# Circulating Tumour Cells (CTCs) as Potential Biomarkers of Clinicopathological and Prognostic Significance in Gastric Cancer Patients: A Systematic Review and Meta-Analysis

**Yiding Li**
Fourth Military Medical University
**Guiling Wu**
Fourth Military Medical University
**Wanli Yang**
Fourth Military Medical University
**Xiaoqian Wang**
Fourth Military Medical University
**Lili Duan**
Fourth Military Medical University
**Liaoran Niu**
Fourth Military Medical University
**Yujie Zhang**
Fourth Military Medical University
**Wei Zhou**
Fourth Military Medical University
**Liu Hong** ( hongliu1@fmmu.edu.com )
Fourth Military Medical University https://orcid.org/0000-0002-8276-2345
**Daiming Fan**
Fourth Military Medical University

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# Abstract

Background: Gastric cancer (GC) is a common highly recurrent malignant tumor that is associated with poor prognosis. Circulating tumor cells (CTCs) have drawn much attention because of their diagnostic value in diverse cancers, including GC. This study aimed to assess the relevance of CTCs in predicting the clinicopathological parameters and prognostic significance of GC.

Methods: We systematically searched PubMed, Medline and Web of Science for relevant studies. Each database was searched from its date of inception through January 22, 2020. The odds ratios (ORs), hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated as effect values using the random-effects model.

Results: In total, 52 articles that reported 68 studies comprising 4158 GC patients were included. The pooled results on TNM stage indicated that the III/IV group had a notably higher CTCs positivity rate than the I/II group (OR=2.73, 95% CI (1.95,3.82), $I^2$=65%). The poorly differentiated group had a significantly higher CTCs positivity rate than the well/moderately differentiated group (overall: OR=1.91, 95% CI (0.77,4.71)), as well as the Lauren classification diffuse/hybrid type group and the intestinal group (overall: OR=1.77, 95% CI (0.70,4.44)). The bulk of analysis revealed that CTC positivity detected in GC patients was correlated with worse overall survival (OS) (HR =1.94, 95% CI (1.64,2.30), P≤0.001), progression-free survival (PFS) (HR =2.45, 95% CI (1.65,3.64), P≤0.001), and disease-free survival (DFS) (HR =2.78, 95% CI (1.89,4.10), P≤0.001). Then, we extracted data and analyzed the DCR of chemotherapy in patients with GC, and the pooled analysis demonstrated that the DCR of the CTC positivity was lower than that of the CTC negativity (RR =0.63, 95% CI (0.44,0.91))

Conclusions: In conclusion, our study demonstrated that the CTCs positivity was correlated with the poor OS, PFS and DFS in GC patients and provided a scientific foundation for gastric cancer staging.


# Background

Gastric cancer (GC) is a common highly recurrent malignant tumor that is the fifth-most prevalent tumor, which was the third-most frequent primary cause of tumor-related death worldwide. In 2018, GC was responsible for over 1,000,000 new cases and 783,000 deaths attracting much attention as an important cancer worldwide [1]. Indeed, men are twice as likely to develop GC than women. The incidence rates of GC have been increasing each year in Asian countries, such as China, Japan and Korea [2, 3]. Due to the initial diagnostic difficulty in GC, a large number of patients were usually diagnosed at advanced or metastatic stages, leading to a low average survival time (the 5-year survival probability was still lower than 30%) [4]. Thus, early diagnostic markers for GC are urgently needed.

An important step in tumor metastasis is when tumor cells enter the blood circulation; thus, for a deeper understanding of tumor metastasis and for the earlier detection of tumors, circulating tumor cells (CTCs) have become an attractive topic in the past decade. The research by Prof. Ashworth in 1869 represents the first description of CTCs [5], which, as cancer cells, circulate in the blood after naturally falling off

from the primary or metastatic tumor [6]. There is ample evidence indicating that CTCs circulate in the blood and flow throughout the body, which is the leading cause of significant metastasis and recurrence [7].

Many studies have shown that CTC positivity may be a poor prognostic marker in breast and colorectal cancer patients [8, 9]. However, due to different study designs and statistical methods, the value of CTC positivity in GC patients remains unclear. Thus, based on the previous studies, we undertook the present meta-analysis mainly to systematically summarize the evidence that comes from CTCs positivity rate regarding the clinical diagnostic and prognostic significance of CTCs detected in GC patients.

# Methods

Search Strategy

Studies from PubMed, Medline and Web of Science were systematically identified using keywords (“Circulating tumor cells”, “CTCs”, and “gastric cancer”). Each database was searched from its date of inception through January 22, 2020. The following key words were used: “Circulating tumor cells”, “CTCs”, and “gastric cancer”. We use the following strategy: ((((((((((Stomach Neoplasms) OR stomach carcinoma) OR gastric tumor) OR gastric carcinoma) OR stomach cancer) OR "Stomach Neoplasms" [Mesh]) OR stomach tumor) OR gastric Neoplasms) OR GC) AND (((((circulating tumor cells detection) OR CTC) OR circulating tumor cells) OR circulating neoplastic cells) OR "Neoplastic Cells, Circulating" [Mesh])).

Eligibility Criteria and Quality Assessment

To be included in the meta-analysis, articles were selected based on the following criteria: (i) the article reported at least one noteworthy outcome indicator of CTCs, or the outcome can be calculated from the published data; (ii) all GC patients were diagnosed with the gold standard test; and (iii) the samples were collected from peripheral blood. Articles were excluded based on the following criteria: (i) the article was published in languages other than English; (ii) the number of GC patients and samples was less than 20; (iii) samples were collected from lymph nodes, bone marrow, or the abdominal cavity; (iv) non-human experiments; (v) reviews, case reports, comments, letters, and meeting records; (vi) GC and CTCs were not studied; and (vii) unable to obtain enough data through article reports and data calculations.

We evaluated the quality of the included literature through the Newcastle-Ottawa Scale(NOS) recommended by the Cochrane Library [10], according to eight items belonging to three categories: (i) study group selection; (ii) comparability of groups; and (iii) outcome of interest. The full score was 9, 1–4 points indicated low quality, while 5–9 points were considered high quality.

Data Extraction

Two reviewers, using a standardized form, independently extracted the data from the included studies, and any disputes or differences were settled by a third independent investigator. The following data were

extracted from each study according to the selection criteria: first author's name, publication year, country of patients, characteristics of patients (number, sex, age), sampling time, detection methods, detection markers, detection rate, Lauren classification, prognostic value, and hazard ratio (HR). The tumors DCR were evaluated in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) guideline. The DCR is calculated as (complete response [CR]+ partial response [PR]+ stable disease [SD])/ (complete response [CR]+ partial response [PR]+ stable disease [SD]+ progressive disease [PD]).

Statistical Analysis

Review Manager software (RevMan, version 5.3, The Nordic Cochrane Centre, The Cochrane Collaboration, London, UK) was used for all analyses in our meta-analysis. We extracted the HR and 95% confidence interval (CI) of overall survival (OS), progression-free survival (PFS), and disease-free survival (DFS) from the included studies to statistically assess the prognostic effects of CTCs. If there were not directly provided the HRs, 95% CIs, or P-values in the original literature, the estimated HR was used to assess prognostic effects based on the method described by Tierney et al[11], and HR>1 reflects more disease progression or deaths in the patients with CTC positivity. We pooled the extracted HRs together in Review Manager. To statistically assess the correlation between clinicopathological characteristics and CTC detection in GC patients, we estimated the odds ratio (OR) from the included studies, and OR>1 implied that the CTC-positive group was associated with a parameter. Furthermore, the estimated risk ratio (RR) was calculated to assess the DCR. All statistical values were combined with 95% CIs and two-sided P-values, the threshold of which was set to 0.05. To obtain more conservative results, the random-effects model was used for analysis, which is more consistent with multicenter clinical research. Heterogeneity between studies was calculated using the Q test and $I^2$ statistic, and $P \leq 0.1$ or $I^2 \geq 50\%$ were considered to indicate significant heterogeneity. Simultaneously, based on the difference in the data retrieved, subgroup analyses were performed, such as for the age of patients, sex of patients, Lauren classification, differentiation of cancer, and detection methods. Publication bias was detected using a funnel plot.

# Results

Study Characteristics

The original search yielded 2566 records in PubMed, Medline and Web of Science. Of these, 1087 duplicate studies were excluded. We excluded 1395 records after reading the titles and abstracts. After reviewing the full texts, 52 articles were finally included in this study [12-63]. The selection flowchart of this study is shown in Figure 1.

In total, 52 articles (ranging from 1997 to 2019) from Asia, Europe and South America (China, Japan, Korea, Taiwan, Iran, Brazil, Korea, Germany, Italy, Turkey, Poland and France) that reported 68 arms comprising 4158 GC patients were included (sample size median: 59(22–810), mean: 85) (Table 1). Of these 68 arms, 40 arms used the RT-PCR method and 8 arms used the CellSearch System. The sampling

time was classified as the preoperative in 45 arms, the intraoperative in 10 arms as well as the postoperative in 9 arms. Meanwhile, four studies did not report the sampling time. Twenty-one articles[12, 16, 20, 21, 23, 24, 26, 29, 30, 32-34, 37-40, 42, 48, 50-52] with 24 arms provided HRs or HRs could be obtained by calculation for OS, PFS or DFS to perform the meta-analysis. Eighteen of the remaining 21 articles [16, 20, 21, 23, 24, 26, 29, 30, 32-34, 38, 40, 42, 48, 50-52] with 21 arms provided HRs for OS. Nine articles [12, 16, 20, 21, 23, 24, 29, 30, 40] with 11 arms provided HRs for PFS, and 3 articles [37, 39, 52] provided HRs for DFS.

Quality Assessment

The quality of the 52 included studies (13 studies in the low-quality categories and the other 39 studies in the high-quality categories) was evaluated on the basis of the NOS (Table 2).

Diagnosis

Diagnosis of CTCs with the parameters of patients

We analyzed the basic parameters of patients, TNM staging, histologic type and invasion in our meta-analysis. The results of the pooled analysis on the parameters of GC patients are summarized in Table 3.

*The CTCs positivity rate according to the basic parameters of patients.* The meta-analysis of all relevant researches on age indicated that the >60 age group had a remarkably higher CTCs positivity rate than the ≤60 age group (OR=1.48, 95% CI (1.13,1.92), $I^2$=0%). Then, we performed subgroup analysis on sample time (preoperative: OR=1.45, 95% CI (1.00,2.11), $I^2$=16%; intra/postoperative: OR=1.45, 95% CI (0.94,2.25), $I^2$=0%). A similar trend was also discovered in the subgroup analysis of sex. The meta-analysis of relevant studies on sex indicated that the male group had a higher CTCs positivity rate than the female group without significant difference (OR=1.07, 95% CI (0.90,1.27), $I^2$=0%). Then, we performed subgroup analysis on sample time (preoperative: OR=1.15, 95% CI (0.94,1.40), $I^2$=0%; intra/postoperative: OR=0.87, 95% CI (0.62,1.22), $I^2$=2%).

*The CTCs positivity rate according to the TNM stage.* To further analyze the correlation between the CTCs positivity rate and TNM stage of GC patients, we extracted the TNM stage data from the included studies. The pooled results on TNM stage indicated that the III/IV group had a notably higher CTCs positivity rate than the I/II group (OR=2.73, 95% CI (1.95,3.82), $I^2$=65%). Then, subgroup analysis was performed on sample time (preoperative: OR=2.41, 95% CI (1.56,3.73), $I^2$=71%; intra/postoperative: OR=3.36, 95% CI (2.34,4.82), $I^2$=0%). In addition, the pT category, $LN^3$ (lymph node metastasis), and pM (distant metastasis) of all studies were also separately analyzed. The pT3/T4 group had a notably higher CTCs positivity rate than the pT1/T2 group (overall: OR=2.20, 95% CI (1.64,2.95), $I^2$=54%; preoperative: OR=1.39, 95% CI (1.05,1.83), $I^2$=22%; intra/postoperative: OR=3.93, 95% CI (2.69,5.75), $I^2$=17%). Simultaneously, there was a significant difference between the lymph node metastasis positive and negative groups (overall: OR=2.45, 95% CI (1.77,3.40), $I^2$=55%; preoperative: OR=1.96, 95% CI (1.34,2.85),

$I^2$=60%; intra/postoperative: OR=3.42, 95%CI (2.08,5.60), $I^2$=53%), as well as the distant metastasis positive and negative groups (overall: OR=1.97, 95% CI (1.43,2.72), $I^2$=2%; preoperative: OR=1.85, 95% CI (1.31,2.61), $I^2$=0%; intra/postoperative: OR=2.77, 95% CI (1.18,6.47), $I^2$=16%).

*TheCTCs positivity rate according to the histologic type.* The degree of tumor differentiation and Lauren classification in GC can be indicated by the detection of CTCs. The degree of tumor differentiation was divided into three degrees: well, moderately, and poorly differentiated. And, Lauren classification was divided into three types: intestinal, diffuse, and hybrid type. The poorly differentiated group had a higher CTCs positivity rate than the well/moderately differentiated group (overall: OR=1.91, 95% CI (0.77,4.71), $I^2$=85%; preoperative: OR=1.00, 95% CI (0.30,3.30), $I^2$=86%; intra/postoperative: OR=5.88, 95% CI (2.25,15.35), $I^2$=65%), as well as the Lauren classification diffuse/hybrid type group and intestinal group (overall: OR=1.77, 95% CI (0.70,4.44), $I^2$=79%; preoperative: OR=0.97, 95% CI (0.56,1.70), $I^2$=34%; intra/postoperative: OR=14.52, 95% CI (0.65,323.45), $I^2$=87%), suggesting an higher CTCs positivity rate in the poorly differentiated group and Lauren classification diffuse/hybrid type group, but had no statistically significant.

*TheCTCs positivity rate according to the invasion.* The lymphatic invasion-positive group had a higher CTCs positivity rate than the lymphatic invasion-negative group (overall: OR=1.80, 95% CI (1.26,2.57), $I^2$=38%; preoperative: OR=1.27, 95% CI (0.99,1.63), $I^2$=0%; intra/postoperative: OR=3.29, 95% CI (1.97,5.47), $I^2$=0%), vessel invasion-positive group (overall: OR=2.29, 95% CI (1.54,3.40), $I^2$=61%; preoperative: OR=1.35, 95% CI (0.88,2.07), $I^2$=37%; intra/postoperative: OR=3.41, 95% CI (2.20,5.29), $I^2$=30%) and perineural invasion-positive group (overall: OR=3.32, 95%CI (1.86,5.93), $I^2$=0%; preoperative: OR=2.45, 95%CI (1.16,5.19), $I^2$=0%; intra/postoperative: OR=5.21, 95%CI (2.09,12.98), $I^2$=30%) than in the negative group, indicating that tumors in GC patients who are CTC positive may be more likely to spread.

The survival of GC patients

To analyze the survival of GC patients, we extracted 21 studies that provided data for OS with 1,673 GC patients, 11 studies for PFS with 811 GC patients, and 3 studies for DFS with 280 GC patients. The pooled results on prognostic effects of CTCs detected in GC patients indicated that CTC-positive status detected in GC patients was associated with poor OS (HR =1.94, 95% CI (1.64,2.30), P≤0.001; $I^2$=33%, Figure 2a), PFS (HR =2.45, 95% CI (1.65,3.64), P≤0.001; $I^2$=71%, Figure 2b), and DFS (HR =2.78, 95% CI (1.89,4.10), P≤0.001; $I^2$=0%, Figure 2c).

According to the difference in the variables (publication year, country, patients' number, sampling time, detection methods, CTC positive rate, quality of the article), we performed subgroup analyses and the results is demonstrated in Table 4. In the included studies for OS, the median publication year was 2013, the median number of patients was 65 and the median positive rate of the patients was 46.2%, respectively. In the included studies for PFS, the median publication year was 2016, the median number of patients was 62 and the median positive rate of the patients was 42.9%, respectively. The summary

analysis results demonstrated that CTC positivity had a significant impact on the prognosis of OS and PFS in most subgroups.

CTC detection and DCR

6 studies assessed the association between CTCs positivity and DCR in patients receiving chemotherapy/chemoradiotherapy. When pooling the estimated RR, CTC-positive patients had a poor response to chemoradiotherapy compared with CTC-negative patients (RR =0.63, 95% CI (0.44,0.91)), as shown in Figure 3.

Subgroup analysis and publication bias

Heterogeneity analysis showed that PFS in the pooled survival effects has significant heterogeneity ($I^2$= 71%, P≤0.001). To explore potential sources of heterogeneity, we used subgroup analysis according to the difference in the variables including the publication year, country, patients' number, sampling time, detection methods, positive rate, and quality of the article. Eventually, we concluded that the main source of the heterogeneity in PFS came from the intra/postoperative set of Matsusaka et al [40] and Pernot et al [20]. When we divided detection methods into PCR, cytological methods, and detected methods (eg. in situ hybridization assay, morphology-based enrichment coupled with RT-PCR, colorimetric membrane-array) subgroups, we found the interesting results. In our Meta-analysis, Compared with the PCR method (OR=1.51, 95% CI (1.37, 1.67)) (Figure 4), the CellSearch™ System (OR=1.38, 95% CI (1.29, 1.48)) had a lower CTCs positivity rate, but it's ability to count CTCs number and correlated with the increased risk of death(OS: (RT-PCR: HR=1.57, 95%CI (1.28,1.92)), (CellSearch™ System: HR=2.02, 95%CI (1.63, 2.50))). It confirms that the CellSearch™ System is a detected method of high reproducibility, accuracy, linearity, and reliability. Non-CellSearch™ System cytological methods (OR=1.72, 95% CI (1.52, 1.96)) had a higher CTCs positivity rate than PR-PCR and CellSearch™ System. The other methods (OR=1.74, 95% CI (1.42, 2.14)) had a higher CTCs positivity rate than RT-PCR(OR=1.51, 95% CI (1.37, 1.67)), as well as its ability to count CTCs number and their correlation with the increased risk of death (OS: (RT-PCR: HR=1.57, 95%CI (1.28,1.92)), (Others: HR= 2.22, 95%CI (1.44, 3.41))). We found that the increased risk of death (OS) for CTC-positive patients in cytological methods and other methods subgroup was more conspicuous compared with PCR subgroup.

We used funnel plots to detect publication bias, as shown in Figure 5. All of the funnel plots of included studies showed the symmetrical distribution. Thus, no significant publication bias was found in the meta-analyses of OS, PFS, and DFS.

# Discussion

GC, an epithelial malignant tumor, is an important cause of tumor death worldwide. Although the treatment of gastric cancer has been developed rapidly in recent years, the 5-year survival rate is still low, estimated at 10% in patients with advanced GC [4]. Because most patients with gastric cancer are

detected at advanced stages and the tumors are highly metastatic, prognosis is not positive and treatment options are limited, contributing to high mortality. In recent years, CTCs have been increasingly investigated and are of great significance in the diagnosis, treatment and prognosis of malignant tumors. The "seed and soil" theory can reasonably explain the metastasis and recurrence of CTCs in peripheral blood [64]. Many studies have aimed to explore the predictive and prognostic significance of CTCs in different tumors, and a majority of CTC-positive patients with solid tumors have been indicated to have a poor prognosis [8, 9, 65]. In addition, due to its advantages of time-saving, money-saving, comfort and comparable repeatability, CTCs, as an effective monitor, can be used to figure out whether the treatment is useful.

For GC, although the prognostic value of CTCs was reported in several previous meta-analyses, most of them only focused on a single detection method or on the clinicopathological significance of CTC detection [66, 67]. Although the selected studies in our meta-analysis matched part of Huang's [68] included studies which explored the clinicopathological significance of CTC detection in GC patients, there are some important distinctions between our meta-analysis and theirs. Compared with the previously published mate-analyses, our meta-analysis still has some innovation. Briefly, we made a more comprehensive and new meta-analysis, including 52 articles (ranging from 1997 to 2019) from Asia, Europe and South America (China, Japan, Korea, Taiwan, Iran, Brazil, Korea, Germany, Italy, Turkey, Poland and France) that reported 68 arms comprising 4158 GC patients. Moreover, to explore potential sources of heterogeneity, we used subgroup analysis according to the difference in the variables including the publication year, country, patients' number, sampling time, detection methods, positive rate, and quality of the article. We concluded that the main source of the heterogeneity in PFS came from the intra/postoperative set of Matsusaka et al and Pernot et al. In fact, multiple articles [20, 35, 40, 41, 44, 49, 50, 53] include multiple independent sets of data that must be included in the meta-analysis, which has not been organized by Huang et al. influencing the precision of their results. Finally, our meta-analysis provided new insights into the advantages and disadvantages of CTCs detected with different methods in GC patients. Importantly, we also assessed the association between CTCs positivity and DCR in patients receiving chemotherapy/chemoradiotherapy, which is rarely reported in previously published mate-analyses. These all indicate that CTCs can be used as a clinical marker for GC and as a guide for chemotherapy.

As a result, our results are more informative than those of previous studies. Our meta-analysis of 52 articles including 4158 GC patients suggested that the CTCs-positivity have potential to stage GC, histological type, and invasion. CTCs can also be used as a risk factor for recurrence/metastasis during the follow-up of GC. The meta-analysis of DFS and OS further supports this conclusion. Patients with CTC positivity have a worse prognosis than those with CTC negativity. For OS, CTC-positive patients had a higher mortality risk than in CTC-negative patients (HR =1.94, 95% CI (1.64,2.30)). For PFS, CTC-positive patients had a higher risk of tumor development than CTC-negative patients (HR =2.45, 95% CI (1.65,3.64)). In addition, CTC-positive patients had poor prognostic effect on DFS than negative patients (HR =2.78, 95% CI (1.89,4.10)). Then, we found that CTC-positive patients in the non-PCR (cytological

methods and other detected methods) group had a higher mortality risk that in the PCR group (HR=2.14 and 1.57).

CTC detection methods comprise two parts: enrichment approaches and detection approaches. Currently, RT-PCR, cytological methods (CellSearch™ System or non-CellSearch™ System), and other detected methods (eg. in situ hybridization assay, morphology-based enrichment (density-based cell separation and/or size based on cell separation) coupled with RT-PCR, colorimetric membrane-array) are the most widely used methods for detecting CTCs [69]. RT-PCR have been applied to detecting CTCs for almost 20 years. As we all know, due to the improper expression of target antigens/genes in normal cells, PCR has high sensitivity but low specificity. The breakthrough in detecting CTCs emerged to identify intact cells. And CellSearch system, as the current gold standard, is the most typical representative. The CellSearch system used for detection of CTCs has more advantages compared to PCR, including high reproducibility, accuracy, linearity, and reliability [70, 71]. However, the sensitivity of the CellSearch™ System is lower, due to less CTCs in blood and their short half-life. In our Meta-analysis, Compared with the PCR method (OR=1.51, 95% CI (1.37, 1.67)) (Figure 4), the CellSearch™ System (OR=1.38, 95% CI (1.29, 1.48)) had a lower CTCs positivity rate, but it's ability to count CTCs number and correlated with the increased risk of death(OS: (RT-PCR: HR=1.57, 95%CI (1.28,1.92)), (CellSearch™ System: HR=2.02, 95%CI (1.63, 2.50))). It confirms that the CellSearch™ System is a detected method of high reproducibility, accuracy, linearity, and reliability. Non-CellSearch™ System cytological methods (OR=1.72, 95% CI (1.52, 1.96)) had a higher CTCs positivity rate than PR-PCR and CellSearch™ System. The other methods (OR=1.74, 95% CI (1.42, 2.14)) had a higher CTCs positivity rate than RT-PCR(OR=1.51, 95% CI (1.37, 1.67)), as well as its ability to count CTCs number and their correlation with the increased risk of death (OS: (RT-PCR: HR=1.57, 95%CI (1.28,1.92)), (Others: HR= 2.22, 95%CI (1.44, 3.41))). We found that the increased risk of death (OS) for CTC-positive patients in cytological methods and other methods subgroup was more conspicuous compared with PCR subgroup (table 4).Interestingly, because a more accurate CTC detection method can identify more patients with poor prognosis, the positive subgroup had a greater risk of death and tumor progression than the negative subgroup. According to the subgroup analysis, we concluded that the main source of the heterogeneity in PFS came from the intra/postoperative set of Matsusaka et al [40] and Pernot et al [20]. All of the funnel plots of included studies showed the symmetrical distribution. Thus, no significant publication bias was found in the meta-analyses of OS, PFS, and DFS.

Several studies indicated that the dynamic monitoring of CTCs positivity might help to predict therapeutic efficacy in cancer [72-74]. Then, we extracted data and analyzed the DCR of chemotherapy in patients with GC, and the pooled analysis demonstrated that the DCR of the CTC positivity was lower than that of the CTC negativity (RR =0.63, 95% CI (0.44,0.91)). Because CTC may enter the peripheral blood from the primary tumor, it is more likely that the tumor will recur after treatment. Therefore, CTCs, as an effective monitor, can be used to figure out whether the treatment is useful.

Furthermore, it is worth noting that the positive rate of postoperative CTCs was higher than that of the preoperative sample. Due to epithelial to mesenchymal transition (EMT), CTCs, transferring from

epithelial state to mesenchymal state, receive more invasive and migratory properties. It is worth noting that CTC migrate towards distant organs or return to the primary tumor through blood vessels and lymph capillaries after EMT [75-79]. , which is called self-seeding. And the self-seeding CTCs is easy to infiltrate the primary tumor but not need notability adaptations. Thus, the primary tumor is enriched with more invasive and migratory self-seeding CTCs [80]. Due to operation, the self-seeding CTCs is released [63]. In addition, postoperative immunosuppression also prevents the cellular immune clearance of CTCs, leading to a higher positive rate of postoperative CTCs [81]. In the intra/postoperative sampling time, the status of CTCs is combined with the preoperative CTC status and the release of tumor cells during the operation [81], which indicates that similar status of intraoperative and postoperative CTCs. Published literatures have identified that intra/postoperative sampling time can reflect the most relevant CTC status [82]. Because, in the intra/postoperative sampling, the status of the CTCs is combined with the preoperative CTC status and the release of tumor cells during the operation [63]. Similarly, in our meta-analysis, from staging, histologic type, invasion and prognostic significance, we found that intra/postoperative sampling time has a higher CTCs positivity rate than preoperative sampling time, and intra/postoperative sampling time has a poorer OS than preoperative sampling time [81, 82]. In addition, postoperative immunosuppression also prevents the cellular immune clearance of CTCs [83], leading to a higher positive rate of postoperative CTCs. Therefore, the CTC status during surgery may provide more information according to the above results.

Several limitations in this meta-analysis should be emphasized. First, rather than primary data from individual patients, the meta-analysis used extracted data, and we could not correct all clinicopathological parameters to a consistent standard. Second, numerous biomarkers were utilized to identify CTCs and the cutoff values for CTC positivity in the studies were diverse, resulting in remarkable heterogeneity. Third, we limited our analysis to studies published in English without other languages such as Chinese, Japanese, and German, the choice of language may bring another bias. Therefore, although our summary analysis shows that CTC positivity is a poor prognostic indicator in patients with GC, our results should be interpreted cautiously, as more detailed and accurate data are needed for verification.

# Conclusions

In conclusion, our study demonstrated that the CTCs positivity was correlated with the poor OS, PFS and DFS in gastric cancer patients and provided a scientific foundation for gastric cancer staging. Additionally, subgroup analysis indicated that patients with CTC positivity have a worse prognosis than CTC-negative patients. Moreover, the intra/postoperative sampling time can reflect the most relevant CTC status. However, the results are certain impacted by the significant heterogeneity of the studies included, particularly due to various timing of sample collection and also the technique utilized for CTC detection. Therefore, the high-quality, well-designed, large-scale multicenter research is needed to verify our results and confirm the clinical value of CTCs in GC patients. Additionally, whether molecular characteristics of CTCs derived from primary gastric tumor and various organ metastases are different is remained to be elucidated. Identifying this information would be very useful for early detection of micro-metastasis and making rational treatment decisions in metastasis GC.

# Abbreviations

GC: Gastric cancer; CTCs: Circulating tumor cells; PB: Peripheral blood; ORs: Odds ratios; RRs: Risk ratios; HRs: Hazard ratios; Cis: confidence intervals; OS: Overall survival; RFS: Relapse-free survival; NOS: Newcastle-Ottawa Scale; DCR: Disease control rate; CR: Complete response; PR: Partial response; SD: Stable disease; PD: progressive disease; ORR: Overall response rate.

# Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LYD and WGL conceived and designed the study. WGL, YWL and WXQ searched literatures. LYD, DLL and NLR selected studies. WGL, ZYJ and ZW extracted data. LYD, WGL performed outcome analysis. LYD, WGL wrote the paper. WGL, YWL, WXQ, HL and FDM reviewed and edited the manuscript. All authors read and approved the manuscript.

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Authors' Information

Yiding Li: liyiding@fmmu.edu.cn, State key Laboratory of Cancer Biology and National Clinical Research Center for Digestive Diseases, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, China, 710032.

Guiling Wu: wguiling@fmmu.edu.cn, School of Aerospace Medicine, Fourth Military Medical University, Xi'an, China, 710032.

Wanli Yang: 1104387844@qq.com, State key Laboratory of Cancer Biology and National Clinical Research Center for Digestive Diseases, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, China, 710032.

Xiaoqian Wang: andywang2014@126.com, State key Laboratory of Cancer Biology and National Clinical Research Center for Digestive Diseases, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, China, 710032.

Lili Duan: 1826305487@qq.com, State key Laboratory of Cancer Biology and National Clinical Research Center for Digestive Diseases, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, China, 710032.

Liaoran Niu: 609702851@qq.com, State key Laboratory of Cancer Biology and National Clinical Research Center for Digestive Diseases, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, China, 710032.

Yujie Zhang: 609183757@qq.com, State key Laboratory of Cancer Biology and National Clinical Research Center for Digestive Diseases, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, China, 710032.

Wei Zhou: 252376698@qq.com, State key Laboratory of Cancer Biology and National Clinical Research Center for Digestive Diseases, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, China, 710032.

Liu Hong: hongliufmmu@163.com, State key Laboratory of Cancer Biology and National Clinical Research Center for Digestive Diseases, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, China, 710032.

Daiming Fan: hlhyhj@126.com, State key Laboratory of Cancer Biology and National Clinical Research Center for Digestive Diseases, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, China, 710032.

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# Tables

Due to technical limitations, table 1, 2, 3 & 4 is only available as a download in the Supplemental Files section.

# Figures

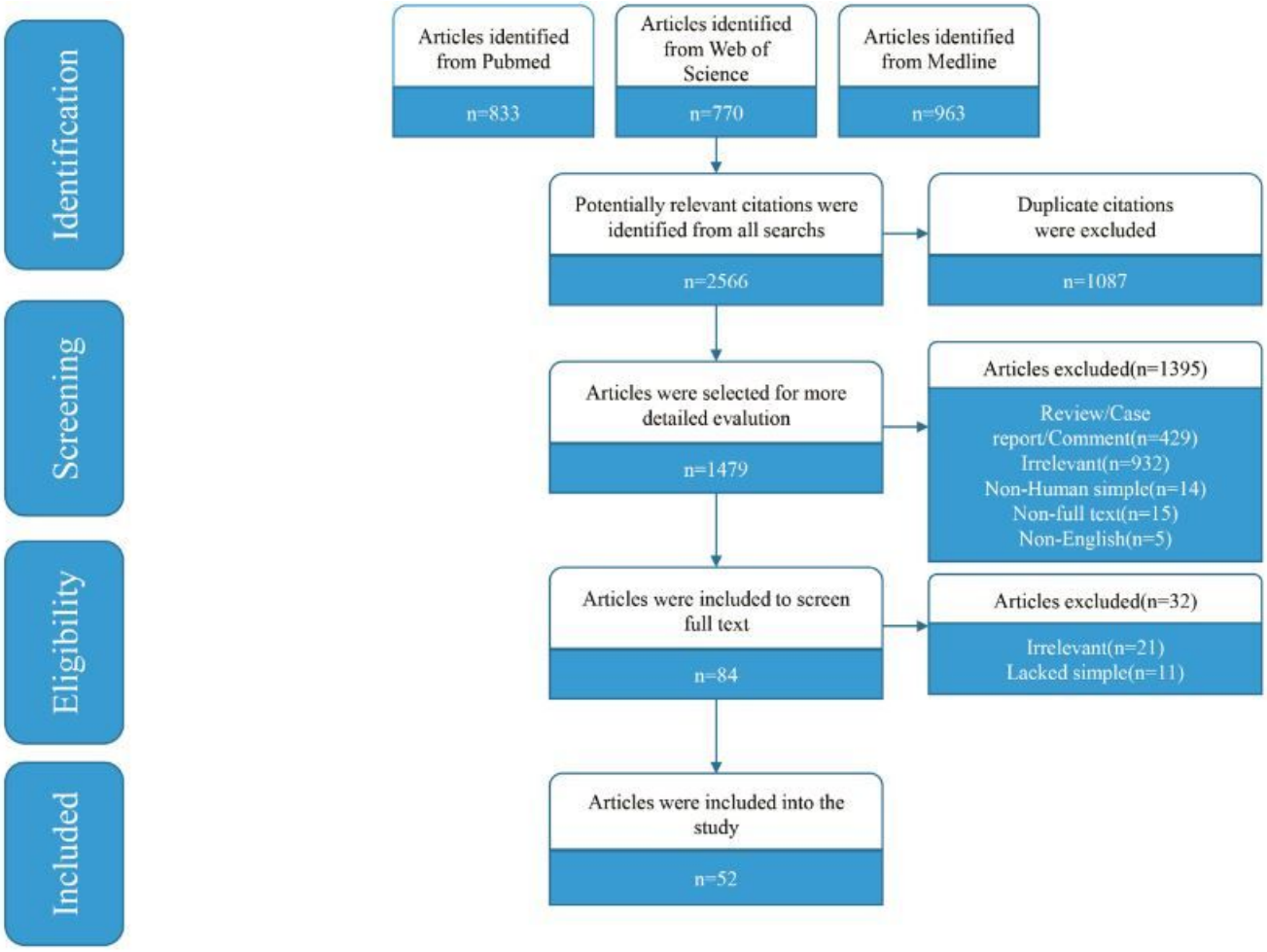
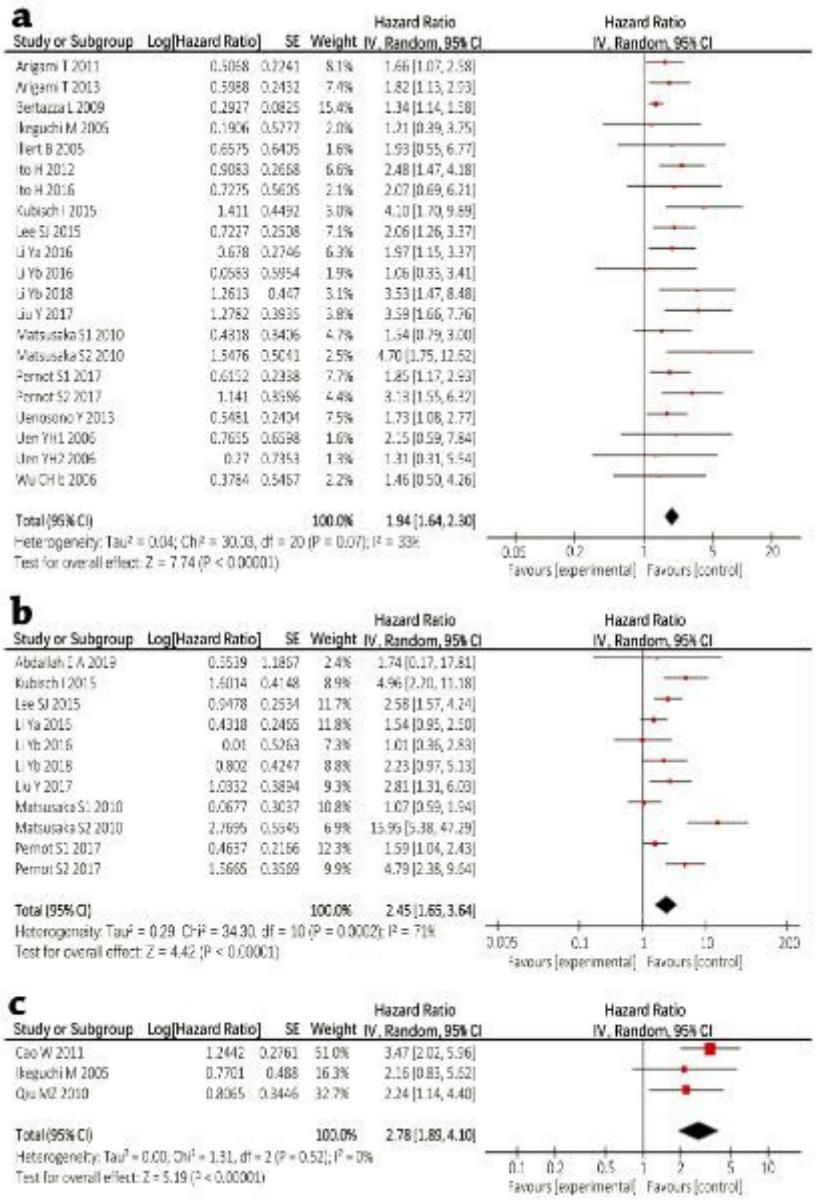


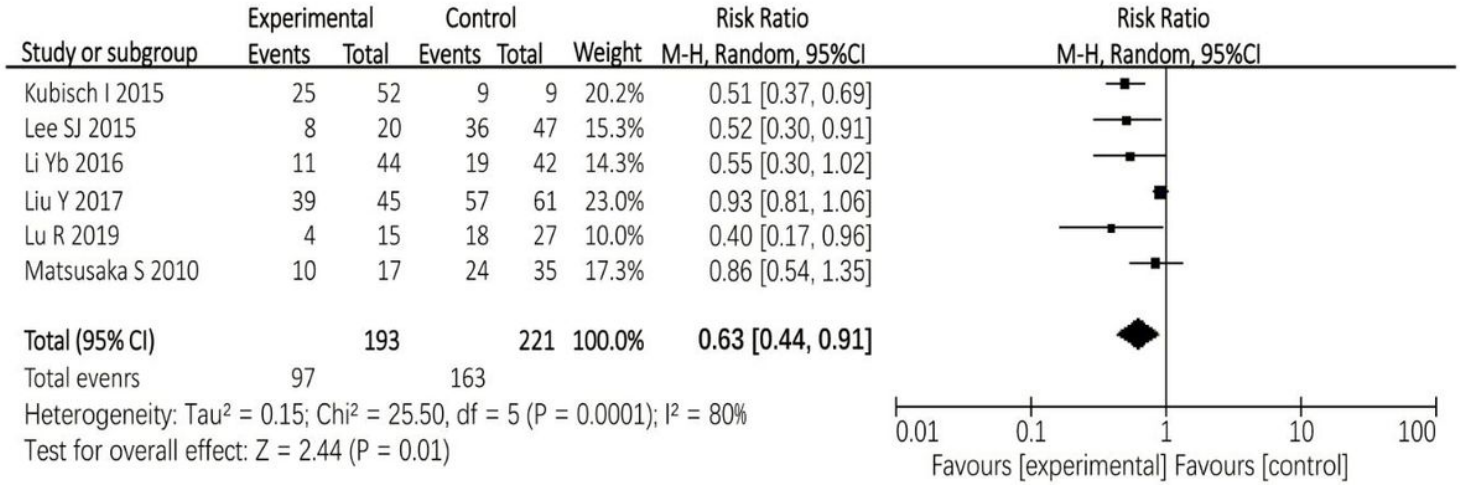

**Figure 1**

Figure 1



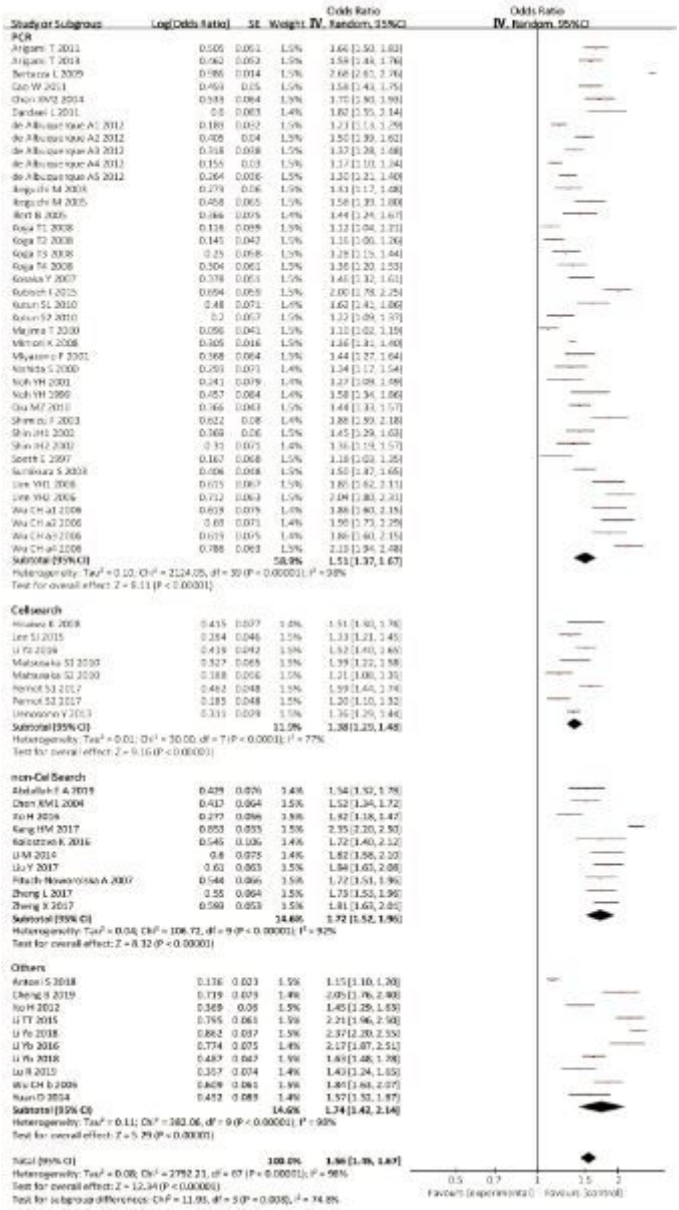

## Figure 2

Figure 2



## Figure 3

Figure 3



## Figure 4

Figure 4

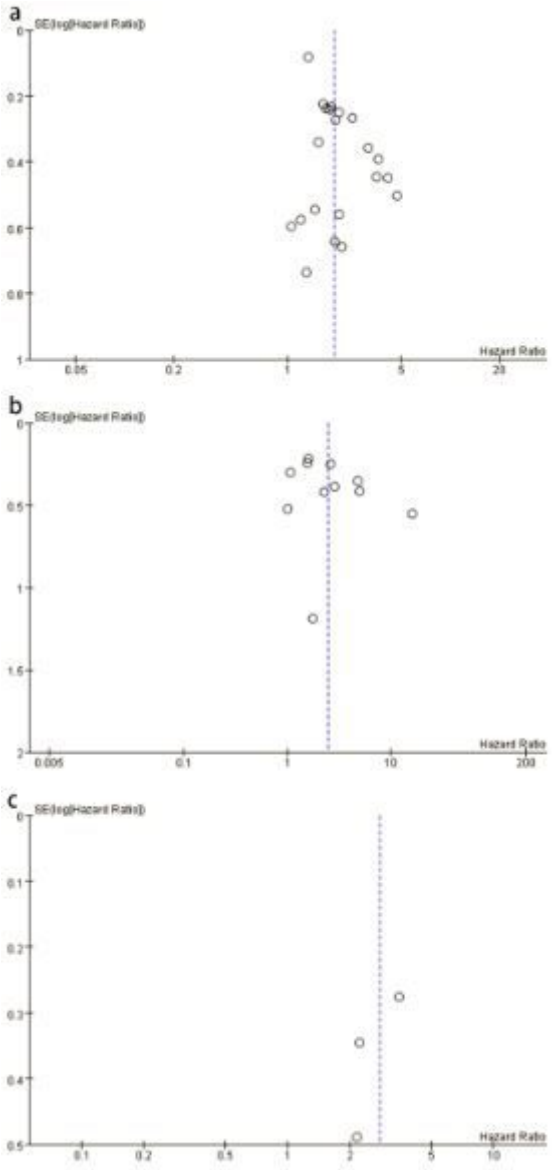



**Figure 5**

Figure 5

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