rates were observed in these patients when preoperative CTC counts ≥ 5 were detected.

A previous study has indicated that postoperative adjuvant TACE treatment could reduce recurrence and prolong survival in HCC patients with so-called high risk factors for residual tumors [36]. Other studies have shown that monitoring CTC changes between pretreatment and post treatment could contribute to the prediction of prognosis in colorectal and breast cancer [37,38]. In our study, we analyzed the dynamic change in CTC count pre-TACE and post-TACE. Our data indicated that CTC counts decreased more significantly in the group of patients who received hepatectomy and TACE sequential therapy than in the group of patients who received hepatectomy alone (mean ΔCTC, -1.73 *vs.* -0.66, P=0.019). In addition, patients who received both surgery and TACE had a significantly longer DFS (median, 16.4 *vs.* 11.4 months) and lower

recurrence rates (46.2% *vs.* 64.9%) than those patients who received surgery alone (P=0.036). This suggested that adjuvant TACE is capable of inhibiting the progress of residual intrahepatic tumors, reducing the postoperative recurrence rate and improving the prognosis for HCC patients. It is feasible to evaluate response to treatment and disease progression via dynamically detecting changes in postoperative CTCs.

Conclusion

Preoperative CTC ≥ 5 is a predictor for tumor recurrence after resection. Preventatively performing TACE after hepatectomy for those patients with so-called high-risk factors enables a decrease in CTC counts and improves DFS.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology. All procedures performed in this study abided with the Declaration of Helsinki. The reference number is TJ-IRB20181101. All patients included in the study wrote informed consent.

Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author.

Authors' Contributions

WX, YNL, RT and JJY collected, analyzed and interpreted the patient data. JLW managed patients which included recruiting patients, performing operations and making follow-up. JMZ analyzed data and wrote the manuscript. ZWZ and XPC designed the experiment and modified the manuscript. All authors read and approved the final manuscript.

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Clinical Trial Registration

ChiCTR, ChiCTR-OOC-16010183, 2016-12-18, http://www.chictr.org.cn/showprojen.aspx?proj=17259.

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References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-E86.
2. Bruix J, Sherman M. Management of hepatocellular carcinoma: An update. Hepatology. 2011;53(3):1020-22.
3. Tsochatzis E, Meyer T, Burroughs A. Hepatocellular carcinoma. Lancet. 1995;3(1):55.
4. Tabrizian P, Jibara G, Shrager B, Schwartz M, Roayaie S. Recurrence of hepatocellular cancer after resection: Patterns, treatments, and prognosis. Ann Surg. 2015;261(5):947-55.
5. Zamcheck N, Pusztaszeri G. CEA, AFP and other potential tumor markers. CA Cancer J Clin. 1975;25(4):204-14.
6. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366(10):883-92.
7. Pantel K, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. Nat Rev Cancer. 2008;8(5):329-40.
8. T AR. A case of cancer in which cells similar to those in the tumors were seen in the blood after death. Australian Med J. 1869;14(3):146-9.
9. Zhang C. The diagnostic value of assays for circulating tumor cells in hepatocellular carcinoma: A meta-analysis. Medicine. 2017;96(29):e7513.
10. Yang JD, Campion MB, Liu MC, Chaiteerakij R, Giama NH, Mohammed HM, et al. Circulating tumor cells are associated with poor overall survival in patients with cholangiocarcinoma. Hepatology. 2016;63(1):148-58.
11. Wang XW. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. Gastroenterology. 2008;136(3):1012-1024.
12. Yin Z, Xu W, Cao L, Li J, Zhang Y. Circulating tumor cells in hepatocellular carcinoma: Detection techniques, clinical implications, and future perspectives. Semin Oncol. 2012;39(4):449-460.
13. Vona G, Sabile A, Louha M, Sitruk V, Romana S, Schütze K, et al. Isolation by size of epithelial tumor cells: A new method for the immunomorphological and molecular characterization of circulating tumor cells. The Am J Pathol. 2000;156(1):57-63.
14. Poon RT, Ng IO, Lau C, Yu W, Yang Z, Fan S, et al. Tumor microvessel density as a predictor of recurrence after resection of hepatocellular carcinoma: a prospective study. J Clin Oncol. 2002;20(7):1775-85.
15. Shuqun C, Mengchao W, Han C, Feng S, Jiahe Y, Guanghui D, et al. Tumor thrombus types influence the prognosis of hepatocellular carcinoma with the tumor thrombi in the portal vein. Hepatogastroenterology 2007;54(74):499-502.
16. Shi J, Lai ECH, Li N, Guo W, Xue J, Lau W, et al. A new classification for hepatocellular carcinoma with portal vein tumor thrombus. J Hepatobiliary Pancreat Sci. 2011;18(1):74-80.
17. Ye JZ, Chen JZ, Li ZH, Bai T, Chen J, Zhu SL, et al. Efficacy of postoperative adjuvant transcatheter arterial chemoembolization in hepatocellular carcinoma patients with microvascular invasion. World J Gastroenterol. 2017;23(41):7415-24.
18. Tong Y, Li Z, Liang Y, Yu H, Liang X, Liu H, et al. Postoperative adjuvant TACE for patients of hepatocellular carcinoma in AJCC stage I: Friend or foe? A propensity score analysis. Oncotarget. 2017;8(16):26671-8.
19. Wang JH, Zhong XP, Zhang YF, Wu XL, Li SH, Jian PE, et al. Cezanne predicts progression and adjuvant TACE response in hepatocellular carcinoma. Cell Death & Disease. 2017;8(9):e3043.
20. Akkiz H, Carr BI, Kuran S, Karaoğullarından Ü, Üsküdar O, Tokmak S, et al. Macroscopic portal vein thrombosis in HCC patients. Can J Gastroenterol Hepatol. 2018;2018:1-8.
21. Fukumoto T, Kido M, Takebe A, Tanaka M, Kinoshita H, Kuramitsu K, et al. New macroscopic classification and back-flow thrombectomy for advanced hepatocellular carcinoma with portal vein tumor thrombus invading the contralateral second portal branch. Surg Today. 2017;47(9):1094-103.
22. Lim KC, Chow PKH, Allen JC, Siddiqui FJ, Chan ESY, Tan SB. Systematic review of outcomes of liver resection for early hepatocellular carcinoma within the Milan criteria. Br J Surg. 2012;99(12):1622-9.

23. Laget S, Broncy L, Hormigos K, Dhingra DM, Ben Mohamed F, Capiod T, et al. Technical insights into highly sensitive isolation and molecular characterization of fixed and live circulating tumor cells for early detection of tumor invasion. PLOS ONE. 2017;12(1):e0169427.

24. Hofman V, Ilie MI, Long E, Selva E, Bonnetaud C, Molina T, et al. Detection of circulating tumor cells as a prognostic factor in patients undergoing radical surgery for non-small-cell lung carcinoma: Comparison of the efficacy of the CellSearch Assay™ and the isolation by size of epithelial tumor cell method. Int J Cancer. 2011;129(7):1651-60.

25. Hofman V, Long E, Ilie M, Bonnetaud C, Vignaud JM, Fléjou JF, et al. Morphological analysis of circulating tumour cells in patients undergoing surgery for non-small cell lung carcinoma using the Isolation by Size of Epithelial Tumour cell (ISET) method. Cytopathology. 2012;23(1):30-38.

26. Llovet JM, Gores GJ. Re: Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst. 2008;100(21):1557-8.

27. Shah SA, Greig PD, Gallinger S, Cattral MS, Dixon E, Kim RD, et al. Factors associated with early recurrence after resection for hepatocellular carcinoma and outcomes. J Am Coll Surg. 2006;202(2):275-83.

28. Meer S, Man RADE, Coenraad MJ, Sprengers Dm, Nieuwkerk KM KHJP, Klupen HZ, et al. Surveillance for hepatocellular carcinoma is associated with increased survival: Results from a large cohort in the Netherlands. J Hepatol. 2015;63(5):1156-1163.

29. Zhang B, Yang B, Tang Z. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol. 2004;130(7):417-22.

30. Cha C, Fong Y, Jarnagin WR, Blumgart LH, DeMatteo RP. Predictors and patterns of recurrence after resection of hepatocellular carcinoma. J Am Coll Surg. 2003;197(5):753-58.

31. Yamanaka N, Okamoto E, Fujihara S, Kato T, Fujimoto J, Oriyama T, et al. Do the tumor cells of hepatocellular carcinomas dislodge into the portal venous stream during hepatic resection? Cancer. 1992;70(9):2263-7.

32. Koo J, Fung K, Siu KF, Lee NW, Lett Z, Ho J, et al. Recovery of malignant tumor cells from the right atrium during hepatic resection for hepatocellular carcinoma. Cancer. 1983;52(10):1952-1956.

33. Zhai W, Lim TK, Zhang T, Phang S, Tiang Z, Guan P, et al. The spatial organization of intra-tumour heterogeneity and evolutionary trajectories of metastases in hepatocellular carcinoma. Nat Commun. 2017;8(1):4565.

34. Huang A, Zhao X, Yang XR, Li FQ, Zhou XL Wu K. Circumventing intratumoral heterogeneity to identify potential therapeutic targets in hepatocellular carcinoma. J Hepatol. 2017;67(2):293-301.

35. Sun YF, Yang XR, Zhou J, Qiu SJ, Fan J, Xu Y. Circulating tumor cells: Advances in detection methods, biological issues, and clinical relevance. J Cancer Res Clin Oncol. 2011;137(8):1151-73.

36. Ren ZG, Lin ZY, Xia JL, Ye SL, Ma ZC, Ye QH, et al. Postoperative adjuvant arterial chemoembolization improves survival of hepatocellular carcinoma patients with risk factors for residual tumor: A retrospective control study. World J Gastroenterol. 2004;10(19):2791-4.

37. Liu MC, Shields PG, Warren RD, Cohen P, Wilkinson M, Ottaviano YL, et al. Circulating tumor cells: A useful predictor of treatment efficacy in metastatic breast cancer. J Clin Oncol. 2009;27(31):5153-9.

38. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26(19):3213-21.