Circulating tumour cells predict survival in gastric cancer patients: a meta-analysis

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Aim of the study: The prognostic value of the detection of circulating tumour cells (CTCs) in gastric cancer has been studied intensely in recent years. However, the application of different technologies led to inconsistent results between the studies. Here, we performed a meta-analysis of published studies to summarise the evidence.

Material and methods: Medline and ISI Web of Knowledge were searched up to March 2013 using "circulating tumor cells" and "gastric cancer" as search terms. Hazard ratio (HR) with 95% confidence intervals (CIs) for prognostic outcomes and clinical characteristics were extracted from each study. Pooled hazard ratios (HR) and odds ratios (OR) were calculated using random or fixed-effects models.

Results: Twelve studies enrolling 774 patients were included. The combined HR estimate for overall survival (OS), disease-free survival (DFS), and progression-free survival (PFS) were 1.41 (95% CI: 1.28–1.62), 2.99 (95% CI: 2.01–4.45) and 1.64 (95% CI: 1.02–2.62), respectively. Subgroup analysis concerning detection methods and sampling time showed that results of RT-PCR for the OS group and RT-PCR for the DFS group suggest a prognostic significance of CTC detection (pooled HR [95% CI]: 1.45 [1.28–1.65], $I^2 = 38\%$, $p = 0.13$; 2.99 [2.01–4.45], $I^2 = 0\%$, $p = 0.32$). In addition, results of the baseline CTC detection group also indicated a significant prognostic value to predict OS and DFS (pooled HR [95% CI]: 1.47 [1.19–1.82], $I^2 = 38\%$, $p = 0.14$; 2.99 [2.01–4.45], $I^2 = 0\%$, $p = 0.32$). We simultaneously found that the detection of CTCs correlated with pathological stage (pooled OR [95% CI]: 2.95 [1.65–5.28], $I^2 = 56\%$, $p = 0.03$), lymph node status (pooled OR [95% CI]: 2.26 [1.50–3.41], $I^2 = 37\%$, $p = 0.09$), the depth of invasion (pooled OR [95% CI]: 3.21 [1.38–7.43], $I^2 = 72\%$, $p = 0.002$), and distant metastasis (pooled OR [95% CI]: 2.68 [1.25–5.73], $I^2 = 43\%$, $p = 0.15$).

Conclusions: Detection of CTCs is associated with poorer prognosis in gastric cancer patients.

Key words: circulating tumour cells, gastric cancer, prognosis, meta-analysis.

Introduction

Gastric cancer (GC) is the fourth most common cancer and the second most common cause of cancer death globally [1]. To date, pathological stage, Lauren's histological type, invasion in lymphatic and vascular system, and residual tumour presence are widely used factors to predict survival outcomes of GC patients [2–6]. However, prediction of the aforementioned factors is clinically insufficient. Though increasing prognostic markers are being discovered, more powerful factors are still needed [7, 8].

The presence of tumour cells in the blood stream was first reported by Ashworth [9] in 1869. The low concentration in peripheral blood makes it difficult to detect circulating tumour cells (CTCs). The CELLSEARCH system, Immunocytochemistry (ICC), and reverse transcriptase polymerase chain reaction (RT-PCR) are widely used methods to detect CTCs currently, and the CELLSEARCH system has been ratified by the FDA (Food and Drug Administration) for the application of prognosis prediction in breast cancer patients. Recently, meta-analyses pooling studies using the above-mentioned approaches have documented the prognostic value of CTC detection in patients with lung cancer [10], breast cancer [11], and colorectal cancer [12]. Pooled HRs of these studies show that the presence of CTCs indicate a poorer prognostic outcome. However, the prognostic relevance of CTC detection in gastric cancer patients remains controversial. Varied CTC detection methods and contrasting survival outcomes can be found in studies focusing on the prognostic value of CTCs [13–24] in gastric cancer.

Here, we conducted the first comprehensive meta-analysis of published literature on this topic to summarise the evidence of the prognostic value of CTC detection in gastric cancer patients.

Material and methods

Search strategy

Medline and the ISI Web of Knowledge database were searched in March 2013. The following keywords were variably combined: "circulating tumor cells", "CTCs" and "gastric cancer". No language or time restrictions were made.

Data extraction

Three reviewers (HY Wang, J Wei, and ZY Zou) independently extracted the primary data and baseline characteristics of the included studies. The primary data were hazard ratio (HR) and its 95% confidence interval (CI) of OS, PFS, and DFS. In nine included articles, only the *p*-value and/or the Kaplan-Meier survival curves, but not HR and its 95% CI, were given. As for these articles [14–22], methods according to the work of Parmar, William-

son, and Tierney were used to calculate the HR [25–27]. The baseline characteristics included first author, publication year, study size, patients' age, pathological stage, sampling time, methods of detection, CTCs markers and positive definition, detection rate, observed survival outcomes, and HR estimation methods. No included studies reported histological subtype data. Therefore, this characteristic is lacking in our analysis. All disagreements were resolved by discussion.

Inclusion criteria

In order to be eligible, studies had to: (i) discuss the relevance of CTC detection in peripheral blood and survival outcomes such as OS, PFS, and DFS; and (ii) provide sufficient data for extracting or estimating HR and its 95% CI. If more than one marker was used in a certain study, the results of each marker were recorded as an independent set.

Exclusion criteria

Studies were excluded from the analysis if: (i) the articles were not written in English, (ii) the articles were reviews or letters, (iii) studies had a sample size < 20 patients, or (iv) studies lacked requisite information to extract or calculate primary data for meta-analysis.

Statistical methods

We calculated the logHR and standard error (SE) by using software designed by Matthew Sydes and Jayne Tierney (Medical Research Council Clinical Trials Unit, London, UK) [27]. The pooled HR was gained using fixed or random-effects models according to the heterogeneity between studies. Heterogeneity was evaluated with the Cochran's Q test as well as the I^2 index and was defined as $p < 0.10$ or $I^2 > 50\%$ [28]. Subgroup research for detection

methods and sampling time were performed if the included studies were ≥ 2. The association of detection of CTCs with clinical variables (sexuality, pathological stage, lymph node metastasis, depth of invasion, and distant metastasis) were also investigated. Four additional articles [29–32] were included in this section because clinical characteristics and CTC conditions were provided. The odds ratio was used as a measure index of the correlation.

We used Forrest plots to show the pooled HR, and HR > 1 indicated worse survival outcome. All calculations were conducted by using Review Manager Version 5.2 (The Cochrane Collaboration, Software Update, Oxford, UK).

Results

Characteristics of eligible studies

The literature search yielded 725 articles. After title reading, abstract reading, and full-text reviewing, a total of 12 articles were included (Fig. 1). Eligible studies encompassed 772 gastric cancer patients and the sample size ranged from 26 to 123 patients. The included studies were conducted between 2005 and 2011. The main features of these studies are listed in Table 1. Uen's study [18] and Koga's study [19] used different markers to detect CTCs, and Matsusaka's study [15] recorded both PFS and OS. Therefore, each result of these studies was analysed independently. Furthermore, four of excluded studies containing investigable data were additionally used in the investigation of the correlation between detection of CTCs and clinical characteristics.

Overall analyses of circulating tumour cells and survival

Twelve HRs for OS extracted from 9 studies accounting for 527 patients were pooled [14–19, 21–23]. The pooled HR was 1.65 (95% CI: 1.32–2.06) ($I^2 = 43\%$, $p = 0.06$). The result showed an increased mortality in patients with positive CTCs (Fig. 2, Table 2).

Two HRs for PFS and two HRs for DFS were extracted from 4 studies accounting for 299 patients [13, 15, 20, 24]. The pooled HRs for PFS and DFS were 1.64 (95% CI: 1.02–2.62) ($I^2 = 29\%$, $p = 0.24$) and 2.99 (95% CI: 2.01–4.45) ($I^2 = 0\%$, $p = 0.32$), respectively. It revealed that patients with CTCs detected had an increased risk of disease progression or recurrence (Fig. 2, Table 2).

Subgroup analyses of detection methods and sampling times

Subgroups were stratified by different detection methods and sampling times (Table 2). Meta-analysis was conducted if the subgroup encompassed more than one study.

We implemented meta-analysis in the subgroups that had sufficient studies. Among these subgroups, the method-stratified ones included RT-PCR for the OS group [14, 18, 19, 22], RT-PCR for the DFS group [13, 24], CELLSEARCH for the OS group [15, 17], and other methods for the OS group [16, 21]. Sampling-time stratified ones included baseline for the OS group [14–17, 19, 22] and during surgery for the OS group [18, 21]. Two subgroups (RT-PCR for the OS group
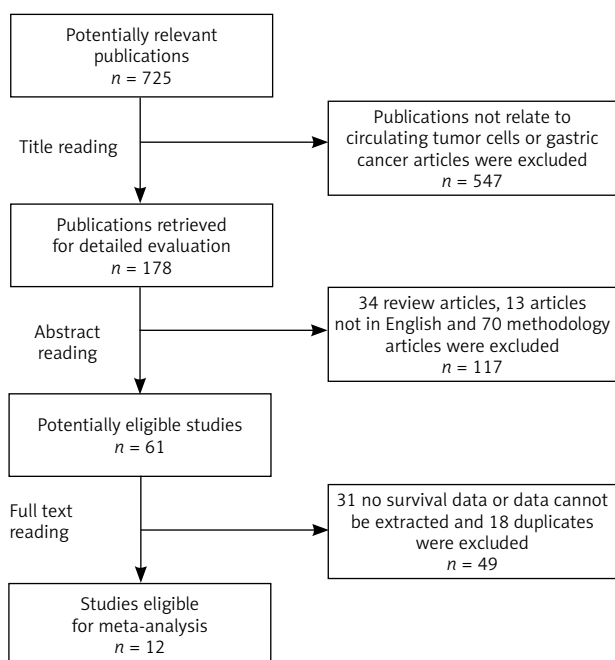

Fig. 1. Flowchart of the selection

Table 1. Baseline characteristics of included studies

Author [ref.], year	No. of patients	Age	Stage	Sampling time	Methods	Markers and positive definition	Detection rate	Outcome	HR estimation
Qiu [13], 2010	123	median, 59	M0, M1	prior to surgery	RT-PCR	CEA mRNA (+)	36.6	DFS	reported in text
Arigami [14], 2011	95	average, 68	M0, M1	prior to surgery	RT-PCR	B7-H3 mRNA (+)	50.5	OS	data extrapolated
Matsusaka [15], 2010	52	median, 62	–	before treatment	CELLSEARCH	≥ 4CTCs/7.5 ml blood	32.7	OS, PFS	data extrapolated
Pituch-Noworolska [16], 2007	57	mean, 57.0	M0, M1	prior to surgery	flow cytometry	≥ 3 cells CK+ per slide	54.4	OS	data extrapolated
Hiraiwa [17], 2008	27	mean, 68.9	M1	before treatment	CELLSEARCH	≥ 2 CTCs/7.5 ml blood	55.6	OS	data extrapolated
Uen [18], 2006	52	mean, 60.0	M0, M1	during surgery	RT-PCR	C-MET, MUC-1 mRNA (+)	61.5 (C-MET) 71.2 (MUC-1) 74.3 (both)	OS	data extrapolated
Koga [19], 2008	69	mean, 65.9	M0, M1	prior to surgery	RT-PCR	CK19, CK20 mRNA (+)	11.6 (CK19+) 15.5 (CK20 +)	OS	data extrapolated
Yie [20], 2008	26	median, 58	M0, M1	–	RT-PCR	survivin mRNA (+)	45.4	PFS	data extrapolated
Wu [21], 2006	64	mean, 60.5	M0, M1	during surgery	high-throughput colorimetric membrane-array	hTERT, CK-19, CEA, MUC1 mRNA (all +)	60.9	OS	data extrapolated
Illert [22], 2005	41	median, 69	M0, M1	prior to surgery	RT-PCR	CK20 mRNA (+)	36.6	OS	data extrapolated
Bertazza [23], 2009	70	median, 68	M0, M1	after surgery	RT-PCR	survivin mRNA (+)	98.6	OS	reported in text
Cao [24], 2011	98	–	M0, M1	prior to surgery	RT-PCR	survivin mRNA (+)	45.9	DFS	reported in text

OS – overall survival; PFS – progression-free survival; DFS – disease-free survival; RT-PCR – reverse transcriptase polymerase chain reaction

and RT-PCR for the DFS group) suggested the prognostic significance of CTC detection (pooled HR [95% CI]: 1.45 [1.28–1.65], I^2 = 38%, p = 0.13; 2.99 [2.01–4.45], I^2 = 0%, p = 0.32). The baseline CTC group also indicated a significant prognostic value to predict OS and DFS (pooled HR [95% CI]: 1.47 [1.19–1.82], I^2 = 38%, p = 0.14; 2.99 [2.01–4.45], I^2 = 0%, p = 0.32). However, the results of the CELLSEARCH group and the other-methods group were not significant (pooled HR [95% CI]: 1.67 [0.57–4.92], I^2 = 67%, p = 0.08; 1.53 [0.40–5.85], I^2 = 79%, p = 0.03).

## Correlation between detection of circulating tumour cells and clinical characteristics

We extracted clinical characteristics from the included studies and four additional articles [29–32]. The potential correlation between detection of CTCs and clinical variables was investigated. The pooled odds ratio demonstrated that CTCs were more likely to be detected in patients with stage III/IV [13, 14, 16, 18, 21, 24], pT3/T4 [13, 14, 16, 18, 21, 24], positive lymph node metastasis [13, 14, 16, 18–20, 24, 29, 30, 32], or positive distant metastasis [16, 21, 24, 29] (Table 3, Fig. 3). Significant correlation was not observed when we stratified the studies by sexuality [13, 14, 16, 18–

20, 24, 30–32]. The odds ratio was 1.02 (95% CI: 0.75–1.37) (I^2 = 0%, p = 0.46).

Discussion

Recently, a series of meta-analysis articles documented that CTC detection had prognostic value in patients with colorectal cancer [12], lung cancer [10], breast cancer [33, 34], melanoma [35], and prostate cancer [36]. It was the first time that a meta-analysis to confirm the prognostic value of CTCs in gastric cancer patients had been conducted. The pooled HRs for OS, PFS, and DFS were all above 1 and no overlap with 1 was observed. Our results indicated that the appearance of CTCs in peripheral blood betokened a poorer survival outcome.

Subgroup analyses were carried out according to different detection methods and sampling times. The CELLSEARCH system, RT-PCR, and immunocytochemistry were the most commonly used methods to detect CTCs. Among them, the CELLSEARCH system is the only one that is certificated by the FDA. The studies were divided into a RT-PCR group, a CELLSEARCH system group, and an other-methods group. The results of the RT-PCR group was in agreement with overall analyses, while those of the CELL-
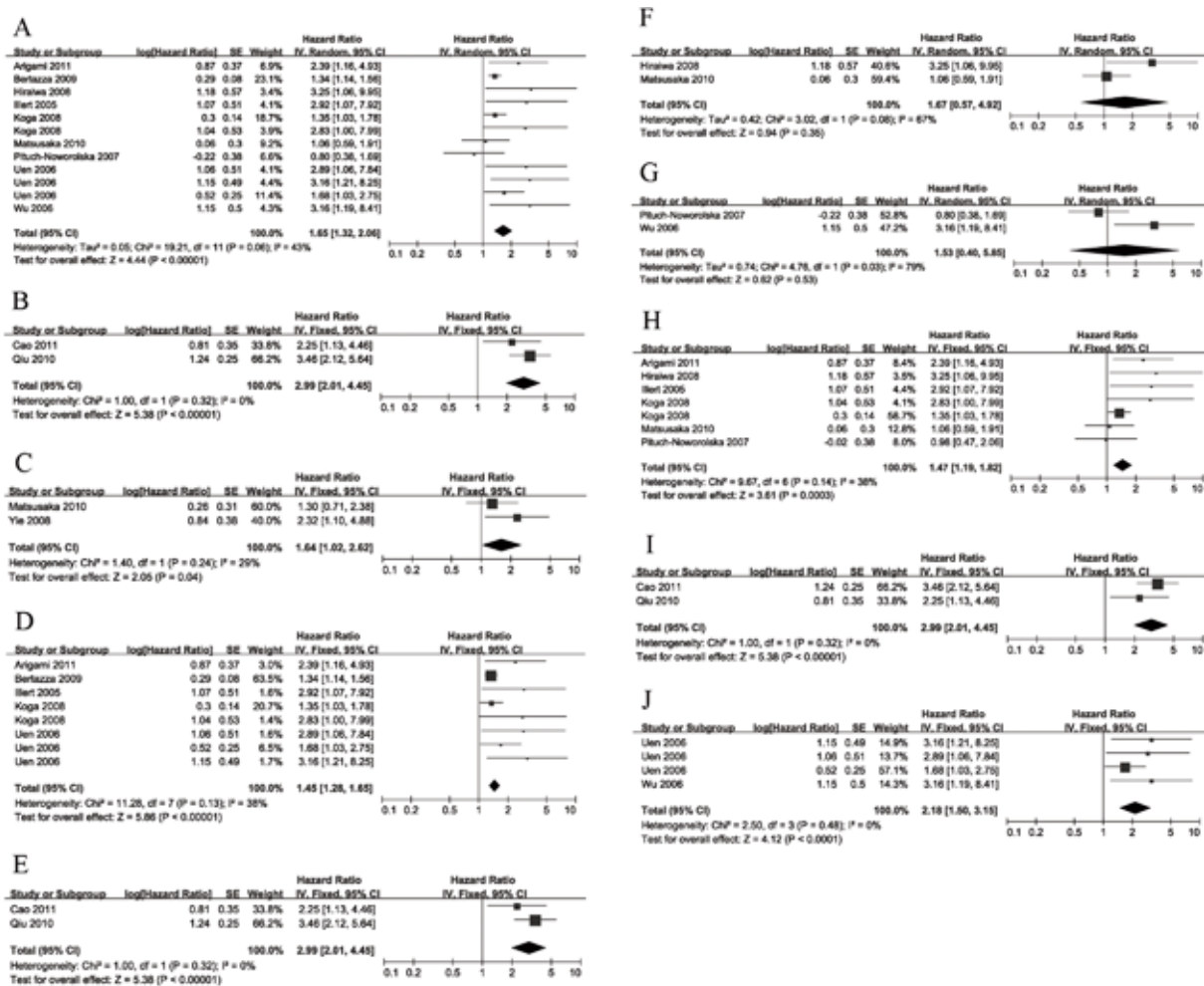
Fig. 2. Forrest plots of estimated hazard ratios (HRs) for (**A**) CTC detection and OS, **B**) CTC detection and PFS, **C**) CTC detection and DFS, **D**) CTC detection using RT-PCR and OS, **E**) CTC detection using RT-PCR and DFS, **F**) CTC detection using CELLSEARCH and OS, **G**) CTC detection using other methods and OS, **H**) baseline CTC detection and OS, **I**) baseline CTC detection and DFS, and (**J**) CTC detection during surgery and OS

CTCs – circulating tumour cells; OS – overall survival; PFS – progression-free survival; DFS – disease-free survival; RT-PCR – reverse transcriptase polymerase chain reaction

Table 2. Results of overall and subgroup meta-analyses

	n	OS			*n*	PFS			*n*	DFS		
		HR [95% CI]	I²	*p*		HR [95% CI]	I²	*p*		HR [95% CI]	I²	*p*
Total	12	1.65 [1.32–2.06]	43%	0.06	2	1.64 [1.02–2.62]	29%	0.24	2	2.99 [2.01–4.45]	0%	0.32
Method												
RT-PCR	8	1.45 [1.28–1.65]	38%	0.13	1	2.32 [1.10–4.88]	–		2	2.99 [2.01–4.45]	0%	0.32
CELLSEARCH	2	1.67 [0.57–4.92]	67%	0.08	1	1.30 [0.71–2.38]	–		0	–	–	
other methods	2	1.53 [0.40–5.85]	79%	0.03	0	–	–		0	–	–	
Sampling time												
baseline	7	1.47 [1.19–1.82]	38%	0.14	1	1.30 [0.71–2.38]	–		2	2.99 [2.01–4.45]	0%	0.32
during surgery	4	2.18 [1.50–3.15]	0%	0.48	0	–	–		0	–	–	
after treatment	1	1.34 [1.14–1.56]	–		0	–	–		0	–	–	

OS – overall survival; PFS – progression-free survival; DFS – disease-free survival; n – study numbers; p – p value; RT-PCR – reverse transcriptase polymerase chain reaction

SEARCH and the other-methods groups were not. Matsusaka *et al.* [15] and Hiraiwa *et al.* [17] used the CELLSEARCH system to detect CTCs. However, our analysis showed that the prognostic value was not significant (Table 2). We pooled the HRs by use of random effects model as I² = 67% and *p* = 0.08. Significant heterogeneity may be caused by different cutoff of CTC detection and relatively small sample size. Similarly, non-significant prognostic value and
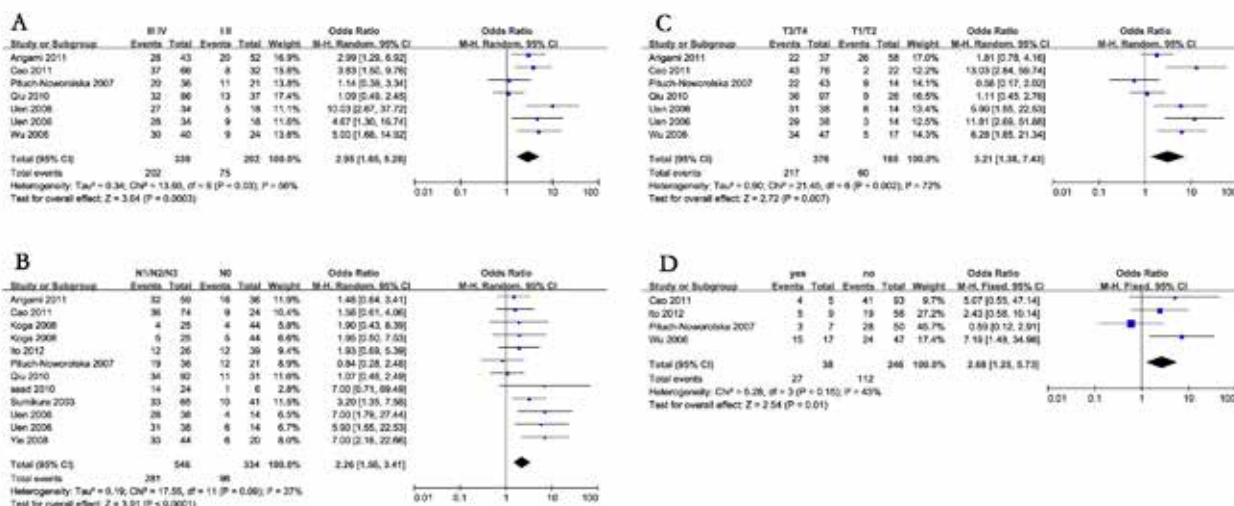
Fig. 3. Forrest plots of estimated odds ratios for correlation of circulating tumour cells appearance and (**A**) pathological stage; **B**) lymph node metastasis; **C**) depth of invasion; and (**D**) distant metastasis

Table 3. Detection of CTCs and clinical characteristics

Clinical characteristics	Study (*n*)	Patient (*n*)	OR [95% CI]	I^2	I value
Sexuality (male vs. female)	13	875	1.02 [0.75–1.37]	0%	0.46
pathological stage(III/IV vs. I/II)	7	541	2.95 [1.65–5.28]	56%	0.03
Lymph node (N1/N2/N3 vs. N0)	12	880	2.26 [1.50–3.41]	37%	0.09
The depth of invasion (pT3/pT4 vs. pT1/pT2)	7	541	3.21 [1.38–7.43]	72%	0.002
Distant metastasis (yes vs. no)	4	284	2.68 [1.25–5.73]	43%	0.15

high heterogeneity were observed in the subgroups of other methods. Ring's research indicated that RT-PCR was more sensitive than other CTC detection methods [37]. The most commonly applied method of included studies was RT-PCR. Hence, we believe that more studies using the CELLSEARCH system and other methods to evaluate the prognostic value of CTCs in gastric cancer patients could help to solve the puzzle. Besides, novel detection methods emerge continuously [38] and should be taken into consideration in future.

According to our results, detection of CTCs at baseline showed the ability to predict OS and DFS. Patients before any treatment with CTC presence in blood had shorter survival time and relapsed earlier if they underwent radical surgery. Interestingly, if CTCs were detected in a blood sample taken during surgery, it also indicated a poorer OS. This might be explained by Hou JM's point of view that CTCs have the ability to promote metastasis [39]. However, heterogeneity was the greatest problem in these subgroup analyses because the therapeutic regimens differed from each other. Therefore, more studies with sufficient key information like surgery type and chemotherapy regimens are needed to obtain further understanding of the CTC detection's prognostic value at different time points in gastric cancer patients.

We found that sexuality was not related to detection of CTCs in gastric cancer patients. Correlations were found

between detection of CTCs and clinical characteristics including pathological stage, lymph node metastasis, depth of invasion, and distant metastasis. To avoid the heterogeneity caused by variant pathological staging version, in the analysis focusing on pathological stage and the depth of invasion, we only enrolled studies adopting UICC version 5 or 6. According to the same reasoning, patients were divided into "III/IV vs. I/II" and "pT3/pT4 vs. pT1/pT2" groups to keep the analysis powerful. The pooled odds ratio were all above 2 and indicated a higher detection rate of CTCs in patients with advanced stage, deeper tumour invasion, and lymph node/distant metastasis. Therefore, researchers suggested that CTCs could provide useful information for tumour staging and even cancer diagnosis [40]. Paterlini-Brechot's article indicated that CTCs were tumour cells from local or metastasis niduses that invaded blood vessels and contaminated peripheral blood [41]. This point of view may explain the correlation of detection of CTCs and clinical characteristics.

The limitations of the present meta-analysis need to be discussed. Firstly, HRs and 95% CI of some included studies were extracted. Log(HR) and se(log(HR)) were then calculated by the software provided by Matthew Sydes and Jayne Tierney. Potential biases may relate to this process. Secondly, heterogeneity existed between studies because of diverse detection methods, different cut off of CTCs, etc. We tried to solve this problem by extracting more informa-

tion from the articles and performing subgroup analyses. However, significant heterogeneity still existed in some subgroups and a random-effects model was used for more conservative estimates. Hence, to validate the prognostic value of CTC detection, large multicentre prospective studies enrolling homogeneous populations are required in future. Thirdly, our meta-analysis only used published data. Updated individual patient data were not obtained. If those data were added to our analyses, the accuracy and determinacy could be better.

Our meta-analysis suggests that detection of CTCs in peripheral blood is a prognostic factor to predict survival outcomes, including OS, PFS, and DFS, in gastric cancer patients. We found that CTCs were inclined to be positive in patients with more advanced disease. This may explain why detection of CTCs is associated with poorer prognosis. To confirm this conclusion and discover further significance of CTCs, like guiding treatment, more multicentre, well-designed prospective studies are needed in future.

The authors declare no conflict of interest.

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