

# Prognostic significance of circulating tumor cells in esophageal carcinoma: a meta-analysis

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**Purpose:** The prognostic significance of circulating tumor cells (CTCs) in esophageal carcinoma (EC) is controversial. We aim to assess its association with clinicopathological and prognostic relevance in EC by using a meta-analysis.

**Methods:** We searched PubMed, Cochrane Database, Embase databases, and the references in relevant studies that assessed the clinicopathological or prognostic relevance of CTCs in peripheral blood of patients with EC. Statistical analyses were conducted by using Stata software to calculate the pooled odds ratio (OR), hazard ratio (HR), and 95% confidence intervals (CIs) using fixed or random-effects models according to the heterogeneity of included studies. The subgroup analyses were performed according to ethnicity, histological type, and detection method.

**Results:** Sixteen trials containing 1,260 patients were included for analysis. Pooled results showed that presence of CTCs was significantly associated with poor overall survival (HR =1.71, 95% CI [1.30, 2.12],  $P<0.001$ ) and progression-free survival (HR =1.67, 95% CI [1.19, 2.15],  $P<0.001$ ) in EC patients. Subgroup analysis indicated that presence of CTCs was closely associated with worse overall survival (Asian: HR =1.66, 95% CI [1.24, 2.08],  $P<0.001$ ; squamous cell carcinoma [SCC]: HR =1.66, 95% CI [1.24, 2.08],  $P<0.001$ ; no polymerase chain reaction [PCR]: HR =2.08, 95% CI [1.40, 2.76],  $P<0.001$ ) and progression-free survival (Asian: HR =1.63, 95% CI [1.15, 2.12],  $P<0.001$ ; SCC: HR =1.63, 95% CI [1.15, 2.12],  $P<0.001$ ; PCR: HR =1.63, 95% CI [1.15, 2.12],  $P<0.001$ ). Additionally, ORs showed that presence of CTCs was significantly correlated with tumor node metastasis (TNM) staging (overall: OR = 1.96, 95% CI [1.34, 2.87],  $P=0.001$ ; Asian: OR =2.09, 95% CI [1.37, 3.19],  $P=0.001$ ; SCC: OR =1.97, 95% CI [1.21, 3.07],  $P=0.003$ ; PCR: OR =2.23, 95% CI [1.43, 3.47],  $P<0.001$ ), venous invasion (overall: OR =2.23, 95% CI [1.46, 3.40],  $P<0.001$ ; Asian: OR =2.23, 95% CI [1.46, 3.40],  $P<0.001$ ; SCC: OR =2.23, 95% CI [1.46, 3.40],  $P<0.001$ ; PCR: OR =2.23, 95% CI [1.46, 3.40],  $P<0.001$ ), lymph node metastasis (overall: OR =2.41, 95% CI [1.50, 3.86],  $P<0.001$ ; Asian: OR =2.89, 95% CI [1.80, 4.65],  $P<0.001$ ; SCC: OR =2.44, 95% CI [1.47, 4.07],  $P=0.001$ ; PCR: OR =2.89, 95% CI [1.80, 4.65],  $P<0.001$ ) and distant metastasis (Asian: OR =2.68, 95% CI [1.01, 7.08],  $P=0.047$ ) in patients with EC.

**Conclusion:** The presence of CTCs indicates a poor prognosis in EC patients, especially in Asian and SCC patients. Further well-designed prospective studies are recommended to explore the clinical applications of CTCs in patients with EC.

**Keywords:** CTCs, esophageal carcinoma, metastasis, Asian, prognosis, meta-analysis

## Introduction

Esophageal carcinoma (EC) is the eighth most common cancer and the sixth leading cause of cancer-related death worldwide.<sup>1</sup> Despite recent progress in diagnostic procedures and multimodality treatment approach, the prognosis of EC patients remains dismal, with 5-year overall survival (OS) rate  $<20\%$ .<sup>2,3</sup> Recurrence and distant metastases are the main cause of treatment failure and cancer-related deaths. In recent

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years, many studies have demonstrated that circulating tumor cells (CTCs) are related to tumor relapse, metastasis, and patients' prognosis.<sup>4-6</sup> In EC patients, detecting CTCs may show clinical benefits in treatment and prognosis.

CTCs are tumor cells that are derived from the primary tumor, releasing into the bloodstream and circulating throughout the body. CTCs may form micrometastase, which is an important initial step leading to recurrence and distant metastases.<sup>7,8</sup> Currently, various new CTCs assays have been developed and used for their detection, including immunocytochemistry, reverse-transcriptase polymerase chain reaction (RT-PCR), and the CellSearch System.<sup>6,9,10</sup> Several meta-analyses have demonstrated the prognostic significance of CTCs in breast, gastric, and colorectal cancer.<sup>5,11,12</sup> However, there still remains controversy regarding the association between CTCs status and clinical significance in EC. Therefore, we performed a meta-analysis to determine the association between CTCs status and clinicopathological characteristics and prognosis, including tumor stage, lymph node metastasis, distant metastasis, and patients' survival.

## Materials and methods

### Search strategy

A literature search for relevant studies was performed systematically by two researchers (up to October 2015). We searched PubMed, Cochrane Database, and Embase databases using the following search terms: circulating tumor cells, CTCs, and esophageal carcinoma. Moreover, we screened the references of the relevant studies (reviews and included studies) to check for potentially relevant articles. For studies with the same population, only the latest published was selected. The search was restricted to articles in English.

### Inclusion criteria

To keep our analysis accurate and reliable, eligible studies were selected according to the following criteria: 1) patients were pathologically confirmed of EC; 2) immunocytochemistry, RT-PCR, or CellSearch detection methods were used to detect tumor specific genes/antigens in peripheral blood (PB); and 3) investigated the association between clinicopathological or prognostic significance and presence of CTCs in EC patients, with at least one of the outcome measures of interest reported in the study or calculable from the published data.

### Exclusion criteria

Studies were excluded from the meta-analysis if: 1) were review articles or letters; 2) the samples came from the bone

marrow, lymph nodes, and mesenteric/portal blood; 3) the results of interest were not reported or it was impossible to calculate results from the original data; 4) studies with fewer than 20 analyzed patients.

### Data extraction

The following data were independently extracted from included studies by two reviewers: author's name, patient's country, publication year, characteristics of the study population (number, age), tumor stage, methods of CTC detection, target antigen and gene, detection rate, sampling time (baseline: the time before operation or chemoradiotherapy), cut-off point, treatment, follow-up period, prognostic outcomes (OS, progression-free survival [PFS]). Disagreements were resolved by discussion between the two reviewers.

### Statistical analysis

The estimated odds ratio (OR) was used to summarize the association between the presence of CTCs and the clinicopathological characteristics of EC. The hazard ratio (HR) was used to summarize the effect measures for the prognostic outcomes (OS, PFS). 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>13</sup> The subgroup analyses were performed on the basis of ethnicity (Asian and European), histological type (squamous cell carcinoma [SCC] and adenocarcinoma) and detection method (PCR and no PCR). All statistical values were reported with 95% confidence intervals (CIs) and the two-sided *P*-value threshold for statistical significance was set at 0.05. Heterogeneity among the studies was calculated with the *Q* test and *I*<sup>2</sup> statistic, and the *I*<sup>2</sup> value indicated the degree of heterogeneity. A *P*-value <0.10 or *I*<sup>2</sup>>50% were considered significant heterogeneity, and a random-effects model (DerSimonian and Laird) was used. Otherwise, a fixed-effects model (Mantel-Haenszel) was used. Subgroup analysis was performed on the basis of ethnicity. Publication bias was assessed by Egger's test and Begg's test. One-way sensitivity analyses were performed to assess the stability of the meta-analysis results. All statistical analyses were performed with STATA 12.0 (StataCorp, College Station, TX, USA), using two-sided *P*-values.

## Results

### Baseline study characteristics

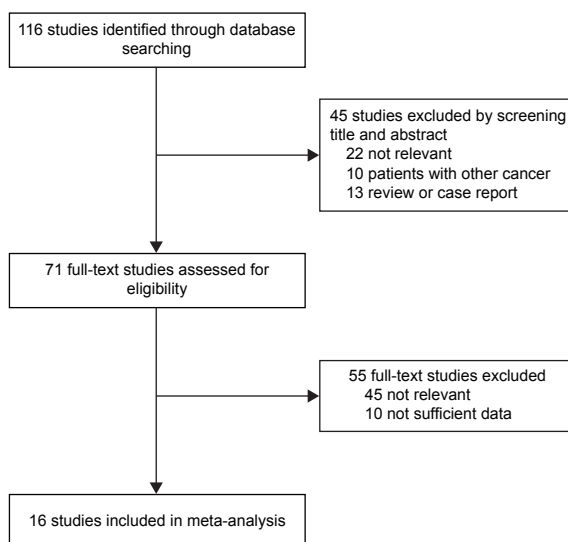
We identified 116 studies in this systematic literature search. By screening the titles and abstracts, 71 potential studies

were retrieved. Then, 55 studies were excluded after they were fully reviewed because they were irrelevant (45 studies) or had insufficient data (ten studies). Finally, 16 articles met the inclusion criteria for analysis, comprising 1,260 patients (Figure 1).<sup>14-29</sup> The studies were from Asia and Europe (China, Japan, Germany, and Czech) and were published between 2002 and 2015. Eight studies provided HRs on OS and PFS to perform the meta-analysis. The main characteristics of the included studies are summarized in Table 1.

## Effect of the presence of CTCs on the prognostic effect (OS and PFS)

Survival analysis was performed on HR for OS and PFS in six (691 patients) and five (571 patients) studies, respectively. The pooled HR showed that the presence of CTCs was highly correlated with poor OS (HR =1.71, 95% CI [1.30, 2.12],  $P<0.001$ , fixed-effect) (Figure 2). Moreover, the presence of CTCs indicated a poor prognostic effect on PFS (HR =1.67, 95% CI [1.19, 2.15],  $P<0.001$ , fixed-effect) (Figure 3).

In the subgroup analysis, the significant prognostic effect of CTC detection was confirmed in Asian (OS: HR =1.66, 95% CI [1.24, 2.08],  $P<0.001$ , fixed-effect; PFS: HR =1.63, 95% CI [1.15, 2.12],  $P<0.001$ , fixed-effect) (Figures 2 and 3), SCC (OS: HR =1.66, 95% CI [1.24, 2.08],  $P<0.001$ , fixed-effect; PFS: HR =1.63, 95% CI [1.15, 2.12],  $P<0.001$ , fixed-effect); PCR (PFS: HR =1.63, 95% CI [1.15, 2.12],  $P<0.001$ , fixed-effect) and no PCR subgroups (OS: HR =2.08, 95% CI [1.40, 2.76],  $P<0.001$ , fixed-effect) (Table 2).



**Figure 1** Flow chart of selecting the eligible publications.

## Correlation of CTCs with clinicopathological features

### Correlation of CTCs with the tumor node metastasis (TNM) stage

The meta-analysis on TNM stage indicated a significantly higher incidence of CTCs in the stage III/IV group relative to the stage I/II group (OR =1.96, 95% CI [1.34, 2.87],  $P=0.001$ , random-effect). In the subgroup analysis, the incidence of CTCs was significantly different between III/IV and I/II group in Asian (OR =2.09, 95% CI [1.37, 3.19],  $P=0.001$ , random-effect), SCC (OR =1.97, 95% CI [1.21, 3.07],  $P=0.003$ , random-effect), and PCR subgroups (OR =2.23, 95% CI [1.43, 3.47],  $P<0.001$ , random-effect) (Table 2).

The pooled analyses on the depth of invasion (pathology tumor category), lymph node metastasis, and distant metastasis were performed separately. The presence of CTCs in pT3/T4 group was significantly higher than pT1/T2 group (overall: OR =1.77, 95% CI [1.02, 3.06],  $P=0.04$ ; SCC: OR =2.14, 95% CI [1.56, 2.94],  $P<0.001$ , random-effect) (Table 2). We also found that the presence of CTCs was associated with a significantly increased risk of lymph node metastasis (overall: OR =2.41, 95% CI [1.50, 3.86],  $P<0.001$ ; Asian: OR =2.89, 95% CI [1.80, 4.65],  $P<0.001$ ; SCC: OR =2.44, 95% CI [1.47, 4.07],  $P=0.001$ ; PCR: OR =2.89, 95% CI [1.80, 4.65],  $P<0.001$ , random-effect) (Table 2). Moreover, we found that the presence of CTCs was correlated with distant metastasis in Asian subgroup (Asian: OR =2.68, 95% CI [1.01, 7.08],  $P=0.047$ , random-effect) (Table 2).

### Correlation of CTCs with histological differentiation

Eight studies were available for investigating the relationship between CTC status and histological differentiation (poor vs well and moderate). The presence of CTCs was not associated with histological differentiation in overall and subgroup analysis (Table 2).

### Correlation of CTCs with venous invasion

Five studies assessed the relationship between CTC status and venous invasion. We found that the presence of CTCs was correlated with a significantly increased risk of venous invasion in Asian, SCC, and PCR subgroups (overall: OR =2.23, 95% CI [1.46, 3.40],  $P<0.001$ ; Asian: OR =2.23, 95% CI [1.46, 3.40],  $P<0.001$ ; SCC: OR =2.23, 95% CI [1.46, 3.40],  $P<0.001$ ; PCR: OR =2.23, 95% CI [1.46, 3.40],  $P<0.001$ , fixed-effect) (Table 2).

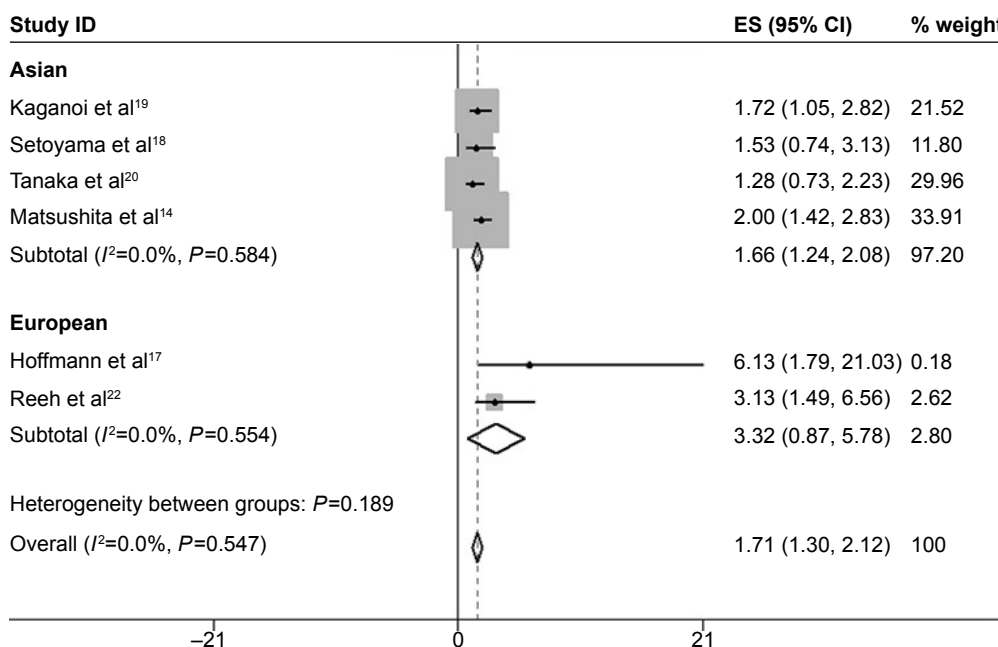
## Publication bias and sensitivity analyses

We performed Begg's test and Egger's test to assess the publication bias. There was no evidence of publication bias

Table 1 Baseline characteristics of the included studies

Study	Country	Ethnicity	Year	Number of patients	Age Mean/median (range)	Technique	Detection Rate %	Target antigen/gene	Tumor stage	Sampling time	Treatment	Cutoff	OM	Follow up Median (range)
Matsushita et al <sup>14</sup>	Japan	Asian	2015	90	65 (46–98)	CellSearch	25 (27.8)	EPCAM, CD45, CK8, CK19	I–IV	Baseline	Surgery + CRT or chemotherapy	≥ 1/7.5 mL	OS	10.3 (0.3–36.4)
Yin et al <sup>15</sup>	People's Republic of China	Asian	2012	72	63 (46–83)	RT-PCR	50 (47.2)	CEA, CK19, Survivin	I–III	Baseline or postradiotherapy	Radiotherapy + chemotherapy	NR	PFS	Max: 48
Nakashima et al <sup>16</sup>	Japan	Asian	2003	54	65.3(38–83)	RT-PCR	31 (57.4)	CEA	I–IV	Baseline	CRT + surgery	NR	NR	30 (2–60)
Hoffmann et al <sup>17</sup>	Germany	European	2010	62	61	RT-PCR	30 (48.4)	Survivin	I–III	Baseline	CRT + surgery	NR	OS	36
Setoyama et al <sup>18</sup>	Japan	Asian	2007	125	65.4 (38–87)	RT-PCR	77 (61.6)	CEA	I–IV	Baseline	Surgery	NR	OS	25.1 (1–73.7)
Kaganoi et al <sup>19</sup>	Japan	Asian	2004	70	NR	RT-PCR	23 (32.9)	SCCA	I–IV	Baseline	Surgery + chemotherapy	NR	OS, PFS	NR
Tanaka et al <sup>20</sup>	Japan	Asian	2010	244	NR	RT-PCR	34 (13.9)	CEA, SCCA	I–IV	Baseline or postoperative	Surgery + CRT or chemotherapy	NR	NR	24.3
Song et al <sup>21</sup>	People's Republic of China	Asian	2012	85	62 (44–83)	RT-PCR	32 (37.6)	STC-1	I–IV	Baseline	Surgery	NR	PFS	24
Reeh et al <sup>22</sup>	Germany	European	2015	100	66 (32–85)	CellSearch	18 (18)	CD45	I–IV	Baseline	Surgery	≥ 1/7.5 mL	OS, PFS	26 (22.18–29.82)
Bobek et al <sup>23</sup>	Czech	European	2014	43	NR	Metacell	27 (62.8)	CK18	I–IV	Baseline	Surgery	NR	NR	NR
Hashimoto et al <sup>24</sup>	Japan	Asian	2008	49	62.1 (39–76)	RT-PCR	14 (28.6)	CEA	I–IV	Baseline	Surgery + CRT or chemotherapy	NR	NR	11
Liu et al <sup>25</sup>	People's Republic of China	Asian	2007	53	58.1	RT-PCR	15 (28.3)	CEA	I–III	Baseline or postoperative	Surgery	NR	NR	12
Ikoma et al <sup>26</sup>	Japan	Asian	2007	44	NR	RT-PCR	12 (27.3)	CEA	I–IV	Baseline	Surgery + CRT	NR	NR	NR
Ito et al <sup>27</sup>	Japan	Asian	2004	28	66.5 (51–83)	RT-PCR	7 (25)	CEA, CK20	I–IV	Baseline	Surgery + CRT or chemotherapy	1	NR	NR
Koike et al <sup>28</sup>	Japan	Asian	2002	33	NR	RT-PCR	17 (51.5)	NP63	II–V	Baseline	Surgery or radiotherapy	CTC/1×10 <sup>7</sup> MNC	NR	NR
Cao et al <sup>29</sup>	People's Republic of China	Asian	2009	108	58.9 (36–82)	RT-PCR	51 (47.2)	Survivin	I–IV	Baseline	Surgery + CRT or chemotherapy	NR	NR	33

**Abbreviations:** CRT, chemoradiotherapy; CTC, circulating tumor cell; NR, not reported; OM, outcome measured; OS, overall survival; PFS, progression-free survival; SD, standard deviation; RT-PCR, reverse transcriptase-polymerase chain reaction; MNC, mononuclear cells.



**Figure 2** Meta-analysis of HR for the correlation of the presence of CTCs with OS.

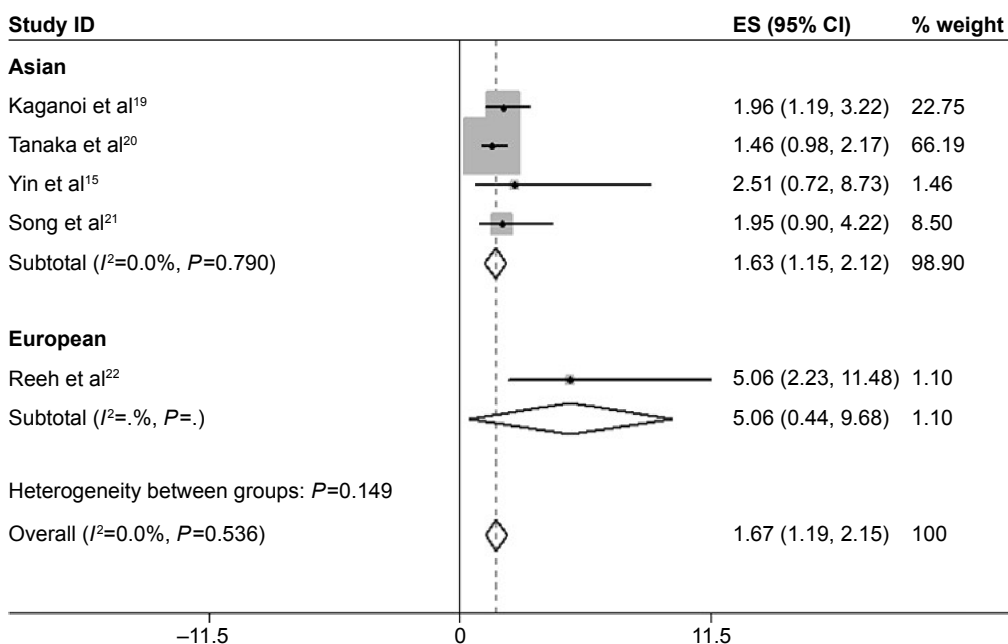
**Notes:** The diamond represents the comprehensive results. The square represents the point estimates. The dotted line represents HR=1.71.

**Abbreviations:** CI, confidence interval; CTCs, circulating tumor cells; HR, hazard ratio; OS, overall survival; ES, effect size.

for the pooled analysis of OS ( $P_{Begg}=0.452$  [Figure 4A],  $P_{Egger}=0.375$ ) and PFS ( $P_{Begg}=0.221$  [Figure 4B],  $P_{Egger}=0.200$ ). We conducted a sensitivity analysis to assess the stability of this meta-analysis. When any single study was deleted, the results were not materially altered (data not shown). These data suggest that our results were stable and credible.

## Discussion

Due to recurrence and metastasis of EC, the 5-year survival rate range remains from 15% to 20%, despite improvements having been made in esophagectomy and chemoradiotherapy.<sup>30-32</sup> The detailed mechanisms of EC metastasis have not yet been clarified. Recently, it has been demonstrated that CTCs have the abilities to intravasate



**Figure 3** Meta-analysis of HR for the correlation of the presence of CTCs with PFS.

**Notes:** The diamond represents the comprehensive results. The square represents the point estimates. The dotted line represents HR=1.67.  $P$  value in the European study is missing as there is only one study.

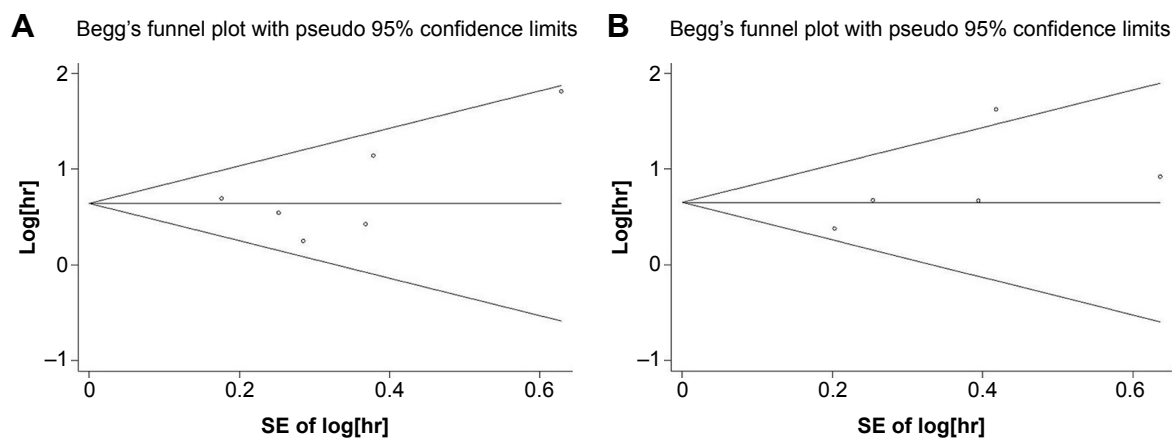
**Abbreviations:** CI, confidence interval; CTCs, circulating tumor cells; HR, hazard ratio; PFS, progression-free survival; ES, effect size.

**Table 2** Detailed results of subgroup analyses for clinicopathological features and prognostic significance

Clinicopathological features	Number of studies	Number of patients	Total		Ethnicity		Histological type		Detection method	
			Asian	European	SCC	Adenocarcinoma	PCR	No PCR		
Stage III/IV vs I/II (OR)	15	1,196	1.96 [1.34, 2.87], I <sup>2</sup> =42.1%, P=0.001	1.25 [0.53, 2.95], I <sup>2</sup> =0%, P=0.605	2.09 [1.37, 3.19], I <sup>2</sup> =46.7%, P=0.001	1.97 [1.27, 3.07], I <sup>2</sup> =48.3%, P=0.003	1.61 [0.54, 4.82], I <sup>2</sup> =0%, P=0.682	2.23 [1.43, 3.47], I <sup>2</sup> =46.7%, P<0.001	1.17 [0.61, 2.26], I <sup>2</sup> =0%, P=0.638	
pT: T3/T4 vs T1/T2 (OR)	12	990	1.77 [1.02, 3.06], I <sup>2</sup> =62.0%, P=0.04	1.62 [0.28, 9.22], I <sup>2</sup> =47.8%, P=0.588	1.76 [0.96, 3.24], I <sup>2</sup> =66.7%, P=0.068	2.14 [1.56, 2.94], I <sup>2</sup> =26.3%, P<0.001	2.54 [0.74, 8.71], I <sup>2</sup> =0%, P=0.138	1.76 [0.96, 3.24], I <sup>2</sup> =66.7%, P=0.068	1.62 [0.68, 9.22], I <sup>2</sup> =47.8%, P=0.588	
LN (+) vs (-) (OR)	12	1,028	2.41 [1.50, 3.86], I <sup>2</sup> =57.4%, P<0.001	0.89 [0.37, 2.10], I <sup>2</sup> =0%, P=0.782	2.89 [1.80, 4.65], I <sup>2</sup> =52.3%, P<0.001	2.44 [1.47, 4.07], I <sup>2</sup> =57.6%, P=0.001	1.25 [0.41, 3.82], I <sup>2</sup> =0%, P=0.691	2.89 [1.80, 4.65], I <sup>2</sup> =52.3%, P<0.001	0.89 [0.37, 2.10], I <sup>2</sup> =0%, P=0.782	
DM (+) vs (-) (OR)	6	482	2.25 [0.81, 6.27], I <sup>2</sup> =66.0%, P=0.12	1.43 [0.05, 39.12], I <sup>2</sup> =86.2%, P=0.832	2.68 [1.01, 7.08], I <sup>2</sup> =51.7%, P=0.047	2.02 [0.81, 5.07], I <sup>2</sup> =49.0%, P=0.132	2.27 [0.05, 106.03], I <sup>2</sup> =75.6%, P=0.675	2.10 [0.59, 7.52], I <sup>2</sup> =50.2%, P=0.253	2.18 [0.32, 14.99], I <sup>2</sup> =80.8%, P=0.426	
Differentiation: poor vs well and moderate (OR)	8	749	1.07 [0.73, 1.58], I <sup>2</sup> =0%, P=0.72	-	0.99 [0.65, 1.51], I <sup>2</sup> =0%, P=0.979	0.99 [0.65, 1.50], I <sup>2</sup> =0%, P=0.961	1.43 [0.42, 4.85], I <sup>2</sup> =0.568	1.00 [0.65, 1.51], I <sup>2</sup> =0%, P=0.979	1.70 [0.60, 4.82], I <sup>2</sup> =0.322	
Venous invasion (+) vs (-) (OR)	5	523	2.23 [1.46, 3.40], I <sup>2</sup> =0.43.7%, P<0.001	-	2.23 [1.46, 3.40], I <sup>2</sup> =0.43.7%, P<0.001	2.23 [1.46, 3.40], I <sup>2</sup> =43.7%, P<0.001	-	2.23 [1.46, 3.40], I <sup>2</sup> =43.7%, P<0.001	-	
OS (HR)	6	691	1.71 [1.30, 2.12], I <sup>2</sup> =0%, P<0.001	3.32 [0.87, 5.78], I <sup>2</sup> =0%, P=0.008	1.66 [1.24, 2.08], I <sup>2</sup> =0%, P<0.001	1.66 [1.24, 2.08], I <sup>2</sup> =0%, P<0.001	-	1.49 [0.97, 2.00], I <sup>2</sup> =0%, P<0.001	2.08 [1.40, 2.76], I <sup>2</sup> =0%, P<0.001	
PFS (HR)	5	571	1.67 [1.19, 2.15], I <sup>2</sup> =0%, P<0.001	5.06 [0.44, 9.68], I <sup>2</sup> =0.032	1.63 [1.15, 2.12], I <sup>2</sup> =0%, P<0.001	1.63 [1.15, 2.12], I <sup>2</sup> =0%, P<0.001	-	1.63 [1.15, 2.12], I <sup>2</sup> =0%, P<0.001	5.06 [0.44, 9.68], I <sup>2</sup> =0.032	

**Abbreviations:** DM, distant metastasis; HR, hazard ratio; LN, lymph node; OR, odds ratio; OS, overall survival; PCR, polymerase chain reaction; PFS, progression-free survival; SCC, squamous cell carcinoma; - symbol, no results due to insufficient studies; pT, pathology tumor.





**Figure 4** Funnel plot analysis (A: OS; B: PFS).

**Abbreviations:** OS, overall survival; PFS, progression-free survival; SE, standard error.

into the circulation, reach the distant organs, evade immune system, and eventually grow into clinically detectable metastasis.<sup>33–35</sup> Several studies have indicated that the presence of CTCs could be significantly correlated with distant metastasis and poor prognosis in lung cancer<sup>36</sup> and colorectal cancer.<sup>6</sup> The presence of CTCs may explain the mechanism of metastasis and relapse by the “seed and soil theory” in colorectal cancer.<sup>37</sup> However, the relationships between presence of CTCs and clinical outcomes in EC patients are not clear. Therefore, a quantitative meta-analysis about the correlations is required. This is the first meta-analysis to evaluate the clinicopathological and prognostic significance of CTCs detection by summarizing all relevant studies.

Our meta-analysis indicates that the presence of CTCs is significantly associated with TNM staging, venous invasion, lymph node metastasis in Asian, SCC, and PCR subgroups. In addition, the Asian population with presence of CTCs is positively correlated with distant metastasis. The Paget’s “seed and soil hypothesis” claims that the metastasis forms when certain tumor cells (seed) are compatible with microenvironment of certain organs (soil).<sup>37,38</sup> The CTCs are shed from the primary tumor, form micrometastatic foci via hematogenous metastasis and eventually develop into distant metastasis.<sup>37,39</sup> This may be one of the mechanisms of metastasis of EC.

The survival analysis reveals that patients with presence of CTCs have worse OS and PFS than those who lack CTCs. We also performed subgroup analyses based on ethnicity, histological type, detection method to further evaluate prognostic value of CTCs. The results suggested that patients with presence of CTCs have poor OS and PFS in Asian and SCC subgroups. This may be attributed to the differences in gene and environment among ethnicity. Several genes have been found to contribute to esophageal squamous cell

carcinoma in Asian population, such as ADH1B, ALDH2, PLCE1, and C20orf54.<sup>40,41</sup> Moreover, nutritional imbalance, nitrosamine-rich and mycotoxin contaminated foods can play important roles in EC among Asian population.<sup>42</sup> The absence of publication bias is confirmed with the funnel plots. These results are consistent with prior reports of meta-analysis in lung cancer,<sup>36</sup> colorectal cancer,<sup>6</sup> and gastric cancer.<sup>5</sup> Thus, we prove that the presence of CTCs at baseline in PB is significantly correlated with tumor metastasis and poor prognosis of Asian patients with EC.

The CTCs are defined as tumor cells originating from primary tumor and circulating freely in PB. Previous studies showed that the CTCs had detached from primary tumor before tumor metastasis was clinically visible.<sup>43</sup> Moreover, detection of CTCs in PB is very convenient and comfortable for patients, and is readily repeatable in a noninvasive manner. Thus, the presence of CTCs in PB could be used as a monitoring tool for tumor metastasis and prognosis of EC.

There are limitations to this meta-analysis. First, several articles did not provide the HRs and 95% CIs, and we calculated them from the reported data. Second, there are varied methods of detecting CTCs in the included studies and different end points and experimental design could have partly affected the results of survival analyses. Third, the heterogeneity could not be eliminated, and we used the random-effects model to obtain more conservative estimates. This heterogeneity may have been caused by differences in population characteristics. Furthermore, we should note that the enrolled studies were both cohort studies and nonrandomized clinical trials, and this difference in experimental design may also have caused heterogeneity. Despite these limitations, our meta-analysis is the first article to analyze the clinical significance and prognostic value of presence of CTCs in EC.

In conclusion, our meta-analysis indicates that presence of CTCs is significantly associated with poor prognosis in EC patients, especially in Asian and SCC patients. Moreover, presence of CTCs is positively correlated with TNM staging, venous invasion and lymph node metastasis in Asian and SCC patients, and with distant metastasis in Asian patients. These results should be confirmed by adequate, high-quality, well-designed multicenter studies.

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## Disclosure

The authors report no conflicts of interest in this work.

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