

Review Article

Meta-analyses of the clinicopathological and prognostic significance of circulating tumor cells in esophageal cancer

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Abstract: In the age of precision medicine, some research on esophageal cancer (EC) has focused on the detection of circulating tumor cells (CTCs), but the results are controversial. Therefore, we evaluated the clinicopathological and prognostic significance of CTCs in EC in comprehensive meta-analyses. We conducted a systematic search of the databases PubMed, Embase, and Cochrane as well as the Science Citation Index for relevant studies. The meta-analyses were performed using a random-effects model with hazard ratio (HR), odds ratio (OR), and 95% confidence intervals (CIs) as effect values. Sixteen studies with 1,256 EC patients were included in the meta-analyses of which 10 were suitable for prognostic analysis and 16 for clinicopathological analysis. CTC-positive EC patients were found to be significantly associated with poor disease-free survival (DFS)/progression-free survival (PFS) (HR, 2.42; 95% CI, 1.72-3.41) and overall survival (OS) (HR, 2.59; 95% CI, 1.88-3.58). The incidence of CTCs was significantly different for stage III/IV vs I/II group (OR, 2.62; 95% CI, 1.80-3.83), lymphatic invasion-positive vs negative group (OR, 2.23; 95% CI, 1.44-3.46), and venous invasion-positive vs negative group (OR, 2.46; 95% CI, 1.50-4.05). In contrast, CTC incidence was not significant in histological type (poorly differentiated vs well and moderately differentiated groups: OR, 1.25; 95% CI, 0.87-1.78) and tumor site (upper and middle groups vs lower group: OR, 1.17; 95% CI, 0.80-1.70). Our meta-analyses exhibited that CTC positivity in the peripheral blood indicated poor prognosis and had clinicopathological significance in EC patients.

Keywords: Circulating tumor cells, esophageal cancer, clinicopathological parameters, prognosis, meta-analyses

Introduction

Esophageal cancer (EC) is the eighth most commonly diagnosed aggressive cancer and the sixth most common cause of death globally [1] with an estimated 455,800 new cases and 400,200 deaths in 2012 worldwide [2]. Although a series of advances has been made in EC diagnosis and systematic therapies in the past decades, prognosis and 5-year survival rate (15-25%) are still not promising [3]. Despite complete surgical resection, local recurrence and distant metastases still present the main causes for EC-related deaths [4]. The limitations of traditional imaging techniques are difficulties in finding occult, minimal residual lesions and the lack of specific tumor markers to indicate the poor prognosis, which present the main causes for poor prognosis of EC. Therefore, it is imperative to find a marker for

detecting micrometastatic lesions, which may enable a better evaluation of EC patients' prognosis.

In recent years, much attention has been focused on research about circulating tumor cells (CTCs). CTCs are shed from primary tumors, recurrent tumors, or metastases into the bloodstream and possess genetic and antigenic specific features of the relevant tumor, which has been first reported by Ashworth in 1869 [5]. Extensive studies on breast, prostate, colorectal, and esophageal cancer have demonstrated that CTCs have a potential association with tumor metastasis and relapse [6-8]. Currently, detection methods for rare CTCs are becoming more and more precise (high sensitivity and specificity), mainly due to the two approaches reverse transcriptase polymerase chain reaction (RT-PCR) and immunocy-

tochemistry (ICC) [9]. Among the CTC detection methods, CellSearch® is the only system currently approved by the United States Food and Drug Administration (FDA) for clinical use. It uses antibodies covered with immunomagnetic beads against the tumor-related antigen [10].

Many studies have demonstrated that CTC presence is a poor prognostic factor in breast, prostate, colorectal, gastric, ovarian, and melanoma cancer, which has been further verified by previous relevant meta-analyses [11-16]. However, the association between CTC presence and cancer prognosis in EC patients are controversial, as some studies reported non-significant results [17]. Therefore, we performed comprehensive meta-analyses to evaluate the clinicopathological and prognostic significance of CTCs in EC patients.

Materials and methods

Search strategy

A comprehensive search of the databases PubMed, Ovid (resource selected: Embase), and Cochrane as well as the Science Citation Index was performed without time and region restrictions in February 2016. The search strategy included the following, variably combined main keywords and MeSH terms: “Neoplastic Cells”, “Circulating [MeSH]”, “circulating tumor cell(s)”, “isolated tumor cell”, “disseminated tumor cell”, “circulating tumor microemboli”, “circulating tumor cell cluster”, “micrometastasis”, “minimal residual disease”, “esophageal neoplasms [MeSH]”, “esophageal carcinoma”, and “esophageal cancer”. We combined the options subject word and random word for a wider search of relevant articles. Furthermore, we searched the reference lists of retrieved articles and review articles manually to check for potentially relevant articles.

Eligibility criteria

(1) Only articles published in a peer-reviewed journal were eligible, whereas data from letters and conference abstracts were considered not eligible. (2) All patients needed to be diagnosed with esophageal cancer, but adenocarcinomas of the esophagogastric junction were excluded. (3) Only peripheral blood was eligible as sample site, whereas bone marrow and lymph node

were excluded. (4) At least one result that presents measures of interest about the prognostic or clinicopathological significance of CTCs in EC patients was reported in the study or calculated from published data. (5) Only studies with more than 20 patients enrolled were eligible. (6) Only English language articles were included.

Data extraction and quality assessment

Two independent reviewers have screened eligible studies by the sequence of title, abstract, and full text. The following information were extracted from included articles: First author's names, year of publication, patient characteristics (sample size, age, male/female ratio), clinicopathological features of patients (tumor site, TNM stage, lymphatic invasion, venous invasion, tumor histology), volume and time of blood withdrawal, CTC detection method, target gene/antigen, cutoff value for defining CTC positivity, duration of follow-ups, and number of CTC-positive and CTC-negative patients in each clinicopathological subgroup. We also recorded hazard ratios (HRs) and confidence intervals (CIs, if available) for overall survival (OS), disease-free survival (DFS), and progression-free survival (PFS). When not directly acquired from the original articles, we calculated these values by the method of Tierney et al. [18]. If the reported HR referred to CTC-negative instead of CTC-positive patients, we calculated the HR's reciprocal to be consistent with other articles. When more than one blood sample per patient was acquired at different sampling times for, e.g., baseline, mid-therapy, or post-therapy, we recorded the results of each time point as independent data for prognostic analyses; However, for the analyses of clinicopathological characteristics, we only extracted CTC baseline data. The quality of the eligible studies was evaluated based on the Newcastle-Ottawa scale (NOS) criteria [19]. In case of any disagreements on data extraction and quality evaluation of the included articles, we solved it by comprehensive discussion.

Statistical analysis

We estimated pooled HRs and 95% CIs for DFS/PFS and OS using the Stata v. 12.0 software, and used the pooled OR to summarize the association between CTC detection and EC

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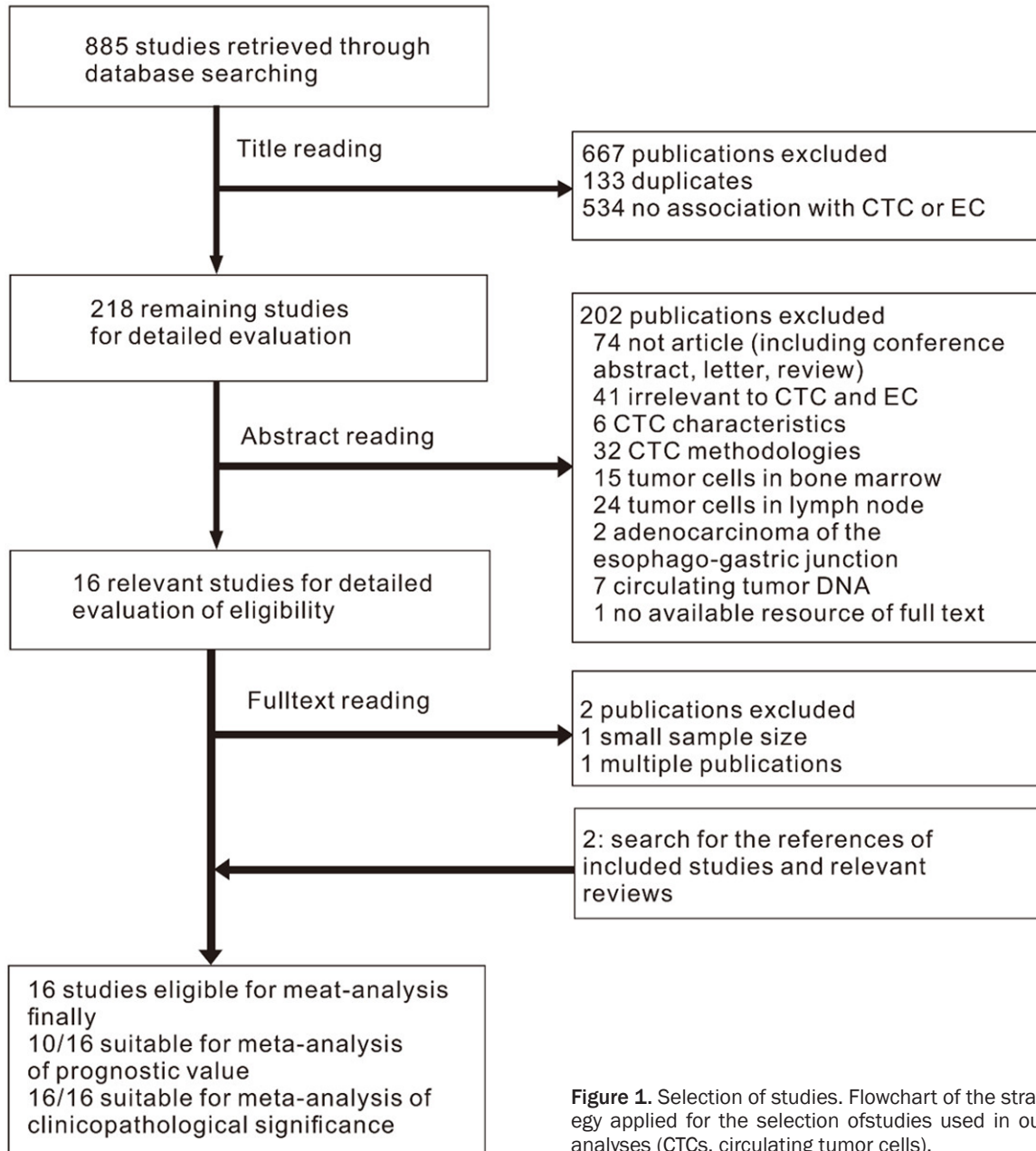


Figure 1. Selection of studies. Flowchart of the strategy applied for the selection of studies used in our analyses (CTCs, circulating tumor cells).

clinicopathological features. In order to take the heterogeneity among studies (such as the different CTC detection methods based on different target genes/antigens) into account, we applied a random-effect model in all meta-analyses below, as this model acquires more conservative results than the fixed-effect model by creating a wider CI around the pooled HR and OR. The heterogeneity among studies was evaluated applying Cochran's Q test and I^2 index. Potential sources of heterogeneity were explored in meta-regression analyses. To en-

sure addressing the different causes of heterogeneity, we also performed subgroup analysis by sample size, detection methods, sampling time, and stage. Publication bias was evaluated using Begg's and Egger's test when more than ten studies were included [20]. When publication bias was statistically significant, we performed a trim-and-fill analysis to further estimate the adjusted HR. To corroborate the results, we evaluated the influence of single studies on the pooled HR by estimating the average HR in the absence of the respective study in a

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Table 1. Main characteristics of included studies in our meta-analyses

Reference	No. of patients (M/F)	Age	Sampling-time	Method	Target gene/antigen	Stage	Rate (+)	Follow-ups (months)	Out-come	NOS
Tanaka 2015 [29]	38 (30/8)	63(43-87)	Baseline Mid-therapy	Cellsearch	EpCAM, CK8, CK19	M ₀ , M ₁	19/38 15/38	19 (Mean)	OS	6
Reeh 2015 [27]	100 (77/23)	66 (32-85)	Baseline	Cellsearch	EpCAM, CK	M ₀ , M ₁	18/100; 14/86 ^a	37.5 (Mean)	OS, DFS	7
Matsushita 2015 [28]	90 (78/12)	65 (46-98)	Baseline	Cellsearch	EpCAM, CK8, CK19	M ₀ , M ₁	25/90	10.3 (0.3-36.4)	OS	7
Yin 2012 [26]	72 (54/18)	63 (46-83)	Baseline Post-therapy	RT-PCR	CEA, CK19, survivin	M ₀	50/72 39/72	24 (Max)	PFS	6
Song 2012 [25]	85 (54/31)	62 (44-83)	Baseline	RT-PCR	STC-1	M ₀ , M ₁	32/85	24 (Max)	PFS	6
Tanaka 2010 [17]	244 (212/32)	NR	Baseline Post-therapy	RT-PCR	CEA, SCC	M ₀ , M ₁	34/244 41/244	24.3 (Mean)	OS, DFS	7
Cao 2009 [24]	108 (85/23)	58.9 (36-82)	Baseline	RT-PCR	Survivin	M ₀ , M ₁	51/108; 27/48 ^b	19.5 (1-33)	DFS	7
Setoyama 2007 [23]	125 (114/11)	65.4 (38-87)	Post-therapy	RT-PCR	CEA	M ₀ , M ₁	77/125	25.1 (1-73.7)	DFS	7
Liu 2007 [35]	53 (38/15)	58.1 (Mean)	Baseline	RT-PCR	CEA	M ₀	15/53	NR	NR	6
Kaganoi 2004 [21]	70 (64/16)	NR	Baseline Mid-therapy	RT-PCR	SCC	M ₀ , M ₁	23/70 24/70	60 (Max)	DFS	7
Ito 2004 [32]	28 (26/2)	66.5 (51-83)	Baseline	RT-PCR	CEA	M ₀ , M ₁	7/28	NR	NR	5
Honma 2006 [22]	46 (41/5)	NR	Baseline	RT-PCR	SCC	M ₀ , M ₁	14/46	34 (Mean)	OS, DFS	6
Koike 2002 [30]	33 (25/8)	NR	Baseline	RT-PCR	ΔNp63	M ₀ , M ₁	17/33	NR	NR	6
Hashimoto 2008 [33]	49 (42/7)	62.1 (39-76)	Baseline Post-therapy	RT-PCR	CEA	M ₀ , M ₁	14 ^c /49	11 (Mean)	NR	6
Li 2015 [34]	61 (54/7)	62.1 (49-78)	Baseline	ISET		M ₀ , M ₁	20/61	NR	NR	6
Nakashima 2003 [31]	54 (52/2)	65.3 (38-83)	Baseline	RT-PCR	CEA	M ₀ , M ₁	31/54	30 (2-60)	NR	7

^aOnly the 86 patients that the stage all are M0 are included in survival analysis. ^bOnly the 48 patients are included in survival analysis. ^cThe 14 patients are CTC positivity in baseline or post-therapy. Abbreviations: Rate (+), Rate of CTC positive patients; NOS, Newcastle-Ottawa Scale; OS, overall survival; DFS, disease-free survival; PFS, progression-free survival; NR: Not reported; RT-PCR: Reverse-transcriptase polymerase chain reaction; ISET, isolation by size of tumor; EpCAM, epithelial cellular adhesion molecule; CK, cytokeratin; CEA, carcinoembryonic antigen; STC-1, stanniocalcin-1; SCC, squamous cell carcinoma; Max: Maximum.

sensitivity analysis. The two-side *P*-value threshold for statistical significance was set at 0.05.

Results

Baseline study characteristics

We identified 887 records by the primary systematic computerized literature search. However, after screening of titles and abstracts, 869 studies were excluded (the reasons are detailed in **Figure 1**). Furthermore, two studies were excluded due to small sample size, multiple publications, or after reading the full text. Among multiple similar publications, we only extracted the publication with the largest number of patients. After application of all eligibility criteria, 16 studies were found eligible for the meta-analyses (**Figure 1**). Among these 16 articles, only ten studies were suitable for prognosis analyses, whereas all the included 16 articles were suitable for clinicopathological analyses. The ten studies suitable for prognosis analyses encompassed 904 EC patients (sample size: Median, 79 [38-244]; Mean, 90) and

were published between 2004 and 2015. The 16 studies suitable for clinicopathological analyses contained 1,256 EC patients (sample size median: 66 [28-1256], mean: 79) and were published between 2002 and 2015. The main characteristics of the eligible studies are summarized in **Table 1**.

Analysis of CTC effects on prognosis

CTCs and DFS/PFS. HR for DFS/PFS were available in eight articles [17, 21-27]. More than one HR for DFS/PFS was extracted in two studies [17, 26] due to the detection of CTCs in multiple time points and the presence of independent HR. The pooled HR for the included studies exhibited a significantly increased risk of progression in CTC-positive patients (HR, 2.42; 95% CI, 1.72-3.41; **Figure 2A**). Heterogeneity among studies was statistically significant (*P* = 0.003, *I*² = 64.3%) and publication bias was present (Begg's test: *P* = 0.210; Egger's test: *P* = 0.008; **Figure 4A**). Trim-and-fill analysis showed that five studies might be missing and that if these were published, the adjusted HR would be 1.615 (95% CI, 1.12-2.33; *P* = 0.01;

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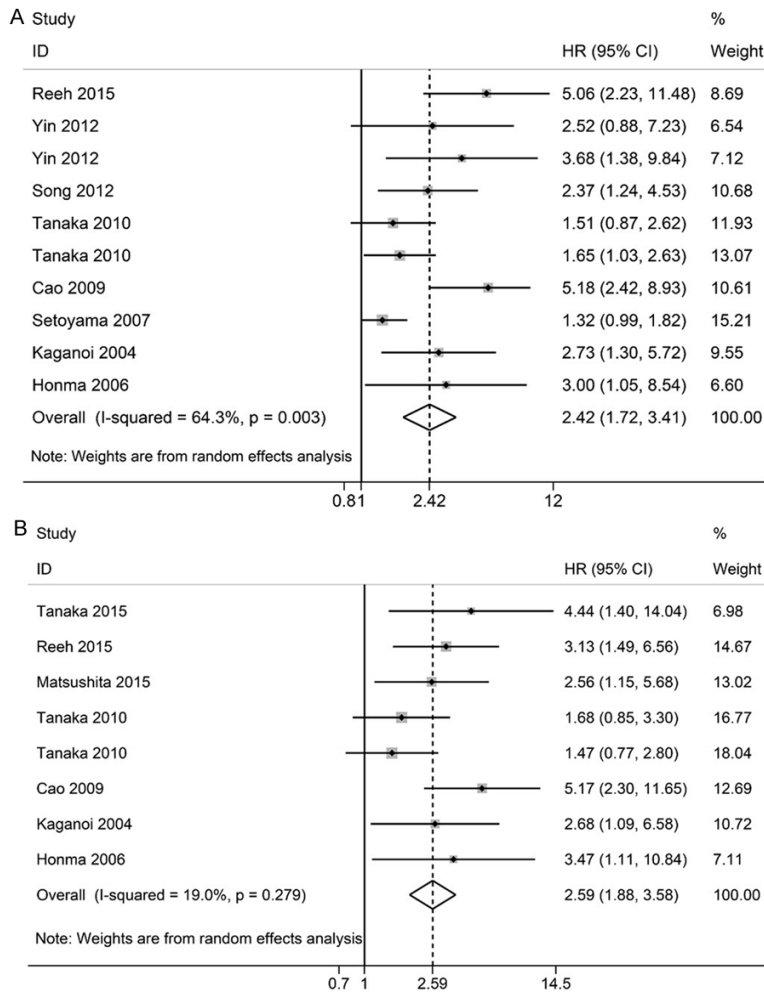


Figure 2. Summary of estimates of hazard ratio (HR) for DFS/PFS (A) and OS (B). (A) The estimated HR is summarized for DFS/PFS with CTC detection. (B) The estimated HR is summarized for OS with CTC detection.

Df = 15; Random effects). Furthermore, sensitivity analysis confirmed the stability of our results (data not shown).

To explore potential sources of heterogeneity, we conducted a meta-regression analysis based on the following covariates: Publication year, sample size, sampling time, detection method, and tumor stage (M_0 vs M_0 , M_1). Univariate analysis revealed tumor stage ($P = 0.006$) and sample size ($P = 0.055$) as potential sources of heterogeneity.

Subgroup analyses. To further explore the causes of heterogeneity, we performed subgroup analyses by sample size (≥ 80 , < 80), sampling time, and tumor stage. As shown in **Table 2**, the pooled HRs for all subgroups were statistically significant, which confirmed that

CTC presence was a strong prognostic factor in all subgroups. Given that only one study used the CellSearch® detection method [27] among all included studies, whereas all others applied the RT-PCR detection method, we did not perform subgroup analyses stratified by the detection method. However, after omitting this study, the remaining RT-PCR subgroup was still statistically significant (HR, 2.23; 95% CI, 1.60-3.11; $I^2 = 59.5\%$, $P = 0.011$).

CTCs and OS. HR for OS was available in seven articles [17, 21, 22, 24, 27-29]. More than one HR for OS was extracted in one study [17] for the same reason as mentioned above. The pooled HRs for the included studies showed a significantly increased risk of death in CTC-positive patients (HR, 2.59; 95% CI, 1.88-3.58; **Figure 2B**). Heterogeneity among studies was not statistically significant ($P = 0.279$; $I^2 = 19\%$) and publication bias was not examined because less than ten studies were included, which was not sufficient for the funnel plot and Begg's and

Egger's tests. Sensitivity analysis confirmed the stability of our results (data not shown).

Although heterogeneity among studies was not statistically significant, to corroborate our results, we conducted a meta-regression analysis considering the following covariates: Publication year, sample size, sampling time, detection method, and tumor stage (M_0 vs M_0 , M_1). Univariate analysis revealed that only the sample size was statistically significant ($P = 0.035$).

Subgroup analyses. We also carried out subgroup analyses by sample size (≥ 80 , < 80), sampling time, detection method, and tumor stage. The pooled HR for all but the "mid-therapy/post-therapy" subgroup were statistically significant, as shown in **Table 2**. In the "baseline"

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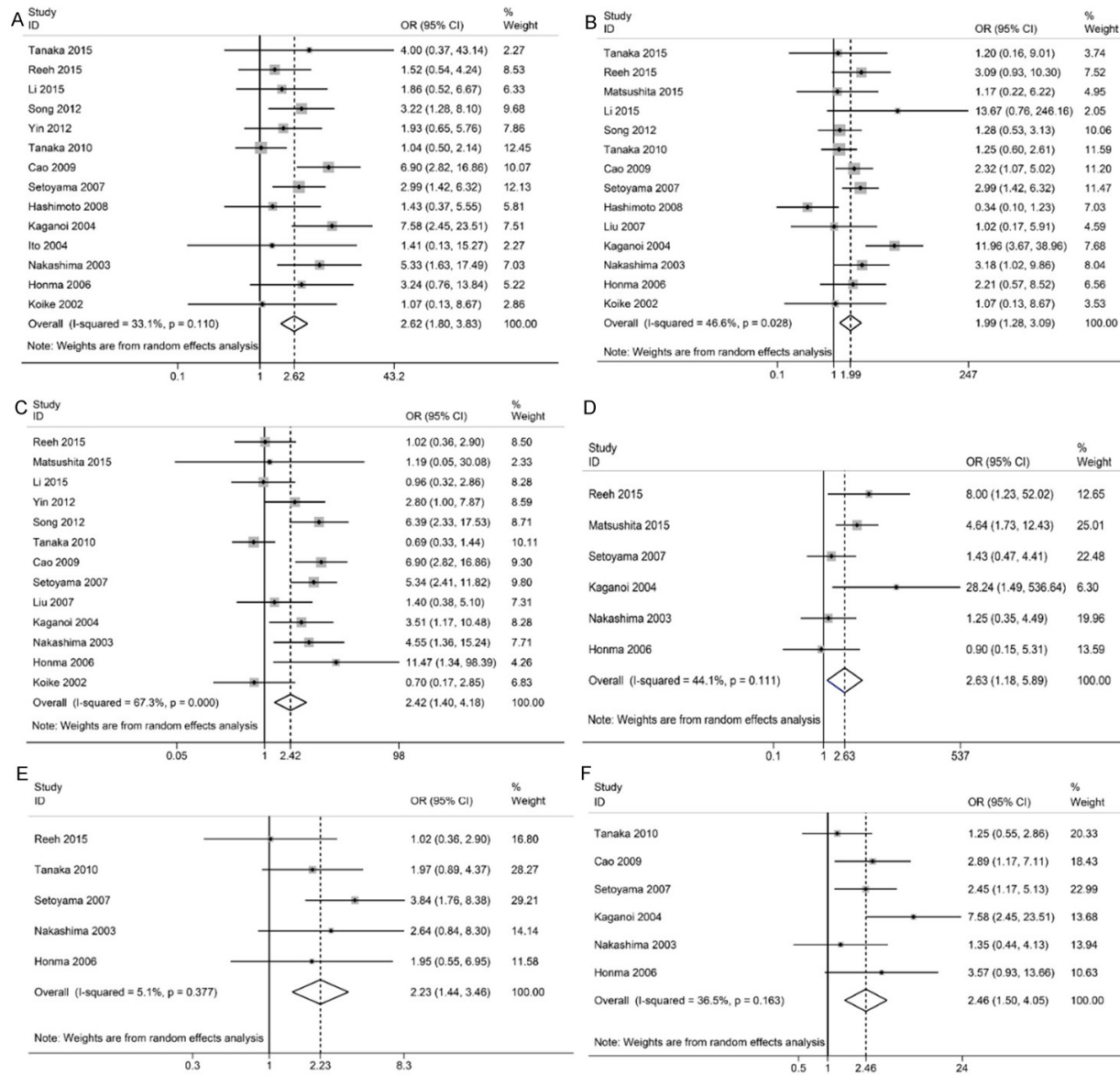


Figure 3. Meta-analyses of the correlation of CTCs with clinicopathological parameters. A. TNM stage; B. T factor; C. M factor; D. N factor; E. Lymphatic invasion; F. Venous invasion.

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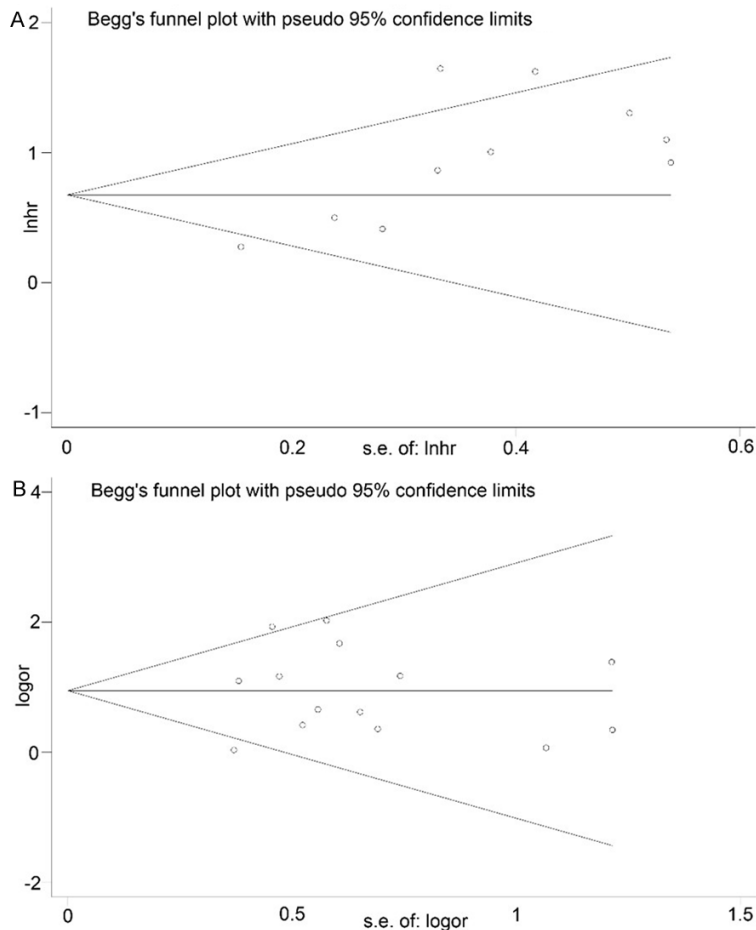


Figure 4. Funnel plot analyses. A. Funnel plot of the studies on disease-free survival/progression free survival; B. Funnel plot of the studies on TNM stage.

subgroup, the pooled HR was statistically significant (HR, 2.84; 95% CI, 1.93-4.19), whereas it was not significant in the mid-therapy/post-therapy subgroup (HR, 2.30; 95% CI, 0.79-6.64). However, the latter subgroup only included two studies.

Correlation of CTCs with clinicopathological parameters

We calculated odds ratios (ORs) to assess the association between CTCs and clinicopathological parameters. We found an odds ratio of OR >1, indicating that CTC is associated with clinicopathological parameters.

CTC incidence in the TNM stage. Fourteen studies reported the incidence of CTCs in TNM stage III/IV and I/II [17, 21-27, 29-34]. The pooled OR indicated a significantly higher incidence of CTCs in the stage III/IV group relative to the stage I/II group (OR, 2.62; 95% CI, 1.80-

3.83; $I^2 = 33.1\%$; $P = 0.110$; **Figure 3A**). No evidence for the existence of publication bias was found (Begg's test: $P = 0.913$; Egger's test: $P = 0.953$; **Figure 4B**). Sensitivity analysis confirmed the stability of our results (data not shown).

Moreover, we performed pooled analyses of included studies of the T factor (depth of tumor invasion), N factor (lymph node metastasis), and M factor (distant metastasis). Altogether, we acquired 14 studies about the T factor [17, 21-25, 27-31, 33-35]. The CTC incidence in the T3/T4 group was significantly different from the T1/T2 group (T3/T4 versus T1/T2: OR, 1.99; 95% CI, 1.28-3.09; $I^2 = 46.6\%$, $P = 0.028$; **Figure 3B**). The publication bias was not statistically significant (Begg's test: $P = 1$; Egger's test: $P = 0.975$; **Figure 5A**). Thirteen articles were included in the N factor group [17, 21-28, 30, 31, 34, 35]. The CTC incidence was significantly different between the factors N+

and N- (N+ vs N-: OR, 2.42; 95% CI, 1.40-4.18; $I^2 = 67.3\%$, $P = 0.000$; **Figure 3C**). The publication bias was also not statistically significant (Begg's test: $P = 0.502$; Egger's test: $P = 0.893$; **Figure 5B**). Six studies about the M factor were included [11, 21-23, 27, 28, 31]. Furthermore, we detected a significant difference in CTC incidence between the tumor stages M_1 and M_0 (M_1 vs M_0 : OR, 2.63; 95% CI, 1.18-5.89; $I^2 = 44.1\%$, $P = 0.111$; **Figure 3D**). As the meta-analyses only included six studies (and not more than ten studies), we did not evaluate the publication bias. Sensitivity analyses also confirmed the stability of our results above (data not shown).

CTC incidence in histological type and tumor site. Ten studies were included for analysis of the tumor histology [17, 21-23, 25-27, 31, 33, 34] and nine studies were included for analysis of the tumor site [17, 22, 24-26, 31, 33-35]

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Table 2. Results of subgroup analyses for values of CTC presence on DFS/PFS and OS

Subgroup selection	df	DFS/PFS	
		HR (95% CI; random effects)	Test for heterogeneity (I ²)
Sample size			
<80	5	3.54 (2.43, 5.17)	0.00%
≥80	5	1.88 (1.28, 2.74)	62.40%
Samplingtime			
Baseline	6	2.91 (1.86, 4.56)	52.60%
Post-therapy	3	1.65 (1.09, 2.51)	50.80%
Baseline or mid-therapy	1	2.73 (1.30, 5.73)	-
Stage			
M ₀	4	4.33 (2.85, 6.56)	0.00%
M ₀ , M ₁	6	1.70 (1.33, 2.19)	22.20%
All	10	2.42 (1.72, 3.41)	64.30%
OS			
Subgroup selection	df	HR (95% CI; random effects)	Test for heterogeneity (I ²)
Sample size			
<80	5	3.87 (2.39, 6.28)	0.00%
≥80	5	2.02 (1.42, 2.88)	0.00%
Samplingtime			
Mid-therapy/post-therapy	2	2.30 (0.79, 6.64)	62.90%
Post-therapy	5	2.84 (1.93, 4.19)	13.90%
Baseline or mid-therapy	1	2.68 (1.09, 6.58)	-
Stage			
M ₀	2	3.93 (2.27, 6.79)	0.00%
M ₀ , M ₁	6	2.15 (1.54, 3.01)	0.00%
Method			
Cellsearch	3	3.09 (1.89, 5.05)	0.00%
RT-PCR	5	2.43 (1.50, 3.92)	43.80%
All	8	2.59 (1.88, 3.58)	19.00%

Abbreviations: CTCs, circulating tumor cells; RT-PCR, reverse transcriptase polymerase chain reaction; DFS, disease-free survival; PFS, progression-free survival; OS, overall survival.

regarding the CTC incidence. The pooled OR exhibited that CTC incidence was neither in the tumor histology group (poorly differentiated vs well and moderately differentiated tumors: OR, 1.25; 95% CI, 0.87-1.78; I² = 0.0%, P = 0.587) nor in the tumor site group (upper and middle vs lower: OR, 1.17; 95% CI, 0.80-1.70; I² = 0.0%, P = 0.880) statistically significant.

CTC incidence in venous and lymphatic invasion. We included five studies for the analysis of lymphatic invasion [17, 22, 23, 27, 31] and six studies for the analysis of venous invasion [17, 21-24, 31] regarding CTC incidence. Higher CTC incidences were observed in the lymphatic invasion-positive and venous invasion-positive groups compared with the corresponding negative groups (lymphatic invasion positive vs negative: OR, 2.23; 95% CI, 1.44-3.46; I² = 5.1%, P

= 0.377; **Figure 3E**; Venous invasion positive vs negative: OR, 2.46; 95% CI, 1.50-4.05; I² = 36.5%, P = 0.163; **Figure 3F**). Sensitivity analyses confirmed the stability of our results above (data not shown).

Discussion

The present meta-analyses exhibited that the presence of CTCs in peripheral blood (PB) is associated with poor DFS/PFS and OS and significantly increases the risk of cancer progression and death. Subgroup and sensitivity analyses further verified these results. Although a certain degree of publication bias was detected, these results were still statistically significant after adjusting for the publication bias by a trim-and-fill analysis. Moreover, we evaluated the correlations between CTCs and main clini-

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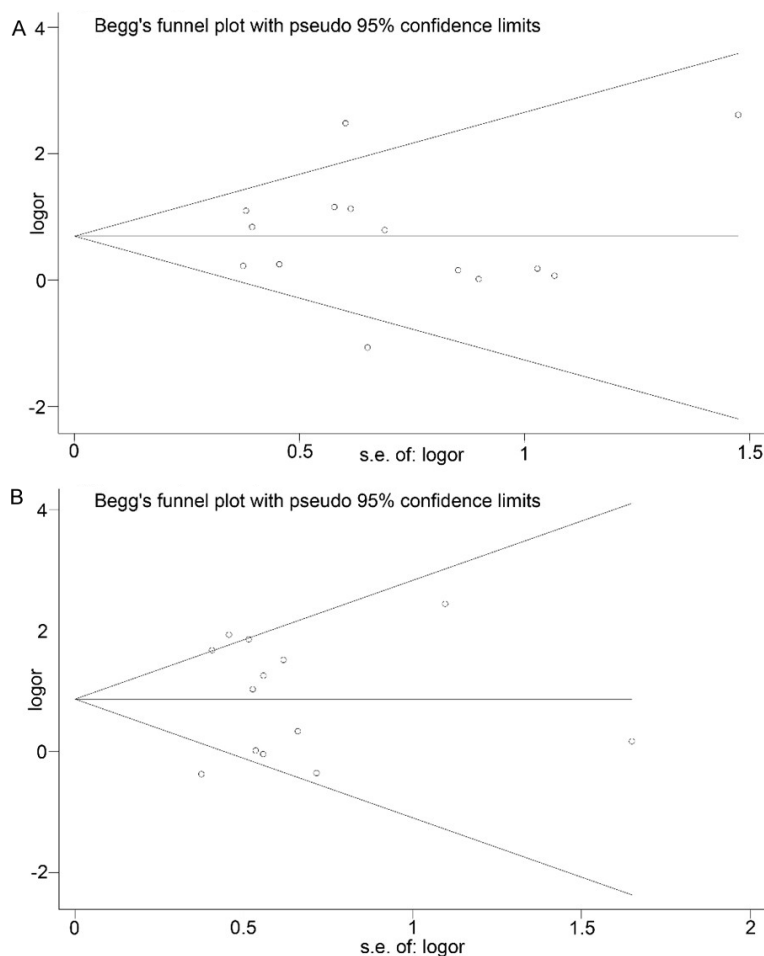


Figure 5. Funnel plot analyses. A. Funnel plot of the studies on the T factor; B. Funnel plot of the studies on the N factor.

cal clinicopathological characteristics, revealing that CTCs are significantly associated with the TNM stage, venous invasion, and lymphatic invasion, but not with the histological type and tumor site. Compared with a similar meta-analysis recently published by Qiao et al., our meta-analyses were based on a more comprehensive search strategy and a more reasonable statistical method, as our subgroup analyses were based on the results of meta-regression analyses [36]. However, despite of some differences, our results were almost consistent with the results of Qiao et al., which further confirms that CTC positivity in PB indicates a poor prognosis in EC patients with clinicopathological significance.

Though a series of studies had demonstrated that the presence of disseminated or isolated tumor cells in bone marrow or lymph nodes indicates poor prognosis [37-39], the respective samples were acquired in an invasive oper-

ation during the EC patient management, which was not easily accepted. Therefore, our meta-analyses did not include bone marrow and lymph node samples. Moreover, detecting CTCs in the PB was not time- or cost-consuming and could be easily repeated during therapy and follow-up, which was more favorable as a monitoring tool during EC patient management. Thus, an increasing number of studies have focused on the detection of CTCs in PB in recent years.

Our results excluded detection methods as a source for heterogeneity. However, we still consider them as a potential source due to the differences in the detection methods and the targeted genes/antigens. Therefore, we performed subgroup analyses based on the different detection methods. In the meta-analysis of CTCs and OS, the pooled results were still statistically significant in the RT-PCR and CellSearch® subgroups. However, in the meta-

analysis of CTCs and DFS/PFS, only one study used CellSearch®, whereas the others used RT-PCR. Due to this limited number of studies using CellSearch®, we could not perform the subgroup analysis and determine whether the CellSearch® subgroup was still significant in CTCs and DFS/PFS. Therefore, a larger number of studies using CellSearch® are needed for future analysis. However, omitting the single study that used CellSearch®, the result of the RT-PCR subgroup was still significant. Despite the different targeted genes/antigens in the RT-PCR subgroup, we could not perform any subgroup analysis based on targeted genes/antigens due to the limited number of included studies. Therefore, it would be necessary to use standardized detection methods in relevant studies for the clinical utility of CTCs in the future.

In theory, given the intraoperative tumor cell release, the rapid apoptosis or necrosis of the

altered internal environment in the systemic circulation after primary tumor resection [35], or the function of systemic treatment (chemoradiotherapy and/or biotherapy), the mid-therapy/post-therapy sampling time might better reflect the most relevant CTC status than the baseline. However, several studies confirmed that the mid-therapy/post-therapy sampling time reflects the CTC status better [40, 41]. Although our results of the univariate meta-regression analysis were not statistically significant on the sampling time, we performed subgroup analyses due to the aforementioned reasons. In the meta-analyses of CTCs and DFS/PFS, the mid-therapy/post-therapy subgroup (three studies included) and the baseline subgroup (six studies included) were both statistically significant. In CTCs and OS, the pooled HR was also significant in the baseline subgroup, whereas it was not significant in the mid-therapy/post-therapy subgroup, which only included two studies. Because of the limited number of included studies in the subgroup analyses, we could not get any conclusive result, especially in the mid-therapy/post-therapy subgroup. Therefore, a larger number of multicenter studies on baseline and mid-therapy/post-therapy time for the same EC patients are needed to determine whether the mid-therapy/post-therapy sampling time reflects the prognostic significance for CTCs better and to assess the prognosis for CTCs.

As it is well known, the cancer stage is the main factor resulting in different prognoses in EC patients. Therefore, we performed a meta-regression analysis on the cancer stage (M_0 vs M_0 , M_1). In CTCs and DFS/PFS, the meta-regression analysis exhibited that the cancer stage was the main source of heterogeneity. In contrast, this result was not statistically significant in CTCs and OS. Therefore, we further performed subgroup analyses, which showed that all relevant subgroups proved the prognosis for CTCs. However, the subgroup analysis had limitations, as the M_0 group only included two studies in CTCs and OS and the analyzed patients of included studies could not be divided into M_0 and M_1 stages. Therefore, in the future, a larger number of multicenter studies with EC patients separated into M_0 or M_1 stages are needed to assess the prognosis for CTCs. Moreover, our results indicated that the sample size of included studies might be the source of heterogeneity in both CTCs and DFS/PFS or OS. However,

subgroup analysis by sample size (≥ 80 , < 80) revealed that all relevant subgroups were still statistically significant, which further confirmed the prognosis of CTCs.

Regarding the association between CTCs and clinicopathological features, our meta-analyses showed a significant association with TNM stage, venous invasion, and lymphatic invasion. Hence, CTC detection in baseline may act as a staging tool for EC patients, which was further supported by the above results on OS and DFS/PFS. The results remained significant in sensitivity analysis and showed no statistically significant publication bias, which was confirmed by Begg's and Egger's test. However, in order to determine whether the CTCs in baseline can act as a staging tool and how exactly they can be applied as a staging tool, more relevant studies with larger sample sizes and more comprehensive data are required.

Besides the aforementioned heterogeneity among studies, our meta-analyses had additional limitations, such as different detection methods, sampling times, and stage factors. First, our meta-analysis was based on published data from eligible studies, and we could not get any detailed, individual patient data. Second, no universal standard exists for the optimal CTC cutoff in CellSearch® and the positivity standard in RT-PCR. Third, no consensus exists on the optimal follow-up time to predict the prognosis significance for CTCs, and some included studies did not report follow-up data and only reported the median follow-up time or no follow-up time at all. Therefore, we could not further analyze the prognosis significance based on different follow-up times. Fourth, the analyzed patients were not homogeneous, as some studies only included squamous esophageal cancer, whereas other studies included polytypic esophageal carcinoma and also applied different treatment regimens. Moreover, the TNM stage versions were different in some studies.

In summary, the results of our meta-analysis supported the notion of a strong prognosis value of CTCs in PB and that CTCs could be used as a staging tool. In future, well-designed, large-scale multicenter studies based on homogeneous populations with more comprehensive data are required to explore the CTCs' prognostic and clinicopathological value for EC patients.

Disclosure of conflict of interest

None.

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