Am J Transl Res 2021;13(12):13590-13598
www.ajtr.org /ISSN:1943-8141/AJTR0137313

Original Article

The longitudinal change of circulating tumor cell during chemotherapy and its correlation with disease features, treatment response and survival profile of advanced gallbladder carcinoma

Yinping Wang[1,2*], Zhiqing Yuan[1,2*], Sibo Zhu[3], Xunxia Bao[3], Zhiliang Fu[3], Timing Zhen[3], Kaichen Xing[3], Yijue Zhang[4], Xinxing Li[5,6], Jianhua Sun[1,2], Qiwei Li[1,2], Linshi Wu[1,2]

[1]Department of General Surgery, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 201112, China; [2]Department of Biliary-Pancreatic Surgery, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200127, China; [3]Cinoasia Institute, Shanghai 200438, China; [4]Department of Anesthesiology, South Campus, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200127, China; [5]Department of General Surgery, Changzheng Hospital, The Second Military Medical University, Shanghai 200003, China; [6]Department of Gastroenterological Surgery, Tongji Hospital, School of Medicine, Tongji University, Shanghai 200065, China. [*]Equal contributors.

Received July 13, 2021; Accepted November 3, 2021; Epub December 15, 2021; Published December 30, 2021

Abstract: The current study aimed to investigate the relation of circulating tumor cell (CTC) with clinicopathological features. In addition, its longitudinal change during chemotherapy and its correlation with prognosis in advanced gallbladder carcinoma (GBC) patients were explored. Totally 45 unresectable, locally advanced or metastatic GBC patients who underwent chemotherapy were enrolled in this prospective study. The CTC in 7.5 ml blood was detected at pre-treatment and 3 months post-treatment. CTC was almost detectable in all advanced GBC patients before treatment, whose count was positively correlated with metastatic disease (vs. local advanced disease) (P=0.002), number of organs with metastases (P=0.006), and CA199 level (P=0.002). After treatment, CTC count declined from 4.0 (range: 0.0-83.0) at pre-treatment to 2.0 (range: 0.0-36.0) at post-treatment (P=0.003). Interestingly, pre-treatment CTC count (P=0.270) was of no difference, while post-treatment CTC count was lower (P=0.038) in objective-response patients compared to that in non-objective-response patients; meanwhile, both pre-treatment CTC count (P=0.017) and post-treatment CTC count (P<0.001) were lower in disease-control patients compared with those in non-disease-control patients. Importantly, pre-treatment CTC count ≥2 (versus <2) was only correlated with worse progression-free survival (PFS) (P=0.014) but not overall survival (OS) (P=0.057); while pre-treatment CTC count ≥5 (versus <5), post-treatment CTC count ≥2 (versus <2), post-treatment CTC count ≥5 (versus <5), CTC count up (versus equal/down) were all correlated with poor PFS and OS (all P<0.050). In conclusion, higher CTC count during chemotherapy correlates with worse treatment response, PFS and OS in advanced GBC patients, which implies that CTC measurement may optimize the prognostication and individualized treatment in these patients.

Keywords: Advanced gallbladder carcinoma, circulating tumor cell, clinicopathological features, chemotherapy, prognosis

Introduction

Gallbladder carcinoma (GBC) is one of the most fatal gastrointestinal malignances, which accounts for 80-95% of biliary tract carcinomas [1]. It is currently considered that screening of risk factors such as gallstones, chronic cholecystitis, chronic bacterial infection, primary sclerosing cholangitis, etc. would improve the early identification of GBC, and then promote the surgical accessibility to facilitate the better prognosis of GBC patients [2]. However, due to the unspecific clinical symptoms, GBCs are commonly diagnosed at advanced stages, which limits the optimal application of resection, and worsens the patients' prognosis [3]. In addition, for the general GBC patients, their 5-year survival rate is already as low as 5%,

not to mention that for those unresectable advanced-stage GBC patients, their survival prognosis is even much worse [1-3]. Therefore, it is of value to explore potential prognostic markers for unresectable, advanced stage GBC patients to improve their survival.

Circulating tumor cell (CTC) is a kind of cancer cells that are detached from primary tumor and then entered blood circulation, which is currently observed to be closely related to tumor metastasis [4]. Recently, CTC detection is proposed to be not only a diagnostic tool, but also a prognostic marker for several carcinomas [5, 6], and it's now recommended as a useful way to monitor progression-free survival (PFS) and overall survival (OS) in metastatic breast, prostate, and colorectal carcinomas by Food and Drug Administration of USA [4]. In terms of hepatobiliary carcinomas, several reports uncover that CTC correlates with advanced disease features and shows a potency for prognostication to facilitate precision medicine in hepatocellular carcinoma patients [7, 8]. However, as for GBC, very limited data about CTC is revealed, and only a single recent study observes that CTC differentiates GBC patients from disease controls (cholecystitis patients) and normal controls, and correlates with more advanced TNM stage of GBC [9]. However, its prognostic role and longitudinal change during treatment in advanced GBC patients are unknown; meanwhile, based on the features of CTC and its previous application in other carcinomas, its investigation in advance GBC might optimize the prognostication of patients.

Therefore, the current study aimed to investigate the relation of CTC with clinicopathological features; furthermore, its longitudinal change during chemotherapy and its correlation with prognosis in advanced GBC patients were explored.

Materials and methods

Patients

This prospective cohort study consecutively enrolled 45 newly-diagnosed advanced GBC patients who were treated in our hospital between March 2016 and April 2020. The enrollment criteria included: (1) confirmed diagnosis of GBC by pathological examination via aspiration biopsy; (2) unresectable, locally advanced or metastatic GBC confirmed by imagine examinations; (3) age ≥18 years; (4) Eastern Cooperative Oncology Group performance status (ECOG PS) score 0-1; (5) adequate liver, renal and bone marrow function to undergo chemotherapy; (6) had measurable lesions on computed tomography (CT) scan or magnetic resonance imaging (MRI). Patients with any of the following conditions were excluded: (1) contraindications to chemotherapy; (2) poor compliance to regular follow-up; (3) pregnant or lactating female patients. This study was approved by the Ethics Committee of Renji Hospital with approval number "Renji Hospital [2014] 51k". All patients signed the informed consents.

Basic clinical data collection

After diagnostic workup and necessary examinations, the following clinical features of patients were recorded in case report form (CRF): age, gender, ECOG PS score, disease status (locally advanced or metastatic), organs with metastases, carbohydrate antigen 199 (CA199) level, and carcinoembryonic antigen (CEA) level.

Treatment regimens

Patients received one of the following gemcitabine-based or fluorouracil-based chemotherapy regimens as first-line therapy: (1) gemcitabine plus cisplatin: gemcitabine 1000 mg/m^2 plus cisplatin 50 mg/m^2 administered intravenously (IV) on days 1 and 8, every 3 weeks; (2) gemcitabine plus oxaliplatin: gemcitabine 1000 mg/m^2 IV on day 1, followed by oxaliplatin 100 mg/m^2 IV on day 2, every 2 weeks; (3) fluorouracil plus cisplatin/leucovorin: leucovorin 200 mg/m^2, fluorouracil 400 mg/m^2 on day 1 followed by fluorouracil 2400 mg/m^2 continuous infusion over 46 h, plus cisplatin 50 mg/m^2, every 2 weeks; (4) fluorouracil plus oxaliplatin/leucovorin: leucovorin 200 mg/m^2 followed by a 400 mg/m^2 bolus fluorouracil followed by a 22-hour infusion of fluorouracil 600 mg/m^2 on two consecutive days, plus a 2-hour infusion of 85 mg/m^2 oxaliplatin, on day 1, every 2 weeks. Chemotherapy was continued until the patient declined further doses or until limiting toxicity or disease progression occurred. The dosages of chemotherapy drugs or the chemotherapy cycles were adjusted by attending physicians when the intolerant toxicity or disease progression occurred.

Table 1. Characteristics of GBC patients

Items	GBC patients (n=45)
Demographics	
Age (years), mean ± SD	61.3±10.0
≤60 years, No. (%)	19 (42.2)
>60 years, No. (%)	26 (57.8)
Gender, No. (%)	
Male	12 (26.7)
Female	33 (73.3)
Disease-related features	
ECOG PS score, No. (%)	
0	28 (62.2)
1	17 (37.8)
Disease status, No. (%)	
Locally advanced	13 (28.9)
Metastatic	32 (71.1)
Number of organs with metastases, No. (%)	
0	13 (28.9)
1	19 (42.2)
2	11 (24.5)
3	2 (4.4)
Metastatic organs, No. (%)	
Liver	25 (55.6)
Peritoneum	13 (28.9)
Bone	5 (11.1)
Lung	4 (8.9)
CA199 (kU/L), median (range)	81.6 (1.4-15238.6)
Normal (≤40 kU/L), No. (%)	14 (31.1)
Abnormal (>40 kU/L), No. (%)	31 (68.9)
CEA (ng/mL), median (range)	6.6 (0.9-613.3)
Normal (≤5 ng/mL), No. (%)	21 (46.7)
Abnormal (>5 ng/mL), No. (%)	24 (53.3)
Treatment regimens	
Gemcitabine plus cisplatin, No. (%)	30 (66.7)
Gemcitabine plus oxaliplatin, No. (%)	8 (17.8)
Fluorouracil plus cisplatin/leucovorin, No. (%)	4 (8.8)
Fluorouracil plus oxaliplatin/leucovorin, No. (%)	3 (6.7)

GBC, gallbladder carcinoma; SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; PS, performance status; CA199, carbohydrate antigen 199; CEA, carcinoembryonic antigen.

CTC analysis

Peripheral blood samples (7.5 mL) of GBC patients were respectively collected before initiation of chemotherapy and after 3-month chemotherapy (4-6 cycles), using tubes containing acid citrate dextrose. All samples were processed within 1 hour after collection. The CTC was isolated and enriched by EasySep™ Direct Human CTC Enrichment Kit (Stem Cells Technologies, Inc., Vancouver, BC, Canada), and the CTC count was detected by flow cytometry. The specific procedures of CTC detection were performed as described in a previous study [9]. In the survival analysis, CTC count was classified using the cut-off value of 2 and 5, according to the previous study [8]. Change of CTC count was calculated by the pre-treatment CTC count minus the post-treatment CTC count.

Follow-up and assessment

After initiation of chemotherapy, imagine examinations, such as MRI or CT scanning, were performed to assess the disease status, every 6 weeks in the first six months of the year and then every 2-4 months. The tumor response was evaluated at 3 months after initiation of chemotherapy, which was classified in accordance with the Response Evaluation Criteria in Solid Tumors criteria [10], including complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Objective response rate (ORR) was calculated as CR+PR and disease control rate (DCR) was calculated as CR+PR+SD. Furthermore, survival status of patients was documented during follow-up, and the progression-free survival (PFS) and the overall survival (OS) were evaluated. PFS was defined as the time interval from the initiation of chemotherapy to disease progression or death, whichever occurred first. OS was defined as the time interval from the initiation of chemotherapy to death.

Statistical analysis

R 4.0.3 and GraphPad Prism 7.02 (GraphPad Software Inc., San Diego, California, USA) were

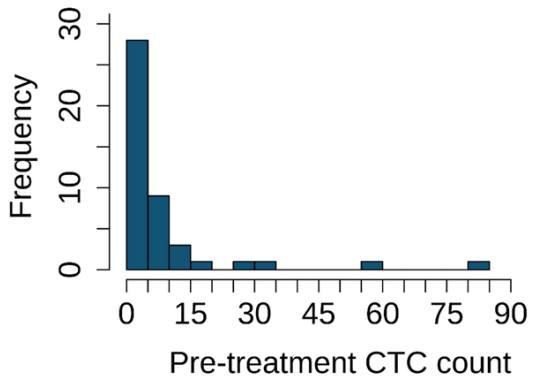

Figure 1. Distribution of pre-treatment CTC count.

used for data analysis and plotting. Data were shown as mean ± standard deviation (SD), median with range, or number with percentage. Correlation of pre-treatment CTC count with clinical features was determined by Wilcoxon rank sum test or Kruskal-Wallis H rank sum test. The change of CTC count before and after treatment was determined by Wilcoxon signed-rank test. Comparison of CTC count between two groups was determined by Wilcoxon rank sum test. Survival curve distribution was displayed by Kaplan-Meier method, and the comparison of survival curve between two groups was determined by Log-rank test. Independent related factors of DFS and OS were determined by multivariate Cox's analysis. *P* value less than 0.050 was considered as statistically significant.

Results

Patients characteristics

Forty-five advanced GBC patients with a mean age of 61.3±10.0 years were enrolled, among which 33 (73.3%) were females and 12 (26.7%) were males (**Table 1**). ECOG PS 0 and 1 appeared in 28 (62.2%) cases and 17 (37.8%) cases, respectively. Besides, 13 (28.9%) cases presented with locally advanced disease, while the other 32 (71.1%) cases presented with metastatic disease. In addition, the information about other detailed clinical features and chemotherapy regimens were shown in **Table 1**.

Pre-treatment CTC and its relation to clinical features

Pre-treatment CTC showed a mean count of 8.6±15.1 and median count of 4.0 (IQR: 1.0-8.0) (Range: 0.0-83.0) in advanced GBC patients. Its distribution in these patients was illuminated in **Figure 1**. Interestingly, pre-treatment CTC count was positively correlated with metastatic disease (vs. locally advanced disease) (P=0.002), number of organs with metastases (P=0.006), liver metastases (P=0.004) and abnormal CA199 level (P=0.002), but not correlated with other clinical features in the advanced GBC patients (**Table 2**).

Treatment response, PFS and OS

After chemotherapy, 0 (0.0%), 15 (33.3%), 16 (35.6%) and 14 (31.1) cases had CR, PR, SD and PD, respectively, with ORR of 33.3% and DCR of 68.9% in advanced GBC patients (**Figure 2A**). Furthermore, median PFS was 5.0 (95% CI: 3.7-6.3) months (**Figure 2B**) and median OS was 10.0 (95% CI: 8.7-11.3) months (**Figure 2C**) in these patients, respectively.

Longitudinal change of CTC count and treatment response

After treatment, CTC count declined from 4.0 (range: 0.0-83.0) at pre-treatment to 2.0 (range: 0.0-36.0) at post-treatment in advanced GBC patients (P=0.003) (**Figure 3**). Importantly, post-treatment CTC count was lower in ORR patients compared to that in non-ORR patients (P=0.038), while pre-treatment CTC count (P=0.270) and change of CTC count (P=0.091) were of no difference between them (**Figure 4A-C**). Furthermore, both pre-treatment CTC count (P=0.017) and post-treatment CTC count (P<0.001) were lower, while change of CTC count (P=0.003) was higher in DCR patients compared with those in non-DCR patients (**Figure 4D-F**).

Correlation of CTC count with PFS and OS

Pre-treatment and post-treatment CTC count was cut off by 2 or 5, meanwhile, change of CTC count was categorized as up or equal/down, so as to assess their correlations with PFS and OS. Pre-treatment CTC count ≥2 (vs. <2, P=0.014, hazard ratio (HR) =2.199) (**Figure 5A**), pre-treatment CTC count ≥5 (vs. <5, P=0.002, HR=2.381) (**Figure 5B**), post-treatment CTC count ≥2 (vs. <2, P<0.001, HR=4.550) (**Figure 5C**), post-treatment CTC count ≥5 (vs. <5, P<0.001, HR=3.151) (**Figure 5D**), and CTC count up (vs. equal/down, P<0.001, HR=3.519) (**Figure 5E**) were all cor-

Table 2. Correlation of pre-treatment CTC count with clinical features of GBC patients

Clinical features	Pre-treatment CTC count, median (range)	*P* value
Age		0.781
≤60 years	3.0 (1.0-83.0)	
>60 years	5.0 (0.0-18.0)	
Gender		0.990
Male	4.0 (1.0-15.0)	
Female	3.0 (0.0-83.0)	
ECOG PS score		0.111
0	3.0 (0.0-83.0)	
1	5.0 (1.0-27.0)	
Disease status		0.002
Locally advanced	1.0 (0.0-12.0)	
Metastatic	5.0 (1.0-83.0)	
Number of organs with metastases		0.006
0	1.0 (0.0-12.0)	
1	5.0 (1.0-31.0)	
2	6.0 (1.0-83.0)	
3	16.5 (15.0-18.0)	
Liver metastases		0.004
No	2.0 (0.0-12.0)	
Yes	5.0 (1.0-83.0)	
Peritoneum metastases		0.067
No	3.0 (0.0-56.0)	
Yes	6.0 (1.0-83.0)	
Bone metastases		0.148
No	3.5 (0.0-83.0)	
Yes	10.0 (2.0-56.0)	
Lung metastases		0.631
No	4.0 (0.0-83.0)	
Yes	7.0 (1.0-15.0)	
CA199		0.002
Normal (≤40 kU/L)	2.0 (0.0-7.0)	
Abnormal (>40 kU/L)	6.0 (1.0-83.0)	
CEA		0.590
Normal (≤5 ng/mL)	4.0 (0.0-83.0)	
Abnormal (>5 ng/mL)	3.5 (1.0-56.0)	

GBC, gallbladder carcinoma; CTC, circulating tumor cell; ECOG, Eastern Cooperative Oncology Group; PS, performance status; CA199, carbohydrate antigen 199; CEA, carcinoembryonic antigen.

related with worse PFS. By comparison of HR, post-treatment CTC count ≥2 (vs. <2) showed best value for predicting PFS.

In addition, pre-treatment CTC count ≥2 (vs. <2, P=0.057, HR=1.942) (**Figure 6A**) did not associate with OS; while pre-treatment CTC count ≥5 (vs. <5, P=0.027, HR=1.961) (**Figure 6B**), post-treatment CTC count ≥2 (vs. <2, P<0.001, HR=4.294) (**Figure 6C**), post-treatment CTC count ≥5 (vs. <5, P<0.001, HR=2.979) (**Figure 6D**), and CTC count up (vs. equal/down, P=0.002, HR=3.222) (**Figure 6E**) were all correlated with worse OS. By comparison of HR, post-treatment CTC count ≥2 (vs. <2) showed best potency for predicting OS.

Independent factor of PFS and OS

Post-treatment CTC ≥2 is independently correlated with both worse PFS (P=0.004, HR=2.913) and OS (P< 0.001, HR=5.791) (**Table 3**). Furthermore, number of organs with metastases, peritoneum metastases, abnormal CA199 and abnormal CEA are also independently related to PFS or OS (all P<0.05).

Discussion

Several interesting findings were discovered in our present study, which padded the data of CTC detection in GBC: (1) CTC was almost detectable in all advanced GBC patients before treatment, whose count was positively correlated with metastatic disease (vs. local advanced disease), number of organs with metastases, and CA199; (2) After treatment, CTC count was obviously declined in advanced GBC patients, especially in DCR patients; (3) Pre-treatment CTC count, post-treatment CTC count and its change all presented potential for predicting prognosis in advanced GBC patients.

Since the introduction of CTC detection in clinical settings, it has been recognized as a potential biomarker for carcinomas diagnosis and progression monitor [11-13]. For instance, CTC capture presents with a certain value for diagnosis of early-stage lung cancer with 52.94% sensitivity and 90.00% specificity [14], and CTC count correlates with lymph node and distant metastases in newly diagnosed lung cancer patients [14]. CTC is also identified in 90.76% non-metastatic breast cancer patients, whose count positively relates to tumor size and hormone receptor negative status

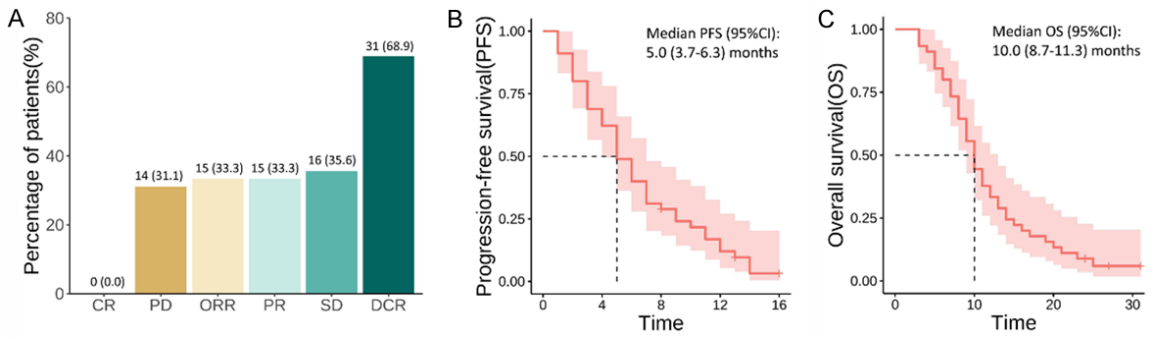

Figure 2. Treatment response and survival data. Treatment response at 3 months (A), PFS (B) and OS (C) in chemotherapy treated advanced GBC patients.

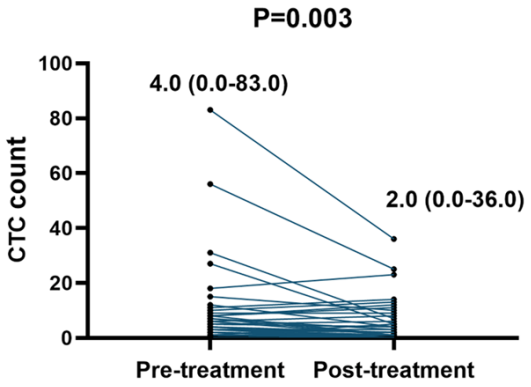

Figure 3. Change of CTC count.

[14]. In aspect of hepatobiliary carcinomas, a recent meta-analysis exhibits that CTC detection achieves 0.60 (95% CI: 0.57-0.63) specificity and 0.95 (95% CI: 0.93-0.96) sensitivity for the diagnosis of hepatocellular carcinoma [15]. Then, another cohort study finds that preoperative CTC count associates with Edmondson stage and postoperative CTC count correlates with TP53 positive status in hepatocellular carcinoma patients [16]. However, possibly due to the low incidence of GBC, its study about CTC detection is very limited. Only a current study reveals that CTC appears 92.6% sensitivity and 91.7% specificity for GBC diagnosis, and its count could discriminate GBC with different TNM stages from each other with cut-off threshold of 3 or 4 [9]. However, the detection of CTC and its clinical relation with disease features in advanced GBC is never investigated. In our current study, we observe that CTC was almost detectable in all advanced GBC patients before treatment, whose count is positively correlated with metastatic disease (vs. local advanced disease), number of organs with metastases, and CA199. The possible explanations were as follows: (1) Higher CTC count increased the possibility of tumor cell planting in other organs apart from the GBC [1-3], therefore it is positively correlated with metastatic disease (vs. local advanced disease) and metastatic organ number. (2) CA199 reflected the worse disease conditions, which is reported to be highly expressed in metastatic GBC. Meanwhile, higher CTCs is related to metastatic GBC. Taken together, CTCs showed a positive correlation with CA199 indirectly.

Most studies about CTC in carcinomas focus on its diagnostic and prognostic potency, while its longitudinal change during treatment is seldom explored [14-16]. In our study, we found that CTC count was decreased after chemotherapy in advanced GBC patients, which might be on account of that after the chemotherapy drug flooded into blood circulation, the CTC was diminished to some extent. Furthermore, we observed that post-treatment CTC count correlated with decreased ORR, and then pre-treatment CTC count, post-treatment CTC count and its change all presented relation to declined DCR in advanced GBC patients, indicating the close association of CTC count with treatment response in these patients. These might result from: (1) for the pre-treatment CTC count, its high level reflected higher severity of GBC (such as more metastatic organs, elevated CA199 level), and therefore, it correlated with chemotherapy response indirectly; (2) for the post-treatment CTC count and change of CTC count, the patients realizing better treatment response reflected the inhibition of disease progression, and therefore, the CTC count was repressed in the meanwhile.

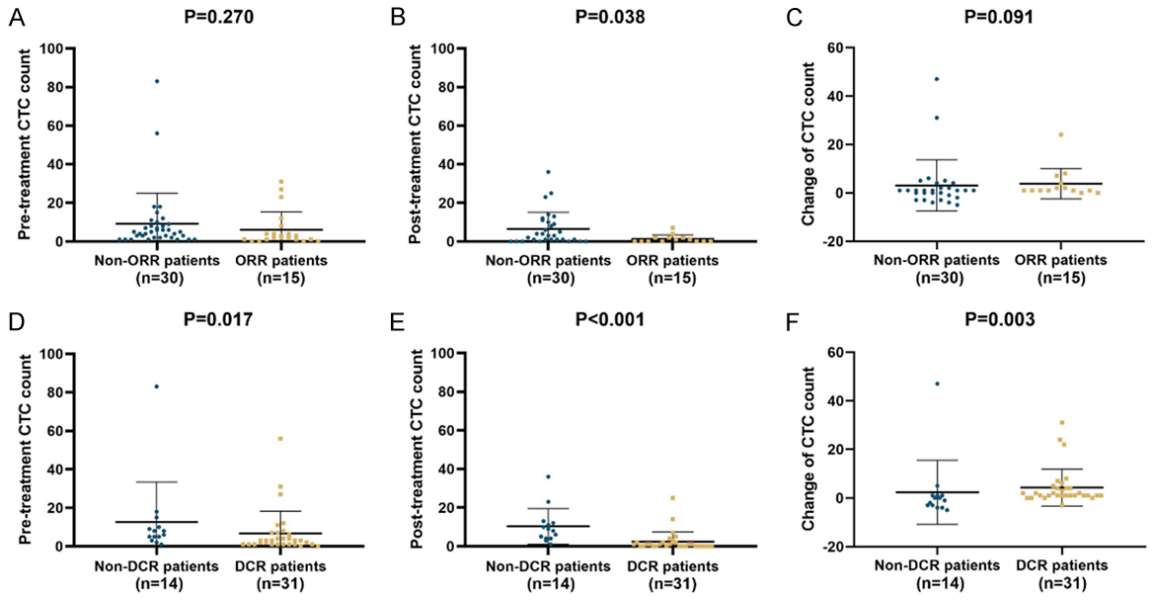

Figure 4. Correlation of pre-treatment/post-treatment/change of CTC count with treatment response. Comparison of pre-treatment CTC count (A), post-treatment CTC count (B) and change of CTC count (C) between ORR patients and non-ORR patients. Comparison of pre-treatment CTC count (D), post-treatment CTC count (E) and change of CTC count (F) between DCR patients and non-DCR patients.

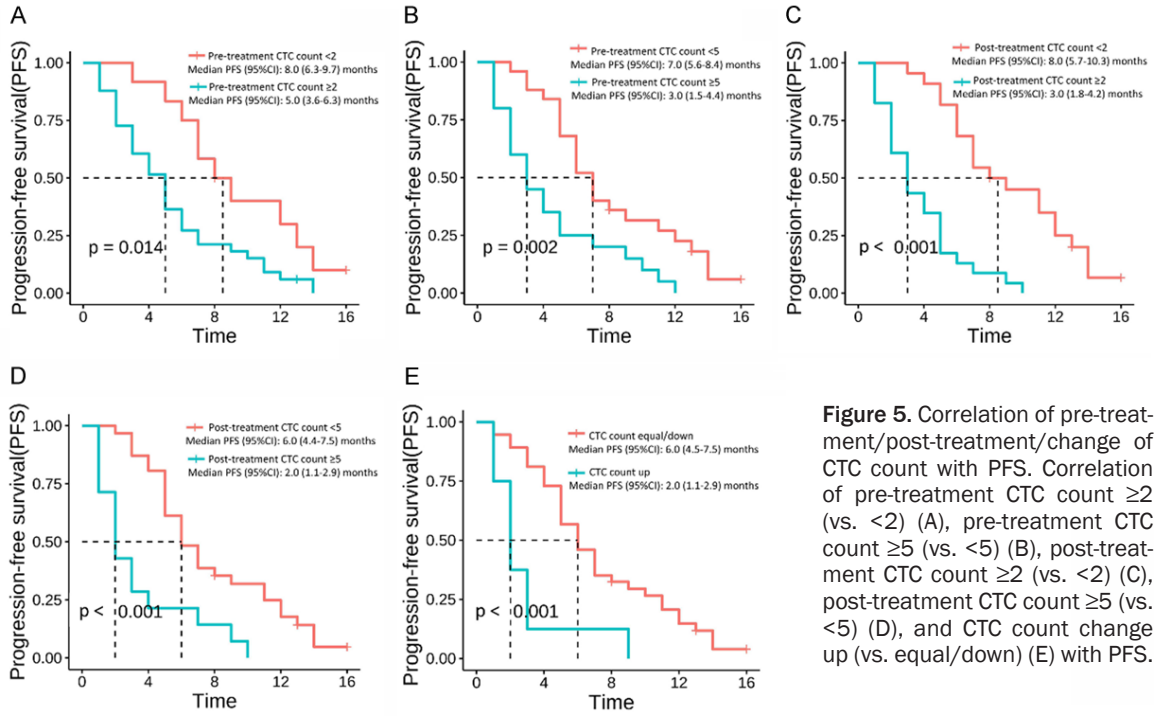

Figure 5. Correlation of pre-treatment/post-treatment/change of CTC count with PFS. Correlation of pre-treatment CTC count ≥2 (vs. <2) (A), pre-treatment CTC count ≥5 (vs. <5) (B), post-treatment CTC count ≥2 (vs. <2) (C), post-treatment CTC count ≥5 (vs. <5) (D), and CTC count change up (vs. equal/down) (E) with PFS.

Apart from the diagnostic, disease monitoring, and treatment response predicting role of CTC in carcinomas, its prognostic role is especially encouraging. For instance, pre-treatment CTC count above or equal to 4 tightly relates to worse PFS, and then the decline of CTC count after treatment predicts favorable PFS and OS in metastatic breast cancer patients [17]; CTC-positive status at each sample time all correlates with poor PFS and OS in gastric cancer patients revealed by a meta-analysis [18]. With regard to hepatobiliary carcinomas, a previous

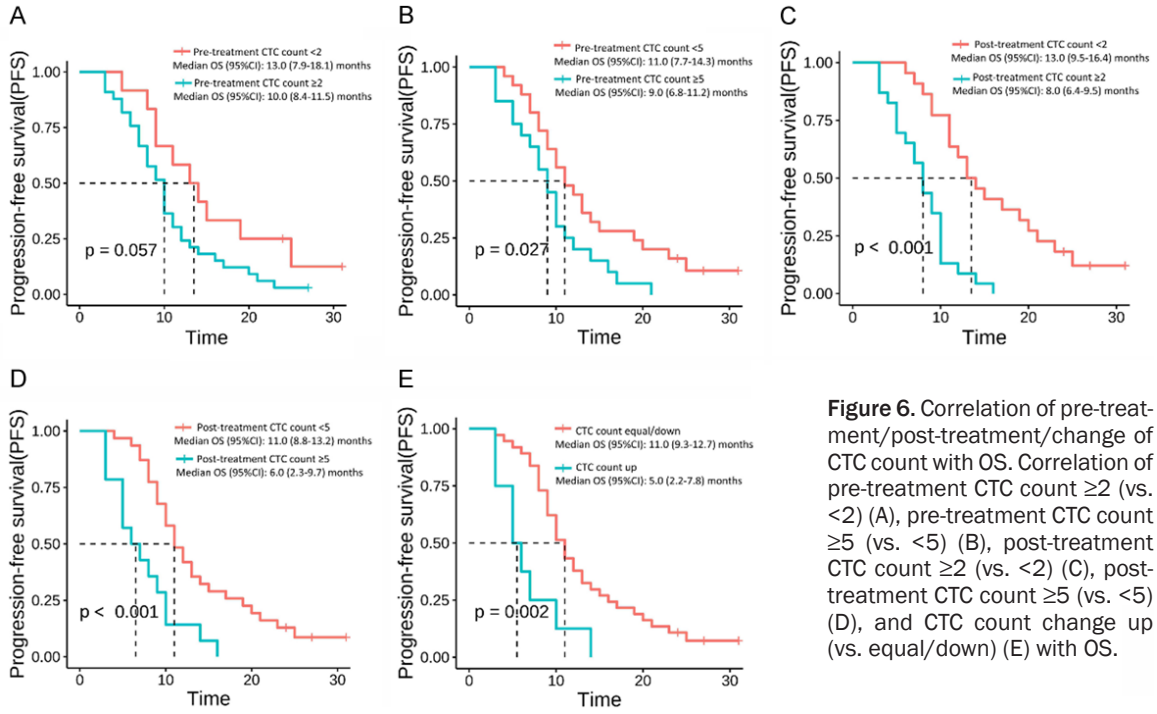

Figure 6. Correlation of pre-treatment/post-treatment/change of CTC count with OS. Correlation of pre-treatment CTC count ≥2 (vs. <2) (A), pre-treatment CTC count ≥5 (vs. <5) (B), post-treatment CTC count ≥2 (vs. <2) (C), post-treatment CTC count ≥5 (vs. <5) (D), and CTC count change up (vs. equal/down) (E) with OS.

Table 3. Cox's proportional hazard regression analysis for PFS and OS

Factors	HR (95% CI)	*P* value
Multivariate Cox's regression for PFS		
Post-treatment CTC ≥2	2.913 (1.416-5.993)	0.004
Number of organs with metastases	2.027 (1.276-3.220)	0.003
CA199 abnormal (>40 kU/L)	2.208 (1.081-4.508)	0.030
Multivariate Cox's regression for OS		
Post-treatment CTC ≥2	5.791 (2.631-12.745)	<0.001
Peritoneum metastases	2.099 (1.055-4.175)	0.035
CEA abnormal (>5 ng/mL)	2.272 (1.157-4.465)	0.017

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; CTC, circulating tumor cell; CA199, carbohydrate antigen 199; CEA, carcinoembryonic antigen.

meta-analysis enrolling 23 studies uncovers that CTC positive status predicts poor relapse free survival and OS in hepatocellular carcinoma [19]. However, there has been no study revealing the prognostic role of CTC in GBC patients until now. In our present study, we observed that pre-treatment CTC count, post-treatment CTC count and its change all presented potential for predicting prognosis (reflected by PFS and/or OS) in advanced GBC patients. The following reasons might explain these results: (1) CTC count correlated with advanced disease conditions such as more metastatic organs and elevated CA199 level, which resulted in the worse PFS and OS indirectly in advanced GBC patients; (2) CTC count was related to worse treatment response of chemotherapy, which directly led to poor PFS and OS in advanced GBC patients.

Some limitations still existed in this present study: (1) Due to the low incidence of GBC, the sample size in the study was relatively small, so the findings needed to be further validated by large sample-sized study; (2) Only unresectable and advanced GBC patients were investigated in this study, so the findings were limited to these patients but not resectable GBC patients, which needed further exploration as well; (3) In order to reduce the bias and compounders, other therapies apart from chemotherapy treated advance GBC patients were not included (such as targeted therapy, immune checkpoint therapy, etc.), which also needed further assessment.

In conclusion, higher CTC count during chemotherapy correlates with worse treatment response, PFS and OS in advanced GBC patients, which implies that CTC measurement may opti-

mize the prognostication and individualized treatment in these patients.

Acknowledgements

This study was registered on Chinese Clinical Trial Registry website (www.chictr.org.cn) with the approval number "ChiCTR2100052139".

Disclosure of conflict of interest

None.

Address correspondence to: Linshi Wu and Qiwei Li, Department of General Surgery, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 201112, China; Department of Biliary-Pancreatic Surgery, Renji Hospital, School of Medicine, Shanghai Jiaotong University, No. 2000 Jiangyue Road, Minhang District, Shanghai 201112, China. Tel: +86-021-34506583; E-mail: wulinshi-renji@163.com (LSW); dr_liqiwei@163.com (QWL)

References

[1] Shukla SK, Singh G, Shahi KS, Bhuvan and Pant P. Staging, treatment, and future approaches of gallbladder carcinoma. J Gastrointest Cancer 2018; 49: 9-15.

[2] Goetze TO. Gallbladder carcinoma: prognostic factors and therapeutic options. World J Gastroenterol 2015; 21: 12211-12217.

[3] Zhu AX, Hong TS, Hezel AF and Kooby DA. Current management of gallbladder carcinoma. Oncologist 2010; 15: 168-181.

[4] Moon DH, Lindsay DP, Hong S and Wang AZ. Clinical indications for, and the future of, circulating tumor cells. Adv Drug Deliv Rev 2018; 125: 143-150.

[5] Cortes-Hernandez LE, Eslami SZ and Alix-Panabieres C. Circulating tumor cell as the functional aspect of liquid biopsy to understand the metastatic cascade in solid cancer. Mol Aspects Med 2020; 72: 100816.

[6] Lin E, Cao T, Nagrath S and King MR. Circulating tumor cells: diagnostic and therapeutic applications. Annu Rev Biomed Eng 2018; 20: 329-352.

[7] Ahn JC, Teng PC, Chen PJ, Posadas E, Tseng HR, Lu SC and Yang JD. Detection of circulating tumor cells and their implications as a novel biomarker for diagnosis, prognostication, and therapeutic monitoring in hepatocellular carcinoma. Hepatology 2020; 73: 422-436.

[8] Yang JD, Campion MB, Liu MC, Chaiteerakij R, Giama NH, Ahmed Mohammed H, Zhang X, Hu C, Campion VL, Jen J, Venkatesh SK, Halling KC, Kipp BR and Roberts LR. Circulating tumor cells are associated with poor overall survival in patients with cholangiocarcinoma. Hepatology 2016; 63: 148-158.

[9] Awasthi NP, Kumari S, Neyaz A, Gupta S, Agarwal A, Singhal A and Husain N. EpCAM-based flow cytometric detection of circulating tumor cells in gallbladder carcinoma cases. Asian Pac J Cancer Prev 2017; 18: 3429-3437.

[10] Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC and Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000; 92: 205-216.

[11] Micalizzi DS, Maheswaran S and Haber DA. A conduit to metastasis: circulating tumor cell biology. Genes Dev 2017; 31: 1827-1840.

[12] Alix-Panabieres C and Pantel K. Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. Cancer Discov 2016; 6: 479-491.

[13] Balic M, Williams A, Lin H, Datar R and Cote RJ. Circulating tumor cells: from bench to bedside. Annu Rev Med 2013; 64: 31-44.

[14] Duan GC, Zhang XP, Wang HE, Wang ZK, Zhang H, Yu L, Xue WF, Xin ZF, Hu ZH and Zhao QT. Circulating tumor cells as a screening and diagnostic marker for early-stage non-small cell lung cancer. Onco Targets Ther 2020; 13: 1931-1939.

[15] Cui K, Ou Y, Shen Y, Li S and Sun Z. Clinical value of circulating tumor cells for the diagnosis and prognosis of hepatocellular carcinoma (HCC): a systematic review and meta-analysis. Medicine (Baltimore) 2020; 99: e22242.

[16] Ye X, Li G, Han C, Han Q, Shang L, Su H, Han B, Gong Y, Lu G and Peng T. Circulating tumor cells as a potential biomarker for postoperative clinical outcome in HBV-related hepatocellular carcinoma. Cancer Manag Res 2018; 10: 5639-5647.

[17] Bahnassy AA, Saber MM, Mahmoud MG, Abdellateif MS, Abd El-Mooti Samra M, Abd El-Fatah RM, Zekri AN and Salem SE. The role of circulating tumor cells in metastatic breast cancer: prognostic and predictive value. Mol Biol Rep 2018; 45: 2025-2035.

[18] Yang C, Zou K, Yuan Z, Guo T and Xiong B. Prognostic value of circulating tumor cells detected with the CellSearch System in patients with gastric cancer: evidence from a meta-analysis. Onco Targets Ther 2018; 11: 1013-1023.

[19] Fan JL, Yang YF, Yuan CH, Chen H and Wang FB. Circulating tumor cells for predicting the prognostic of patients with hepatocellular carcinoma: a meta analysis. Cell Physiol Biochem 2015; 37: 629-640.