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Review Article

A meta-analysis of circulating tumor cells (CTCs) correlation with the prognosis in metastatic breast cancer

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Abstract: A number of studies have confirmed that circulating tumor cells (CTCs) are closely related with metastatic breast cancer (MBC) prognosis. However, whether CTCs can act as an independent prognostic indicator in MBC remains unclear. Therefore, a meta-analysis of published literatures was carried out to assess the specific role of CTCs in MBC prognosis. Relevant articles were searched online between May 1995 to May 2015 through different databases, and data were extracted to assess the information on the correlation of CTCs with overall survival (OS) and progression-free survival (PFS) in MBC, together with hazard ratios (HRs) and 95% confidence intervals (CIs). Data were pooled from different studies as an effective measure. The identification of five CTCs was significantly and positively associated with short survival time in all MBC patients. OS was observed with HR=2.71 (95% CI: 2.41, 3.05) and PFS was observed with HR=1.88 (95% CI: 1.74, 2.03). Moreover, this correlation was not associated with blood sampling time (OS: HR=2.50, 95% CI [1.73, 3.61]; PFS: HR=2.33, 95% CI [1.80, 3.01]) and treatment methods (OS: HR=1.98, 95% CI [1.53, 2.56]; PFS: HR=1.66, 95% CI [1.36, 2.02]). This present study confirms that the number of CTCs is positively associated with MBC prognosis. CTCs detection can be used as a significant index to guide treatment strategies and evaluate MBC prognosis. Further clinical studies are warranted to establish clinical criteria for the use of CTCs in breast cancer prognosis.

Keywords: Circulating tumor cells (CTCs), metastatic breast cancer (MBC), prognosis, progression-free survival (PFS), overall survival (OS)

Introduction

According to data obtained from the American Cancer Society, breast cancer accounted for 29% (231,840 cases) of all the new cancer cases in 2015 and resulted in 15% (40,290 cases) of cancer related deaths among females in the United States [1]. In China, the overall incidence rate of breast cancer was 32.43 per 100,000 individuals, which accounts for 16.20% (208,000 cases) of all cancer cases in women; ranking first among all cancer incidences. Furthermore, overall mortality due to breast cancer was 8.65 per 100,000 individuals, which accounts for 7.90% (55,500 cases) of all cancer related deaths in women; ranking fifth among all cancer deaths [2]. Therefore, it is obvious that breast cancer continues to pose a threat to women's health, and specifically, its metastasis results in poor prognosis.

Breast cancer eventually metastasizes and spreads to organs such as the brain, bone, lungs and liver via the hematopoietic system. Based on this fact, the scientific community has recently focused on the possibility of CTCs detection in blood, with an idea of providing a simpler and quicker method of evaluating a patient's prognosis; enabling clinicians to choose a more rational treatment based on this snapshot information. François-Clément Bidard suggested, through their systematic review of CTCs detection methods, that CTC enumeration/characterization may improve the management of breast cancer patients [3]. The standard method for CTC isolation is the CellSearch® system (Veridex), which has been approved by the Food and Drug Administration (FDA) in the USA for clinical applications [4].

During the year 2011 and 2012, two meta-analysis articles focused on the relationship of CTCs and breast cancer in general without specifically focusing on metastatic samples [5, 6]. Thereafter, many researchers [7-23] have pro-

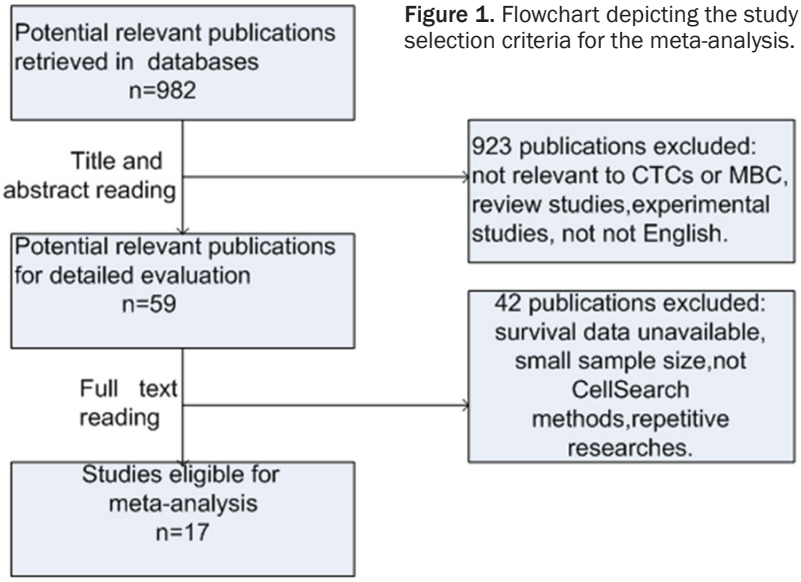


Figure 1. Flowchart depicting the study selection criteria for the meta-analysis.

posed that numerical CTCs ≥5 in 7.5 mL of blood positively correlate with poor prognosis in MBC. Consequently, CTCs detection may act as an important indicator for the diagnosis and treatment of MBC. Nevertheless, some authors [8, 9] have proven that ≥1 CTCs, and not ≥5 CTCs, in 7.5 mL of blood is sufficient to establish a link with poor prognosis. Therefore, this confirms that the number of CTCs positively associate with the outcome and prognosis of MBC; however, the exact number of CTCs, which could be defined as a standard, remains controversial. The reason for this unambiguity may be attributed to limited multi-ethnic samples and small sample sizes in these studies. At the same time, the different testing methods used in different studies may have also caused these diversified results. Therefore, in order to have a better understanding of the association between CTCs and MBC prognosis, we performed this meta-analysis by analyzing the prognostic value of CTCs through analyzing the correlation between progression-free survival (PFS) and overall survival (OS) in MBC patients. In addition, we also carried out subgroup analyses on the effect of CTCs in different MBC subtypes and in different time points of CTCs detection.

Materials and methods

Publication search

A methodical search for all English language literatures from May 1995 to May 2015 was performed in the following databases: PubMed, Web of Science, and Embase. The search strategy used included the following Medical Subject Headings (MeSH terms)/keywords: "circulating tumor cell(s)" or "CTCs", "metastatic breast cancer" or "MBC" or "advanced breast cancer". Additionally, the reference list of relevant articles, review articles and published meta-analyses were also inspected.

Inclusion and exclusion criteria

The title and abstract of all the identified articles were screened according to the following inclusion criteria: (1) subjects of the study should be patients with MBC; (2) all CTCs detection methods used in assessing the correlation between CTCs status and prognostic outcome such as PFS or OS, as well as the relevant prognostic index in MBC, are valid; (3) the study has enough information to compute a hazard ratio (HR) with 95% confidence interval (CI) to estimate PFS and/or OS; (4) at least 50 patients are enrolled in the study; (5) the articles were in English language. Articles were excluded according to the following criteria: (1) patients in the article were also enrolled in other studies; (2) histopathology-based diagnosis of breast cancer was inflammatory breast cancer or sarcoma. If there were disagreements in the selection of articles, further analysis was conducted by accessing the full-text of the articles and by discussion with the other researchers, if necessary. An attempt to contact the actual authors was also carried out to obtain more detailed information.

Data extraction and outcomes

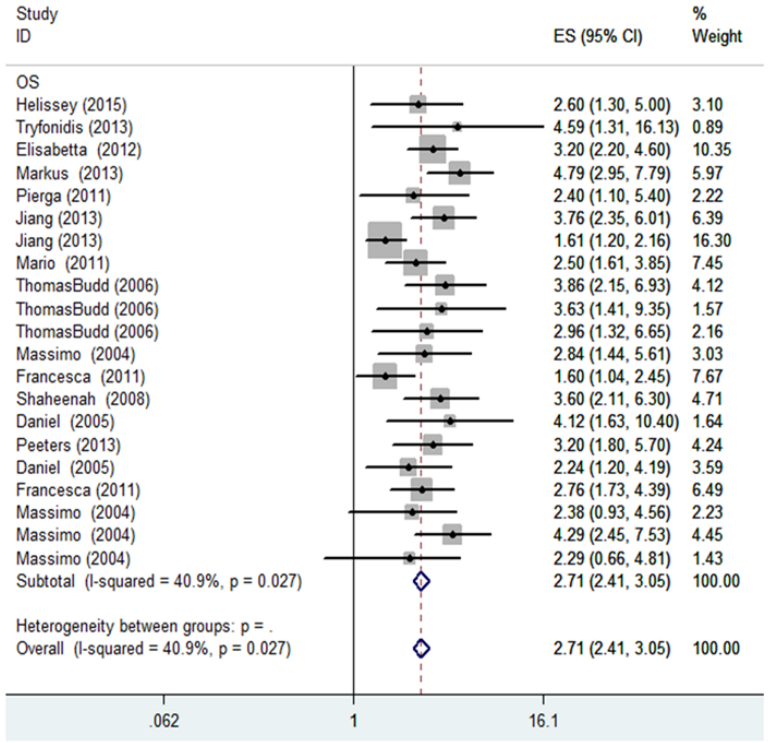
The full text of each eligible study was reviewed and the following information was extracted: first author's name, year of publication, number of patients in the study, and time of blood collection. Survival curves, PFS, OS, HR and 95% CI were also extracted. If HRs and 95% CIs were not reported in the study, these were calculated according to the method previously reported

Table 1. Detailed information of studies included for the meta-analysis

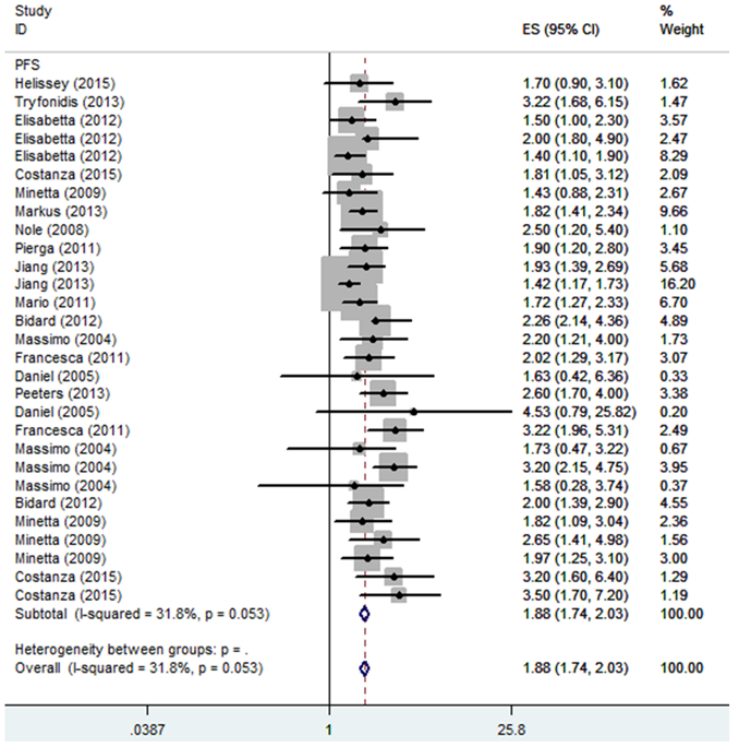
Author	Year	Age (range), month	Median-follow up (range), month	No. of Patients	Cut-off of CTC+ (n/N, %)	Sampling time	Intervention	Outcome
Helissey [7]	2015	60 (NM)	20 (8-30)	56	5 CTC/7.5 ml (24/54, 44.4)	Baseline	Third-line chemotherapy	PFS, OS
					5 CTC/7.5 ml (14/21, 66.7)	Mid-follow-up		
Tryfonidis [8]	2013	62 (23-75)	28 (0.23-42.4)	83	5 CTC/7.5 ml (20/68, 29.4)	Baseline	DEB	PFS, OS
					1 CTC/7.5 ml (39/68, 57.4)			
Elisabetta [9]	2012	57 (31-78)	42 (2-71)	203	5 CTC/7.5 ml (92/203, 45.3)	Baseline	Retrospective analysis	PFS, OS
					1 CTC/7.5 ml (150/203, 73.9)			
Costanza [10]	2015	51 (32-75)	NM (0.6-34.2)	64	5 CTC/7.5 ml (19/52, 36.5)	Baseline	Nab-paclitaxe+/-tigatuzumab	PFS, OS
					5 CTC/7.5 ml (14/52, 26.9)	15 days		
					5 CTC/7.5 ml (13/49, 26.5)	29 days		
Minetta [11]	2009	51.5 (33-88)	13.3 (0.69-39)	74	5 CTC/7.5 ml (25/72, 34.7)	Baseline	Endocrine therapy and Chemotherapy	PFS
					5 CTC/7.5 ml (13/62, 21.0)	3-5 weeks		
					5 CTC/7.5 ml (17/55, 30.9)	7-9 weeks		
Markus [12]	2012	55 (23-91)	11.13 (NM)	445	5 CTC/7.5 ml (189/445, 42.5)	Baseline	Different type of therapy	PFS, OS
Nole [13]	2008	52 (29-77)	8.5 (1.0-19.75)	80	5 CTC/7.5 ml (49/80, 61.3)	Baseline	New treatment	PFS
Pierga [14]	2011	57 (NM)	14.9 (1.5-33)	267	5 CTC/7.5 ml (114/267, 42.7)	Baseline	First-line Chemotherapy	PFS, OS
Jiang [15]	2013	50 (NM)	NM	294	5 CTC/7.5 ml (115/294, 39.1)	Baseline	Retrospective analysis	PFS, OS
Mario [16]	2011	53 (28-82)	18 (1-65)	235	5 CTC/7.5 ml (94/235, 40.0)	Baseline	Chemotherapy	PFS, OS
Thomas [17]	2006	59 (NM)	10.1 (1.9-34.1)	138	5 CTC/7.5 ml (35/138, 25.0)	Baseline	Chemotherapy	OS
Massimo [18]	2004	58 (NM)	NM	177	5 CTC/7.5 ml (87/177, 49.0)	Baseline	Chemotherapy	PFS, OS
					5 CTC/7.5 ml (49/163, 30.0)	First follow-up		
Francesca [19]	2011	58 (33-80)	7.0 (1.0-20.0)	93	5 CTC/7.5 ml (44/93, 47.3)	Baseline	Chemotherapy	PFS, OS
Shaheenah [20]	2008	48.5 (23-84)	15 (NM)	185	5 CTC/7.5 ml (71/185, 38.4)	Baseline	Retrospective analysis	PFS, OS
Cristofanilli [21]	2005	59 (NM)	12.2 (NM)	83	5 CTC/7.5 ml (43/83, 52.0)	Baseline	Chemotherapy	PFS, OS
Peeters [22]	2013	77 (NM)	17.6 (14.7-20.5)	154	5 CTC/7.5 ml (70/154, 45.5)	Baseline	Retrospective analysis	PFS, OS

Abbreviations: DEB, docetaxel (D) plus epirubicin (E) in combination with bevacizumab (B); PFS, progression-free survival; OS, overall survival; NM, not mentioned in the study.

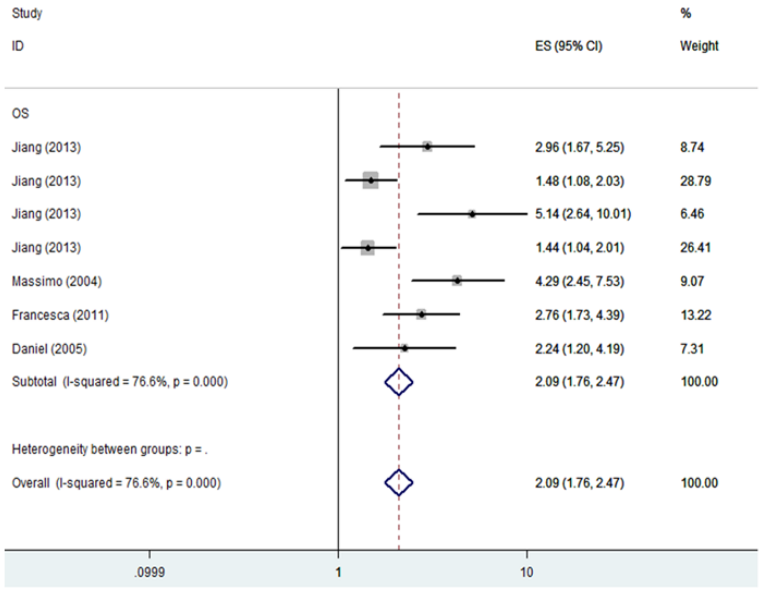
A


Study ID
ES (95% CI)
% Weight
OS
Helissey (2015) 2.60 (1.30, 5.00) 3.10
Tryfonidis (2013) 4.59 (1.31, 16.13) 0.89
Elisabetta (2012) 3.20 (2.20, 4.60) 10.35
Markus (2013) 4.79 (2.95, 7.79) 5.97
Pierga (2011) 2.40 (1.10, 5.40) 2.22
Jiang (2013) 3.76 (2.35, 6.01) 6.39
Jiang (2013) 1.61 (1.20, 2.16) 16.30
Mario (2011) 2.50 (1.61, 3.85) 7.45
ThomasBudd (2006) 3.86 (2.15, 6.93) 4.12
ThomasBudd (2006) 3.63 (1.41, 9.35) 1.57
ThomasBudd (2006) 2.96 (1.32, 6.65) 2.16
Massimo (2004) 2.84 (1.44, 5.61) 3.03
Francesca (2011) 1.60 (1.04, 2.45) 7.67
Shaheenah (2008) 3.60 (2.11, 6.30) 4.71
Daniel (2005) 4.12 (1.63, 10.40) 1.64
Peeters (2013) 3.20 (1.80, 5.70) 4.24
Daniel (2005) 2.24 (1.20, 4.19) 3.59
Francesca (2011) 2.76 (1.73, 4.39) 6.49
Massimo (2004) 2.38 (0.93, 4.56) 2.23
Massimo (2004) 4.29 (2.45, 7.53) 4.45
Massimo (2004) 2.29 (0.66, 4.81) 1.43
Subtotal (I-squared = 40.9%, p = 0.027) 2.71 (2.41, 3.05) 100.00
Heterogeneity between groups: p = .
Overall (I-squared = 40.9%, p = 0.027) 2.71 (2.41, 3.05) 100.00
.062
1
16.1

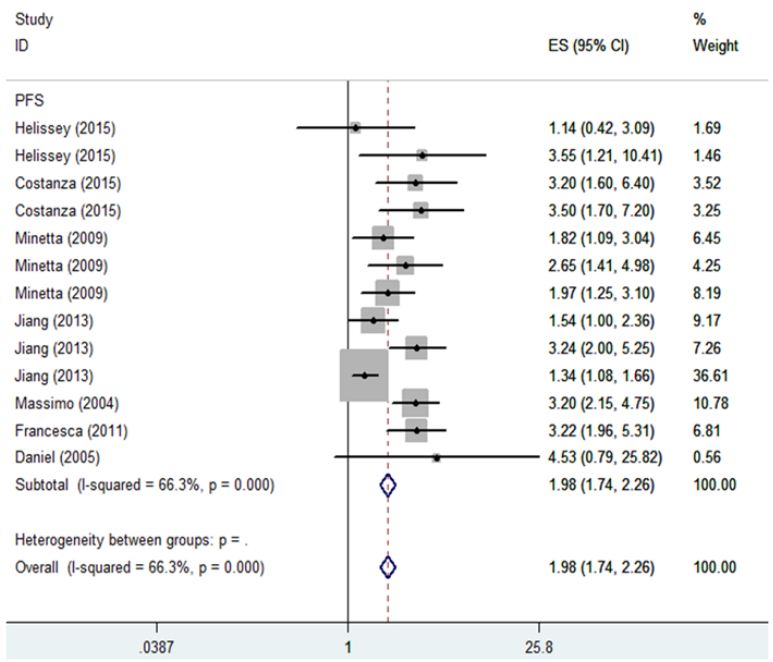
B


Study ID
ES (95% CI)
% Weight
PFS
Helissey (2015) 1.70 (0.90, 3.10) 1.62
Tryfonidis (2013) 3.22 (1.68, 6.15) 1.47
Elisabetta (2012) 1.50 (1.00, 2.30) 3.57
Elisabetta (2012) 2.00 (1.80, 4.90) 2.47
Elisabetta (2012) 1.40 (1.10, 1.90) 8.29
Costanza (2015) 1.81 (1.05, 3.12) 2.09
Minetta (2009) 1.43 (0.88, 2.31) 2.67
Markus (2013) 1.82 (1.41, 2.34) 9.66
Nole (2008) 2.50 (1.20, 5.40) 1.10
Pierga (2011) 1.90 (1.20, 2.80) 3.45
Jiang (2013) 1.93 (1.39, 2.69) 5.68
Jiang (2013) 1.42 (1.17, 1.73) 16.20
Mario (2011) 1.72 (1.27, 2.33) 6.70
Bidard (2012) 2.26 (2.14, 4.36) 4.89
Massimo (2004) 2.20 (1.21, 4.00) 1.73
Francesca (2011) 2.02 (1.29, 3.17) 3.07
Daniel (2005) 1.63 (0.42, 6.36) 0.33
Peeters (2013) 2.60 (1.70, 4.00) 3.38
Daniel (2005) 4.53 (0.79, 25.82) 0.20
Francesca (2011) 3.22 (1.96, 5.31) 2.49
Massimo (2004) 1.73 (0.47, 3.22) 0.67
Massimo (2004) 3.20 (2.15, 4.75) 3.95
Massimo (2004) 1.58 (0.28, 3.74) 0.37
Bidard (2012) 2.00 (1.39, 2.90) 4.55
Minetta (2009) 1.82 (1.09, 3.04) 2.36
Minetta (2009) 2.65 (1.41, 4.98) 1.56
Minetta (2009) 1.97 (1.25, 3.10) 3.00
Costanza (2015) 3.20 (1.60, 6.40) 1.29
Costanza (2015) 3.50 (1.70, 7.20) 1.19
Subtotal (I-squared = 31.8%, p = 0.053) 1.88 (1.74, 2.03) 100.00
Heterogeneity between groups: p = .
Overall (I-squared = 31.8%, p = 0.053) 1.88 (1.74, 2.03) 100.00
.0387
1
25.8

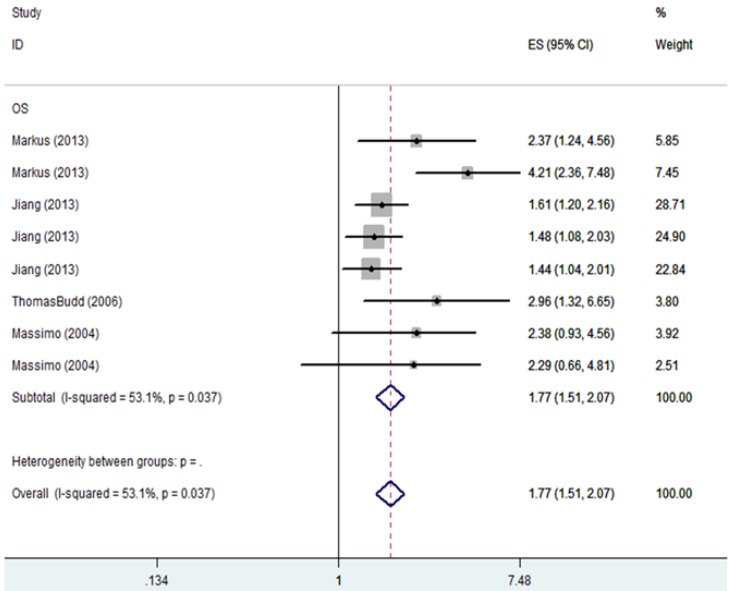
C


Study
ID
ES (95% CI)
%
Weight
OS
Jiang (2013) 2.96 (1.67, 5.25) 8.74
Jiang (2013) 1.48 (1.08, 2.03) 28.79
Jiang (2013) 5.14 (2.64, 10.01) 6.46
Jiang (2013) 1.44 (1.04, 2.01) 26.41
Massimo (2004) 4.29 (2.45, 7.53) 9.07
Francesca (2011) 2.76 (1.73, 4.39) 13.22
Daniel (2005) 2.24 (1.20, 4.19) 7.31
Subtotal (I-squared = 76.6%, p = 0.000) 2.09 (1.76, 2.47) 100.00
Heterogeneity between groups: p = .
Overall (I-squared = 76.6%, p = 0.000) 2.09 (1.76, 2.47) 100.00
.0999
1
10

D


Study
ID
ES (95% CI)
%
Weight
PFS
Helissey (2015) 1.14 (0.42, 3.09) 1.69
Helissey (2015) 3.55 (1.21, 10.41) 1.46
Costanza (2015) 3.20 (1.60, 6.40) 3.52
Costanza (2015) 3.50 (1.70, 7.20) 3.25
Minetta (2009) 1.82 (1.09, 3.04) 6.45
Minetta (2009) 2.65 (1.41, 4.98) 4.25
Minetta (2009) 1.97 (1.25, 3.10) 8.19
Jiang (2013) 1.54 (1.00, 2.36) 9.17
Jiang (2013) 3.24 (2.00, 5.25) 7.26
Jiang (2013) 1.34 (1.08, 1.66) 36.61
Massimo (2004) 3.20 (2.15, 4.75) 10.78
Francesca (2011) 3.22 (1.96, 5.31) 6.81
Daniel (2005) 4.53 (0.79, 25.82) 0.56
Subtotal (I-squared = 66.3%, p = 0.000) 1.98 (1.74, 2.26) 100.00
Heterogeneity between groups: p = .
Overall (I-squared = 66.3%, p = 0.000) 1.98 (1.74, 2.26) 100.00
.0387
1
25.8

E


Study
ID
ES (95% CI)
%
Weight
OS
Markus (2013) 2.37 (1.24, 4.56) 5.85
Markus (2013) 4.21 (2.36, 7.48) 7.45
Jiang (2013) 1.61 (1.20, 2.16) 28.71
Jiang (2013) 1.48 (1.08, 2.03) 24.90
Jiang (2013) 1.44 (1.04, 2.01) 22.84
ThomasBudd (2006) 2.96 (1.32, 6.65) 3.80
Massimo (2004) 2.38 (0.93, 4.56) 3.92
Massimo (2004) 2.29 (0.66, 4.81) 2.51
Subtotal (I-squared = 53.1%, p = 0.037) 1.77 (1.51, 2.07) 100.00
Heterogeneity between groups: p = .
Overall (I-squared = 53.1%, p = 0.037) 1.77 (1.51, 2.07) 100.00
.134
1
7.48

F

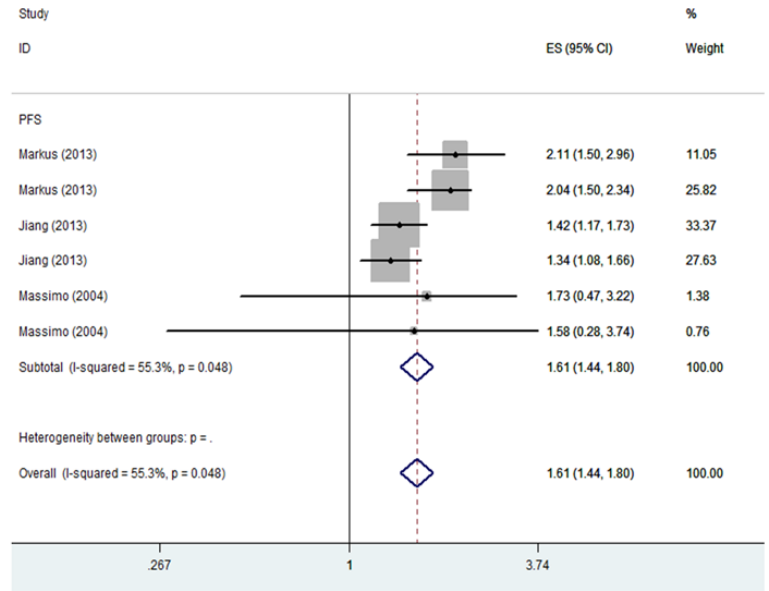

Study
ID
ES (95% CI)
%
Weight
PFS
Markus (2013) 2.11 (1.50, 2.96) 11.05
Markus (2013) 2.04 (1.50, 2.34) 25.82
Jiang (2013) 1.42 (1.17, 1.73) 33.37
Jiang (2013) 1.34 (1.08, 1.66) 27.63
Massimo (2004) 1.73 (0.47, 3.22) 1.38
Massimo (2004) 1.58 (0.28, 3.74) 0.76
Subtotal (I-squared = 55.3%, p = 0.048) 1.61 (1.44, 1.80) 100.00
Heterogeneity between groups: p = .
Overall (I-squared = 55.3%, p = 0.048) 1.61 (1.44, 1.80) 100.00
.267
1
3.74

Figure 2. The forest plot represents the correlation of five CTCs with OS/PFS in MBC. A and B. Describes the HR value of five CTCs with OS and PFS, respectively. C. Represents the subgroup analysis of CTCs with OS for mid-therapy. D. Represents the subgroup analysis of CTCs with PFS for mid-therapy. E. Represents the subgroup analysis of CTCs with OS for the second or subsequent therapies. F. Represents the subgroup analysis of CTCs with PFS for the second or subsequent therapies.

in 1988 and 2007 [24, 25]. If CTCs detection was conducted at different time points, these time points were categorized as "baseline", "mid-follow-up" and "post therapy"; and data at each time point was extracted. In addition, if there was a comparison between the different number of CTCs and prognosis in MBC, or the relationship between CTCs and different subtypes or other markers were described, all of these results were recorded as independent data sets. OS and PFS were the two significant clinical outcome indicators used as endpoints in our study.

Statistic analysis

Literature quality evaluation was analyzed as described by Mc Shane, among which they reported the recommendations for the research on tumor markers of prognosis [26]. Heterogeneity between these studies was assessed by Q-test and the I^2-index, and potential publication bias was evaluated by the Egger and Begg's test and funnel plot. All meta-analyses were conducted using STATA version 12.0 and pooled HR for survival using the random-effects model when I^2>50% and the fixed-effects model when I^2≤50%. Additionally, subgroup analyses on the correlation of CTCs with MBC subtypes and different therapeutic methods were also performed. All statistical tests were two-sided, and a *P*-value <0.05 was considered statistically significant.

Results

General characteristics of included studies

After the initial search based on the inclusion criteria, a total of 17 studies [7-23] that involved 2,631 patients were included for the final meta-analysis. All these studies employed the same technique (CellSearch System) for blood sample detection. CTCs detection were conducted at baseline time before treatment implementation in 11 studies [7-9, 12-14, 16-18, 21, 23], while the remaining studies [10, 11, 15, 19, 20, 22] carried out CTCs testing at baseline and mid-therapy time points. Two studies [8, 9] analyzed the association between five CTCs or one CTC and their correlation with patient survival. A flowchart that describes the selection process of studies for this meta-analysis is presented in **Figure 1**, and the general characteristics of these selected studies are summarized in **Table 1**.

Analysis of the correlation between the identification of five CTCs and survival in MBC patients

The effect of CTCs on patient survival in all 17 studies was first analyzed. OS and PFS were assessed as outcome indicators based on HR. CTCs detection included baseline and mid-therapy time points. Eight studies [10, 11, 15, 17-20, 22] recorded a >1 HR value due to multiple time points or various treatment methods. The overall assessment of the HR value indicates that the identification of CTCs was significantly associated with short survival time in all MBC patients. HR value of the correlation between five CTCs and OS was 2.71 (95% CI: 2.41, 3.05; *P*=0.027; fixed effects model), as shown in **Figure 2A**; while HR value of the correlation between five CTCs and PFS was 1.88 (95% CI: 1.74, 2.03; *P*=0.053; fixed effects model), as shown in **Figure 2B**. In addition, a series of hierarchical analysis were also carried out including the correlation of five CTCs with patient prognosis when CTCs were detected at the mid-therapy time point or after the second or subsequent therapies. These subgroup analysis has again demonstrated that the association between five CTCs and poor prognosis was remarkably affirmative and strong, irrespective of the time of blood specimen collection for CTCs detection (OS: HR=2.50 [95% CI: 1.73, 3.61], *P*=0.000, random effects; PFS: HR=2.33 [95% CI: 1.80, 3.01], *P*=0.000, random effects), as shown in **Figure 2C** and **2D**. Similarly, this association was positive, irrespective of the kind of chemotherapeutic treatment used (OS: HR=1.98 [95% CI: 1.53, 2.56], P=0.037, random effects; PFS: HR=1.66 [95% CI: 1.36, 2.02], P=0.048, random effects), as shown in **Figure 2E** and **2F**.

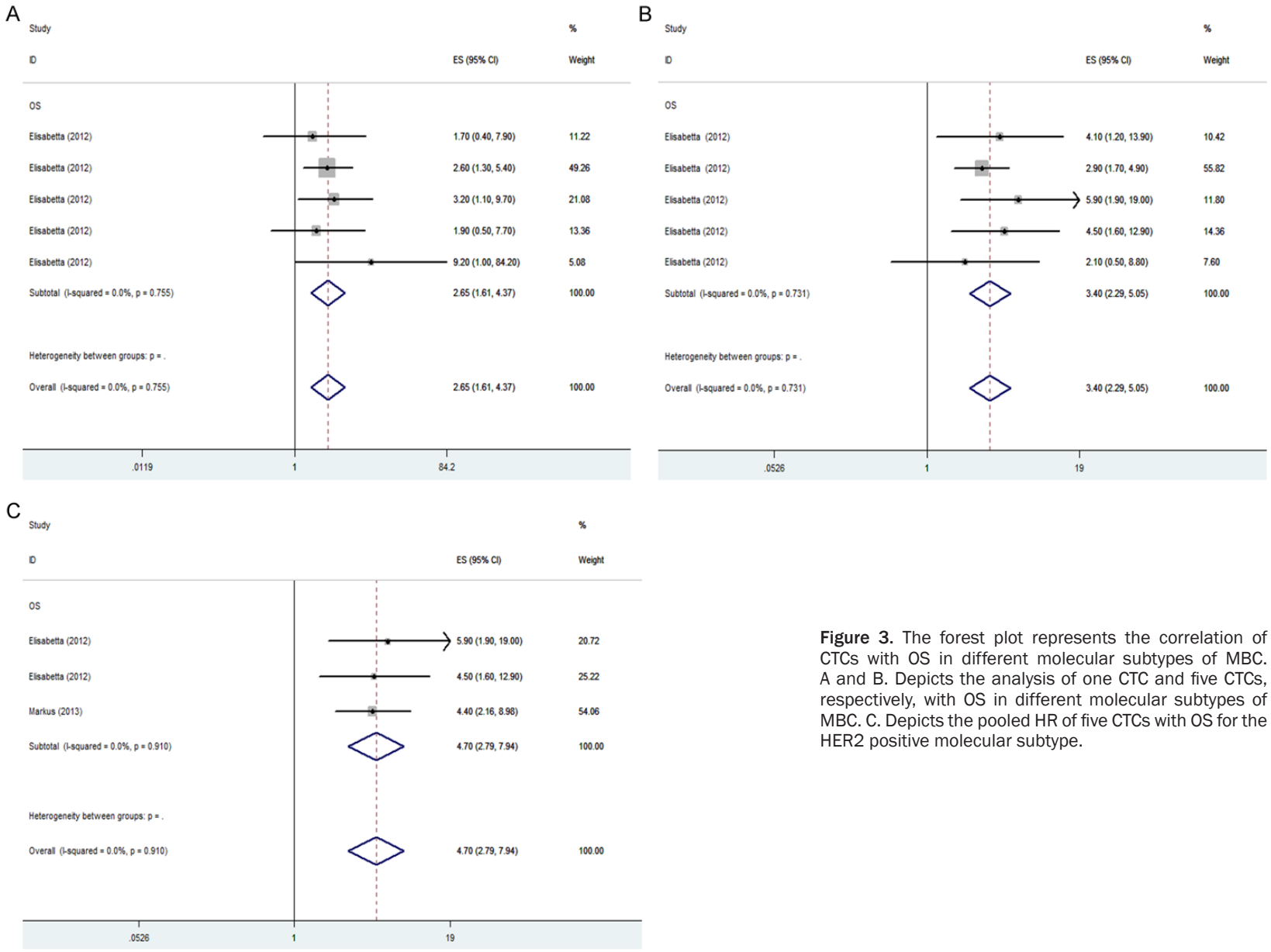
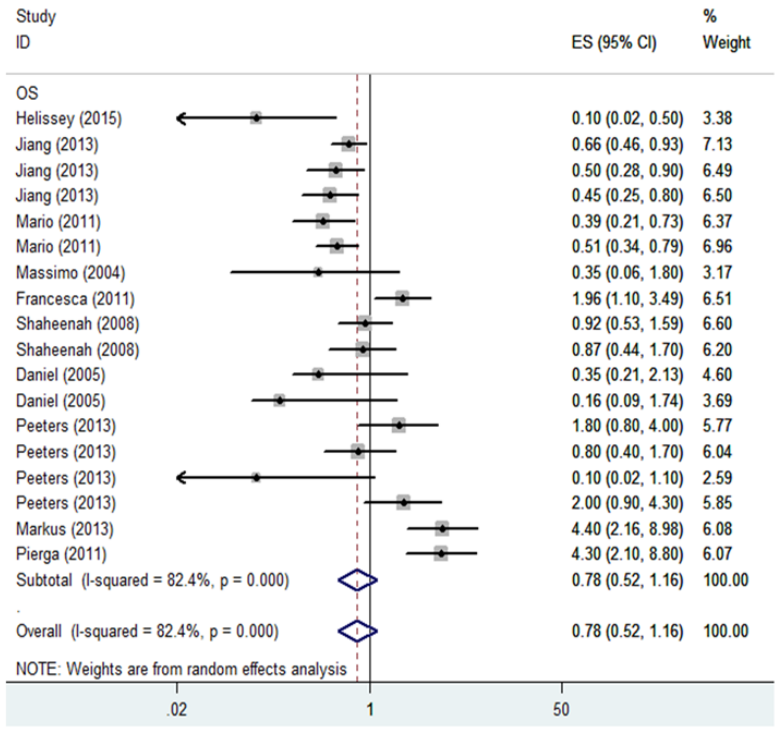
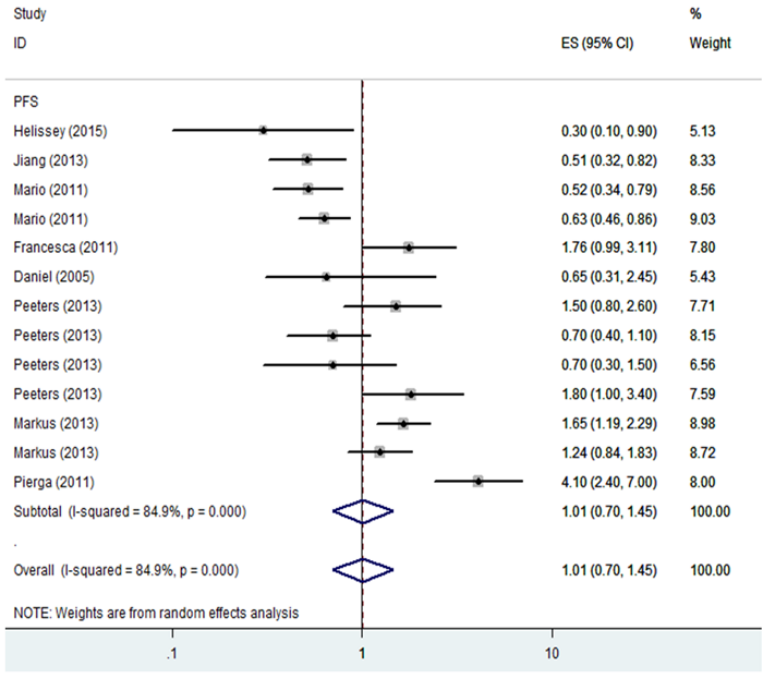


Figure 3. The forest plot represents the correlation of CTCs with OS in different molecular subtypes of MBC. A and B. Depicts the analysis of one CTC and five CTCs, respectively, with OS in different molecular subtypes of MBC. C. Depicts the pooled HR of five CTCs with OS for the HER2 positive molecular subtype.

A


Study
ID
ES (95% CI)
%
Weight
OS
Helissey (2015) 0.10 (0.02, 0.50) 3.38
Jiang (2013) 0.66 (0.46, 0.93) 7.13
Jiang (2013) 0.50 (0.28, 0.90) 6.49
Jiang (2013) 0.45 (0.25, 0.80) 6.50
Mario (2011) 0.39 (0.21, 0.73) 6.37
Mario (2011) 0.51 (0.34, 0.79) 6.96
Massimo (2004) 0.35 (0.06, 1.80) 3.17
Francesca (2011) 1.96 (1.10, 3.49) 6.51
Shaheenah (2008) 0.92 (0.53, 1.59) 6.60
Shaheenah (2008) 0.87 (0.44, 1.70) 6.20
Daniel (2005) 0.35 (0.21, 2.13) 4.60
Daniel (2005) 0.16 (0.09, 1.74) 3.69
Peeters (2013) 1.80 (0.80, 4.00) 5.77
Peeters (2013) 0.80 (0.40, 1.70) 6.04
Peeters (2013) 0.10 (0.02, 1.10) 2.59
Peeters (2013) 2.00 (0.90, 4.30) 5.85
Markus (2013) 4.40 (2.16, 8.98) 6.08
Pierga (2011) 4.30 (2.10, 8.80) 6.07
Subtotal (I-squared = 82.4%, p = 0.000) 0.78 (0.52, 1.16) 100.00
Overall (I-squared = 82.4%, p = 0.000) 0.78 (0.52, 1.16) 100.00
NOTE: Weights are from random effects analysis
.02
1
50

B


Study
ID
ES (95% CI)
%
Weight
PFS
Helissey (2015) 0.30 (0.10, 0.90) 5.13
Jiang (2013) 0.51 (0.32, 0.82) 8.33
Mario (2011) 0.52 (0.34, 0.79) 8.56
Mario (2011) 0.63 (0.46, 0.86) 9.03
Francesca (2011) 1.76 (0.99, 3.11) 7.80
Daniel (2005) 0.65 (0.31, 2.45) 5.43
Peeters (2013) 1.50 (0.80, 2.60) 7.71
Peeters (2013) 0.70 (0.40, 1.10) 8.15
Peeters (2013) 0.70 (0.30, 1.50) 6.56
Peeters (2013) 1.80 (1.00, 3.40) 7.59
Markus (2013) 1.65 (1.19, 2.29) 8.98
Markus (2013) 1.24 (0.84, 1.83) 8.72
Pierga (2011) 4.10 (2.40, 7.00) 8.00
Subtotal (I-squared = 84.9%, p = 0.000) 1.01 (0.70, 1.45) 100.00
Overall (I-squared = 84.9%, p = 0.000) 1.01 (0.70, 1.45) 100.00
NOTE: Weights are from random effects analysis
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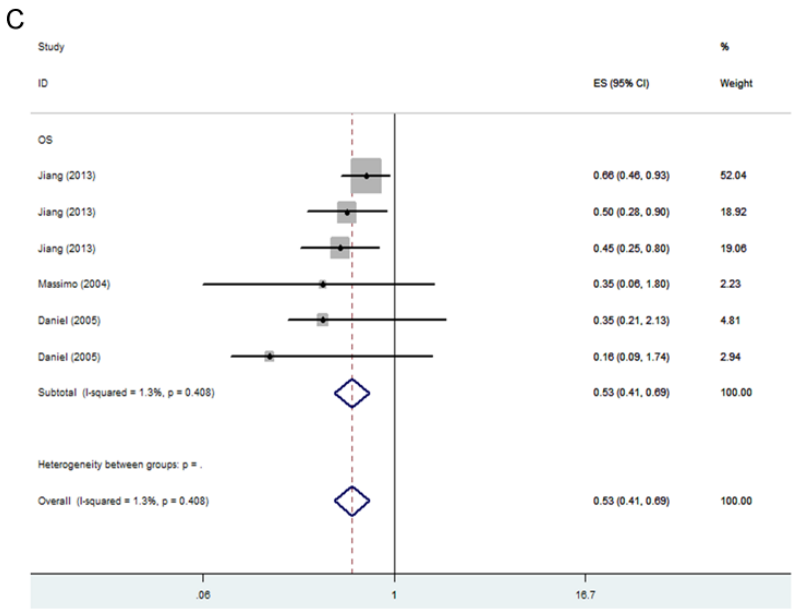

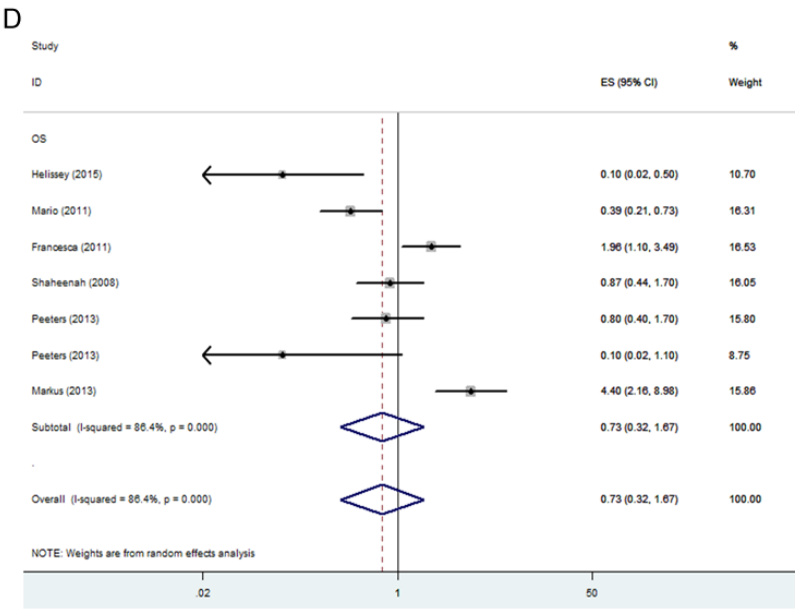

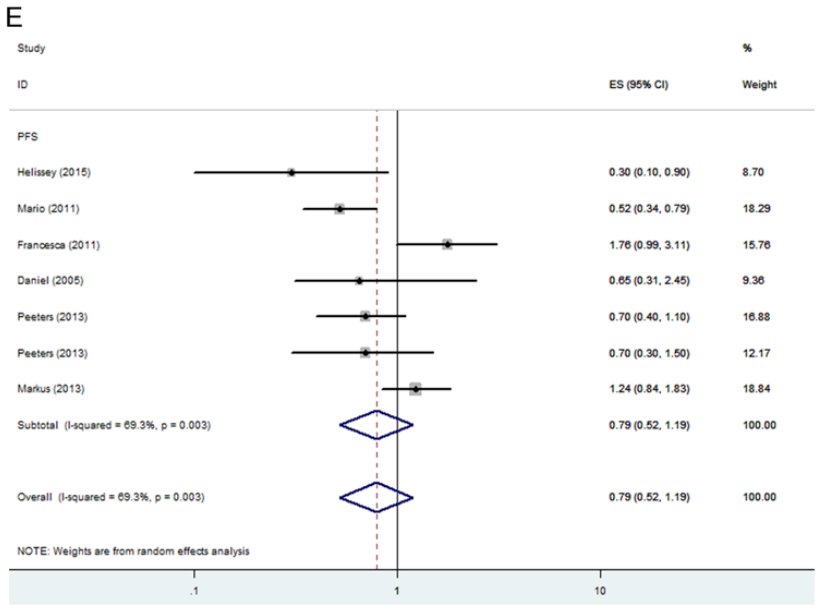

Figure 4. Forest plot of HR for OS/PFS in clinicopathological parameters influence on the survival. (A and B) Independently describes HR for molecular subtypes with OS and PFS. Significant factors analysis in (C) (PR/ER+ and OS); (D) (HER2+ and OS); (E) (HER2+ and PFS).

Thus, this meta-analysis consistently reveals that the presence of five CTCs was significantly associated with poor OS and PFS, but was not associated with blood sampling time and treatment methods.

Analysis of the correlation between the identification of CTCs and survival in MBC patients from different molecular subtypes

The analysis of five CTCs in 7.5 mL of blood has been generally used as a positive reference by many researchers, despite the contrary belief of smaller research communities that the identification of one CTC in 7.5 mL of blood is sufficient to act as a positive indicator. This in fact led to the publication of very few studies that correlated data based on the identification of only one CTC. In this meta-analysis, only two studies [8, 9] were identified to be consistent with our inclusion criteria. Thus, we assessed the association between the identification of one CTC and OS in different MBC molecular subtypes such as Luminal A (ER/PR+, HER2-), Luminal B (ER/PR+, HER2+), Non-luminal HER2 (ER-/PR-/HER2+), Triple Negative (ER-/PR-/HER2-). The pooled HR value was 2.65 (95% CI: 1.61, 4.37; *P*=0.755; fixed effects), as shown in **Figure 3A**.

Similarly, the pooled HR value of the association between the identification of five CTCs and OS in different molecular subtypes was also assessed. Cumulative HR value was 3.40 (95% CI: 2.29, 5.05; *P*=0.731, fixed effects), as seen in **Figure 3B**. Moreover, a separate analysis on human epidermal growth factor receptor-2 (HER2) molecular subtypes based on two different studies has also suggested that the identification of five CTCs was correlated with shorter OS, with a HR value of 4.70 (95% CI: 2.79, 7.94; *P*=0.910; fixed effects), as shown in **Figure 3C**. These results conclude that CTCs ≥1 and ≥5 display a similar correlation with serious outcome in MBC in all molecular subtypes.

HER2 positive 5CTCs/7.5 ml and the significant clinicopathological parameters influence on the survival in MBC

Intriguingly, HER2 positive independently impersonated a significantly negative impact on poor short time with OS and PFS (OS: HR=0.73, 95% CI [0.32, 1.67], *P*=0.000, random effects; PFS: HR=0.79 95% CI [0.52, 1.19], *P*=0.003, random effects) (**Figure 4D** and **4E**). Simultaneously, PR/ER+ was not negatively associated with survival time for OS (HR=0.53 95% CI [0.41, 0.69], *P*=0.408, fixed effects). (**Figure 4C**) Moreover, we generally analyzed the connection between MBC molecular subtypes and the survival, the pooled HR for OS revealed a negatively relevant with overall survival (HR=0.78 95% CI [0.52, 1.16], *P*=0.000, random effects) (**Figure 4A**). However, for PFS, there showed a statistically irrelevant (HR= 1.01, 95% CI [0.70, 1.45], *P*=0.000, random effects) (**Figure 4B**). Therefore, more studies are needed to evaluate the relationship between each subtype and the survival situation.

Other targets effects on the survival in the MBC

Location of metastasis and how many metastatic sites are probably associative with the survival in tumor patients. Here, We analyzed whether ≥2 metastatic sites had an effects on PFS and OS and the results were not all consistent with our preconception (OS: HR=1.47 95% CI [1.17, 1.86], *P*=0.453, fixed effects; PFS: HR=1.50, 95% CI [1.15, 1.94], *P*=0.025, random effects) (**Figure 5A** and **5B**). Analysis of the reasons for this result may be due to not enough statistically significant studies. Carcinoembryonic antigen (CEA) as a spectrum tumor marker abnormally appeared in periphery blood of tumor patients like colorectal cancer, breast cancer and lung cancer, and acted as a monitoring tumor marker to estimate the prognosis. In our study, 3 researches concluded that CEA is not as important as previously thought (HR=1.73 95% CI [1.34, 2.25], *P*=0.779, fixed effects) (**Figure 5C**).

Publication bias analysis

Publication bias among studies that described the relationship between five CTCs and OS or PFS using Begg's funnel plot were analyzed, as shown in **Figure 6A** and **6B**. Results revealed no obvious publication bias among these studies.

Discussion

There are multitudinous methods of treatment for breast cancer, and the therapeutic efficiency affected by many factors, including the occurrence of metastasis which is one of the

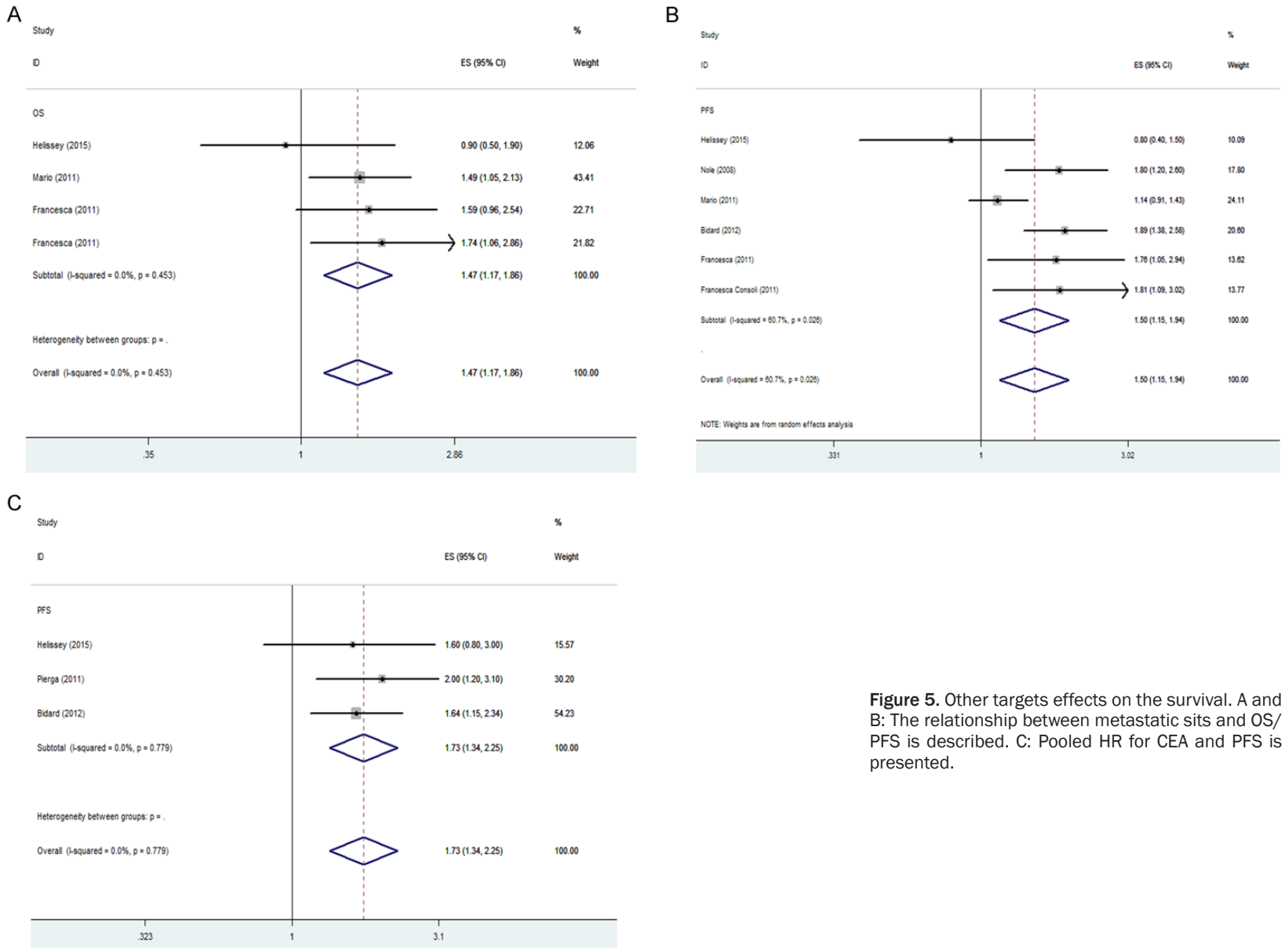

Figure 5. Other targets effects on the survival. A and B: The relationship between metastatic sits and OS/PFS is described. C: Pooled HR for CEA and PFS is presented.

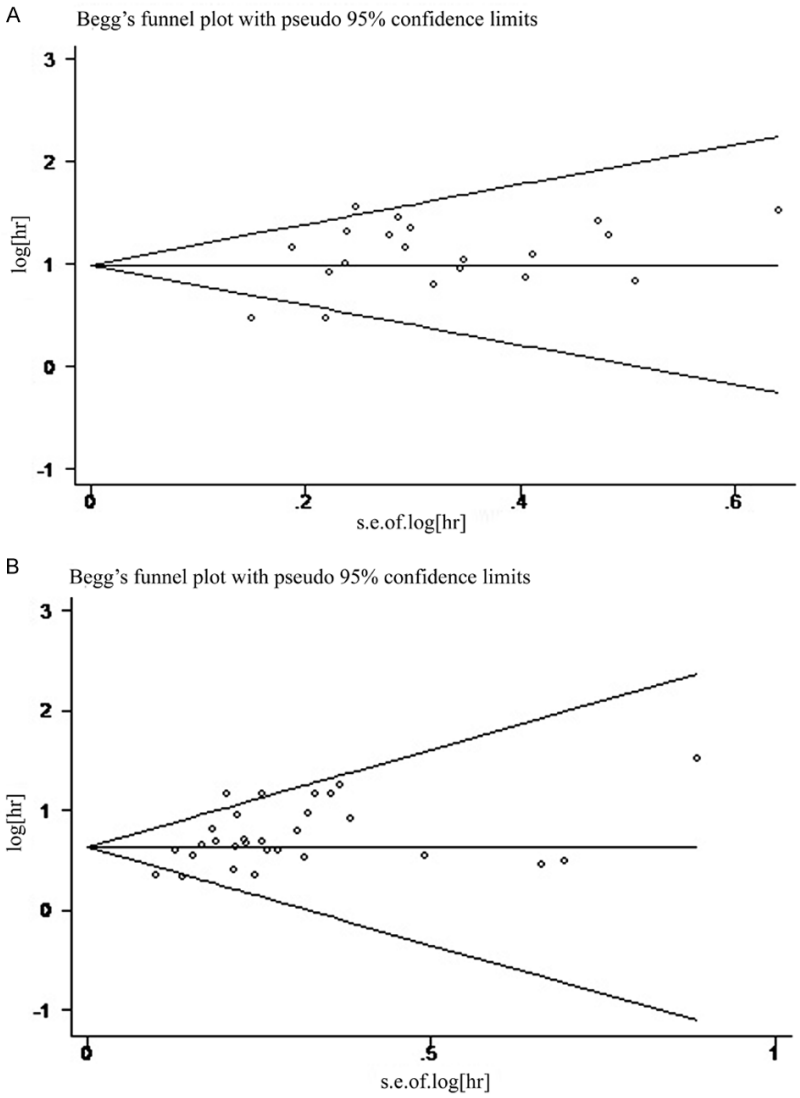

Figure 6. Funnel plot of publication bias for OS and PFS.

important factors influencing the treatments. Based on this problem, to discover metastasis of breast cancer as early as possible and adapt treatments have been urgent requirement of clinicians. From previous studies, we know that CTCs play a key role in tumor of distant metastasis, which used as a biomarker affords to predict the prognosis of patients with cancer [27]. In existing literatures, only two meta-analysis studies explored the role of CTCs in breast cancer prognosis. These studies were conducted based on the assumption that the appearance of CTCs in blood could be used as an independent prognostic indicator in breast cancer, without analyzing MBC patients [5, 6]. Therefore, in our meta-analysis, we focused on testing the ability of CTCs to predict MBC prognosis. Our study performed a comprehensive analysis of relevant and high-quality clinical studies that included 17 articles and 2,631 patients. The association of CTCs was correlated with PFS and OS in MBC patients. Furthermore, miscellaneous methods have been described for the detection of CTCs including the CellSearch® system, which is the only method approved by the FDA in the USA for clinical application. Thus, in our analysis, only studies that employed the CellSearch® system for detection were included to eliminate interference due to diverse testing methods. Simultaneously, we also performed a pooled data analysis to assess the relationship between CTCs detection and survival in different molecular breast cancer subtypes, especially HER2; which is regarded as an important poor prognostic factor in breast cancer [28].

Our meta-analysis results indicate that the appearance of CTCs was significantly associated with short survival time for PFS and OS in MBC, which is consistent with previous published studies. This observation led us to conclude that as long as CTCs can be easily detected in blood in MBC patients, this can be directly used as an independent noninvasive biomarker that would undoubtedly benefit patients. Current studies have employed common methods such as RT-PCR and CellSearch techniques to detect CTCs in patient samples. Based on a review by Van der Auwera I *et al.*, only 36% of MBC patients were identified positive for CTCs using the CellSearch system, 26% of MBC patients were identified positive for CK-19 gene using RT-PCR, and 54% of MBC patients were identified positive for mammaglobin gene using RT-PCR [29].

However, detection efficiency was not very high. Thus, further research is required to improve the sensitivity of these detection techniques.

The specific association between CTCs and poor prognosis was further confirmed by the subgroup meta-analysis of CTCs collected at different time points and their association with OS and PFS. This subgroup analysis suggests that CTCs can act as a reliable prognostic indicator at every stage of treatment, starting from detection at the baseline time point. This also led the investigators of the present study to speculate that CTCs can be developed for clinical applications. Generally, CTCs are defined as positive when five or more are detected in 7.5 ml of blood. A study by Tryfonidis [8] evaluated the influence of identifying one CTC on survival at baseline time before treatment initiation and its consequences. They suggested that this had a statistically significant correlation with worse prognosis in MBC (OS: HR=2.4, 95% CI [0.7, 8.5], *P*=0.027; PFS: HR=2.1, 95% CI [0.9, 4.6], *P*=0.004). In addition, Elisabetta further discussed the conceivable relationship between ≥1 CTCs and survival [OS: HR=2.7 (1.7-4.3); PFS: HR=1.6 (1.1-2.3); *P*<0.01], and demonstrated that the definition of positive CTCs cannot be re-defined [9].

In addition, a series of subgroup meta-analysis including the subtypes and the second or further lines of therapy were carried out in this study. Subtypes were classified by immunohistochemistry based on expression of progesterone receptor (PR), estrogen receptor (ER), and human epidermal growth factor receptor-2 (HER-2). Respectively named as Luminal A (ER/PR+, HER2-), Luminal B (ER/PR+, HER2+), Non-luminal HER2 (ER-/PR-/HER2+), Triple Negative (ER-/PR-/HER2-). The CTC enumeration were detected in all molecular subtypes, including HER2+ (Luminal B and Non-luminal) and ER/PR+ (Luminal A and Luminal B) [30], but the related studies in our review were few and the specific correlation between HER2+ or ER/PR+ and the prognosis is always controversial, hence we only performed analysis regarding whether the molecular subtypes have an effect on survival. Particularly, among those studies, the results demonstrate that HER2+ and ER/PR+ were not associated with prognosis in MBC, in accordance with the previous studies [5]. With the use of combination chemotherapy patterns [31]: different combinations, doses, or sequencing could well produce moderate but worthwhile additional benefits, and the appropriate duration of treatment with current chemotherapeutic and hormonal regimens remains uncertain, especially among patients at substantial risk of late recurrence [32]. Chemotherapy is designed to kill as many tumor cells as possible with the use of 'maximum tolerated doses' or MTDs and is most frequently-used in cancer patients [33]. Currently, first line, second line, third line and further fourth line therapy are implemented in clinical work. Is the first line therapy superiorly better than others? With this question, the meta-analysis of the effect of second or further line therapy on survival was examined [HR: OS, 0.66 (0.30, 1.43); PFS, 0.86 (0.57, 1.30), P=0.007]. In a way, the results could suggest that the clinical response of first line drugs is not always the most effective; this could make modification treatment of dependant on some of patient clinical features.

There are many reasons for bias. On one hand, it could be due to the methodology of the study, as before mentioned, some studies reported less detailed results that the hazard ratio of 95% CI, so there studies were unlikely to be evaluated. Of 10 studies excluded for the meta-analysis due to a lack of enough data, some researches acted as if significant estimated values were not included. On the other hand, the heterogeneity of the patient populations can lead to bias, the potential population bias makes it hazardous to generalize the results of the meta-analysis of subgroups of patients that were not included in the data aggregation the same as previous description [34]. Our research also has certain limitation including the appearance of bias, secondly a considerable degree of difference in each study were regarded as another limitation reason. Such as the demographic study samples, breast cancer types, and the blood testing time, the difference of equipment, the therapeutic methods, all of these should be considered as potential sources of heterogeneity. However, we disposed the heterogeneity by a strict methodological method that uses a random-effects model for more conservative estimates. At least a minimum of 3 researches to implement amalgamative analyses and only with a minimum sample of 50 patients brought into studies. On the other side, respective subgroup analysis was conducted to appraise possibility

initiators of bias and the scanning inters study heterogeneity. It is undeniable that the present studies are limited. More studies are needed to support the conclusion.

In summary, our study supports the viewpoint that CTCs indeed have a stable prognostic value in blood sample analysis. Consequently, it may be speculated that CTCs detection during therapeutic management could guide towards treatment adjustment based on the individual prognosis of MBC patients. In order to realize the clinical application of CTCs in breast cancer, more clinical trials, especially uninterrupted multicenter studies, are required to further evaluate the significance of using CTCs as a primitive approach for monitoring its response to systemic therapy.

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Disclosure of conflict of interest

None.

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