

# Association between circulating tumor cells in the peripheral blood and the prognosis of gastric cancer patients: a meta-analysis

Tao Jin\*, Pan-Ping Liang\*, Ze-Hua Chen, Feng-Jun He, Ze-Dong Li, Zheng-Wen Chen, Jian-Kun Hu and Kun Yang 

*Ther Adv Med Oncol*

2023, Vol. 15: 1–17

DOI: 10.1177/  
17588359231183678

© The Author(s), 2023.  
Article reuse guidelines:  
[sagepub.com/journals-permissions](https://sagepub.com/journals-permissions)

## Abstract

**Background:** Research on the correlation between circulating tumor cells (CTCs) and gastric cancer (GC) has increased rapidly in recent years. However, whether CTCs are associated with GC patient prognosis is highly controversial.

**Objective:** This study aims to evaluate the value of CTCs to predict the prognosis of GC patients.

**Design:** A meta-analysis.

**Data Sources and Methods:** We searched the PubMed, Embase, and Cochrane Library databases for studies that reported the prognostic value of CTCs in GC patients before October 2022. The association between CTCs and overall survival (OS) and disease-free survival (DFS)/recurrence-free survival (RFS) and progression-free survival (PFS) of GC patients was assessed. Subgroup analyses were stratified by sampling times (pre-treatment and post-treatment), detection targets, detection method, treatment method, tumor stage, region, and HR (Hazard Ratio) extraction methods. Sensitivity analysis was performed by removing individual studies to assess the stability of the results. Publication bias was evaluated using funnel plots, Egger's test, and Begg's test.

**Results:** We initially screened 2000 studies, of which 28 were available for further analysis, involving 2383 GC patients. The pooled analysis concluded that the detection of CTCs was associated with poor OS (HR=1.933, 95% CI 1.657–2.256,  $p < 0.001$ ), DFS/RFS (HR=3.228, 95% CI 2.475–4.211,  $p < 0.001$ ), and PFS (HR=3.272, 95% CI 1.970–5.435,  $p < 0.001$ ). Furthermore, the subgroup analysis stratified by tumor stage ( $p < 0.01$ ), HR extraction methods ( $p < 0.001$ ), detection targets ( $p < 0.001$ ), detection method ( $p < 0.001$ ), sampling times ( $p < 0.001$ ), and treatment method ( $p < 0.001$ ) all showed that CTC detection was associated with poor OS and DFS/RFS for GC patients. Furthermore, the study showed that CTCs were associated with the poor DFS/RFS of GC when CTCs were detected for patients from Asian or No-Asian regions ( $p < 0.05$ ). In addition, higher CTCs predicted poorer OS for GC patients who are from Asian regions ( $p < 0.001$ ), but without statistical difference for GC patients from No-Asian regions ( $p = 0.490$ ).

**Conclusion:** CTC detection in peripheral blood was associated with poor OS, DFS/RFS, and PFS in patients with GC.

**Keywords:** circulating tumor cells, gastric cancer, prognosis

Received: 2 December 2022; revised manuscript accepted: 31 May 2023.

## Highlights

1. The association between CTCs and GC is still controversial.
2. The subgroup analysis stratified by sampling times, detection targets, detection method, treatment method, tumor stage, and HR extraction methods was conducted to explore the source of heterogeneity.
3. The study showed that the detection of CTCs was associated with a poor prognosis of GC.

Correspondence to:

**Kun Yang**

Department of General Surgery & Laboratory of Gastric Cancer, State Key Laboratory of Biotherapy/ Collaborative Innovation Center of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China

Gastric Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China

[yangkun068@163.com](mailto:yangkun068@163.com)

Tao Jin

Pan-Ping Liang

Ze-Hua Chen

Feng-Jun He

Ze-Dong Li

Zheng-Wen Chen

Jian-Kun Hu

Department of General Surgery & Laboratory of Gastric Cancer, State Key Laboratory of Biotherapy/ Collaborative Innovation Center of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan, China Gastric Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan, China

\*Co-First Author

## Introduction

Gastric cancer (GC) remains the fifth most prevalent tumor worldwide and is the fourth leading cause of oncological death.<sup>1,2</sup> Despite the advanced progress achieved in the treatment of GC by various methods, such as laparoscopic surgery,<sup>3</sup> robotic surgery,<sup>4</sup> and perioperative chemotherapy,<sup>5</sup> its prognosis remains poor due to late detection, the aggressiveness of tumor cells, and high recurrence/metastasis rates.<sup>6</sup> Recurrence and metastasis may involve a range of cells, including circulating tumor cells (CTCs), which have been progressively recognized to play an important role in distant metastasis, according to the ‘seed and soil theory’.<sup>7</sup>

CTCs are tumor cells that originate from primary or metastatic tumors and travel through the systemic circulation to distant organs, where they can initiate metastatic lesions.<sup>8</sup> CTCs can be isolated from a patient’s blood sample *via* different technologies that take advantage of their physical and biological properties. Recent studies have shown that CTCs can be found in many solid tumor types, such as breast, prostate, lung, and colorectal cancers,<sup>9–12</sup> potentially enabling early detection of metastatic progression, treatment response, and tumor recurrence.<sup>13</sup> CTC analyses are considered a real-time ‘liquid biopsy’ for patients with cancer, and several studies have also demonstrated the clinical significance of isolated CTCs in cancer prognosis, treatment response, and recurrence,<sup>14,15</sup> which enables minimally invasive and repetitive sampling and is clinically useful in early diagnosis, monitoring of therapeutic efficacy, and prognosis.

In recent years, CTCs have been approved by the FDA as a prognostic biomarker for monitoring patients with breast, prostate, lung, hepatocellular, and colorectal cancer.<sup>16–18</sup> Several studies have shown that the detection of CTCs in GC patients might be used for staging and assessing treatment outcomes.<sup>19,20</sup> However, the prognostic value of CTC in GC is still controversial, because the previous studies were almost retrospective studies, with a small sample size and high heterogeneity. Furthermore, two meta-analyses evaluated the clinical significance of CTCs in GC.<sup>21,22</sup> However, samples of one meta-analysis were drawn from peripheral blood and bone marrow, with few studies.<sup>21</sup> Another meta-analysis may have been inaccurate because the included studies utilized different detection assays; moreover, it selectively reported their positive results with high heterogeneity, but without further subgroup

analysis stratified by detection method, detection target, and treatment, which might be a source of heterogeneity, and led to unstable outcomes; therefore, we conducted subgroup analysis stratified by detection method, detection target, and treatment to avoid the heterogeneity due to different detection assays, targets, and treatment.<sup>22</sup> In addition, with many novel studies emerging, it was necessary to review the value of CTCs in predicting the prognosis of GC. Thus, this meta-analysis aims to further discuss the association between CTCs and the prognosis of GC.

## Methods

We used MOOSE (Meta-analysis Of Observational Studies in Epidemiology)<sup>23</sup> and PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)<sup>24</sup> as guidelines to conduct the systematic review. A comprehensive search of relevant studies (English language) published from inception to October 2022 was retrieved in the following databases: PubMed, EMBASE, and the Cochrane Library. By using the terms ‘Circulating tumor cells’, ‘CTCs’, ‘Stomach neoplasm’, and ‘GC’, we also checked the bibliographies of all relevant papers for further qualified studies. The detailed search strategy for the electronic database is shown in Supplemental Material 1.

### Eligibility criteria

#### Inclusion criteria

- (1) Patients with pathologically confirmed GC including stage I–IV;
- (2) Patients accepting surgery or perioperative chemotherapy were all included;
- (3) Samples collected at baseline or intra/post-treatment were all included;
- (4) Reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemistry, immunofluorescence, flow cytometry, immunostaining, fluorescence *in situ* hybridization (FISH) staining, and CellSearch detection methods were used to detect tumor-specific genes/antigens.
- (5) Sufficient GC patient survival data with CTC status;
- (6) The most recent study or the study with the most subjects was chosen if overlapping research studies were found.

#### Exclusion criteria

- (1) Samples were not drawn from peripheral blood (e.g. from urine or bone marrow);

- (2) The histology type of the included GC patients was neuroendocrine carcinoma or other types of GC;
- (3) Patients with other tumors;
- (4) The outcomes of interest were not reported, and it was impossible to calculate outcomes from the originally published data;

#### *Data extraction and assessment of study quality*

Two authors independently reviewed the qualified studies and extracted the following information: author, region, publication year, study design, number of patients, gender ratio, age, cancer stage, treatment status, sample volume and time, follow-up, cutoff, HR estimate, outcomes (OS, DFS/RFS, PFS), target, and detection method. The risk of bias for individual studies was assessed using the Newcastle–Ottawa scale (NOS).<sup>25</sup> Disagreements were resolved by a joint review of the manuscript to reach a consensus.

#### *Data synthesis and analysis*

The HRs were used to evaluate the prognostic effect (OS, DFS/RFS, and PFS), which were extracted from multivariate Cox regression. Generally, an  $HR > 1$  indicated a worse outcome of GC patients with positive detection of CTCs. If the HR and its variance were not reported directly in the original study, these values were calculated from available reported data using software designed by Tierney *et al.*<sup>26</sup> All statistical values were combined with a 95% confidence interval (95% CI). Pooled HRs and corresponding 95% CIs were used to evaluate the relationship between the level of CTCs and the prognosis of patients with GC by  $\ln HR$ ,  $\text{selnHR}$ .  $p < 0.05$  was considered statistically significant. Heterogeneity between studies was assessed using Cochran's  $Q$ -test and Higgins  $I$ -squared statistics to measure the extent of variation not due to chance alone. If  $I^2 \leq 25\%$ , it indicates low heterogeneity; if  $25\% < I^2 \leq 50\%$ , it indicates moderate heterogeneity; and if  $50\% < I^2 \leq 75\%$ , it indicates substantial heterogeneity. If  $I^2 \geq 75\%$ , it indicates considerable heterogeneity. If  $p < 0.05$ , a random-effects model was used; otherwise, the pooled analysis was performed with the fixed-effects model. In the presence of heterogeneity, subgroup analyses were performed to assess the prognostic value of CTCs based on characteristics such as sampling times, detection markers, CTC detection methods, treatment methods, tumor stage, region, and HR (Hazard Ratio) extraction methods (data were calculated or

provided by the article). Sensitivity analysis was also performed by sequential omission of individual studies to assess the stability of the results. Publication bias was evaluated using funnel plots, Egger's test, and Begg's test. All statistical analyses were performed using STATA version 15.0. (College Station, TX 77845, USA).

#### *Results*

##### *Search results and basic characteristics*

In all, 28 eligible studies<sup>19,20,27–52</sup> containing 2383 patients were enrolled for analysis (Figure 1). Table 1 shows the basic characteristics and experimental design variables of all included studies. All studies were performed using various cellular and molecular methods, including RT-PCR ( $n = 8$ ), CellSearch ( $n = 8$ ), immunohistochemistry ( $n = 2$ ), immunofluorescent staining ( $n = 3$ ), FISH ( $n = 2$ ), chromosomal microarray analysis (CMA;  $n = 1$ ), flow cytometry ( $n = 2$ ), RNA in situ hybridization (RNA-ISH;  $n = 1$ ), and ligand-targeted enzyme-linked polymerase chain reaction (LT-PCR;  $n = 1$ ). The most common detection target was EpCAM+, CK+, CD45–, and DAPI+ by CellSearch, followed by CK, CEA, and other targets. Three studies were conducted in Western populations, and 25 studies were conducted in Asian populations.

##### *Quality evaluation of the included literature*

The assessment of the risk of bias for individual studies showed 20 studies assessed with a score  $\geq 6$  and the remaining eight studies were evaluated with a score  $< 6$ . The quality evaluation of the included literature is shown in Table 2.

#### **Association between CTCs and prognosis of GC**

There were 22 articles (17 retrospective studies and five prospective studies) covering HR, and the pooled HR showed a significant association between CTCs and the OS of GC ( $HR = 1.933$ , 95% CI 1.657–2.256,  $p < 0.001$ ), with low heterogeneity. (Figure 2). Furthermore, 11 studies (eight retrospective studies and three prospective studies) reported DFS/RFS, and the pooled HR revealed that CTCs were related to the DFS/RFS of GC patients ( $HR = 3.228$ , 95% CI 2.475–4.211,  $p < 0.001$ ) with low heterogeneity. (Figure 3). Furthermore, the outcomes of five prospective studies showed that CTC detection was associated with poor OS ( $HR = 2.655$  95% CI 1.831–3.850,  $p < 0.001$ ) and three prospective studies showed

**Table 1.** The basic characteristic and experimental design variables of included studies of meta-analysis.

Author	Region	Year	Number	M/F	Age	Cancer stage	Target	Detection references method	Treatment
Majima <i>et al.</i> <sup>27</sup>	Japan	2000	52	NR	NR	I-IV	CK	RT-PCR	Surgery
Illert <i>et al.</i> <sup>28</sup>	Germany	2005	70	48/22	69 [41-87]	I-IV	CK20	RT-PCR	Surgery
Ikeguchi and Kaibara <sup>29</sup>	Japan	2005	59	38/21	66.3[26-86]	I-IV	CEA (mRNA)	RT-PCR	Surgery
Uen <i>et al.</i> <sup>30</sup>	China	2006	52	31/21	30 (>60)	I-IV	MUC1/C-Met	RT-PCR	Surgery
Wu <i>et al.</i> <sup>31</sup>	China	2006	64	41/23	60.5 (36-84)	I-IV	hTERT/CK19/ CEA/MUC1	CMA	Surgery
Noworolska <sup>32</sup>	Poland	2007	57	44/13	NR	I-IV	CK	flow cytometry	Surgery + chemo
Hiraiwa <i>et al.</i> <sup>33</sup>	Japan	2008	27	NR	68.9 ± 9.6	IV	EpCAM	CellSearch	NR
Yie <i>et al.</i> <sup>34</sup>	China	2008	26	NR	NR	I-IV	Survivin	RT-PCR	Surgery + chemo
Matsusaka <i>et al.</i> <sup>45</sup>	Japan	2010	52	8/44	62 [24-78]	IV	CellSearch	CellSearch	Chemo
Saad <i>et al.</i> <sup>35</sup>	Egypt	2010	30	16/14	NR	I-IV	E-cadherin	Immunohistochemistry	Surgery + chemo
Qiu <i>et al.</i> <sup>36</sup>	China	2010	123	82/41	59 [28-84]	I-IV	CEA (mRNA)	RT-PCR	Surgery + chemo
Arigami <i>et al.</i> <sup>37</sup>	Japan	2010	95	64/31	47 (>70)	I-IV	B7-H3	RT-PCR	Surgery
Cao <i>et al.</i> <sup>38</sup>	China	2011	98	63/35	NR	I-IV	Survivin	RT-PCR	Surgery
Uenosono <i>et al.</i> <sup>39</sup>	Japan	2013	148	99/49	57 (>70)	I-IV	CellSearch	CellSearch	Surgery
Li <i>et al.</i> <sup>20</sup>	China	2015	136	89/47	59 [25-80]	II-IV	CellSearch	CellSearch	Chemo
Okabe <i>et al.</i> <sup>46</sup>	Japan	2015	136	87/49	NR	II-IV	CellSearch	CellSearch	NR
Ito <i>et al.</i> <sup>47</sup>	Japan	2016	65	46/19	58.8[33-76]	I-IV	Telomerase	Immunofluorescence staining	Surgery
Liu <i>et al.</i> <sup>48</sup>	China	2017	59	35/24	59 (median)	III-IV	EpCAM	Immunofluorescence staining	Chemo
Pernot <i>et al.</i> <sup>19</sup>	Japan	2017	106	70/36	NR	I-IV	CellSearch	CellSearch	Chemo
Li <i>et al.</i> <sup>52</sup>	China	2018	115	93/22	68(≥60)	III-IV	hHER2+	FISH	Chemo
Zhang <i>et al.</i> <sup>40</sup>	China	2018	93	68/25	77(≥45)	I-III	CellSearch	CellSearch	Surgery
Miki <i>et al.</i> <sup>41</sup>	Japan	2020	126	NR	70[28-92]	I-IV	CEA	Flow cytometry	Surgery
Liu <i>et al.</i> <sup>42</sup>	China	2020	70	51/19	38(≥63)	I-IV	PD-1+	Immunohistochemistry	Chemo/chemo + surgery
Ning <i>et al.</i> <sup>43</sup>	China	2021	59	49/10	29(≥60)	I-IV	CellSearch	CellSearch	Chemo/chemo + surgery
Zhu <i>et al.</i> <sup>44</sup>	China	2021	114	87/27	52(≥65)	I-IV	DAPI +/CD45-/ CEP 8 +/CEP17+	FISH	Chemo/chemo + surgery
Zeng <i>et al.</i> <sup>49</sup>	China	2022	132	91/41	59.2 ± 10.5	I-IV	Folate receptor (FR+)	LT-PCR	Surgery
Jhi <i>et al.</i> <sup>50</sup>	Korea	2021	31	22/9	63 [42-87]	IV	CK+ or EpCAM+, CD45- and DAPI+	Immunofluorescence staining	Chemo
Qiu <i>et al.</i> <sup>51</sup>	China	2022	188	128/60	65(≥65)	I-III	EpCAM and CK8/18/19	RNA-ISH	Surgery

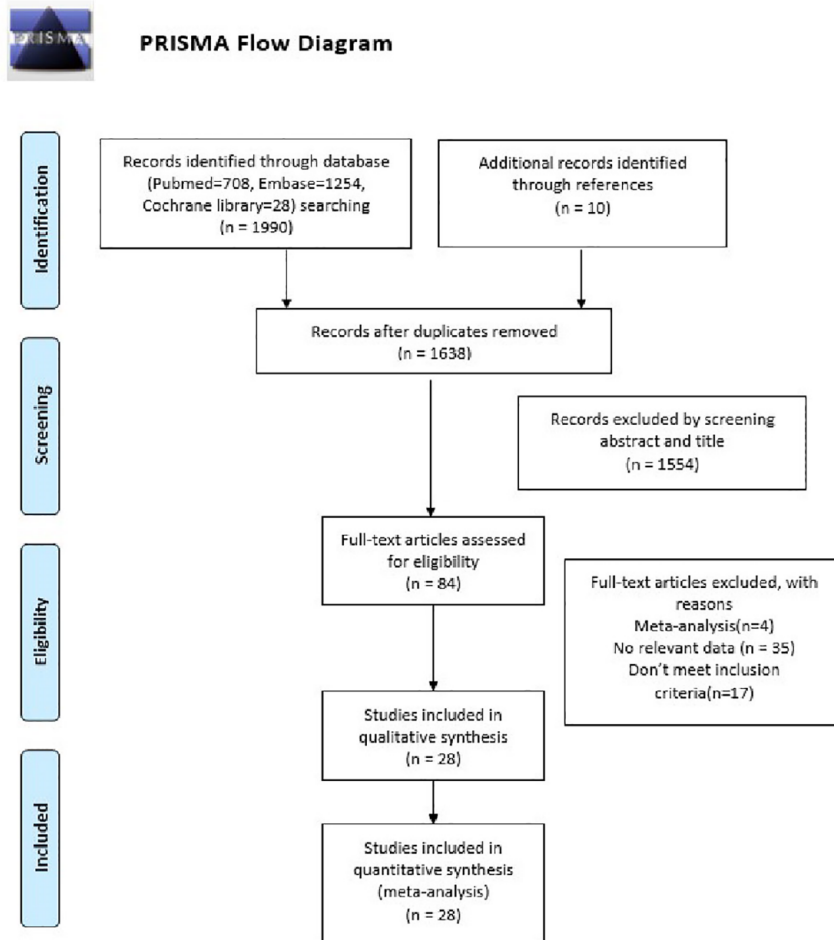
Detection time	Sample volume	Positive ratio	Cutoff	HR estimate	Follow-up	Outcomes	Design	CA
Preoperative	10	5/52	HC	FC	NR	OS	Retrospective	NR
Preoperative	9	28/70	HC	FC	20 (1–57)	OS	Retrospective	NR
Postoperative	1.5	27/59	P	FC	20.1 (2–31)	OS/DFS	Retrospective	NR
Postoperative	5	32/52	5	FC	NR	OS	Retrospective	NR
Postoperative	4	25/64	ROC	FC	28 (20–33)	OS/DFS	Retrospective	NR
Postoperative + chemo	NR	31/57	3	FC	NR	OS	Retrospective	NR
Pre/post-treatment	7.5	15/27	2	FC	5.8 (1.0–15.0)	OS	Retrospective	NR
Postoperative + chemo	2	NR	ROC	ML	36	RFS	Retrospective	Age, sex, grade, location, stage
Post	7.5	17/52	4	ML	NR	PFS, OS	Retrospective	Stage, treatment
Postoperative	2	15/30	HC	ML	NR	OS, RFS	Retrospective	Stage, grade, PPMM
Preoperative + chemo	5	45/123	HC	ML	37 (3.0–73.6)	DFS	Retrospective	Stage, grade, age
Postoperative	5	48/95	ROC	ML	24 (1–74)	OS	Retrospective	T, N stage
Postoperative	6	45/98	ROC	ML	47.5 (36.5–56)	DFS	Retrospective	Lauren classification, stage
Preoperative	7.5	16/148	1	ML	31.6 (4–72)	OS	Retrospective	Stage, lymphatic, vessel invasion
Post-chemo	7.5	57/136	3	ML	28.3 (median)	RFS, OS	Prospective	PM, HER2, treatments
Preoperative	7.5	25/136	1	ML	26	PFS, OS	Prospective	CA19-9, CEA, sex, stage
Postoperative	7.5	18/65	ROC	ML	60	OS/RFS	Retrospective	Stage
Post-chemo	5	36/59	2	ML	NR	OS/DFS	Retrospective	CA19-9, CEA
Post-chemo	7	65/106	2	ML	24.9 (median)	PFS, OS	Prospective	Location, age, ECOG PS, stage
Post-chemo	6	56/115	2	ML	NR	PFS, OS	Prospective	Stage
Postoperative	7.5	64/93	5	FC	36.4 (33.9–38.7)	DFS, OS	Prospective	NR
Postoperative	8	NR	ROC	ML	36	RFS	Retrospective	Age, stage, lymphatic invasion, grade
Postoperative	10	50/70	8	ML	12.9 (median)	PFS, OS	Retrospective	Age, sex, stage, treatment
Postoperative	7.5	16/59	1	ML	48	PFS, OS	Prospective	Stage, size, vascular invasion
Preoperative	3.2	66/114	3	ML	23.5 (median)	OS	Retrospective	Grade, treatment response
Postoperative	3	126/132	12.6	ML	NR	RFS	Prospective	CEA, CA1-99, stage
Pre-chemo	5	25/31	7.5	ML	12(1–30)	OS	Prospective	Stage, treatment response
Postoperative	5	75/188	5	ML	18.5(4–30)	OS	Retrospective	Age, gender, tumor stage

CA, covariates adjusted; Chemo, chemotherapy; DFS, disease-free survival; ECOG PS, Eastern Cooperative Oncology Group performance status; FC, HR calculate by figure; HC: healthy control; M/F, male/female; ML, multivariate Cox regression; NR, not report; P, provided by article; PFS, progression-free survival; PM, peritoneal metastasis; PPMM, peripheral blood micrometastasis; Pre, preoperative; Post, postoperative; OS, overall survival; RFS, recurrence-free survival; ROC, receiver operating characteristic curve; RT-PCR, reverse transcription-polymerase chain reaction.

**Table 2.** The quality assessment of included studies of meta-analysis.

Selection (0–4), comparability (0–2), outcome (0–3)										
Study	REC	SNEC	AE	DO	SC	AF	AO	FU	AFU	Total
Majima <i>et al.</i> <sup>27</sup>	0	1	1	1	0	0	0	0	0	3
Illert <i>et al.</i> <sup>28</sup>	1	1	1	1	0	0	1	1	1	7
Ikeguchi and Kaibara <sup>29</sup>	0	1	1	1	0	0	1	0	1	5
Uen <i>et al.</i> <sup>30</sup>	1	1	1	1	0	0	1	0	1	6
Noworolska <sup>32</sup>	1	1	1	1	0	0	1	1	1	7
Hiraiwa <i>et al.</i> <sup>33</sup>	0	1	1	1	0	0	1	0	0	4
Yie <i>et al.</i> <sup>34</sup>	1	1	1	1	0	0	0	1	0	5
Saad <i>et al.</i> <sup>35</sup>	1	1	1	1	0	0	1	0	1	6
Cao <i>et al.</i> <sup>38</sup>	1	1	1	1	0	0	0	1	0	5
Ito <i>et al.</i> <sup>47</sup>	1	1	1	1	0	0	1	0	0	5
Uenosono <i>et al.</i> <sup>39</sup>	1	1	1	1	0	0	1	1	0	6
Wu <i>et al.</i> <sup>31</sup>	1	1	1	1	0	0	1	0	0	6
Matsusaka <i>et al.</i> <sup>45</sup>	1	1	1	1	0	0	1	0	1	6
Qiu <i>et al.</i> <sup>36</sup>	1	1	1	1	0	0	1	1	1	7
Arigami <i>et al.</i> <sup>37</sup>	1	1	1	1	1	0	1	0	1	7
Li <i>et al.</i> <sup>20</sup>	1	1	1	1	0	0	0	1	1	6
Okabe <i>et al.</i> <sup>46</sup>	1	1	1	1	0	0	1	1	1	7
Liu <i>et al.</i> <sup>48</sup>	1	1	1	1	0	0	1	1	1	7
Pernot <i>et al.</i> <sup>19</sup>	1	1	1	1	0	0	1	0	1	6
Li <i>et al.</i> <sup>52</sup>	1	1	1	1	0	0	1	1	1	7
Zhang <i>et al.</i> <sup>40</sup>	1	1	1	1	0	0	1	1	1	7
Miki <i>et al.</i> <sup>41</sup>	1	1	1	1	0	0	0	1	0	5
Liu <i>et al.</i> <sup>48</sup>	1	1	1	1	0	0	1	0	0	5
Ning <i>et al.</i> <sup>43</sup>	1	1	1	1	1	0	1	1	1	8
Zhu <i>et al.</i> <sup>44</sup>	1	1	1	1	1	0	1	1	1	8
Zeng <i>et al.</i> <sup>49</sup>	1	1	1	1	0	0	1	0	1	6
Jhi <i>et al.</i> <sup>50</sup>	1	1	1	1	1	0	1	1	1	8
Qiu <i>et al.</i> <sup>51</sup>	1	1	1	1	0	0	1	1	1	7

AE, ascertainment of exposure; AF, additional factors; AO, assessment of outcome; AFU, adequacy of follow-up; DO, outcome not present at the start of the study; FU, length of follow-up; REC, representativeness of exposed cohort; SNEC, selection of nonexposed cohort; SC, control for important factors.



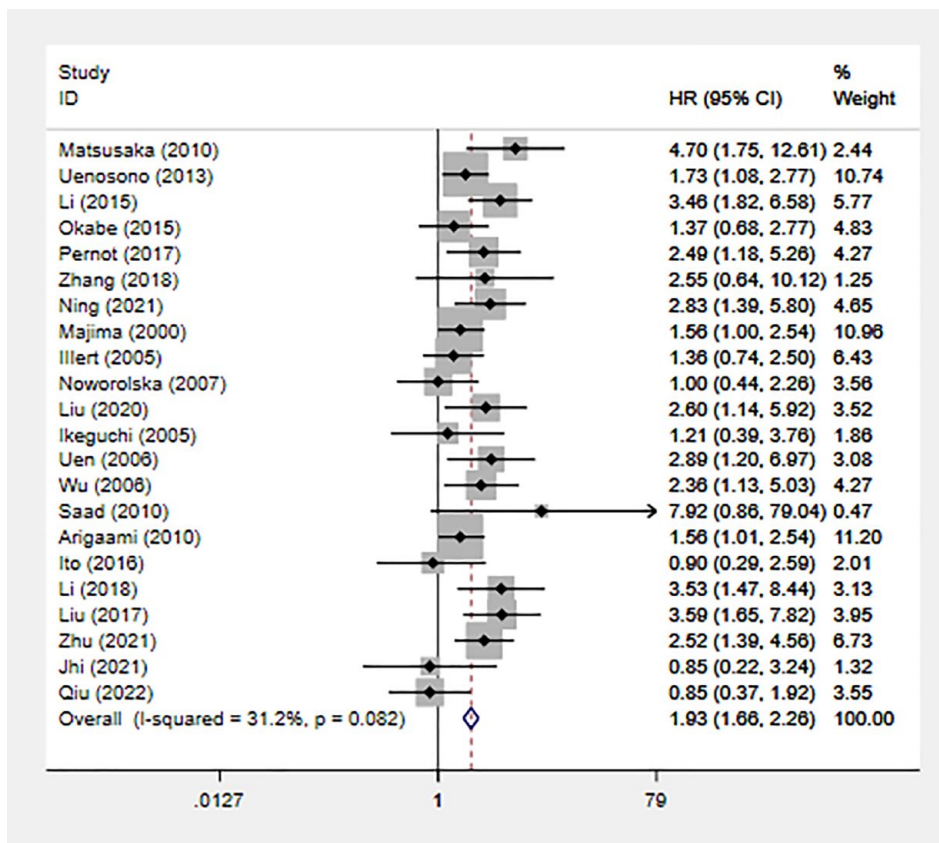
**Figure 1.** Flow chart of selection studies and specific reasons for exclusion.

that DFS/RFS (HR=2.853, 95% CI 1.702–4.782,  $p < 0.001$ ), with low heterogeneity. There were six articles reporting PFS, with a statistically significant pooled HR (HR=3.272, 95% CI 1.970–5.435,  $p < 0.001$ ) and low heterogeneity.

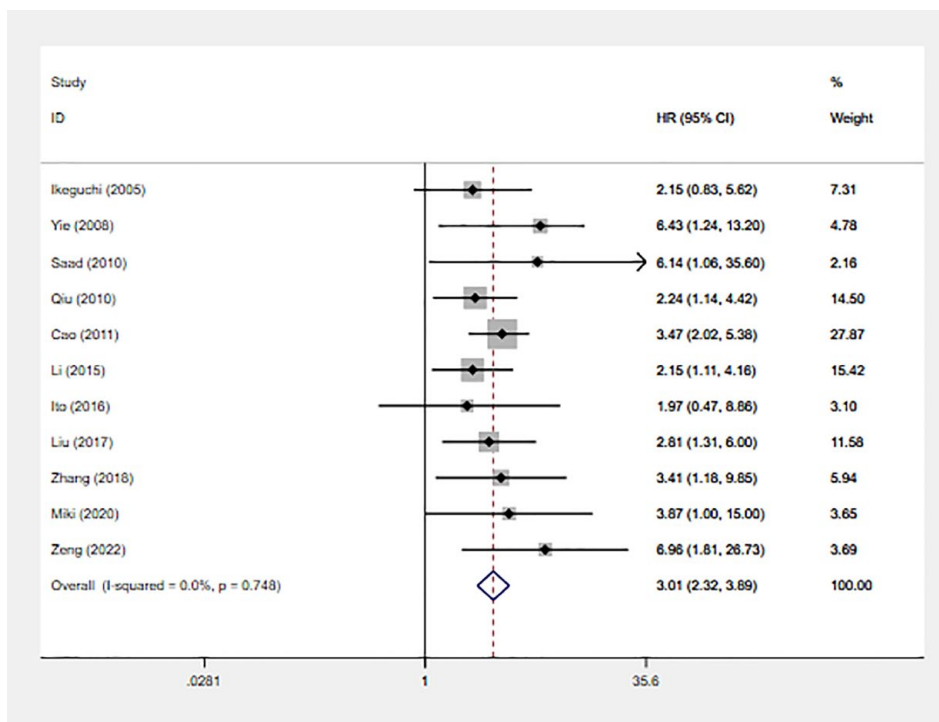
#### *The outcomes of subgroup analysis with OS*

The subgroup analysis on the tumor stage (I + II/III + IV means a proportion of the total number of people in stage I and II to stage III and IV) of studies in the study revealed that higher CTCs were associated with shorter DFS/RFS (I + II/III + IV > 1: HR=1.549, 95% CI: 1.206–1.991,  $p = 0.004$ ; I + II/III + IV < 1: HR=2.212; 95% CI: 1.819–2.691;  $p < 0.001$ ; Tables 3 and 4). Furthermore, the subgroup analysis on the HR extraction method of studies in the study revealed that higher CTCs were associated with shorter OS (Figure calculation: HR=1.717, 95% CI: 1.317–2.238,  $p < 0.001$ ; extracted by multivariate

Cox regression HR=2.091, 95% CI: 1.736–2.520,  $p < 0.001$ ). The subgroup analysis of CTC detection with different methods still showed a poor prognostic effect for GC patients. Higher CTCs predicted poorer OS of GC patients where CTCs were detected by CellSearch (HR=2.299; 95% CI 1.764–2.996;  $p < 0.001$ ), RT-PCR (HR=1.586; 95% CI: 1.215–2.070;  $p = 0.001$ ), and other methods (HR=1.979; 95% CI 1.510–2.594;  $p < 0.001$ ). In addition, the subgroup analysis of different detection targets showed a poor prognostic effect for GC patients of CTCs expressing CK (HR=1.378; 95% CI: 0.990–1.943;  $p = 0.057$ ); EpCAM+, CK+, CD45–, and DAPI+ (HR=2.299; 95% CI: 1.764–2.996;  $p < 0.001$ ); and EpCAM (HR=2.881; 95% CI: 1.538–5.396;  $p = 0.001$ ). The subgroup analysis on the sampling times of GC patients in the study revealed that higher CTCs significantly occurred with shorter OS both in pre-treatment (HR=1.639 95% CI 1.288–2.085,  $p < 0.001$ ), post-chemotherapy (HR=3.382 95% CI 2.386–4.795,



**Figure 2.** The meta-analysis forest plot of pooled OS.  
OS, overall survival.



**Figure 3.** The meta-analysis forest plot of pooled DFS/RFS.  
DFS, disease-free survival; RFS, recurrence-free survival.



**Table 3.** The outcome of pooled OS of meta-analysis.

Overall	N	HR	95% CI	p	I <sup>2</sup>	P	Model
OS	22	1.933	1.657–2.256	<0.001	31.20%	0.082	fixed
HR calculation method							
P	15	2.091	1.736–2.520	<0.001	40.10%	0.055	fixed
FC	7	1.717	1.317–2.238	<0.001	0.00%	0.439	fixed
Detection method							
CellSearch	7	2.299	1.764–2.996	<0.001	19.60%	0.280	fixed
RT-PCR	5	1.586	1.215–2.070	0.001	0.00%	0.688	fixed
Target							
EpCAM+, CK+, CD45–, and DAPI+	7	2.299	1.764–2.996	<0.001	19.60%	0.28	fixed
CK	3	1.387	0.990–1.943	0.057	0.00%	0.65	fixed
Detection time							
Pre	6	1.639	1.288–2.085	<0.001	0.00%	0.603	fixed
Post	16	2.169	1.774–2.651	<0.001	37%	0.068	fixed
Treatment							
Post-chemo	5	3.382	2.386–4.795	<0.001	0.00%	0.893	fixed
Post-surgery	7	1.591	1.184–2.139	0.002	12.40%	0.335	fixed
Post-chemo, surgery	4	2.122	1.364–3.301	0.001	44.60%	0.144	fixed
Stage							
I + II/III + IV > 1	7	1.549	1.206–1.991	0.004	20.9%	0.270	fixed
I + II/III + IV < 1	15	2.212	1.819–2.691	<0.001	22.7%	0.201	fixed
Region							
Asia	19	1.986	1.694–2.329	<0.001	30.1%	0.105	fixed
No-Asia	3	1.251	0.662–2.365	0.490	29.9%	0.240	fixed
Chemo, chemotherapy; FC, HR calculate by figure; N, number of studies; P, provided by article; Pre, preoperative; Post, postoperative; OS, overall survival; RT-PCR, reverse transcription-polymerase chain reaction.							

$p < 0.001$ ), post-surgery (HR = 1.591 95% CI 1.184–2.139,  $p = 0.002$ ), and post-chemotherapy and surgery (HR = 2.122 95% CI 1.364–3.301,  $p = 0.001$ ). The subgroup analysis on CTC detection showed a poor prognostic effect for GC patients from different regions. Higher CTCs predicted poorer OS for GC patients who are

from Asian regions (HR = 1.986; 95% CI 1.694 to 2.329;  $p < 0.001$ ), but without significant statistical difference for patients from No-Asian regions (HR = 1.251; 95% CI 0.662–2.365;  $p < 0.001$ ). Furthermore, no significant differences were observed in the test for subgroup analysis ( $p > 0.05$ ; Supplemental Table A).

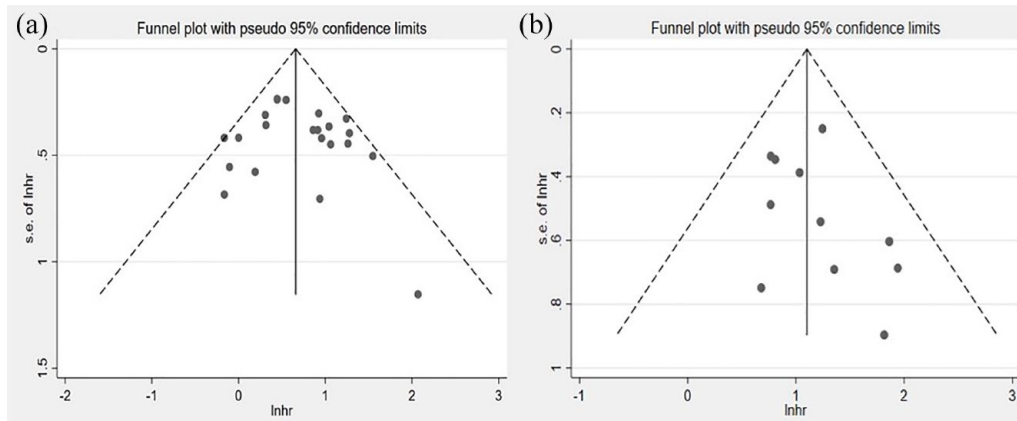
**Table 4.** The outcome of pooled DFS/RFS of meta-analysis.

Overall	N	HR	95% CI	p	I <sup>2</sup>	P	Model
DFS/RFS	11	3.228	2.475–4.211	<0.001	0.00%	0.464	fixed
HR calculation method							
P	9	3.006	2.322–3.893	<0.001	0.00%	0.623	fixed
FC	2	2.644	1.299–5.379	0.007	0.00%	0.527	fixed
Detection method							
CellSearch	2	2.446	1.398–4.279	0.002	0.00%	0.470	fixed
RT-PCR	4	3.059	2.155–4.342	<0.001	3.10%	0.377	fixed
Target							
EpCAM+, CK+, CD45-, and DAPI+	2	2.446	1.398–4.279	0.002	0.00%	0.470	fixed
CEA	3	2.396	1.435–4.000	0.001	0.00%	0.753	fixed
Survivin	2	3.799	2.416–5.972	<0.001	0.00%	0.345	fixed
Detection time							
Pre	1	2.243	1.138–4.422	0.02	NA	NA	NA
Post	10	3.160	2.389–4.179	<0.001	0.00%	0.748	fixed
Treatment							
Post-chemo	1	2.152	1.114–4.157	0.023	NA	NA	NA
Post-surgery	7	3.635	2.587–5.108	<0.001	0.00%	0.558	fixed
Post-chemo, surgery	3	3.140	1.797–5.487	<0.001	31.4%	0.233	fixed
Stage							
I + II/III + IV > 1	4	3.778	2.035–7.013	<0.001	0.0%	0.666	fixed
I + II/III + IV < 1	7	2.865	2.155–3.807	<0.001	0.0%	0.602	fixed
Region							
Asia	9	3.289	2.485–4.352	<0.001	6.0%	0.385	fixed
No-Asia	2	2.733	1.180–6.330	0.019	5.4%	0.304	fixed

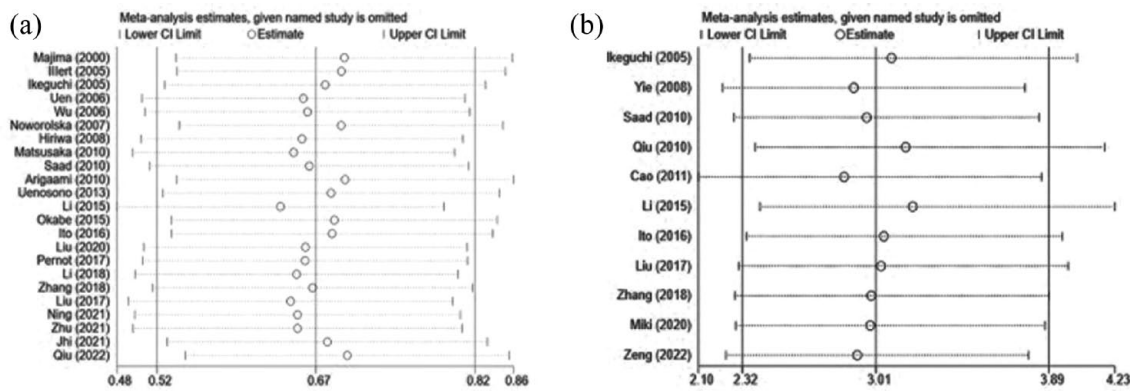
Chemo, chemotherapy; FC, HR calculate by figure; N, number of studies; P, provided by article; Pre, preoperative; Post, postoperative; RT-PCR, reverse transcription-polymerase chain reaction.

*The outcomes of subgroup analysis with DFS/RFS*  
The subgroup analysis on the tumor stage of studies in the study revealed that higher CTCs were related to shorter DFS/RFS (I + II/III + IV > 1: HR = 3.778; 95% CI: 2.035–7.013,  $p < 0.001$ ; I + II/III + IV < 1: HR = 2.865, 95%

CI: 2.155–3.807,  $p < 0.001$ ). Furthermore, the subgroup analysis on the HR extraction method of studies in the study revealed that higher CTCs were associated with shorter DFS/RFS (figure calculation: HR = 2.644, 95% CI: 1.299–5.379,  $p < 0.001$ ; extracted by multivariate Cox



**Figure 4.** The meta-analysis funnel plot of OS and DFS/RFS: (a) the meta-analysis funnel plot of OS and (b) the meta-analysis funnel plot of DFS/RFS.  
DFS, disease-free survival; OS, overall survival; RFS, recurrence-free survival.



**Figure 5.** The sensitivity analysis of OS: (a) the sensitivity analysis of OS and (b) the sensitivity analysis of DFS/RFS.  
DFS, disease-free survival; OS, overall survival; RFS, recurrence-free survival.

regression: HR=3.066, 95% CI: 2.322–3.893,  $p < 0.001$ ). The subgroup analysis of CTC detection with different methods still showed a poor prognostic effect for GC patients. Higher CTCs predicted poorer DFS/RFS of GC patients where CTCs were detected by Cellserach (HR=2.446; 95% CI 1.398–4.279;  $p = 0.002$ ) and RT-PCR (HR=3.059; 95% CI: 2.155–4.342;  $p < 0.001$ ). In addition, the subgroup analysis of different detection targets showed a poor prognostic effect for GC patients of CTCs expressing CEA (HR=2.396; 95% CI: 1.435–4.000;  $p = 0.001$ ); EpCAM+, CK+, CD45–, and DAPI+ (HR=2.446; 95% CI: 1.398–4.279;  $p = 0.002$ ); Survivin (HR=3.799; 95% CI: 2.416–5.972;  $p < 0.001$ ). The subgroup analysis on the sampling times of GC patients in the study revealed that higher CTCs significantly occurred with

shorter DFS/RFS (posttreatment; HR=3.160 95% CI 2.389–4.179,  $p < 0.001$ ). The subgroup analysis on CTC detection showed a poor prognostic effect for GC patients receiving different treatments. Higher CTCs predicted poorer DFS/RFS of GC patients when CTCs were detected post-surgery (HR=3.635; 95% CI 2.587–5.108;  $p < 0.001$ ), post-chemotherapy (HR=2.152; 95% CI: 1.114–4.157;  $p = 0.001$ ), and post-chemotherapy and surgery (HR=3.140; 95% CI 1.797–5.487;  $p = 0.001$ ). Higher CTCs predicted poorer DFS/RFS for GC patients who are from Asian regions (HR=3.289; 95% CI 2.485–4.352;  $p < 0.001$ ), and from No-Asian regions (HR=2.733; 95% CI 1.180–6.330;  $p < 0.001$ ). Furthermore, no significant differences were observed in the test for subgroup analysis ( $p > 0.05$ ; Supplemental Table B).

### Publication bias, sensitivity analysis

The funnel plots, Egger's, and Begg's tests were utilized to gauge publication bias for the studies related to OS (Egger's  $p=0.10$  and Begg's  $p=0.05$ , respectively) and DFS/RFS (Egger's  $p=0.073$  and Begg's  $p=0.731$ , respectively), which showed that there was no significant publication bias found in the HR of either OS (Figure 4(a)) or DFS/RFS (Figure 4(b)). Sensitivity analysis was performed by sequentially removing a study and then calculating its pooled HR and 95% CI. Furthermore, after excluding articles with NOS scores  $<6$ , the remaining studies concluded similar results. This demonstrates that after removing anyone, the HR of OS and DFS/RFS was always distributed within the original 95% CI, thus showing imperceptible variability between studies (Figure 5). This revealed that our outcomes were robust.

### Discussion

CTCs have recently been applied to provide further information to support clinical treatment response assessment for GC patients with poor prognosis despite R0 resection combined with perioperative chemotherapy. Our meta-analysis concluded that the detection of CTCs was associated with poorer OS and could act as a risk factor for relapse/metastasis in patients with GC during the follow-up period, with low heterogeneity and no publication bias. Furthermore, the meta-analysis of prospective studies reported similar results. CTCs cause tumor metastasis or recurrence because they flow with blood circulation to various tissues and organs throughout the body and then grow, causing distant metastasis of the tumor.<sup>53</sup> To better understand the relationship between CTCs and the prognosis of GC, subgroup analyses stratified by estimated HR, detection target, detection method, treatment, sample time, and stage were conducted. Our study included patients with stage I–IV, subgroup analysis showed that CTCs could predict both patients diagnosed at an early or advanced stages, which could assist the clinician to make appropriate treatment strategies for patients. Furthermore, we found that regardless of whether the data were calculated or provided by the article, the results of the association between CTCs and the OS and RFS/DFS of GC patients were consistent.

To date, several detection methods have been applied to detect CTCs; however, it remains challenging to identify CTC in whole blood due to

their extremely low concentrations. Previous studies reported that a traditional method of isolating cells, such as flow cytometry, density gradient centrifugation, and immunocapture by magnetic beads, cannot detect rare CTCs.<sup>32,41,42</sup> The CellSearch system has been approved by the US FDA for the detection of CTCs in patients with cancer and is the first and only system approved for these purposes with high specificity and reproducibility, but cannot capture cells that do not express EpCAM and cytokeratin on the cell membrane.<sup>54</sup> The use of real-time RT-PCR assays, based on mRNA expression, is an alternative to detect CTCs with high specificity and moderate sensitivity. Our study demonstrated that the two methods could both predict the prognosis of GC. However, there is still controversy about whether RT-PCR is superior to CellSearch.<sup>55,56</sup> Furthermore, a novel CTC-biopsy detection system based on the principle of filtration and isolation of tumor cells according to cell size has been developed, with a 93% of CTC detection rate, which was more sensitive than CellSearch, several studies have reported the efficacy of filtration principle for CTC detection in breast cancer,<sup>57</sup> esophageal squamous cell carcinoma,<sup>58</sup> and renal cancer.<sup>59</sup> Chen *et al.* and his colleagues<sup>60</sup> used pre-established surface molecule-independent subtraction enrichment (SE)-iFISH strategy, maintained at high viability and suitable for primary tumor cell culture, to detect CTC for GC, with a detection rate of 33.3%. A previous study also reported that researchers used Cytelligen to detect CTCs.<sup>52</sup> In addition, few studies with small sample sizes reported other detection methods (CMA, flow cytometry, RNA-ISH, and LT-PCR). Thus, it was necessary to further validate the superiority of the detection methods. The most frequently detected targets of CTCs were EpCAM+, CK+, CD45–, and DAPI+ by CellSearch. Moreover, as cell surface markers for the detection of cancer cells, cytokeratins (CK8, CK18, and CK19) play an important role. Other detection targets, such as CEA, cMET, Survivin, and B7-H3, could be used to detect CTCs by RT-PCR. Our study showed that CTCs were associated with a poor prognosis of GC when CTCs were detected by EpCAM+, CK+, CD45–, and DAPI+, CEA and Survivin. Three articles reported the results of CK target, however, it showed no association between CTCs and prognosis of GC patients. As we know, the ToGA study showed that HER2-targeted therapy using trastuzumab has clinical benefits in patients with advanced HER2-positive

GC.<sup>61</sup> Recently, one study demonstrated the potential clinical utility of trastuzumab combined with chemotherapy in patients with HER2-positive CTCs even if they are histologically HER2-negative.<sup>62</sup> Thus, the assessment of CTCs may be useful for monitoring real-time tumor response during HER2-targeted therapy, which required more high-quality studies to validate it. Our study showed that EpCAM was associated with poor OS. Furthermore, CEA, biomarkers in CellSearch and Survivin were associated with poor DFS/PFS. Miki *et al.* and colleagues<sup>41</sup> demonstrated that CEA-positive CTCs will be a clinically beneficial biomarker in patients with GC. Furthermore, a previous study<sup>38</sup> also reported detection of CTCs expressing Survivin mRNA could be a good clinical biomarker used to predict the prognosis of patients with curatively resected GC. There are relatively few studies on other targets, and more research is needed to explore the relationship between targets and the prognosis of GC. Thus, no matter what detection method and detection marker, our meta-analysis showed that higher CTCs were associated with poor OS and DFS/PFS for GC patients.

According to our study, there was a high risk of poor prognosis when CTCs were detected pre- or intra/during treatment. A previous study demonstrated that RFS and OS for patients with CTCs were significantly lower than those for patients without CTCs by preoperative peripheral blood sample.<sup>39</sup> A prospective study designed by Zhang *et al.*<sup>40</sup> evaluated preoperative and postoperative CTC changes in resectable GC and possible correlations with postoperative recurrence and found that preoperative CTCs were not correlated with the recurrence pattern. Patients with hematogenous metastases who had postoperative CTCs  $\geq 2/7.5$  ml had markedly shorter DFS, which confirmed that postoperative CTCs may be a more direct marker of postoperative recurrence, especially in patients who experience hematogenous recurrence. Our study showed that postoperative HR has a better ability to predict OS (post; HR: 2.169 > pre; HR: 1.639), DFS/RFS (post; HR: 3.160 > pre; HR: 2.243) than preoperative HR. Theoretically, a patient's postoperative CTCs may be more responsive to the patient's prognosis, as the surgical procedure may cause the spread of CTCs, thereby altering CTCs in the blood. Furthermore, the surgery itself can also change the survival of cancer patients. Thus, we suggest that the predictive ability of postoperative CTCs might be superior

to that of preoperative CTCs. In addition, Liu *et al.* and colleagues reported that CTC count may be a good monitoring parameter for chemotherapy and an ideal prognostic parameter for palliative chemotherapy patients.<sup>48</sup> Neoadjuvant chemotherapy has been proven to reduce the tumor volume, reduce the tumor stage, and improve the R0 resection rate to improve the prognosis of patients.<sup>63,64</sup> However, the relationship between the detection of CTCs in blood after neoadjuvant chemotherapy and tumor prognosis remains to be further verified due to the lack of sufficient research.

Recently, another liquid biopsy for GC, ctDNA, has been demonstrated to be associated with the diagnosis, treatment response, and prognosis of GC.<sup>65</sup> ctDNA and CTC may focus on different clinical applications due to their different sources, evaluation criteria, and biological characteristics. ctDNA has significant advantages in accurately reflecting tumor cell micro-metastases in the blood circulation and can effectively assess the state of tumor development, and therefore has a unique position in early screening, adjuvant diagnosis, recurrence monitoring, and efficacy assessment. In contrast, ctDNA is more focused on reflecting the variation of DNA within tumor cells, and thus it plays an important role in therapeutic decision-making (driver gene detection) and targeted drug resistance monitoring. CTC analysis provides complete genomic information of living cells, but ctDNA does not provide complete genomic and protein expression information of each cell. From a clinical perspective, CTC is mostly used for prognosis and recurrence monitoring; furthermore, the information obtained from simultaneous detection of CTC and ctDNA may be complementary. The coexistence of the two liquid biopsy technologies may be the future direction of development.<sup>65-67</sup> Furthermore, the study showed that CTCs were associated with the poor DFS/RFS of GC when CTCs were detected for patients from Asian or No-Asian regions. In addition, higher CTCs predicted poorer OS for GC patients who are from Asian regions, but without significant statistical difference for patients from No-Asian regions, which might account for only three studies from No-Asian regions with a small sample size.

There were several limitations in our meta-analysis. First, the heterogeneity between our included studies with different detection methods, markers, and treatment had impact on our

results. We did a careful subgroup analysis stratified by the above factors, and the results were consistent. Furthermore, to a certain extent, the effect value of pooled HR calculated by lnHR and selnHR could avoid some heterogeneity between different studies. Moreover, the whole heterogeneity of our research was slight according to the heterogeneity test. Furthermore, most included studies did not provide detailed data on stage I, II, III, IV separately, only providing mixed stages I–II, I–III, I–IV, II–IV, and so on, thus, we could not distinguish each stage of GC. In addition, the detection methods of CTCs have different sensitivities and specificities, which would cause bias in our research. For example, a panel of molecular markers can improve the sensitivity of CTC detection compared to the single markers being used. In addition, CTCs could be heterogeneous, and it remains unknown what subpopulations are best for predicting survival and monitoring treatment response. Future well-designed prospective studies with larger sample sizes should be conducted to answer these questions. Finally, we have tried our best and failed to register in PROSPERO. However, our meta-analysis was made in strict accordance with the PRISMA statement to ensure the quality of the article.

### Conclusion

CTCs were associated with a poor prognosis in patients with GC. The use of CTCs in clinical practice is promising and could provide help for clinical decisions.

### Declarations

#### *Ethics approval and consent to participate*

All analyses were based on previously published studies; thus, no ethical approval or patient consent was needed.

#### *Consent for publication*

Not applicable.

#### *Author contribution(s)*

**Tao Jin:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.

**Pan Liang:** Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Validation; Writing – original draft.

**Ze Chen:** Conceptualization; Data curation; Formal analysis.

**Feng He:** Data curation; Resources; Software; Validation; Writing – original draft.

**Ze Li:** Conceptualization; Methodology; Project administration; Validation; Writing – original draft.

**Zheng Chen:** Data curation; Formal analysis; Investigation.

**Jian-Kun Hu:** Validation; Writing – review & editing; Funding acquisition.

**Kun Yang:** Conceptualization; Funding acquisition; Supervision; Visualization; Writing – review & editing.

#### *Acknowledgements*

None.

#### *Funding*

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Domestic support from (1) National Natural Science Foundation of China (No. 81772547); (2) Foundation of Science & Technology Department of Sichuan Province (No. 2019YFS0256); (3) 1. 3. 5 project for disciplines of excellence, West China Hospital, Sichuan University (No. ZYJC21006); (4) Foundation of Science & Technology Department of Sichuan Province (2023YFS0060). This work was supported by Gastric cancer center, West China Hospital.


#### *Competing interests*

The authors declare that there is no conflict of interest.

#### *Availability of data and material*

All data generated or analyzed during this study are included in this published article.

#### ORCID iD

Kun Yang  <https://orcid.org/0000-0002-8891-5736>

#### Supplemental material

Supplemental material for this article is available online.

## References

1. Smyth EC, Nilsson M, Grabsch HI, *et al.* Gastric cancer. *Lancet* 2020; 396: 635–648.
2. Sung H, Ferlay J, Siegel RL, *et al.* Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; 71: 209–249.
3. Yu J, Huang C, Sun Y, *et al.* Effect of laparoscopic vs open distal gastrectomy on 3-Year disease-free survival in patients with locally advanced gastric cancer: the CLASS-01 Randomized Clinical Trial. *JAMA* 2019; 321: 1983–1992.
4. Ojima T, Nakamura M, Hayata K, *et al.* Short-term outcomes of robotic gastrectomy vs laparoscopic gastrectomy for patients with gastric cancer: a randomized clinical trial. *JAMA Surg* 2021; 156: 954–963.
5. Joshi SS and Badgwell BD. Current treatment and recent progress in gastric cancer. *CA Cancer J Clin* 2021; 71: 264–279.
6. Banks M, Graham D, Jansen M, *et al.* British Society of Gastroenterology guidelines on the diagnosis and management of patients at risk of gastric adenocarcinoma. *Gut* 2019; 68: 1545–1575.
7. Fidler IJ. The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. *Nat Rev Cancer* 2003; 3: 453–458.
8. Xue J, Xie VK, Wang P, *et al.* Interrelationships of circulating tumor cells with metastasis and thrombosis: role of microRNAs. *Curr Pharm Des* 2014; 20: 5298–5308.
9. Sperger JM, Emamekhoo H, McKay RR, *et al.* Prospective Evaluation of clinical outcomes using a multiplex liquid biopsy targeting diverse resistance mechanisms in metastatic prostate cancer. *J Clin Oncol* 2021; 39: 2926–2937.
10. Mazard T, Cayrefourcq L, Perriard F, *et al.* Clinical relevance of viable circulating tumor cells in patients with metastatic colorectal cancer: the COLOSPOT Prospective Study. *Cancers* 2021; 13: 2966.
11. Cristofanilli M, Budd GT, Ellis MJ, *et al.* Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *New Engl J Med* 2004; 351: 781–791.
12. Hiltermann TJN, Pore MM, van den Berg A, *et al.* Circulating tumor cells in small-cell lung cancer: a predictive and prognostic factor. *Ann Oncol* 2012; 23: 2937–2942.
13. Yu M, Stott S, Toner M, *et al.* Circulating tumor cells: approaches to isolation and characterization. *J Cell Biol* 2011; 192: 373–382.
14. Warkiani ME, Khoo BL, Wu L, *et al.* Ultra-fast, label-free isolation of circulating tumor cells from blood using spiral microfluidics. *Nat Protoc* 2016; 11: 134–148.
15. Chen JY, Tsai WS, Shao HJ, *et al.* Sensitive and specific biomimetic lipid coated microfluidics to isolate viable circulating tumor cells and microemboli for cancer detection. *PLoS One* 2016; 11: e0149633.
16. Tellez-Gabriel M, Knutsen E and Perander M. Current status of circulating tumor cells, circulating tumor DNA, and exosomes in breast cancer liquid biopsies. *Int J Mol Sci* 2020; 21: 9457.
17. Ueo H, Sugimachi K, Gorges TM, *et al.* Circulating tumour cell-derived platin3 is a novel marker for predicting long-term prognosis in patients with breast cancer. *Br J Cancer* 2015; 112: 1519–1526.
18. Zhang W, Xia W, Lv Z, *et al.* Liquid biopsy for cancer: circulating tumor cells, circulating free DNA or exosomes? *Cell Physiol Biochem* 2017; 41: 755–768.
19. Pernot S, Badoual C, Terme M, *et al.* Dynamic evaluation of circulating tumour cells in patients with advanced gastric and oesogastric junction adenocarcinoma: prognostic value and early assessment of therapeutic effects. *Eur J Cancer* 2017; 79: 15–22.
20. Li Y, Gong J, Zhang Q, *et al.* Dynamic monitoring of circulating tumour cells to evaluate therapeutic efficacy in advanced gastric cancer. *Br J Cancer* 2016; 114: 138–145.
21. Huang X, Gao P, Sun J, *et al.* Clinicopathological and prognostic significance of circulating tumor cells in patients with gastric cancer: a meta-analysis. *Int J Cancer* 2015; 136: 21–33.
22. Gao Y, Xi H, Wei B, *et al.* Association between liquid biopsy and prognosis of gastric cancer patients: a systematic review and meta-analysis. *Front Oncol* 2019; 9: 1222.
23. Stroup DF, Berlin JA, Morton SC, *et al.* Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of observational studies in Epidemiology (MOOSE) group. *JAMA* 2000; 283: 2008–2012.
24. Moher D, Shamseer L, Clarke M, *et al.* Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015; 4: 1.

25. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010; 25: 603–605.
26. Tierney JF, Stewart LA, Ghersi D, *et al.* Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 2007; 8: 16.
27. Majima T, Ichikura T, Takayama E, *et al.* Detecting circulating cancer cells using reverse transcriptase-polymerase chain reaction for cytokeratin mRNA in peripheral blood from patients with gastric cancer. *Jpn J Clin Oncol* 2000; 30: 499–503.
28. Illert B, Fein M, Otto C, *et al.* Disseminated tumor cells in the blood of patients with gastric cancer are an independent predictive marker of poor prognosis. *Scand J Gastroenterol* 2005; 40: 843–849.
29. Ikeguchi M and Kaibara N. Detection of circulating cancer cells after a gastrectomy for gastric cancer. *Surg Today* 2005; 35: 436–441.
30. Uen YH, Lin SR, Wu CH, *et al.* Clinical significance of MUC1 and c-Met RT-PCR detection of circulating tumor cells in patients with gastric carcinoma. *Clin Chim Acta* 2006; 367: 55–61.
31. Wu CH, Lin SR, Yu FJ, *et al.* Development of a high-throughput membrane-array method for molecular diagnosis of circulating tumor cells in patients with gastric cancers. *Int J Cancer* 2006; 119: 373–379.
32. Pituch-Noworolska A, Kolodziejczyk P, Kulig J, *et al.* Circulating tumour cells and survival of patients with gastric cancer. *Anticancer Res* 2007; 27: 635–640.
33. Hiraiwa K, Takeuchi H, Hasegawa H, *et al.* Clinical significance of circulating tumor cells in blood from patients with gastrointestinal cancers. *Ann Surg Oncol* 2008; 15: 3092–3100.
34. Yie SM, Lou B, Ye SR, *et al.* Detection of survivin-expressing circulating cancer cells (CCCs) in peripheral blood of patients with gastric and colorectal cancer reveals high risks of relapse. *Ann Surg Oncol* 2008; 15: 3073–3082.
35. Saad AA, Awed NM, Abd Elkerim NN, *et al.* Prognostic significance of E-cadherin expression and peripheral blood micrometastasis in gastric carcinoma patients. *Ann Surg Oncol* 2010; 17: 3059–3067.
36. Qiu MZ, Li ZH, Zhou ZW, *et al.* Detection of carcinoembryonic antigen messenger RNA in blood using quantitative real-time reverse transcriptase-polymerase chain reaction to predict recurrence of gastric adenocarcinoma. *J Transl Med* 2010; 8: 107.
37. Arigami T, Uenosono Y, Hirata M, *et al.* B7-H3 expression in gastric cancer: a novel molecular blood marker for detecting circulating tumor cells. *Cancer Sci* 2011; 102: 1019–1024.
38. Cao W, Yang W, Li H, *et al.* Using detection of survivin-expressing circulating tumor cells in peripheral blood to predict tumor recurrence following curative resection of gastric cancer. *J Surg Oncol* 2011; 103: 110–115.
39. Uenosono Y, Arigami T, Kozono T, *et al.* Clinical significance of circulating tumor cells in peripheral blood from patients with gastric cancer. *Cancer* 2013; 119: 3984–3991.
40. Zhang Q, Shan F, Li Z, *et al.* A prospective study on the changes and clinical significance of pre-operative and post-operative circulating tumor cells in resectable gastric cancer. *J Transl Med* 2018; 16: 171.
41. Miki Y, Yashiro M, Kuroda K, *et al.* Circulating CEA-positive and EpCAM-negative tumor cells might be a predictive biomarker for recurrence in patients with gastric cancer. *Cancer Med* 2021; 10: 521–528.
42. Liu M, Wang R, Sun X, *et al.* Prognostic significance of PD-L1 expression on cell-surface vimentin-positive circulating tumor cells in gastric cancer patients. *Mol Oncol* 2020; 14: 865–881.
43. Ning D, Cui K, Liu M, *et al.* Comparison of CellSearch and circulating tumor cells (CTC)-Biopsy systems in detecting peripheral blood circulating tumor cells in patients with gastric cancer. *Med Sci Monit* 2021; 27: e926565.
44. Zhu Y, Chen N, Chen M, *et al.* Circulating tumor cells: a surrogate to predict the effect of treatment and overall survival in gastric adenocarcinoma. *Int J Biol Markers* 2021; 36: 28–35.
45. Matsusaka S, Chìn K, Ogura M, *et al.* Circulating tumor cells as a surrogate marker for determining response to chemotherapy in patients with advanced gastric cancer. *Cancer Sci* 2010; 101: 1067–1071.
46. Okabe H, Tsunoda S, Hosogi H, *et al.* Circulating tumor cells as an independent predictor of survival in Advanced Gastric Cancer. *Ann Surg Oncol* 2015; 22: 3954–3961.
47. Ito H, Sato J, Tsujino Y, *et al.* Long-term prognostic impact of circulating tumour cells in gastric cancer patients. *World J Gastroenterol* 2016; 22: 10232–10241.



48. Liu Y, Ling Y, Qi Q, *et al.* Prognostic value of circulating tumor cells in advanced gastric cancer patients receiving chemotherapy. *Mol Clin Oncol* 2017; 6: 235–242.
49. Zeng CDD, Jin CC, Gao C, *et al.* Preoperative folate receptor-positive circulating tumor cells are associated with occult peritoneal metastasis and early recurrence in gastric cancer patients: a Prospective Cohort Study. *Front Oncol* 2022; 12: 769203.
50. Jhi JH, Kim GH, Park SJ, *et al.* Circulating tumor cells and TWIST expression in patients with metastatic gastric cancer: a Preliminary Study. *J Clin Med* 2021; 10: 4481.
51. Qiu Y, Zhang X, Deng X, *et al.* Circulating tumor cell-associated white blood cell cluster is associated with poor survival of patients with gastric cancer following radical gastrectomy. *Eur J Surg Oncol J Eur Society Surg Oncol Br Assoc Surg Oncol* 2022; 48: 1039–1045.
52. Li Y, Zhang X, Liu D, *et al.* Evolutionary expression of HER2 conferred by chromosome aneuploidy on circulating gastric cancer cells contributes to developing targeted and chemotherapeutic resistance. *Clin Cancer Res* 2018; 24: 5261–5271.
53. Li TT, Liu H, Yu J, *et al.* Prognostic and predictive blood biomarkers in gastric cancer and the potential application of circulating tumor cells. *World J Gastroenterol* 2018; 24: 2236–2246.
54. Riethdorf S, Fritsche H, Müller V, *et al.* Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. *Clin Cancer Res* 2007; 13: 920–928.
55. Van der Auwera I, Peeters D, Benoy IH, *et al.* Circulating tumour cell detection: a direct comparison between the CellSearch system, the AdnaTest and CK-19/mammaglobin RT-PCR in patients with metastatic breast cancer. *Br J Cancer* 2010; 102: 276–284.
56. Helo P, Cronin AM and Danila DC. Circulating prostate tumor cells detected by reverse transcription-PCR in men with localized or castration-refractory prostate cancer: concordance with CellSearch assay and association with bone metastases and with survival. *Clin Chem* 2009; 55: 765–773.
57. Kallergi G, Politaki E, Alkahtani S, *et al.* Evaluation of isolation methods for circulating tumor cells (CTCs). *Cell Physiol Biochem* 2016; 40: 411–419.
58. Li H, Song P, Zou B, *et al.* Circulating tumor cell analyses in patients with esophageal squamous cell carcinoma using epithelial marker-dependent and -independent approaches. *Medicine* 2015; 94: e1565.
59. Bai M, Zou B, Wang Z, *et al.* Comparison of two detection systems for circulating tumor cells among patients with renal cell carcinoma. *Int Urol Nephrol* 2018; 50: 1801–1809.
60. Chen Y, Yuan J, Li Y, *et al.* Profiling heterogenous sizes of circulating tumor microemboli to track therapeutic resistance and prognosis in advanced gastric cancer. *Hum Cell* 2021; 34: 1446–1454.
61. Bang YJ, Van Cutsem E, Feyereislova A, *et al.* Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; 376: 687–697.
62. Matsushita D, Uenosono Y, Arigami T, *et al.* Clinical significance of circulating tumor cells in the response to trastuzumab for HER2-negative metastatic gastric cancer. *Cancer Chemother Pharmacol* 2021; 87: 789–797.
63. Kang YK, Yook JH and Park YK. PRODIGY: a phase III study of neoadjuvant docetaxel, oxaliplatin, and S-1 plus surgery and adjuvant S-1 versus surgery and adjuvant S-1 for Resectable Advanced Gastric Cancer. *J Clin Oncology* 2021; 39: 2903–2913.
64. Coccolini F, Nardi M, Montori G, *et al.* Neoadjuvant chemotherapy in advanced gastric and esophago-gastric cancer. Meta-analysis of randomized trials. *Int Surg J* 2018; 51: 120–127.
65. Ma X, Ou K, Liu X, *et al.* Application progress of liquid biopsy in gastric cancer. *Front Oncol* 2022; 12: 969866.
66. Zhang Z, Wu H, Chong W, *et al.* Liquid biopsy in gastric cancer: predictive and prognostic biomarkers. *Cell Death Discov* 2022; 13: 903.
67. Kilgour E, Rothwell DG, Brady G, *et al.* Liquid biopsy-based biomarkers of treatment response and resistance. *Cancer Cell* 2020; 37: 485–495.